

Microalgal cultivation for biomass production, carbon dioxide capture and nutrients uptake

Ana Luísa da Cunha Gonçalves

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Ana Luísa da Cunha Gonçalves

Supervision

Manuel José Vieira Simões (PhD) and José Carlos Magalhães Pires (PhD)
LEPABE, Department of Chemical Engineering, Faculty of Engineering, University of
Porto

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Thesis outputs

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A.L. Gonçalves, J.C.M. Pires, M. Simões, 2016. Wastewater polishing by consortia of *Chlorella vulgaris* and activated sludge native bacteria. *Journal of Cleaner Production* 133 pp. 348-357.

A.L. Gonçalves, M.C.M. Alvim-Ferraz, F.G. Martins, M. Simões, J.C.M. Pires, 2016. Integration of microalgae-based bioenergy production into a petrochemical complex: techno-economic assessment. *Energies* 9 pp. 224-240.

A.L. Gonçalves, J.C.M. Pires, M. Simões, 2016. The effects of light and temperature on microalgal growth and nutrient removal: an experimental and mathematical approach. *RSC Advances* 6 pp. 22896-22907.

A.L. Gonçalves, C.M. Rodrigues, J.C.M. Pires, M. Simões, 2016. The effect of increasing CO₂ concentrations on its capture, biomass production and wastewater bioremediation by microalgae and cyanobacteria. *Algal Research* 14 pp. 127-136.

A.L. Gonçalves, J.C.M. Pires, M. Simões, 2016. Biotechnological potential of *Synechocystis salina* co-cultures with selected microalgae and cyanobacteria: nutrients removal, biomass and lipid production. *Bioresource Technology* 200 pp. 279-286.

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Ethics statement

Except elemental analysis of biomass, all the experiments and data analysis were performed by the PhD student. Elemental analysis of biomass was performed in REQUIMTE (Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa) by the technician Carla Rodrigues. Since our laboratory did not have the facilities to perform these experiments, a collaboration was established between our research group and REQUIMTE. Accordingly, the lyophilized samples were sent to REQUIMTE, where elemental analysis of biomass was carried out.

Within the scope of this thesis, the following manuscripts have been accepted in scientific periodicals with referees:

- A.L. Gonçalves, J.C.M. Pires, M. Simões, 2016. A review on the use of microalgal consortia for wastewater treatment. *Algal Research*. In press.

Authors' contributions: ALG wrote the manuscript; JCMP helped in manuscript revision; MS helped in manuscript revision.

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The student

The Supervisors

Ana Gonçalves

Manuel Simões

José Pires

Abstract

The accumulation of carbon dioxide (CO₂) in the atmosphere, as well as the enrichment of water courses in nutrients are environmental issues associated to numerous impacts on ecosystems. Several attempts have been made to address these issues, but the cost and sustainability of current methodologies are still a concern. Cultivation of photosynthetic microorganisms appears as a sustainable solution. Accordingly, the aim of this study was to develop a microalgal production system able to achieve increased biomass productivities and effectively uptake CO₂ and nutrients from the culture medium. To accomplish these goals, two different strategies were evaluated: (i) the use of microalgal monocultures with the optimization of cultivation parameters; and (ii) the use of microalgal and microalgal-bacterial consortia. The photosynthetic microorganisms selected to perform these experiments were *Chlorella vulgaris*, *Pseudokirchneriella subcapitata*, *Synechocystis salina* and *Microcystis aeruginosa*. Taking into account the results obtained at laboratory-scale, the best photosynthetic cultivation systems and conditions were performed in a 60-L open raceway pond. The achieved results were then used to perform a techno-economic assessment (TEA) of a microalgal production unit integrated in a petrochemical complex, aiming to reduce its associated environmental impact.

Regarding the first strategy, the selected microorganisms were grown in monocultures and the influence of light supply, temperature and CO₂ concentration in the air stream were evaluated. Regarding light supply, microalgal growth was performed under different light irradiance values (36, 60, 120 and 180 $\mu\text{E m}^{-2} \text{s}^{-1}$) and different light:dark ratios (10:14, 14:10 and 24:0). This study has demonstrated that higher light irradiance values and light periods resulted in higher average biomass productivities, CO₂ uptake rates and nutrients removal efficiencies. Furthermore, results have shown that *C. vulgaris*, *S. salina* and *M. aeruginosa* presented the highest average biomass productivities and CO₂ uptake rates. In terms of nitrogen removal efficiencies, all microalgal strains showed high removal efficiencies (close to 100%). Phosphorus removal increased with light irradiance and with light:dark ratio, but maximum value achieved was $67.6 \pm 7.1\%$. To evaluate the combined effect of light and temperature, experiments were performed at different average daily light irradiances (15, 21, 36, 75, 105 and 180 $\mu\text{E m}^{-2} \text{s}^{-1}$) and temperatures (15, 25 and 35 °C). In this study, a mathematical model relating specific growth rates with these variables was also developed. Among the studied temperatures, all microorganisms presented higher biomass

productivities, CO₂ uptake rates and nutrients removal efficiencies at 25 °C. Regarding the results from the mathematical model, the optimal temperature determined for the selected microorganisms was 25.3±1.1 °C. On the other hand, the optimal average daily light irradiances varied with the species in the range of 140-258 μE m⁻² s⁻¹. In the study of the effect of CO₂ concentrations in the air stream (0.04-10% v/v), *C. vulgaris*, *S. salina* and *M. aeruginosa* have reached the highest biomass productivities and CO₂ fixation rates. These microorganisms have also been effective in nutrients uptake, reaching removal efficiencies close to 100%. Through mathematical modelling, it was possible to conclude that optimal CO₂ concentration for these microorganisms is 5.4±0.3% (v/v).

In the second strategy, dual-species microalgal consortia and dual-species microalgal-bacterial consortia of *C. vulgaris* and activated sludge native bacteria were performed. In the study regarding microalgal consortia, co-cultivation of the selected microorganisms with *S. salina* has resulted in increased average biomass productivities, CO₂ uptake rates and nutrients removal efficiencies than those obtained with single-species cultures. The consortium composed by *S. salina* and *C. vulgaris* (SC consortium) was considered the most promising one. The study regarding microalgal-bacterial consortia has shown that consortia of *C. vulgaris* with selected bacterial isolates from a municipal wastewater treatment plant (MWTP) resulted in an increased biomass production and nutrients removal. The best performance was achieved by the consortium composed by *C. vulgaris* and *Enterobacter asburiae* (CE consortium).

The cultivation of *C. vulgaris* and the two referred consortia was evaluated in an open raceway pond in indoor and outdoor conditions. The results obtained in indoor conditions were not satisfactory due to the poor light distribution inside the bioreactor. However, *C. vulgaris* growth in this system with natural light conditions has confirmed the potential of this microalga for biomass production, CO₂ uptake and nitrogen and phosphorus removal from the culture medium. Taking into account the experimental results, a TEA of a 100-ha microalgal biomass production unit was performed. This assessment has resulted in the proposal of an economically viable process of microalgal production in Portugal concerning wastewater treatment (1.9×10⁷ m³ of wastewater per year), CO₂ emission saving (1.1×10⁴ t yr⁻¹) and bioenergy production purposes (annual electric energy production of 1.6×10⁷ kWh and annual lipids productivity of 1.9×10³ m³).

Keywords: CO₂ capture; Microalgae; Microalgal biomass production; Microalgal monocultures; Microalgal consortia; Nutrients removal; Techno-economic assessment.

Resumo

A acumulação de dióxido de carbono (CO₂) na atmosfera assim como o aumento da concentração de nutrientes nos cursos de água constituem problemas ambientais graves, devido ao seu impacto negativo nos ecossistemas. No sentido de atenuar estes problemas, diferentes metodologias têm sido propostas, mas o custo e sustentabilidade das metodologias utilizadas atualmente limitam a sua aplicação. Recentemente, a cultura de microrganismos fotossintéticos surgiu como uma alternativa sustentável. O objetivo deste estudo foi desenvolver um sistema de produção de microalgas capaz de atingir elevadas produtividades de biomassa e, ao mesmo tempo, capturar CO₂ adicionado ao meio de cultura e remover nutrientes presentes no mesmo. Duas estratégias diferentes foram avaliadas: (i) utilização de monoculturas de microalgas com otimização das variáveis operacionais; e (ii) utilização de consórcios de microalga-microalga ou microalga-bactéria. Os microrganismos fotossintéticos selecionados para estes ensaios foram *Chlorella vulgaris*, *Pseudokirchneriella subcapitata*, *Synechocystis salina* e *Microcystis aeruginosa*. Tendo por base os resultados obtidos à escala laboratorial, os sistemas e condições de cultura que mostraram maior potencial foram reproduzidos num reator aberto com 60 L de capacidade. Os resultados obtidos foram utilizados no desenvolvimento de um estudo tecno-económico que pretendia avaliar a viabilidade económica da produção de microalgas integrada num complexo petroquímico, com o objetivo de reduzir os seus impactos ambientais.

Na primeira estratégia os microrganismos selecionados foram cultivados em monoculturas, tendo-se avaliado o efeito dos seguintes parâmetros de cultura: luz, temperatura e concentração de CO₂ na corrente gasosa. Relativamente ao efeito da luz, o crescimento das microalgas foi realizado sob diferentes valores de irradiância (36, 60, 120 e 180 $\mu\text{E m}^{-2} \text{s}^{-1}$) e diferentes fotoperíodos (10:14, 14:10 e 24:0). Este estudo demonstrou que maiores valores de irradiância e maiores períodos de luz resultam em maiores produtividades de biomassa, taxas de remoção de CO₂ e eficiências de remoção de nutrientes. Adicionalmente, os resultados mostraram que as espécies *C. vulgaris*, *S. salina* e *M. aeruginosa* foram as que apresentaram maiores produtividades de biomassa e taxas de remoção de CO₂. Em termos de remoção de nutrientes, todas as espécies apresentaram elevadas eficiências de remoção de azoto (próximas de 100%). A remoção de fósforo aumentou com o aumento da irradiância e do período de luz, sendo o máximo valor obtido $67.6 \pm 7.1\%$. Para avaliar o efeito combinado da luz e temperatura, foram realizados ensaios sob diferentes irradiâncias (15,

21, 36, 75, 105 e 180 $\mu\text{E m}^{-2} \text{s}^{-1}$) e temperaturas (15, 25 e 35 °C). Neste estudo foi desenvolvido um modelo matemático que relaciona as taxas específicas de crescimento com estas duas variáveis. Das temperaturas estudadas, todos os microrganismos apresentaram maiores produtividades de biomassa, taxas de remoção de CO_2 e eficiências de remoção de nutrientes a 25 °C. De acordo com os resultados do modelo matemático, a temperatura ótima para os microrganismos selecionados foi de 25.3 ± 1.1 °C. Por outro lado, a irradiância ótima variou de acordo com as espécies: 240-258 $\mu\text{E m}^{-2} \text{s}^{-1}$. No estudo sobre o efeito da concentração de CO_2 na corrente gasosa (0.04-10% v/v), as espécies *C. vulgaris*, *S. salina* e *M. aeruginosa* foram as que apresentaram maiores produtividades de biomassa e taxas de remoção de CO_2 . Estes microrganismos foram também eficientes na remoção de nutrientes, tendo atingido eficiências de remoção próximas de 100%. Através do desenvolvimento de um modelo matemático, foi possível determinar a concentração de CO_2 ótima: $5.4 \pm 0.3\%$ (v/v).

Na segunda estratégia, diferentes consórcios foram estudados: consórcios constituídos por duas espécies de microalgas e consórcios constituídos pela microalga *C. vulgaris* e uma bactéria isolada de uma amostra de lamas ativadas. No estudo correspondente aos consórcios de microalgas, a co-cultura dos microrganismos referidos anteriormente com *S. salina* resultou em maiores produtividades de biomassa, taxas de remoção de CO_2 e eficiências de remoção de nutrientes, sendo o consórcio SC (composto por *S. salina* e *C. vulgaris*) o mais promissor. No estudo relativo aos consórcios de microalga-bactéria, verificou-se que a co-cultura de *C. vulgaris* com bactérias resultantes de lamas ativadas resultou num aumento da produtividade de biomassa e da remoção de nutrientes, sendo que o consórcio mais eficiente foi o CE (composto por *C. vulgaris* e *Enterobacter asburiae*).

Por último, foram realizados ensaios num reator aberto de 60 L (em laboratório e em condições exteriores) utilizando *C. vulgaris* e os dois consórcios referidos anteriormente. Neste estudo, o crescimento em laboratório não foi satisfatório devido a uma pobre distribuição da luz no reator. Contudo, o crescimento de *C. vulgaris* em condições de luz natural confirmou o potencial desta espécie para a produção de biomassa, captura de CO_2 e remoção de nutrientes. Tendo em conta os resultados obtidos experimentalmente, fez-se um estudo tecno-económico para uma unidade de

produção de microalgas. Com a realização deste estudo, foi possível propor um processo economicamente viável de produção de microalgas, remoção de nutrientes (1.9×10^7 m³ de água residual processada por ano), captura de CO₂ (1.1×10^4 t ano⁻¹) e produção de bioenergia (produção elétrica anual de 1.6×10^6 kWh e produção lipídica anual de 1.9×10^3 m³).

Palavras-chave: Captura de CO₂; Microalgas; Produção de biomassa; Monoculturas de microalgas; Consórcios de microalgas; Remoção de nutrientes; Estudo tecno-económico.

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Nomenclature

Acronyms

AD	Anaerobic digestion
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
ATPase	Adenosine triphosphate synthase
BOD	Biochemical oxygen demand
CCAP	Culture Collection of Algae and Protozoa
CCS	Carbon capture and storage
CE	<i>Chlorella vulgaris</i> + <i>Enterobacter asburiae</i> consortium
CF	Cash-flow
CFD	Computational Fluid Dynamics
CFU	Colony forming units
CHP	Combined heat and power
CK	<i>Chlorella vulgaris</i> + <i>Klebsiella</i> sp. consortium
COD	Chemical oxygen demand
CR	<i>Chlorella vulgaris</i> + <i>Raoultella ornithinolytica</i> consortium
DL	Detection limit
DO	Dissolved oxygen
DW	Dry weight
EC	European Commission
EROEI	Energy returned on energy invested
EU	European Union
FAS	Ferrous ammonium sulphate
GHG	Greenhouse gas
HRAP	High rate algal pond
IEA	International Energy Agency
IRR	Internal rate of return
JRC	Joint Research Centre
LEGE	Laboratory of Ecotoxicology, Genomic and Evolution
MWTP	Municipal wastewater treatment plant
NADP ⁺	Nicotinamide adenine dinucleotide phosphate

NADPH	Reduced form of NADP ⁺
NH ₃ -N	Ammonia-nitrogen
NH ₄ -N	Ammonium-nitrogen
NO ₂ -N	Nitrite-nitrogen
NO ₃ -N	Nitrate-nitrogen
NPV	Net present value
OD	Optical density
OECD	Organization for Economic Co-operation and Development
PBR	Photobioreactor
PCI	Plant cost index
PE	Population equivalent
PEF	Pulsed electric field
P _i	Inorganic phosphate
PMMA	Poly(methyl methacrylate)
PO ₄ -P	Phosphate-phosphorus
PSI	Photosystem I
PSII	Photosystem II
QL	Quantification limit
RMSE	Root mean squared error
rpm	Rotations per minute
SC	<i>Synechocystis salina</i> + <i>Chlorella vulgaris</i> consortium
SM	<i>Synechocystis salina</i> + <i>Microcystis aeruginosa</i> consortium
SP	<i>Synechocystis salina</i> + <i>Pseudokirchneriella subcapitata</i> consortium
TEA	Techno-economic assessment
TN	Total nitrogen
TOC	Total organic carbon
TP	Total phosphorus
UK	United Kingdom
USA	United States of America
vvm	Volume of gas per working volume per minute
WWTP	Wastewater treatment plant

Notation

C	CO ₂ concentration in the air stream (% v/v)
C_{opt}	Optimal CO ₂ concentration in the air stream (% v/v)
I	Average daily light irradiance ($\mu\text{E m}^{-2} \text{s}^{-1}$)
I_{opt}	Optimal average daily light irradiance ($\mu\text{E m}^{-2} \text{s}^{-1}$)
k	Nutrients uptake rate (d^{-1})
M_{CO_2}	CO ₂ molecular weight (g mol^{-1})
M_C	Carbon molecular weight (g mol^{-1})
M_{FAS}	FAS molecular weight (g mol^{-1})
n	Data size
N	Cell concentration (cells mL^{-1} or CFU mL^{-1})
N_1	Cell concentration in the beginning of the exponential growth phase (cells mL^{-1} or CFU mL^{-1})
N_2	Cell concentration at the end of the exponential growth phase (cells mL^{-1} or CFU mL^{-1})
P	Average biomass productivity ($\text{mg DW L}^{-1} \text{d}^{-1}$)
r	Interest rate
R	Nutrients removal efficiency (%)
R^2	Coefficient of determination
R_C	Average CO ₂ uptake rate ($\text{mg CO}_2 \text{L}^{-1} \text{d}^{-1}$)
R_N	Average nitrogen uptake rate ($\text{mg N L}^{-1} \text{d}^{-1}$)
R_P	Average phosphorus uptake rate ($\text{mg P L}^{-1} \text{d}^{-1}$)
R_S	Average nutrients uptake rate ($\text{mg S L}^{-1} \text{d}^{-1}$)
$S(t)$	Time-course evolution of nutrients concentration (mg S L^{-1})
S_i	Nutrients concentration in the beginning of the cultivation time (mg S L^{-1})
S_f	Nutrients concentration at the end of the cultivation time (mg S L^{-1})
t	Time (d)
t_1	Beginning of the exponential growth phase (d)
t_2	End of the exponential growth phase (d)
t_i	Beginning of the cultivation time (d)
t_f	End of the cultivation time (d)
T	Temperature ($^{\circ}\text{C}$)
T_{opt}	Optimal temperature ($^{\circ}\text{C}$)
$V_{\text{FAS}; B}$	Volume of FAS used in the titration of the blank (mL)

$V_{FAS; S}$	Volume of FAS used in the titration of the samples (mL)
V_S	Sample's volume (mL)
X	Biomass concentration (mg DW L ⁻¹)
X_1	Biomass concentration in the beginning of the exponential growth phase (mg DW L ⁻¹)
X_2	Biomass concentration at the end of the exponential growth phase (mg DW L ⁻¹)
X_i	Biomass concentration in the beginning of the cultivation time (mg DW L ⁻¹)
X_f	Biomass concentration at the end of the cultivation time (mg DW L ⁻¹)
y	Dependent variable
z	Experimental values
\hat{z}	Predicted values by the model
\bar{z}	Average of the experimental values
α_C	Carbon mass fraction in microalgal biomass (% w/w)
α_N	Nitrogen mass fraction in microalgal biomass (% w/w)
α_P	Phosphorus mass fraction in microalgal biomass (% w/w)
β_0	Constant term
β_i	Coefficients of the linear parameters
β_{ii}	Coefficients of the quadratic parameters
β_{ij}	Coefficients of the interaction parameters
ε	Residual associated to the experiments
λ	Lag time (d)
μ	Specific growth rate (d ⁻¹)
μ_{max}	Maximum specific growth rate (d ⁻¹)
σ	Standard deviation associated with the optimal temperature (°C)

1. Work outline

1.1. Scientific relevance and motivation

In the last decades, CO₂ emissions to the atmosphere have drastically increased, raising its concentration to approximately 40% higher than pre-industrial period levels (Tans and Keeling, 2015). This increase has been associated with several negative environmental impacts, such as the increase of greenhouse effect and ocean acidification; therefore, it becomes urgent for world economies to reduce their CO₂ emissions (IEA, 2011; O'Neill and Oppenheimer, 2002). Additionally, since this greenhouse gas (GHG) presents long residence times (Keith, 2009; Moss et al., 2010), new strategies to reduce CO₂ concentration in the atmosphere and in flue gas emissions are required (Pielke, 2009). At the same time, the population increase and anthropogenic activities (agricultural practices, urbanization and industrialization) have contributed to the scarcity of freshwater and to an excessive discharge of wastes into water bodies. These practices have contributed to the contamination of freshwater resources and to an increase in nutrients (mainly nitrogen and phosphorus) levels (Aslan and Kapdan, 2006; Rawat et al., 2011; Renuka et al., 2013). Nutrients enrichment or eutrophication is responsible for the development of algal blooms, spread of aquatic plants, oxygen depletion, loss of key species and degradation of freshwater ecosystems (Renuka et al., 2013; Ruiz et al., 2013). To avoid these problems European Union (EU) adopted policies regarding climate action and nutrients discharge in water bodies. To ensure that the global average temperature does not exceed pre-industrial levels by more than 2 °C (EC, 2008), 20% reduction in GHG emissions was proposed for developing countries by 2020. Decision 406/2009/EC (2009) defines for each EU Member State the individual targets for GHG emissions during the period from 2013 to 2020. According to this decision, Portugal has a

positive limit, i.e. its GHG emissions can increase by 1% by 2020 compared to 2005 levels. In the case of discharged effluents, EU has defined limits for nutrients (nitrogen and phosphorus) concentration and imposed minimum percentage load reductions in sensitive areas which are subject to eutrophication (Directive 1991/271/EEC, 1991; Directive 1998/15/EC, 1998). According to these Directives, the limits for effluent discharge per population equivalent (PE) are: (i) 15 mg N L⁻¹ (10 to 100 thousand PE) or 10 mg N L⁻¹ (more than 100 thousand PE) for total nitrogen, or a minimum percentage of reduction of 70-80%; and (ii) 2 mg P L⁻¹ (10 to 100 thousand PE) or 1 mg P L⁻¹ (more than 100 thousand PE) for total phosphorus, or a minimum percentage of reduction of 80%.

To achieve these goals, different methodologies have been extensively studied to reduce CO₂ levels in the atmosphere and to promote the treatment of wastewaters. Regarding CO₂ capture, currently applied methods include absorption, adsorption, gas-separation membranes and cryogenic distillation (Gibbins and Chalmers, 2008; Pires et al., 2011). However, these methodologies, applied in carbon capture and storage (CCS) technologies, are considered short-term solutions, as there are still high processing costs and concerns about the environmental sustainability of CO₂ storage (Pires et al., 2011). On the other hand, nitrogen and phosphorus removal from wastewaters is performed as part of the tertiary treatment step of wastewater treatment plants (WWTPs) and is commonly known as wastewater polishing. The most commonly used methods in this step include anaerobic digestion followed by nitrification and denitrification (Fulazzaky et al., 2015; Piao et al., 2015). However, several nitrification and denitrification cycles are required to achieve the nutrient levels accepted by EU legislation. Additionally, these methods require several tanks, internal recycles of activated sludge and long hydraulic residence times, resulting in an overall increase of process costs, complexity and energy input (Foess et al., 1998; Jeyanayagam, 2005; Singh et al., 2005). Alternatively, nitrogen and phosphorus removal may be achieved by chemical methods, such as precipitation using aluminium and iron salts. However, these methods are costly and produce large amounts of sludge contaminated with chemical compounds, requiring further treatment (Bernard and Rémond, 2012; Malhotra et al., 1964; Wang et al., 2006; Zang et al., 2015).

As an alternative to the commonly applied techniques for CO₂ sequestration and nutrients removal from wastewaters, cultivation of photosynthetic microorganisms, such as the eukaryotic microalgae and the prokaryotic cyanobacteria, has emerged in the last decades.

When growing autotrophically, microalgae assimilate CO₂ from the atmosphere or from flue gas emissions, reducing the concentrations of this GHG in the atmosphere (Allen et al., 2009; Ho et al., 2011; Tang et al., 2011). These microorganisms also require inorganic sources of nitrogen and phosphorus for their growth, meaning that they can play an important role in the tertiary treatment step of wastewater remediation (Rawat et al., 2011; Silva-Benavides and Torzillo, 2012). In addition to the remediation potential described for these microorganisms, microalgal biomass can be further used for human food and animal feed and in the production of drugs, cosmetics, functional food, biofuels and fertilizers (Allen et al., 2009; Brennan and Owende, 2010; Parmar et al., 2011; Singh et al., 2005; Spolaore et al., 2006). In addition to the wide variety of biotechnological applications described for microalgae, cultivation of these microorganisms has several advantages. They present higher growth rates and higher biomass productivities than other photosynthetic organisms, such as terrestrial crops (Chisti, 2007), and also higher lipid contents and nutritional values (Pulz and Gross, 2004). Additionally, microalgae can be grown in non-arable land and require far less land than terrestrial crops, thus not competing with agricultural practices and not compromising food production and supply. These microorganisms can also grow in a wide variety of environmental conditions and in low quality waters, reducing the requirements for freshwater and nutrients (Chisti, 2007). However, cultivation of these microorganisms still presents some challenges regarding the achievement of high biomass productivities and removal (of both CO₂ and nutrients) efficiencies at reduced costs.

To improve biomass productivities in microalgal cultures, one possible alternative is to study the process variables that can influence the success of microalgal cultivation. Microalgal growth can be influenced by several factors, such as light, temperature, pH, salinity, nutrient qualitative and quantitative profiles, dissolved oxygen concentration and the presence of toxic compounds (Barsanti and Gualtieri, 2006; Hu, 2004a; Kumar et al., 2010; Yen et al., 2013). Among these parameters, light supply, temperature and CO₂ concentration appear as the most important factors influencing microalgal and cyanobacterial growth. In fact, photoautotrophic growth is driven by light supply, the energy source that is used to convert inorganic carbon into organic matter, and CO₂, the most commonly used inorganic carbon source. At the same time, changes in temperature can easily disturb microalgal growth, since the metabolic activity of these photosynthetic microorganisms can be ceased by extreme temperatures. Another possibility is to evaluate the potential of different microalgal consortia (microalgal and microalgal-bacterial) on biomass production, in order to establish

an effective system in terms of CO₂ capture and nutrients removal. Recently, several studies have reported the potential of these consortia in different applications, including biomass production, CO₂ capture and nutrients removal (Muñoz and Guieysse, 2006; Olguín, 2012; Ramanan et al., 2016; Rawat et al., 2011; Subashchandrabose et al., 2011; Unnithan et al., 2014). The use of polycultures for these purposes can be very advantageous because combining microorganisms with different metabolic activities and adapted to different environmental conditions results in a robust biological system that can operate under different environmental conditions (Boonma et al., 2014; Fouilland, 2012; Johnson and Admassu, 2013). Additionally, cooperative interactions can be established between the microorganisms integrating the consortia, which can result in higher biomass productivities and uptake rates (Renuka et al., 2013).

1.2. Main objectives

The present work aimed the development of a microalgal production system able to achieve increased biomass productivities and effectively uptake CO₂ and nutrients from the culture medium. To accomplish this goal three different strategies (schematically represented in Figure 1.1) were adopted:

(i) Evaluation of the effects of light supply, temperature and CO₂ concentration in the air stream on biomass production, CO₂ capture and nutrients uptake

In this first step, monocultures of *Chlorella vulgaris*, *Pseudokirchneriella subcapitata*, *Synechocystis salina* and *Microcystis aeruginosa* were performed to evaluate the effect of light supply (light irradiance and light:dark ratio), the combined effect of light and temperature and the effect of different CO₂ concentrations in the air stream on biomass production, CO₂ capture and nutrients uptake and to determine optimal light, temperature and CO₂ concentrations for microalgal growth. The experimental assays were performed at laboratory-scale, using 500-mL flasks. To determine optimal growth conditions for the studied microorganisms, mathematical models were established using the obtained experimental data. Due to the wide variety of microalgal species, the study and optimization of culture parameters for all these microorganisms can be costly and time-consuming. Accordingly, mathematical modelling constitutes an important tool for growth prediction and characterization.

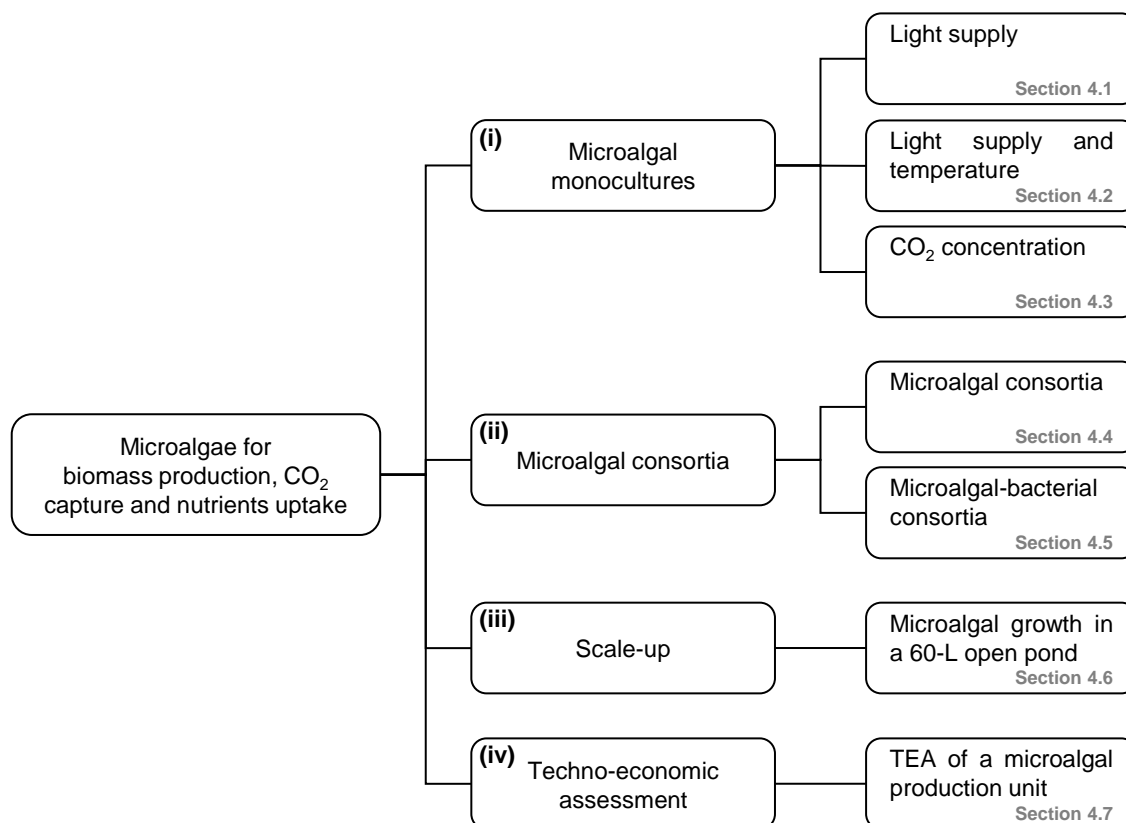


Figure 1.1. Schematic representation of the topics covered in this thesis.

(ii) Evaluation of the performance of different microalgal and microalgal-bacterial consortia in biomass production, CO₂ capture and nutrients uptake

Using the above described microalgae and taking into account the determined optimal growth conditions, different combinations were performed to obtain dual-species cultures and to evaluate their performance in biomass production, CO₂ capture and nutrients uptake. Additionally, microalgal-bacterial dual-species cultures were evaluated. In these experiments, *C. vulgaris* was co-cultured with different bacteria isolated and identified from the activated sludge tank of a MWTP located in Rabada (Santo Tirso, Portugal). The experiments regarding microalgal consortia were also performed at laboratory-scale.

(iii) Evaluation of the best systems in a pilot-scale unit

The microorganism and microalgal consortia presenting the highest efficiencies in terms of biomass production, CO₂ capture and nutrients uptake were selected to validate their applicability at a higher scale. In these experiments, the selected cultures were performed in

a 60-L open raceway pond, a system commonly used in large-scale production of microalgae (Borowitzka, 1999; Jiménez et al., 2003; Tredici and Materassi, 1992).

(iv) Techno-economic assessment of microalgal production

Taking into account the obtained results, a TEA of a microalgal production unit for wastewater treatment, CO₂ capture and bioenergy production integrated in a petrochemical complex was performed taking into account local weather conditions.

The remainder of this thesis is outlined as follows: in Section 2 a review of the literature is presented; in Section 3 the methods used during this work are presented; in Section 4 the results obtained in this study and respective discussion are presented; in Section 5 the main conclusions and suggestions for future work are presented.

2. Literature review

2.1. Microalgae

Microalgae are a broad category of photosynthetic microorganisms that can be found in aquatic or terrestrial ecosystems (Gouveia, 2011; John et al., 2011). The number of microalgal species is not known, but the numbers estimated in the literature round the 200,000 to some millions of species (Norton et al., 1996). Due to their unicellular or multicellular simple structure, microalgae are able to grow in a wide variety of environmental conditions, even in the most extreme ones (Cellamare et al., 2010; de Morais and Costa, 2007c; Mutanda et al., 2011; Pereira et al., 2011; Richardson et al., 2000; Valladares, 2004): (i) they can grow in dimly lit sites, as well as in salt-marsh algal mats exposed to full sunlight; (ii) although most microalgae are mesophilic, some species can be found in mountain snowfields, hot springs and desert soil, experiencing extreme temperature ranges; (iii) microalgae can also be found in hypersaline environments; and (iv) some microalgal species are also able to grow in extreme acidic and extreme alkaline media. Being submerged in an aqueous environment is also favourable for microalgal development, since their access to water, CO₂ and other nutrients becomes easier, thus promoting an efficient conversion of solar energy into biomass (Gouveia, 2011; John et al., 2011).

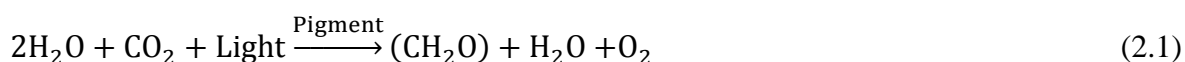
These organisms are classified in different groups, according to some parameters, such as morphologic characteristics, cell wall and photosynthetic pigments composition and chemical nature of their by-products (Brennan and Owende, 2010; Tomaseli, 2004). Prokaryotic cells, known as cyanobacteria (*Cyanophyceae*), resemble more to bacteria rather than algae. They do not have membrane-bound organelles, such as plastids, mitochondria, nuclei, Golgi bodies and flagella. On the other hand, eukaryotic cells have the above

mentioned organelles, which are responsible for cell control, reproduction and survival. These microorganisms are taxonomically rooted with the ancestor of land plants, an organism formed by the endosymbiosis between a heterotrophic eukaryotic host cell and a cyanobacterium, which was thought to act as a plastid (Yoon et al., 2004). Examples of eukaryotic microalgae include green algae (*Chlorophyta*), red algae (*Rhodophyta*) and diatoms (*Bacillariophyta*).

Microalgae are mainly autotrophic organisms: they reproduce themselves through photosynthesis, requiring only inorganic compounds, such as CO₂, to convert solar light energy into chemical energy. However, some microalgae can grow mixotrophically, using facultatively an organic carbon source in addition to CO₂, or even heterotrophically, using only organic carbon as carbon source (Brennan and Owende, 2010; Leite et al., 2013).

2.2. Microalgal photosynthesis

Photosynthesis is described as the biological conversion of light energy into chemical energy in the form of organic carbon compounds. In this oxidation-reduction reaction, represented in Equation 2.1, organic compounds are formed from the reduction of CO₂. CO₂ is an oxidized and inorganic carbon source that requires an energy input to form organic compounds (Falkouski and Raven, 2007).



In Equation 2.1 light acts as a substrate, which means that solar light is the energy source that allows the conversion of inorganic carbon into high energetic organic compounds (Falkouski and Raven, 2007).

In eukaryotic organisms, photosynthesis takes place in chloroplasts. This process is divided into two phases (Figure 2.1): light reactions and dark reactions (Falkouski and Raven, 2007; Ho et al., 2011). Light reactions occur in the presence of light, whereas dark or carbon-fixation reactions occur both in the presence or absence of light. For light reactions, two consecutive photosystems located in the thylakoid membrane of chloroplasts are required (Ho et al., 2011). These photosystems are photochemical reaction centres energetically coupled with antennae, which harvest the light energy and transfer it to the reaction centre.

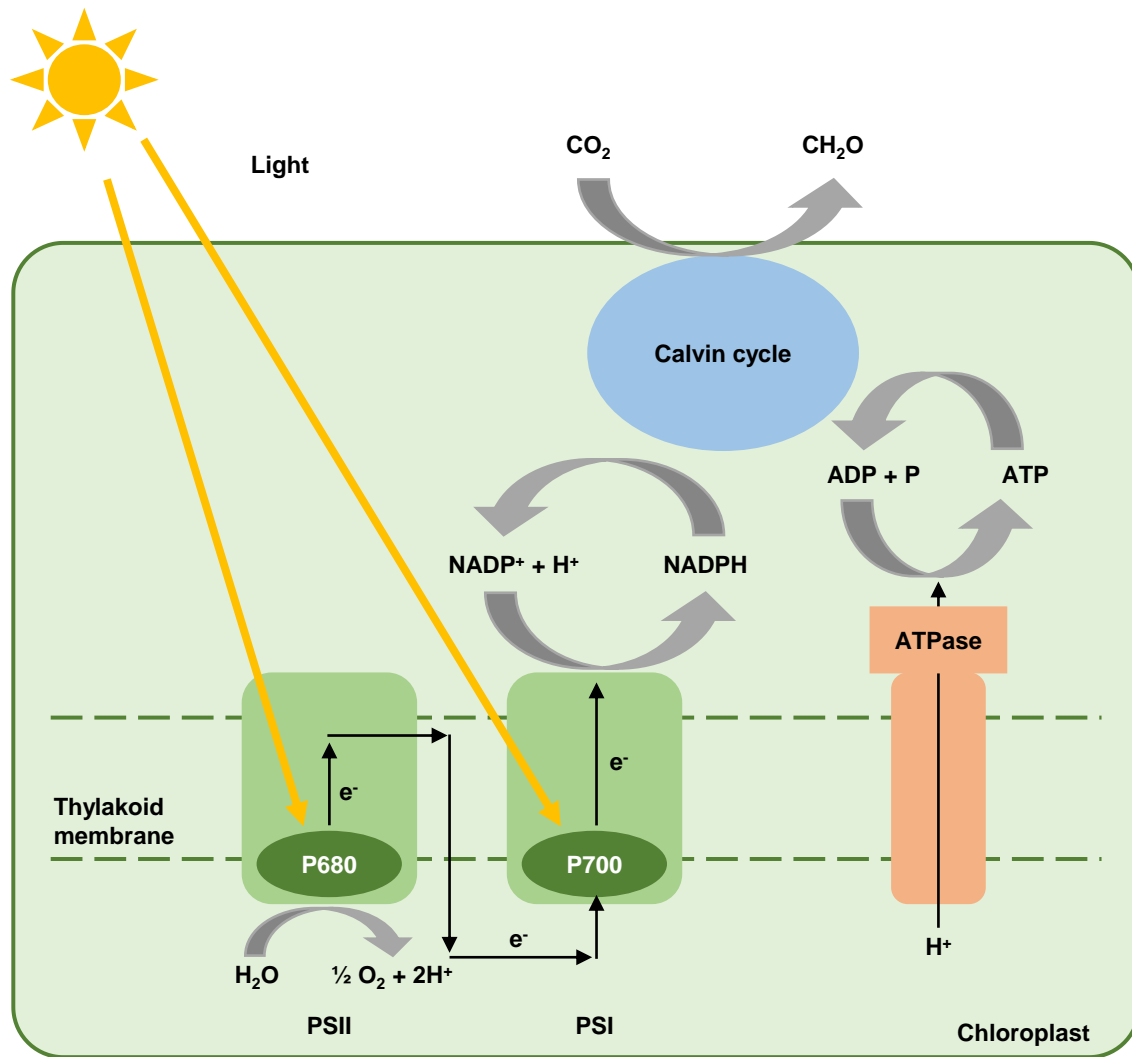
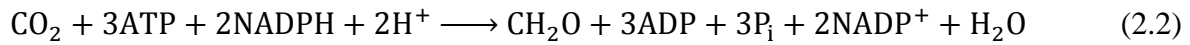


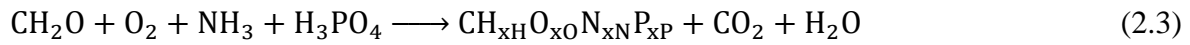
Figure 2.1. Schematic representation of photosynthesis: energy from sun light is acquired by PSI and PSII where electron transport takes place with the formation of NADPH and ATP that are further used in CO_2 fixation reactions, represented by Calvin cycle (adapted from Ho et al. (2011)).

Pigment composition of the two photosystems is different, which explains the difference between the two action spectra (Falkowski and Raven, 2007). The process begins in photosystem II (PSII), which absorbs photons at 680 nm. In this step, a chlorophyll molecule is excited by solar light and the energy produced helps to break down a water molecule into $\frac{1}{2} \text{O}_2$ and 2H^+ . Electrons resulting from the water break down replace those removed by excitation of PSII and are transferred through an electron transport chain towards the photosystem I (PSI), which absorbs light at 700 nm. In PSI, electrons are excited again and electrons resulting from the transport chain in PSII replace the excited ones. These electrons follow a novel electron transport chain, which finishes by the reduction of $\text{NADP}^+ + \text{H}^+$ into nicotinamide adenine dinucleotide phosphate (NADPH), a biological reduction agent. During the electron transport chains, a proton gradient is formed. This gradient is coupled

with an ATP synthase (ATPase) that synthesizes adenosine triphosphate (ATP) from ADP + P via an oxidative phosphorylation process. The highly energetic molecules NADPH and ATP are then used in dark reactions. These reactions take place in the stroma of the chloroplast and are known as Calvin cycle. In this cycle, CO₂ molecules are fixed to form high energetic organic molecules (Equation 2.2). NADPH and ATP produced in the light reactions are essential in this step, as they provide the reductant power and energy required for the synthesis of organic compounds (Ho et al., 2011).



The sugar (CH₂O) produced in the chloroplast is then converted into real biomass (CH_{xH}O_{xO}N_{xN}P_{xP}), as it is demonstrated in Equation 2.3 (Janssen, 2016):



Elements, such as S, K, Mg, Ca, Fe and others present in microalgal biomass are not included in this stoichiometric equation, since they do not contribute a lot on a mass basis.

2.3. Microalgal culturing

Microalgal cultivation can be performed in suspended or immobilized-cell systems (Gómez-Serrano et al., 2015; Hoffmann, 1998; Moreno-Garrido, 2008; Zhu et al., 2013). Table 2.1 presents the main advantages and limitations of these systems. For an effective cultivation process, some parameters, such as light, temperature, pH, nutrients supply and mixing should be carefully controlled (Barsanti and Gualtieri, 2006; Hu, 2004a; Kumar et al., 2010; Yen et al., 2013).

2.3.1. Suspended-cells cultivation systems

Cultivation in suspension is the most commonly used form for microalgal growth (Pires et al., 2013a). Systems typically used for microalgal growth in suspension include closed or open bioreactors. Closed photobioreactors (PBRs) for microalgal growth can be more advantageous because: (i) culture conditions and growth parameters, such as pH, temperature, mixing, CO₂ and oxygen concentrations, can be strictly controlled; (ii) evaporation and contaminations can be easily avoided; and (iii) higher cell concentrations

Table 2.1. Advantages and limitations of suspended- and immobilized-cells cultivation systems

Cultivation systems	Advantages	Limitations
Suspended-cells cultivation systems	<ul style="list-style-type: none"> • Widely studied and optimized • Increased biomass productivities • Applicable in large-scale operations 	<ul style="list-style-type: none"> • Microalgal harvesting can be more expensive and/or time-consuming
Immobilized-cells cultivation systems	<ul style="list-style-type: none"> • Microalgal harvesting is simpler • Aging cultures are more protected against photoinhibition • Immobilization matrix confers cells higher resistance to harsh environments, such as salinity, metal toxicity and pH 	<ul style="list-style-type: none"> • High costs associated to the polymeric matrix (in the case of cell entrapment) • High surface area required (in the case of microalgal adhesion and biofilm formation) • Light limitation may occur • Applicable only for small and pilot-scale operations

can be achieved (Posten, 2009; Posten and Schaub, 2009; Ugwu et al., 2008). Despite these advantages, PBRs have some limitations in terms of overheating, difficulties in scale-up and higher construction costs (Posten and Schaub, 2009). The most commonly used PBRs include flat plate reactors, bubble-column reactors and tubular reactors (Posten, 2009; Ugwu et al., 2008; Xu et al., 2009). Microalgal production in open systems is less expensive in terms of construction and operation and has a larger production capacity (Borowitzka, 1999; Posten and Schaub, 2009; Pulz, 2001). However, biomass productivities and nutrients removal efficiencies are lower than those achieved in closed PBRs due to insufficient mixing, oscillations in the culture conditions and higher susceptibility to contaminations. Additionally, these systems are more prone to CO₂ diffusion to the atmosphere, evaporative losses of water and poor light utilization by cells (Lee, 2001; Posten and Schaub, 2009; Pulz, 2001; Ugwu et al., 2008). Open systems can be divided into two categories: natural ponds, which include lakes, lagoons and ponds, and artificial ponds or containers (Lee, 2001; Posten, 2009; Ugwu et al., 2008). The most commonly used systems include shallow big ponds, tanks, circular ponds and raceway ponds (Lee, 2001; Parmar et al., 2011; Ugwu et al., 2008).

2.3.2. Immobilized-cells cultivation systems

Although suspended-cells cultivation systems have been effectively applied in microalgal biomass production, further separation of microalgal biomass is required. To overcome the problems associated to the time-consuming and energy-demanding harvesting methods

currently applied for microalgal separation, cultivation systems based on microalgal immobilization have appeared as an alternative to the suspended-cells cultivation systems (He and Xue, 2010; Mallick, 2002; Pires et al., 2013a; Ruiz-Marin et al., 2010). According to Tampion and Tampion (1987), an immobilized cell consists in a cell that by natural or artificial techniques is prevented from moving independently of its neighbours to all parts of the aqueous system in study. Natural or passive immobilization occurs through the innate ability of microalgal cells to attach to a specific surface, resulting in biofilm formation. On the other hand, artificial or active immobilization techniques include adsorption, confinement in liquid-liquid emulsions, capturing with semi-permeable membranes, covalent coupling and entrapment within polymers (De-Bashan and Bashan, 2010; Eroglu et al., 2015; Hameed and Ebrahim, 2007; Mallick, 2002). The most commonly used methods for microalgal immobilization include cell entrapment within a polymeric matrix, normally alginate and carrageenan, and cell adhesion and biofilm formation in a solid surface (Christenson and Sims, 2011; Eroglu et al., 2015; Hameed and Ebrahim, 2007; Mallick, 2002). In cell entrapment, microalgal cells are confined in the polymeric matrix and substrates and products diffuse to and from the cells through the pores present in the matrix. However, the high costs associated to the immobilization matrix can be a limiting factor when the aim is to process large amounts of wastewater. Regarding algal biofilms, it is thought that if enough surface area is provided, microalgal growth can be higher than in suspended-cells cultivation systems. These systems result in biomass productivities similar to those reported for cell entrapment at reduced costs: algal biofilm formation presents lower water requirements and does not require an expensive immobilization matrix (Christenson and Sims, 2011). Currently, microalgal immobilized systems are used for different applications, such as metabolites production, toxicity measurements and remediation processes (Moreno-Garrido, 2008).

2.3.3. Main factors influencing microalgal growth

Microalgal growth can be influenced by several factors, both biotic and abiotic. Biotic factors include the presence of pathogens, such as bacteria, fungi and viruses, and the competition by other microalgae, whereas abiotic factors include light (quality and quantity), temperature, pH, salinity, nutrient qualitative and quantitative profiles, dissolved oxygen concentration and the presence of toxic compounds. Additionally, microalgal growth can be influenced by operational conditions, such as hydraulic residence time, harvesting rates, gas

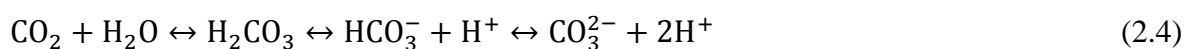
transfer and mixing, since these parameters control CO₂ availability, shear rates and light exposure (Barsanti and Gualtieri, 2006; Hu, 2004a; Kumar et al., 2010; Yen et al., 2013).

Photoautotrophic growth of microalgae is driven by light supply, as this is the energy source that is used to convert inorganic carbon, usually CO₂, into organic carbon. For light irradiances below the light saturation point, photosynthetic activity is proportional to light irradiance. However, for high light irradiance values, the photosynthetic receptor system can be damaged, resulting in the inhibition of photosynthesis and therefore, microalgal growth (Degen et al., 2001; Pires et al., 2012; Pulz, 2001). This behaviour has been extensively described in the literature for several microalgal species. For example, in the study performed by Bouterfas et al. (2006), cultivation of the microalgae *Coelastrum microporum f. astroidea*, *Selenastrum minutum* and *Cosmarium subprotumidum* under light irradiances ranging from 0 to 450 $\mu\text{E m}^{-2} \text{s}^{-1}$ has resulted in a positive relationship between specific growth rates and light supply until a certain point (light irradiances between 150 and 300 $\mu\text{E m}^{-2} \text{s}^{-1}$), at which specific growth rates started decreasing. Selection of an adequate light source for microalgal growth should also take into account spectral quality and intensity. According to Ho et al. (2011), blue (400-500 nm) and red (600-700 nm) lights are preferable than other light sources in terms of cell growth and CO₂ capture. Therefore, a powerful light source should be supplied to increase microalgal productivities. Commonly used light sources include natural light, incandescent and discharge lamps, light-emitting diodes and lasers (Suh and Lee, 2003).

Another parameter that strongly influences microalgal growth is temperature (Davison, 1991). Optimal temperature for microalgal growth is species specific (Robarts and Zohary, 1987): (i) some species, commonly found in polar environments, tolerate temperatures below 10 °C (Michel et al., 1989; Teoh et al., 2004); (ii) others grow in moderate temperatures (10-20 °C); and (iii) others, from tropical regions, tolerate temperatures above 30 °C (Renaud et al., 1995). However, an increase in temperature normally results in an increased metabolic activity, whereas lower temperatures contribute to the inhibition of microalgal growth (Robarts and Zohary, 1987; Xin et al., 2011). Ideally, microalgal cultures should be maintained at temperatures similar to those observed in the environments where they were collected (Hu, 2004a; Larsdotter, 2006). Teoh et al. (2013) have evaluated the response of Antarctic, temperate and tropical microalgae to different temperatures, concluding that optimal growth temperatures were lower (5-10 °C) for the Antarctic species, moderate (15-

20 °C) for the temperate and high (30-35 °C) for the tropical ones. Additionally, the authors have observed that, in general, an increase in temperature (until the optimal value) results in increased specific growth rates. Furthermore, temperature has a strong impact on biochemical reactions, being one of the most important factors influencing the biochemical composition of microalgal biomass. For example, a decrease in temperature below the optimal levels increases the degree of unsaturated lipids, thus promoting the stability and fluidity of thylakoid membranes and protecting the photosynthetic machinery from photoinhibition due to low temperatures. Increased enzyme production can also be observed in response to low temperatures, as an adaptive mechanism to maintain photosynthetic and respiration rates. On the other hand, an increase in temperature above the optimal levels results in the formation of oxygen radicals in algal cells, resulting in oxidative stress-induced carotenogenesis with an increase in carotenoids contents (Hu, 2004a).

The majority of microalgal species are grown in a pH range between 7.0 and 9.0 (Hu, 2004a; Kumar et al., 2010; Yen et al., 2013). However, some microalgae are alkalophilic, whereas others are acidophilic. For example, the microalga *Spirulina platensis* is able to grow in environments with a pH ranging between 9.0 and 10.0 (Belkin and Boussiba, 1991), while the microalga *Chlorococcum littorale* prefers acidic environments, with pH values ranging between 5.0 and 6.0 (Iwasaki et al., 1996; Ota et al., 2009). The pH of the culture medium is responsible for physiological changes in microalgae and, therefore, it is crucial to maintain microalgal cultures in the optimal range to avoid culture loss by extreme pH values. Hansen (2002) has evaluated the effect of culture medium pH (the studied values ranged between 7.5 and 10.0) on specific growth rates of *Ceratium lineatum*, *Heterocapsa triquetra* and *Prorocentrum minimum*, demonstrating that pH values higher than 8.5 (in the case of *C. lineatum*) and 9.0 (in the case of *H. triquetra* and *P. minimum*) have led to a drastic decrease in specific growth rates of the studied microorganisms. An increase in the pH can also induce microalgal flocculation, a process normally referred to as autoflocculation (Vandamme et al., 2012). Culture medium pH can also be associated to the supplied CO₂ concentration, due to the chemical equilibria established between the following species: CO₂, H₂CO₃, HCO₃⁻ and CO₃²⁻ (Hu, 2004a; Kumar et al., 2010; Yen et al., 2013):



The increase of CO₂ concentration in gaseous input stream results in a decrease in pH values in the culture. Therefore, the CO₂ fed to microalgal cultures should be strictly controlled, so

that microalgal cells are not affected by a decrease in the pH of the culture medium. On the other hand, during the cultivation time, it is common to observe an increase in pH, due to CO₂ uptake (Kumar et al., 2010; Yen et al., 2013). Thus, daily monitoring of the pH in the culture medium is very important to assess microalgal growth and to define procedures to maintain the cultures in the optimal pH range.

Optimal salinity levels differ according to microalgal species (Hu, 2004a; Yen et al., 2013). Modifications in the salinity of the culture medium can have adverse effects on microalgal growth and composition, due to: (i) osmotic stress; (ii) ion (salt) stress; and (iii) alterations in the membrane permeability to ions (Glass, 1983). Evaporative losses and rainfalls (in open systems) are the main contributors to modifications in culture medium salinity. The negative effect of these alterations on microalgal growth has been extensively discussed by Tredici and Materassi (1992) and García-González et al. (2003). In the study performed by Yeesang and Cheirsilp (2011), an increase in salt concentration from 0 to 83 mM (in cultures grown in nitrogen-rich media) has resulted in a decrease in specific growth rates of the microalga *Botryococcus* spp. Similar results were reported by Araújo and Garcia (2005) when studying the effect of salinity levels (25 and 35) on biomass concentrations achieved by the microalga *Chaetoceros* cf. *wighamii*. For this reason, the control of salinity levels is crucial for the achievement of high density microalgal cultures. This control can be performed by adding either freshwater or salts to the culture medium.

The main nutrient required for autotrophic microalgal growth is inorganic carbon, since it is the precursor of photosynthetic reactions. However, microalgal growth is dependent on other nutrients, such as nitrogen and phosphorus: these nutrients are required for the synthesis of nucleic acids and proteins. Their presence at limiting concentrations can result in reduced growth rates and biomass productivities. Aiming to evaluate the effect of nitrogen supplementation of the culture medium on the growth of *Tetraselmis subcordiformis*, *Nannochloropsis oculata* and *Pavlova viridis*, Huang et al. (2013) have determined a positive relationship between microalgal growth and nitrogen concentrations (studied values ranged between 0 and 1.76 mM). Specific growth rates determined for *T. subcordiformis*, *N. oculata* and *P. viridis* in the absence of nitrogen ranged between 0.014 and 0.033 h⁻¹, whereas the same values determined for the highest nitrogen concentration were statistically higher. Similarly, when growing the microalgae *Nitzschia* sp., *Sphaerocystis* sp. and *Phormidium* sp. under phosphorus-sufficient and phosphorus-limited conditions, Litchman

et al. (2003) have reported increased specific growth rates (between 0.23 and 0.87 d⁻¹) for cultures grown under phosphorus-sufficient conditions. Regarding nitrogen, microalgae incorporate inorganic nitrogen. The most commonly forms of inorganic nitrogen supplied are nitrate-nitrogen (NO₃-N), ammonium-nitrogen (NH₄-N) and urea (Grobbelaar, 2004; Li et al., 2008; Lin and Lin, 2011; Xu et al., 2001). Concerning phosphorus uptake, this nutrient is required in the form of soluble phosphates and should be supplied in large quantities, since not all the phosphorus compounds are bioavailable for microalgae (Barsanti and Gualtieri, 2006; Kumar et al., 2010). Apart from these nutrients, commonly referred as macronutrients, microalgal growth still requires the presence of trace elements, particularly metals, such as Mg, Ca, Mn, Zn, Cu and Mo (Grobbelaar, 2004; Kumar et al., 2010). Additionally, some studies have reported the use of vitamins to improve microalgal growth (Kumar et al., 2010).

Mixing is also a key growth parameter in microalgal culture because it allows an equal distribution of light and nutrients among microalgal cells, avoiding the existence of stagnant zones, and improves gas transfer between the culture medium and the air (Barsanti and Gualtieri, 2006; Eriksen, 2008; Kumar et al., 2010; Qiang and Richmond, 1996). Gas transfer should not be compromised, since the air bubbled into the cultures contains the CO₂ required for photosynthesis and removes the produced oxygen. Mixing of microalgal cultures is also important in preventing microalgal settling (Barsanti and Gualtieri, 2006; Eriksen, 2008; Kumar et al., 2010) and in avoiding thermal stratification (Barsanti and Gualtieri, 2006). When growing microalgae present in an effluent resulting from oxidation ponds (mainly *Euglena* sp. and *Chlorella* sp.) under shaken and unshaken conditions, Bosca et al. (1991) have reported higher biomass productivities in shaken cultures. Richmond and Vonshak (1978) have also reported that an increase in mixing velocities from 15 to 30 cm s⁻¹ in *Spirulina* sp. ponds has increased biomass yields by 50%. However, high mixing rates can damage microalgal cells (Kumar et al., 2010). For example, Richmond (1988) has reported that high mixing velocities can be harmful to *Spirulina* sp. cultures in open ponds due to the high shear stress values associated. The most commonly used methods for mixing include pumping, mechanical stirring and gas injection (Thomas and Gibson, 1990). Selection of an appropriate method depends on the scale and cultivation system. For example, in open ponds agitation is normally provided by means of a paddlewheel (Qiang and Richmond, 1996).

2.4. Environmental applications of microalgae

Due to the huge taxonomic diversity of microalgae and to their extensive environmental distribution, these photosynthetic microorganisms have numerous applications in diversified areas, such as environment (CO_2 removal and wastewater treatment), energy (biofuels production), pharmaceutical and cosmetics industries, aquaculture, animal feed and human food (Allen et al., 2009; Brennan and Owende, 2010; Spolaore et al., 2006). Figure 2.2 illustrates the main applications described for microalgae. Through photosynthesis, microalgae are able to assimilate CO_2 from the atmosphere, as well as from flue gas emissions. Thus, microalgae can be applied to mitigate the increasing tendency of CO_2 atmospheric concentration that has been observed since Industrial Revolution (Allen et al., 2009; Ho et al., 2011; Tang et al., 2011). Additionally, these photosynthetic microorganisms

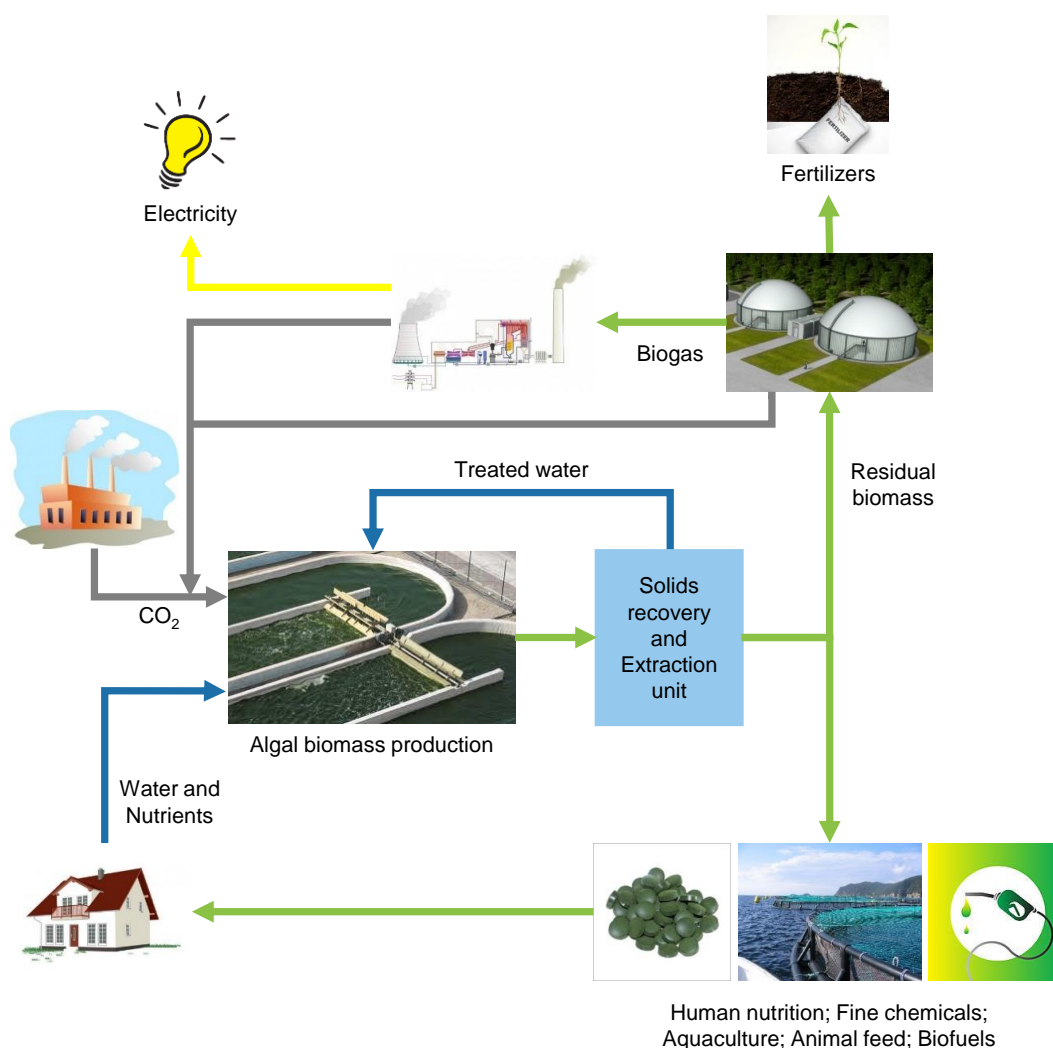


Figure 2.2. Schematic representation of the main applications described for microalgae.

assimilate other compounds, such as nitrogen and phosphorus. These nutrients are frequently found in wastewaters, meaning that microalgae can be a promising alternative in wastewater treatment processes (Rawat et al., 2011; Silva-Benavides and Torzillo, 2012). Furthermore, microalgal biomass can have a lot of applications, in such different areas (Parmar et al., 2011; Spolaore et al., 2006). Algal biomass is suitable for human food and animal feed, as they are an important source of natural vitamins, minerals and fatty acids. They can be used to feed different animals, such as cats, dogs, aquarium fish, birds, horses and cows (Hu, 2004b; Spolaore et al., 2006). Several compounds, such as pigments, antioxidants, β -carotenes, proteins, polysaccharides, triglycerides, fatty acids and vitamins, can be extracted and used as raw materials for the production of cosmetics, drugs and functional food (Hu, 2004b; Singh et al., 2005). Finally, the fatty acids produced by microalgae can be extracted and used for biodiesel production and products, such as proteins and residual biomass, can be fermented to produce ethanol or methane and fertilizers (Brennan and Owende, 2010; John et al., 2011; Parmar et al., 2011). Although microalgae can be used in all these applications, only a few of them are currently applied at a commercial-scale (Table 2.2). This is a result of the high costs associated to microalgal biomass production, which limits

Table 2.2. Current status of large-scale applications of microalgae (adapted from Spolaore et al. (2006) and Paul et al. (2013))

Microalgae	Applications	Country	Status
<i>Aphanizomenon flos-aquae</i>	Human nutrition	USA	Commercial
<i>Chaetoceros muelleri</i>	Aquaculture feed	Global	Commercial
<i>Chlorella</i> spp.	Human nutrition, aquaculture feed, cosmetics	Japan, Taiwan, Czech Republic, Germany	Commercial
<i>Cryptocodinium cohnii</i>	Docosahexaenoic acid	USA	Commercial
<i>Dunaliella salina</i>	Human nutrition, aquaculture feed, β -carotene	Australia, Israel, China, India, USA	Commercial
<i>Dunaliella tertiolecta</i>	Aquaculture feed	Global	Commercial
<i>Haematococcus pluvialis</i>	Aquaculture feed, astaxanthin	USA, Sweden, Israel	Commercial
<i>Isochrysis</i> spp.	Aquaculture feed	Global	Commercial
<i>Monochrysis lutheri</i>	Aquaculture feed	Global	Commercial
<i>Nannochloropsis</i> spp.	Aquaculture feed	Global	Commercial
<i>Pavlova</i> spp.	Aquaculture feed	Global	Commercial
<i>Shizochytrium</i> sp.	Docosahexaenoic acid	USA	Commercial
<i>Skeletonema</i> spp.	Aquaculture feed	Global	Commercial
<i>Spirulina platensis</i>	Human and animal nutrition, cosmetics, phycobiliproteins	Thailand, USA, China, India, Vietnam, Japan	Commercial
<i>Tetraselmis suecica</i>	Aquaculture feed	Global	Commercial
<i>Thalassiosira pseudonana</i>	Aquaculture feed	Global	Commercial

microalgal biomass applications to the commercialization of high-valued products.

Among the applications described for microalgae, this study focuses on the use of microalgae for environmental applications, such as CO₂ capture and nutrients removal from wastewaters. The following sections describe in further detail the state of the art of CO₂ capture and nutrients removal using these photosynthetic microorganisms.

2.4.1. CO₂ capture

Carbon is the most important element for microalgal growth, followed by nitrogen and phosphorus (microalgal biomass contains approximately 50% w/w of carbon, which is all derived from CO₂). Accordingly, the production of 1 g of microalgal biomass corresponds to a CO₂ fixation of 1.83 g, which means that these microorganisms can be effectively applied in CO₂ capture (Cheah et al., 2015).

Microalgae are able to uptake soluble carbonates as a source of CO₂. For low pH values (ranging between 5.0 and 7.0), CO₂ uptake occurs through diffusion. On the other hand, for pH values higher than 7.0, bicarbonate (HCO₃⁻) is the most common form of inorganic carbon present in solution, which enables the external carbonic anhydrase and promotes active transport of this carbon source into microalgal cells (Picardo et al., 2013; Sayre, 2010; Sydney et al., 2014). Once inside the cells, HCO₃⁻ is converted into CO₂ that can be fixed by rubisco (ribulose biphosphate carboxylase oxygenase), producing two molecules of 3-phosphoglycerate (Sayre, 2010; Sydney et al., 2014).

Microalgal culturing can be performed using atmospheric CO₂ or CO₂ resulting from flue gases. Table 2.3 presents CO₂ uptake rates determined for different microalgae using both CO₂ sources. The use of atmospheric CO₂ allows higher flexibility when selecting the location of the microalgal facility, since it does not need to be located close to a CO₂ emission source. Additionally, using this CO₂ source does not require CO₂ transporting systems (Cheah et al., 2015; Moreira and Pires, 2016). Several studies have reported the use of microalgae in CO₂ capture from the atmosphere (Arbib et al., 2014; Cheng et al., 2006; González López et al., 2009; Hulatt and Thomas, 2011). In the study performed by González López et al. (2009), the growth of the cyanobacterium *Anabaena* sp. in bubble-column PBRs (working volume of 1.8 L) using atmospheric air has resulted in a CO₂ fixation rate of 1.45×10³ mg CO₂ L⁻¹ d⁻¹. Arbib et al. (2014) have evaluated the potential of three microalgal

Table 2.3. Application of microalgae in CO₂ capture from the atmosphere and flue gases

Microorganisms	CO ₂ source	%CO ₂ (v/v)	System and operation mode	Time (d)	CO ₂ fixation rate (mg CO ₂ L ⁻¹ d ⁻¹)	R (%)	References
<i>Anabaena</i> sp.	Atmospheric air	≈0.04	Closed suspended system, continuous mode; V=1.8 L	-	1.45×10 ³	-	(González López et al., 2009)
<i>Anabaena</i> sp.	Atmospheric air + pure CO ₂	10	Closed suspended system, batch mode; V=5 L	4	1.01×10 ³	-	(Chiang et al., 2011)
<i>Botryococcus braunii</i>	Atmospheric air + pure CO ₂	5	Closed suspended system, batch mode; V=8 L	15	496.98	-	(Sydney et al., 2010)
<i>Chlorella</i> sp.	Atmospheric air + pure CO ₂	0.106	Closed suspended system, batch mode; V=2 L	15	-	80	(Ramkrishnan et al., 2014)
<i>Chlorella</i> sp.	Atmospheric air + pure CO ₂	2	Closed suspended system, semi-continuous mode; V=0.8 L	8	-	58	(Chiu et al., 2008)
<i>Chlorella</i> sp.	Atmospheric air + pure CO ₂	5	Closed suspended system, batch mode; V=0.6 L	7	583	-	(Ryu et al., 2009)
<i>Chlorella</i> sp.	Atmospheric air + pure CO ₂	10	Closed suspended system, batch mode; V=8 L	25	-	46	(Ramanan et al., 2010)
<i>Chlorella</i> sp.	Atmospheric air + pure CO ₂	15	Closed suspended system, batch mode; V=0.3 L	7	-	85.6	(Cheng et al., 2013)
<i>Chlorella</i> sp.	Flue gas	6-8	Closed suspended system, continuous mode; V=330 L	3	-	10-50	(Doucha et al., 2005)
<i>Chlorella</i> sp.	Flue gas	20-25	Closed suspended system, batch mode; V=50 L	6	-	60	(Chiu et al., 2011)
<i>Chlorella kessleri</i>	Atmospheric air	≈0.04	Closed suspended system, batch mode, V=2 L	10	319.99	-	(Arbib et al., 2014)
<i>Chlorella sorokiniana</i>	Flue gas	12	Closed suspended system, batch mode, V=1 L	4	-	23-45	(Lizzul et al., 2014)
<i>Chlorella sorokiniana</i>	Flue gas	15.6	Closed suspended system, batch mode, V=1.4 L	8	124	-	(Kumar et al., 2014)

V - working volume.

Table 2.3. (Continued)

Microorganisms	CO ₂ source	%CO ₂ (v/v)	System and operation mode	Time (d)	CO ₂ fixation rate (mg CO ₂ L ⁻¹ d ⁻¹)	R (%)	References
<i>Chlorella vulgaris</i>	Atmospheric air	≈0.04	Closed suspended system, batch mode, V=2 L	10	296.75	-	(Arbib et al., 2014)
<i>Chlorella vulgaris</i>	Atmospheric air + pure CO ₂	2	Closed suspended system, batch mode; V=1 L	10	430	-	(Yeh and Chang, 2011)
<i>Chlorella vulgaris</i>	Atmospheric air + pure CO ₂	5	Closed suspended system, batch mode; V=8 L	15	251.64	-	(Sydney et al., 2010)
<i>Chlorella vulgaris</i>	Atmospheric air + pure CO ₂	6.5	Closed suspended system, batch mode; V=0.09 L	7	2.22 ×10 ³	-	(Anjos et al., 2013)
<i>Dunaliella tertiolecta</i>	Atmospheric air + pure CO ₂	5	Closed suspended system, batch mode; V=8 L	15	272.40	-	(Sydney et al., 2010)
<i>Nannochloropsis oculata</i>	Atmospheric air + pure CO ₂	5	Closed suspended system, semi-continuous mode; V=0.8 L	8	-	47	(Chiu et al., 2009)
<i>Scenedesmus obliquus</i>	Atmospheric air	≈0.04	Closed suspended system, batch mode, V=2 L	10	436.67	-	(Arbib et al., 2014)
<i>Scenedesmus obliquus</i>	Atmospheric air + pure CO ₂	6	Closed suspended system, batch mode, V=1.8 L	21	-	28.08	(de Moraes and Costa, 2007a)
<i>Scenedesmus obliquus</i>	Atmospheric air + pure CO ₂	10	Closed suspended system, batch mode, V=1 L	12	549.90	-	(Ho et al., 2010b)
<i>Scenedesmus obliquus</i>	Flue gas	6-18	Closed suspended system, batch mode, V=100 L	17	-	40.2	(Li et al., 2011a)
<i>Spirulina</i> sp.	Atmospheric air + pure CO ₂	6	Closed suspended system, batch mode, V=1.8 L	21	-	53.29	(de Moraes and Costa, 2007a)
<i>Spirulina platensis</i>	Atmospheric air + pure CO ₂	5	Closed suspended system, batch mode; V=8 L	15	318.16	-	(Sydney et al., 2010)
<i>Spirulina platensis</i>	Atmospheric air + pure CO ₂	10	Closed suspended system, batch mode; V=8 L	25	-	39	(Ramanan et al., 2010)
Microalgal consortium obtained from a river	Atmospheric air	≈0.04	Closed suspended system, batch mode, V=2 L	10	417.52	-	(Arbib et al., 2014)

V - working volume.

species (*Chlorella kessleri*, *C. vulgaris* and *Scenedesmus obliquus*) in CO₂ capture from the atmosphere using 2-L flasks as PBRs. After ten days of culturing, CO₂ fixation rates determined for the studied microorganisms were 320, 297 and 418 mg CO₂ L⁻¹ d⁻¹, respectively. However, atmospheric CO₂ concentration (approximately 0.04% v/v) can be limiting to microalgal growth, due to limitations in mass transfer of CO₂ from the gaseous stream to the liquid medium. According to McGinn et al. (2011), CO₂ diffuses into the liquid medium 10⁴ times slower than through the gaseous medium. Accordingly, costly CO₂ sparging might be required to increase the retention time of CO₂ in the culture medium. As an alternative to the use of pure CO₂ to feed microalgal cultures, several authors have reported the use of flue gases. CO₂ concentration in flue gases typically ranges between 6-15% (v/v). For this reason, several authors have evaluated the effect of different CO₂ concentrations on microalgal growth and CO₂ uptake. For example, in the study performed by de Moraes and Costa (2007a), the effect of CO₂ concentrations of 0, 6 and 12% (v/v) on biomass productivities and CO₂ fixation rates of *Spirulina* sp. and *S. obliquus* was evaluated. In this study, the authors have reported higher biomass productivities, specific growth rates and CO₂ fixation rates in cultures performed at 6 and 12% (v/v) of CO₂, with maximum values obtained at 6% (v/v). As a result, current microalgal production plants are being projected near large CO₂ emission sources, such as power plants and refineries (Moreira and Pires, 2016). However, flue gases usually contain large amounts of nitrogen oxides (NO and NO_x) and sulphur oxides (SO_x), which can significantly reduce the pH of the culture medium, thus inhibiting microalgal growth (Cheah et al., 2015; Moreira and Pires, 2016; Pires et al., 2011). In the case of NO and NO_x, these compounds can be used by microalgae as nitrogen source, thus not presenting such a negative impact for microalgal growth. On the other hand, high SO₂ concentrations (between 100 and 250 mg L⁻¹) can be harmful to microalgae, due to the formation of bisulphite (HSO₃⁻), sulphite (SO₃²⁻) and sulphate (SO₄²⁻), which drastically decrease the pH of the culture medium to values between 2.5 and 3.5 (Cheah et al., 2015; Lam et al., 2012). Additionally, high CO₂ concentrations (between 10 and 20% v/v) also contribute to a decrease in pH to approximately 5.5. Although some microalgal species are able to perform photosynthesis in these conditions, thus counterbalancing the pH decrease due to high CO₂ levels, other species are unable to withstand this acidic environment (Cheah et al., 2015). Due to these constraints, only a few studies have reported the use of real flue gases for microalgal growth. When growing *Chlorella* sp. in an outdoor open thin-layer PBR using a flue gas containing 6-8% (v/v) of CO₂, Doucha et al. (2005) have reported CO₂ removal efficiencies between 10 and 50%. A

CO₂ removal efficiency of 40.2% was obtained by Li et al. (2011a) when growing *S. obliquus* in a flue gas containing 6-18% (v/v) of CO₂.

CO₂ uptake by microalgae is strongly dependent on the selected microalgal species. An ideal microalgal species should present the following characteristics: (i) high sinking capacity; (ii) high tolerance to high CO₂ concentrations; and (iii) high tolerance to some pollutants, such as NO, NO_x and SO_x (Cheah et al., 2015). According to Singh and Ahluwalia (2013), *S. obliquus*, *Botryococcus braunii*, *C. vulgaris* and *N. oculata* are the most promising species for carbon sequestration. In addition to the selected microalgal species, CO₂ uptake can be influenced by other parameters, such as CO₂ concentration, temperature and light intensity. Regarding CO₂ concentration, several studies have already demonstrated that microalgal growth in low CO₂ concentrations (atmospheric CO₂ concentrations, for example) can be lower than microalgal growth in CO₂-enriched air streams. On the other hand, high CO₂ concentrations can be toxic for microalgae (Cheah et al., 2015). Temperature is also a key factor on CO₂ uptake because it determines CO₂ solubility in the culture medium: an increase in temperature is responsible for the decrease of CO₂ solubility, which means that microalgae should be grown in moderate temperatures (Moreira and Pires, 2016). Light supply also influences CO₂ uptake by microalgae because this process is directly related to light utilization efficiency by microalgal cells. Accordingly, higher light intensities favour CO₂ uptake because they ensure a better penetration of light into high density cultures. Hydraulic residence time and mass transfer are operational parameters that also influence CO₂ uptake. In general, a decrease in the hydraulic residence time results in an increased CO₂ fixation. On the other hand, an increase in mass transfer coefficient results in increased CO₂ uptake rates (Judd et al., 2015).

2.4.2. Nutrients removal from wastewaters

As referred in Section 2.3.3, the main nutrients required for microalgal growth are carbon, nitrogen and phosphorus. Since wastewaters present considerable concentrations of these nutrients, several studies have reported the potential of microalgal growth in wastewaters from different sources: (i) domestic (Posadas et al., 2013; Yang et al., 2011); (ii) leachate (Lin et al., 2007; Mustafa et al., 2012); (iii) agricultural (González et al., 1997; Hernández et al., 2013; Lefebvre et al., 1996); (iv) refinery (Chojnacka et al., 2004); and (v) industrial (Safonova et al., 2004; Tarlan et al., 2002). These studies have revealed that using

wastewaters for microalgal cultivation promotes an effective treatment of these effluents (nitrogen and phosphorus removal efficiencies reported correspond to 60-99 and 54-95%, respectively), while contributing to the production of microalgal biomass at reduced costs and with lower environmental impact, since nutrients supply and freshwater are not required (Boelee et al., 2011; Park et al., 2011; Silva-Benavides and Torzillo, 2012).

To improve wastewater remediation processes using microalgae, it is very important to understand the mechanisms involved in nutrients removal. Table 2.4 summarizes the mechanisms involved in carbon, nitrogen and phosphorus removal by microalgae.

Although microalgae are mainly autotrophic, some microalgae are heterotrophic, using only organic carbon (e.g. acetate, glucose, glycerol and ethanol) as carbon source, whereas others are mixotrophic, using facultatively an organic carbon source in addition to CO₂ (Brennan and Owende, 2010; Leite et al., 2013; Neilson and Lewin, 1974). In this growth regime, both respiratory and photosynthetic mechanisms can be adopted by microalgae (Lee, 2004).

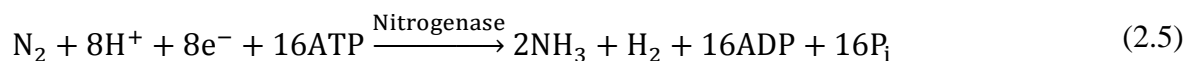
The most common inorganic nitrogen forms include nitrate, nitrite, nitric acid, ammonium, ammonia, molecular nitrogen, nitrous oxide, nitric oxide and nitrogen dioxide

Table 2.4. Mechanisms involved in nutrients (carbon, nitrogen and phosphorus) removal by microalgae

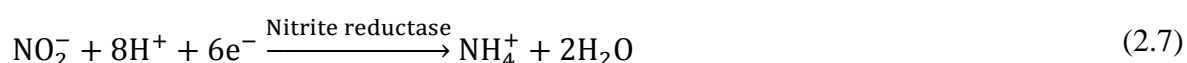
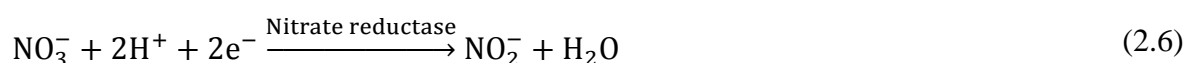
Nutrients	Mechanisms	Cell incorporation
<u>Carbon</u>		
CO ₂	Integration in the Calvin cycle	Diffusion (5.0<pH<7.0) or active transport (pH>7.0)
Organic carbon	Integration in the respiration metabolism	Diffusion or active transport (depending on the size of the molecules)
<u>Nitrogen</u>		
N ₂ -N	Fixation by prokaryotic microalgae (cyanobacteria) into ammonia, followed by conversion into amino acids	
NO ₃ -N and NO ₂ -N	Reduction into ammonium, followed by conversion into amino acids	Active transport
NH ₄ -N	Direct conversion into amino acids	Active transport
	Stripping due to volatilisation (high pH values and temperatures)	n.a.
<u>Phosphorus</u>		
PO ₄ -P	Phosphorylation	Active transport
	Chemical precipitation (high pH values and dissolved oxygen concentrations)	n.a.

n.a. - not applicable.

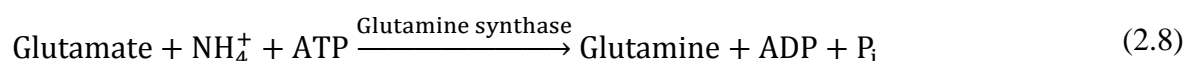
(Barsanti and Gualtieri, 2006). Microalgae play an important role in both nitrogen fixation and assimilation. Prokaryotic microalgae (cyanobacteria) are able to fix atmospheric molecular nitrogen ($\text{N}_2\text{-N}$), converting it into ammonia-nitrogen ($\text{NH}_3\text{-N}$), which can either be incorporated into amino acids and proteins or excreted to the environment (Barsanti and Gualtieri, 2006; Cai et al., 2013):



Eukaryotic microalgae, in turn, are able to assimilate fixed nitrogen, such as $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$ and nitrite-nitrogen ($\text{NO}_2\text{-N}$). These nitrogen sources enter microalgal cells through active transport at the plasma membrane. $\text{NO}_3\text{-N}$ (the most oxidized form) is thermodynamically more stable than $\text{NH}_4\text{-N}$ and, hence, it is more common to find this inorganic nitrogen form in aquatic environments (Barsanti and Gualtieri, 2006; Grobbelaar, 2004). However, assimilation of this nitrogen source requires previous reduction into $\text{NH}_4\text{-N}$, in a two-step process catalysed by the enzymes nitrate reductase and nitrite reductase (Barsanti and Gualtieri, 2006; Crofcheck et al., 2012; Hellebust and Ahmad, 1989). In the first step (Equation 2.6), nitrate reductase catalyses the reduction of $\text{NO}_3\text{-N}$ into $\text{NO}_2\text{-N}$ using NADPH as reducing agent. Further reduction into $\text{NH}_4\text{-N}$ is catalysed by nitrite reductase, which uses ferredoxin to catalyse the six-electron transfer reaction (Equation 2.7) (Barsanti and Gualtieri, 2006; Hellebust and Ahmad, 1989):



$\text{NH}_4\text{-N}$ resulting from $\text{NO}_3\text{-N}$ and $\text{NO}_2\text{-N}$ reduction and actively incorporated into microalgal cells is directly converted into amino acids via the glutamine synthetase-glutamate synthase pathway, where glutamine synthase catalyses glutamine formation from glutamate and ATP, according to Equation 2.8 (Barsanti and Gualtieri, 2006; Crofcheck et al., 2012; Hellebust and Ahmad, 1989):



Since $\text{NH}_4\text{-N}$ assimilation does not require previous reduction steps, it is thought that this is the preferred nitrogen form for microalgae. However, according to Grobbelaar (2004), the

effect of both $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ on microalgal growth is not well defined, since no significant differences were observed in terms of microalgal productivity. In addition to microalgal uptake, $\text{NH}_4\text{-N}$ removal may occur in response to an increase of pH and temperature, where large amounts of $\text{NH}_4\text{-N}$ can be volatilized (Aslan and Kapdan, 2006; Cai et al., 2013; Wang et al., 2014).

Energy transfer and nucleic acid synthesis are mediated by phosphorus. This nutrient enters microalgal cells through active transport at the plasma membrane in the forms of H_2PO_4^- and HPO_4^{2-} . Incorporation of phosphate-phosphorus ($\text{PO}_4\text{-P}$) into organic compounds is performed through the following processes: (i) phosphorylation at the substrate level; (ii) oxidative phosphorylation; and (iii) photophosphorylation. In these processes ATP is produced from adenosine diphosphate (ADP) and an energy input, which can be obtained from the oxidation of the respiratory substrates or from the electron transport system of the mitochondria (in the case of the first two processes) and from light energy transformation (in the case of the third step). The general reaction is represented by Equation 2.9 (Martinez et al., 1999):



$\text{PO}_4\text{-P}$ removal can also be ruled by environmental conditions, such as pH and dissolved oxygen concentration. For pH values above 8.0 and high oxygen concentrations, phosphorus precipitation may occur (Aslan and Kapdan, 2006; Cai et al., 2013; Su et al., 2012b; Wang et al., 2014).

According to their nature and composition, different wastewaters can be used as a source for the above referred nutrients. Wastewaters can be defined as disposable liquids or water-carried waste resulting from domestic, agricultural, urbanization and industrial practices. Usually, wastewaters may contain large quantities of oxygen demanding wastes, pathogenic organisms, organic pollutants, nutrients, such as nitrogen and phosphorus, inorganic compounds and sediments (Sonune and Ghate, 2004). Table 2.5 presents typical compositions of wastewaters from different sources in terms of nitrogen, phosphorus and organic carbon, demonstrating that wastewater composition strongly depends on its source. In general, wastewaters from the swine industry present higher levels of nitrogen and phosphorus than municipal wastewaters. On the other hand, wastewaters resulting from brewery, starch and dairy industries and potato-processing wastewaters present high

Table 2.5. Typical compositions of different wastewaters already used for microalgal growth in terms of nitrogen, phosphorus and carbon

Wastewater type	Source	Nitrogen (mg L ⁻¹)	Phosphorus (mg L ⁻¹)	Carbon (mg L ⁻¹)	References
Agricultural	Potato-processing effluent	12 NH ₄ -N 54 NO ₃ -N	48 PO ₄ -P	745 COD	(Hernández et al., 2013)
Agricultural	Rice effluent	25-95 TN	12-94 PO ₄ -P	2578-6480 COD	(Queiroz et al., 2007)
Anaerobically-digested	Dairy manure	1279-1961 NH ₄ -N <1 NO ₃ -N	240 TP	4855-4945 COD	(Wilkie and Mulbry, 2002)
Anaerobically-digested	Municipal sewage	646 NH ₄ -N	101 PO ₄ -P	76 TOC	(Posadas et al., 2013)
Anaerobically-digested	Piggery manure	303-495 NH ₄ -N	n.s.	n.s.	(González-Fernández et al., 2011)
Industrial	Brewery	3-106 NH ₄ -N 2-11 NO ₃ -N	57-326 TP	565-7837 COD	(Raposo et al., 2010)
Industrial	Carpet mill	18-26 NH ₄ -N 0-28 NO ₃ -N	20-35 PO ₄ -P	1412 COD	(Chinnasamy et al., 2010)
Industrial	Dairy	120-350 NH ₄ -N <1 NO ₃ -N	35-350 TP	2000-20213 COD	(Ince, 1998; Qin et al., 2016; Wilkie and Mulbry, 2002; Woertz et al., 2009)
Industrial	Oil, metal and chemical	1.1 NH ₄ -N 1.9 NO ₃ -N	n.s.	1200 COD	(Safonova et al., 2004)
Industrial	Piggery	324-656 NH ₄ -N	117 PO ₄ -P	1247 TOC	(de Godos et al., 2009; González-Fernández et al., 2011)
Industrial	Starch	49-115 NH ₄ -N	50-385 TP	2470-15440 COD	(Sklyar et al., 2003)
Industrial	Wood-based pulp and paper	n.s.	n.s.	1248 COD	(Tarlán et al., 2002)
Municipal	Domestic sewage	25-55 NH ₄ -N	7-12 PO ₄ -P	400-500 COD	(Park and Craggs, 2011; Posadas et al., 2013; Singh and Thomas, 2012)
Municipal	Landfill leachate	112-192 NH ₄ -N	7-9 PO ₄ -P	3725-4861 COD	(Mustafa et al., 2012)
Municipal	Sewage	23-219 NH ₄ -N 1 NO ₃ -N	1-12 PO ₄ -P	183-380 COD	(He et al., 2013; Hernández et al., 2013; Samorì et al., 2013; Silva-Benavides and Torzillo, 2012; Su et al., 2011, 2012b; Tripathi and Shukla, 1991; Van Den Hende et al., 2011; Wang et al., 2010; Woertz et al., 2009)

NH₄-N - ammonium-nitrogen; NO₃-N - nitrate-nitrogen; TN - total nitrogen; PO₄-P - phosphate-phosphorus; TP - total phosphorus; COD - chemical oxygen demand; TOC - total organic carbon; n.s. - not specified.

concentrations of soluble chemical oxygen demand (COD). To consider the use of microalgae in wastewater treatment, the composition of the effluent to be treated should be previously assessed, since nitrogen to phosphorus molar ratios (N:P) strongly influence microalgal biomass production and hence, nutrients uptake. According to the average elemental composition of microalgal biomass, N:P molar ratios lower than 5:1 result in nitrogen limitation, whereas N:P molar ratios higher than 30:1 result in phosphorus limitation (Larsdotter, 2006).

N:P molar ratios determined for the wastewaters described in Table 2.5 range between 1:1 (for brewery and starch industry wastewaters) and 42:1 (for landfill leachate). With N:P molar ratios of 9:1, 11:1, 14:1 and 15:1, piggery industry and domestic wastewaters, as well as dairy manure and municipal sewage anaerobically-digested wastewaters are considered the most appropriate for microalgal growth. In fact, microalgal nutrients removal from domestic and piggery industry wastewaters has been extensively reported in the literature (Alcántara et al., 2015b; Cai et al., 2013; de Godos et al., 2009; González-Fernández et al., 2011; Hernández et al., 2013; Posadas et al., 2013). Table 2.6 presents nitrogen and phosphorus removal rates determined when culturing microalgae in different wastewaters.

Although the majority of the studies refer to the use of suspended-growth systems, Shi et al. (2007) have assessed nitrogen and phosphorus removal from a municipal wastewater collected in Cologne (Germany) using an immobilization method – the twin-layer system. In this method, the microalgae *C. vulgaris* and *Scenedesmus rubescens* were immobilized through self-adhesion on a substrate layer and another layer provided the growth medium required for microalgal growth. Using this system, microalgae remained 100% immobilized, being able to completely remove $\text{NO}_3\text{-N}$ (initial concentration between $3.7\text{-}6.2\text{ mg N L}^{-1}$) after an exposure period of four days. Other studies have focused on the use of municipal wastewaters to improve microalgal biomass and lipid productivities (Caporgno et al., 2015; Gómez-Serrano et al., 2015; Gouveia et al., 2016; Kong et al., 2009; Mennaa et al., 2015; Nayak et al., 2016). For example, Kong et al. (2009) have grown *Chlamydomonas reinhardtii* in a municipal wastewater obtained from St. Paul Metro plant (St. Paul, Minnesota, USA), achieving nutrients removal rates of $3.44\text{ mg N L}^{-1}\text{ d}^{-1}$ and $0.56\text{ mg P L}^{-1}\text{ d}^{-1}$ for TN and $\text{PO}_4\text{-P}$, respectively. Using a municipal wastewater, average biomass productivities achieved by *C. reinhardtii* ranged between $0.82\text{ and }2.00\text{ g L}^{-1}\text{ d}^{-1}$, with 25.25% (w/w) of microalgal biomass corresponding to lipids. More recently,

Table 2.6. Application of microalgae in nitrogen and phosphorus removal from different wastewaters and respective removal efficiencies

Microorganisms	Waste stream	System and operation mode	Removal time (d)	Nitrogen		Phosphorus		References
				S _i (mg N L ⁻¹)	R (%)	S _i (mg P L ⁻¹)	R (%)	
<i>Chlorella vulgaris</i> / <i>Scenedesmus rubescens</i>	Municipal wastewater	Immobilized system, continuous mode, V=2 L	4	3.7-6.2 NO ₃ -N	≈100	0.4-0.7 TP	n.a.	(Shi et al., 2007)
<i>Chlamydomonas reinhardtii</i>	Municipal wastewater	Closed suspended system, batch mode, V=2 L	10	128.6 TN	33-43	120.6 TP	10-13	(Kong et al., 2009)
<i>Chlorella vulgaris</i> / <i>Scenedesmus obliquus</i>	Municipal wastewater	Closed suspended system, semi-continuous mode, V=150 L	13	119.3-346.6 NH ₄ -N	84-95	4.6-8.3 TP	92-95	(Gouveia et al., 2016)
<i>Chlorella vulgaris</i>	Ethanol and citric acid industry wastewater	Closed suspended system, batch mode, V=16.5 L	6	3-8 NH ₄ -N	71.6	1.5-3.5 PO ₄ -P	28	(Valderrama et al., 2002)
<i>Chlorella vulgaris</i>	Textile industry wastewater	Open suspended system, batch mode, V=36 L	10	6.50 NH ₄ -N	44.4-45.1	7.14 PO ₄ -P	33.1-33.3	(Lim et al., 2010)
<i>Chlorella sorokiniana</i>	Potato-processing wastewater	Closed suspended system, batch mode, V=5 L	30	12.1 NH ₄ -N	>95	3.4 PO ₄ -P	80.7	(Hernández et al., 2013)
<i>Klebsormidium</i> sp. / <i>Stigeoclonium</i> spp.	Horticultural wastewater	Immobilized system, semi-continuous mode, V=65 L	9	47.2 NO ₃ -N	≈100	11.6 PO ₄ -P	70-84	(Liu et al., 2016)
<i>Chlorella zofingiensis</i>	Piggery wastewater	Closed suspended system, batch mode, V=1.37 L	10	148.0 TN	78.72	156.0 TP	85.00	(Zhu et al., 2013)
Native microalgae	Anaerobically-digested dairy industry wastewater	Immobilized system, semi-continuous mode, V=75 L	23	78 TN	62	7 TP	70	(Wilkie and Mulbry, 2002)
<i>Spirulina</i> sp.	Anaerobically-digested piggery wastewater	Open suspended system, semi-continuous mode	6-7	1209-1481 NH ₄ -N	84-96	164-620 PO ₄ -P	72-87	(Olguín et al., 2003)

S_i - initial concentration (in mg L⁻¹); R - removal efficiency (in %); V - working volume; NO₃-N - nitrate-nitrogen; NH₄-N - ammonium-nitrogen; TN - total nitrogen; PO₄-P - phosphate-phosphorus; TP - total phosphorus; n.a. - not applicable.

Gouveia et al. (2016) have cultured *C. vulgaris*, *S. obliquus* and a native consortium in a municipal wastewater collected from Figueira da Foz (Portugal), aiming to determine the best candidate in terms of wastewater remediation and biomass productivity and quality for further uses, such as biofuels, biofertilizers and bioplastics production. With this study, biomass productivities achieved were 0.1, 0.4 and 0.9 g L⁻¹ d⁻¹ for *C. vulgaris*, *S. obliquus* and the consortium, respectively, with 8.1, 10.0 and 13.1% (w/w) corresponding to lipids. Additionally, the studied cultures have effectively removed nitrogen and phosphorus from the wastewater, reaching nitrogen removal efficiencies of 84-98% and phosphorus removal efficiencies of 95-100%. Taking into account these results, the authors have proposed the native consortium as the best option for nutrients removal and biomass production.

Valderrama et al. (2002) have cultured *C. vulgaris* in an industrial effluent resulting from ethanol and citric acid production, achieving NH₄-N and PO₄-P removal efficiencies of 71.6 and 28%, respectively (initial NH₄-N concentration in this effluent ranged between 3 and 8 mg N L⁻¹, whereas initial PO₄-P concentration ranged between 0 and 0.36 mg P L⁻¹). Similarly, Lim et al. (2010) have grown *C. vulgaris* in high rate algal ponds (HRAPs) fed with a textile industry wastewater to evaluate the potential of this microalga in nitrogen and phosphorus removal. Although *C. vulgaris* was able to grow in the textile wastewater (NH₄-N and PO₄-P initial concentrations of 6.50 and 7.14 mg L⁻¹, respectively), nitrogen and phosphorus removal efficiencies achieved were not very high: 44.4-45.1% and 33.1-33.3%, respectively.

In the study performed by Hernández et al. (2013), *C. sorokiniana* was grown in a potato-processing wastewater presenting an initial NH₄-N concentration of 12.1 mg N L⁻¹ and PO₄-P concentration of 3.4 mg P L⁻¹. After a cultivation period of ten days, nitrogen and phosphorus removal efficiencies achieved were 95 and 80.7%, respectively. Liu et al. (2016) have determined the remediation potential of the filamentous microalgae *Klebsormidium* sp. and *Stigeoclonium* spp. grown in an outdoor Algal Turf Scrubber using horticultural wastewater as culture medium (NO₃-N and PO₄-P initial concentrations of 47.2 and 11.6 mg L⁻¹, respectively). With this study, the authors have demonstrated that these microalgae can effectively remove nitrogen and phosphorus from this wastewater, since nitrogen removal efficiencies achieved oscillated between 88 and 99% and phosphorus removal efficiencies were higher than 99%.

The use of microalgae for the remediation of different anaerobically-digested effluents has also been reported in the literature. For example, Wilkie and Mulbry (2002) have evaluated nutrients recovery in an anaerobically-digested dairy industry effluent using native microalgae, reporting TN and TP removal efficiencies of 62 and 70%, respectively (initial TN and TP concentrations were 78 and 7 mg L⁻¹, respectively). On the other hand, Olguín et al. (2003) and Ledda et al. (2015) have focused on nutrients removal and biomass production in anaerobically-digested piggery wastewaters. In the study performed by Olguín et al. (2003), cultivation of *Spirulina* sp. in outdoor conditions and semi-continuous mode has resulted in biomass productivities between 11.8 and 15.1 g m⁻² d⁻¹. In the same conditions, NH₄-N removal efficiencies ranged between 84 and 96% and PO₄-P removal efficiencies ranged between 72 and 87% (initial NH₄-N and PO₄-P concentrations were about 1209-1481 and 164-620 mg L⁻¹, respectively). Similarly, Ledda et al. (2015) have grown *Chlorella* sp. in an anaerobically-digested piggery wastewater (with NH₄-N and PO₄-P initial concentrations of 60 and 18 mg L⁻¹, respectively), reporting biomass productivities of 0.10 g L⁻¹ d⁻¹ and nitrogen and phosphorus removal efficiencies of 95 and 85%, respectively.

There are several factors that influence nutrients removal by microalgae. The pH of microalgal cultures constitutes one of the most important factors influencing nutrients removal. In the case of CO₂, the pH of the culture medium influences its solubility and hence, the carbon concentrating mechanisms adopted by microalgal cells. Additionally, high pH values are responsible for NH₄-N stripping and PO₄-P precipitation. However, other factors, such as light and temperature, also play an important role in nutrients uptake by microalgae. In general, an increase in light supply results in increased nutrients removal. In the study performed by Hu et al. (2000), NO₃-N uptake rates determined for *Synechococcus* sp. grown in nitrate-contaminated groundwater increased proportionally to increasing average daily light irradiance up to 100 μE m⁻² s⁻¹. Similarly, in the study performed by Li et al. (2012), an increase in average daily light irradiance from 0 to 200 μE m⁻² s⁻¹ increased TP removal efficiencies from 65.8 to 87.0% (for *C. kessleri*) and from 79.3 to 83.0% (for *Chlorella protothecoides*). Regarding the temperature effect, higher metabolic activities and hence nutrients uptake rates can be observed for increased temperatures, reaching its maximum when cultivation temperature is close to the optimal values reported for the microorganisms in study. Talbot and De la Noüe (1993) have demonstrated that cultivation of *Phormidium bohneri* in a secondary effluent resulting from an activated sludge treatment plant at 30 °C

for three days has resulted in an effective $\text{NH}_4\text{-N}$ removal, whereas the same culture performed at 10 °C has resulted in an almost negligible $\text{NH}_4\text{-N}$ removal.

2.4.3. The role of microalgal consortia in CO_2 capture and nutrients removal

Although microalgae have been successfully applied in CO_2 capture and nutrients removal from different wastewaters, it is difficult to maintain a microalgal monoculture in these processes. Accordingly, several studies have reported the advantages of using microalgal consortia over single-species cultures (González-Fernández et al., 2011; He et al., 2013; Muñoz and Guieysse, 2006; Subashchandrabose et al., 2011; Wilkie and Mulbry, 2002). Complex degradation processes, which would be difficult to accomplish using monocultures, can benefit from the use of microalgal consortia. Additionally, application of these consortia may result in the development of a robust system, able to resist to environmental fluctuations and invasion by other species (Paerl and Pinckney, 1996; Subashchandrabose et al., 2011). These consortia can naturally occur in the environment or be artificially engineered, by combination of microorganisms that do not necessarily co-occur, for a specific purpose (Jagmann and Philipp, 2014). Among the different possibilities of consortia that can be established, the most commonly used for CO_2 capture and wastewater treatment include microalgal consortia, which are constituted exclusively by photosynthetic microorganisms (eukaryotic and/or prokaryotic), and microalgal-bacterial consortia, which are constituted by photosynthetic microorganisms and heterotrophic bacteria. This section describes the type of interactions that can be established between the microorganisms integrating these consortia and how these interactions can improve CO_2 capture and nutrients uptake.

In microalgal consortia, interactions between photosynthetic microorganisms are not well documented in the literature (Qin et al., 2016). It is thought that growing these microorganisms in a consortium may result in both cooperative and competitive interactions. On the one hand, these microorganisms may establish cooperative interactions through the exchange of metabolites, resulting in an overall increase in biomass productivities and hence, nutrients removal efficiencies (Mendes and Vermelho, 2013). On the other hand, co-cultivation of photosynthetic microorganisms may result in the excretion of secondary metabolites, also known as allelochemicals, that exhibit a negative effect on the co-cultivated microorganisms (Cembella, 2003; Gross, 2003; Mendes and Vermelho, 2013). For example,

when growing a microalgal consortium composed by *C. vulgaris* and *P. subcapitata*, Fergola et al. (2007) have demonstrated that *P. subcapitata* growth was strongly inhibited by chlorellin, a fatty acids mixture excreted by the co-cultivated *C. vulgaris*. Allelochemicals production may be enhanced or suppressed by both abiotic and biotic factors. The most important abiotic factors that enhance allelochemicals production include nutrients starvation, low light intensities and temperatures and high pH values. On the other hand, excessive nutrients concentrations, high light intensities and temperatures and low pH values can inhibit allelochemicals production. Biotic factors influencing allelochemicals production are the concentrations of the involved microorganisms (the ones producing toxic compounds and target cells) (Mendes and Vermelho, 2013). In CO₂ capture and in wastewater treatment processes, interactions between photosynthetic microorganisms can have the following advantages: (i) enhancement of the overall nutrients uptake, providing that sufficient nutrients are supplied; (ii) resistance to contaminants and predators through the induction of allelochemicals production; and (iii) the development of a settleable system (by combining single cell microorganisms with flocculating ones), thus avoiding the requirements for a harvesting method. Additionally, the use of microalgal consortia for these purposes ensures the viability of the remediation process because the loss of one microorganism can be compensated by the other microorganisms integrating the consortia (Renuka et al., 2013). Although microalgal consortia can be applied to both CO₂ capture and nutrients removal, the majority of the studies refers to the application of these consortia in wastewater polishing (Table 2.7). For example, a non-native consortium composed by *Chlorella* sp. and *Scenedesmus* sp. was effectively applied in nitrogen and phosphorus removal from a primary-treated municipal wastewater by Koreivienė et al. (2014). These authors have reported total nitrogen (TN) and TP removal efficiencies ranging between 88.6-96.4 and 99.7-99.9%, respectively. Using a native microalgal consortium from a carpet mill industry effluent, Chinnasamy et al. (2010) have reported an almost complete removal of NO₃-N and PO₄-P, with removal efficiencies ranging between 96.6 and 99.8%. In order to obtain a self-flocculating culture, Renuka et al. (2013) have evaluated the potential of a non-native consortium (composed by unicellular and filamentous microalgae) and two native consortia (one composed by filamentous microalgae and the other composed by unicellular microalgae) in the treatment of a primary-treated sewage, reaching high nitrogen and phosphorus removal efficiencies in all studied consortia: NO₃-N removal efficiencies ranged between 81.5 and 83.3%, NH₄-N removal efficiencies were about 100% and PO₄-P removal efficiencies ranged between 94.9 and 97.8%.

Table 2.7. Application of microalgal consortia in nitrogen and phosphorus removal from different wastewaters and respective removal efficiencies

Microorganisms	Waste stream	System and operation mode	Removal time (d)	Nitrogen		Phosphorus		References
				S _i (mg P L ⁻¹)	R (%)	S _f (mg P L ⁻¹)	R (%)	
<i>Calothrix</i> sp., <i>Lyngbya</i> sp., <i>Ulothrix</i> sp. and <i>Chlorella</i> sp.	Primary-treated sewage water	Closed suspended system, batch mode, V=0.8 L	14	83.7 NO ₃ -N 21.1 NH ₄ -N	83.3 100	3.1 PO ₄ -P	97.7	(Renuka et al., 2013)
<i>Chlamydomonas reinhardtii</i> , <i>Scenedesmus rubescens</i> and <i>Chlorella vulgaris</i>	Primary-treated municipal wastewater	Closed suspended system, batch mode, V=5 L	5-14	52.8-98.7 TN	41.2-100	3.9-11.5 PO ₄ -P	12.2-100	(Su et al., 2012a)
<i>Chlorella</i> sp. and <i>Scenedesmus</i> sp.	Primary-treated municipal wastewater	Closed suspended system, batch mode, V=0.23 L	5-14	14.1-56.5 TN	88.6-96.4	1.5-6.1 TP	99.7-99.9	(Koreivienė et al., 2014)
<i>Chlorella</i> sp., <i>Scenedesmus</i> spp. and <i>Chlorella zofigiensis</i>	Dairy wastewater	Closed suspended system, batch mode, V=0.4 L	3-4	176.0 TN	87.0-91.0	39.6 TP	91.2-96.0	(Qin et al., 2016)
<i>Chlorella vulgaris</i> and <i>Planktothrix isothrix</i>	Municipal wastewater	Closed suspended system, batch mode, V=0.25 L	9	79.3 NH ₄ -N	43.9-81.5	7.5 PO ₄ -P	98.4-100	(Silva-Benavides and Torzillo, 2012)
<i>Chlorococcum</i> sp., <i>Scenedesmus</i> sp., <i>Chlorella</i> sp. and <i>Phaeodactylum tricornutum</i>	Synthetic municipal wastewater	Closed suspended system, batch mode; V=0.4 L	14	n.a.	n.a.	10 PO ₄ -P	80	(Johnson and Admassu, 2013)
Microalgal consortium composed by the families Chlorophyta, Cyanobacteria, Euglenozoa and <i>Microcystis aeruginosa</i> , <i>Scenedesmus quadricauda</i> , <i>Chlorella vulgaris</i> and <i>Euglena viridis</i>	Primary-treated municipal wastewater	Closed suspended system, batch mode V=1 L	7	23.3 NH ₄ -N	100	1.7 TP	100	(Samorì et al., 2013)
Carpet mill industry wastewater native microalgal consortium	Carpet mill industry wastewater	Open suspended system, batch mode, V=60 L	5	0.64 NO ₃ -N 39.4 NH ₄ -N	45.3 58.1	4.6 PO ₄ -P	32.6	(Tripathi and Shukla, 1991)
<i>Rhodobacter sphaeroides</i> and <i>Chlorella sorokiniana</i>	Synthetic high strength organic wastewater	Closed suspended system, batch mode, V=0.5 L	3	1.4-3.9 NO ₃ -N	99.7-99.8	17.6-22.0 PO ₄ -P	96.6-99.1	(Chinnasamy et al., 2010)
		Closed suspended system, batch mode, V=0.2 L	1	<0.1 NO ₃ -N 0.1 NH ₄ -N	100 100	<0.1 PO ₄ -P	100	(Ogboma et al., 2000)

S_i - initial concentration (in mg L⁻¹); R - removal efficiency (in%); V - working volume; NO₃-N - nitrate-nitrogen; NH₄-N - ammonium-nitrogen; TN - total nitrogen; PO₄-P - phosphate-phosphorus; TP - total phosphorus; n.a. - not applicable.

In microalgal-bacterial consortia, competitive and cooperative interactions between microalgae and bacteria have already been reported in the literature (Fukami et al., 1997; Muñoz and Guieysse, 2006; Natrah et al., 2014; Unnithan et al., 2014). Figure 2.3 evidences the possible interactions established between these microorganisms. Regarding competitive interactions, both microalgae and bacteria can have adverse effects on each other. Several studies have already reported the excretion of microalgal metabolites presenting a bactericidal effect (Kellam and Walker, 1989; Najdenski et al., 2013; Natrah et al., 2014). For example, chlorellin presents bactericidal activity against Gram-positive and Gram-negative bacteria, such as *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* (Pratt et al., 1944). Additionally, the increase in pH resulting from the photosynthetic activity can have a detrimental effect on the co-cultivated bacteria (Muñoz and Guieysse, 2006; Unnithan et al., 2014). Similarly, it has already been reported that bacteria can excrete metabolites presenting an algicidal effect towards microalgae (Natrah et al., 2014). On the other hand, a symbiotic relationship may be found in microalgal-bacterial interactions. During photosynthesis, microalgae release organic compounds that can be used by bacteria as carbon and energy source and O₂ that is used for the oxidation of organic matter. On the other hand, bacteria release the CO₂ required for photosynthetic reactions (Bordel et al., 2009; de Godos et al., 2009; Fouilland, 2012; Moriarty, 1997; Paerl and Pinckney, 1996). However, cooperative interactions between microalgae and bacteria are more complex than a simple nutrients exchange. Microalgae can serve as a habitat for bacteria, protecting them from adverse environmental conditions (Unnithan et al., 2014) and can enhance bacterial growth through the release of extracellular metabolites. Mandal et al. (2011) have demonstrated that extracellular polymeric substances produced by the microalga *Amphidinium carterae* have stimulated the growth of the bacterium *Bacillus pumilus*.

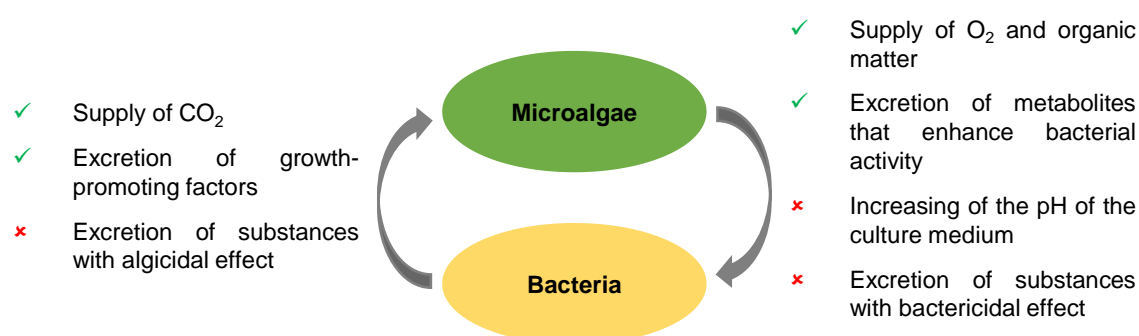


Figure 2.3. Cooperative (✓) and competitive (✗) interactions established between microalgae and bacteria (adapted from Muñoz and Guieysse (2006)).

Likewise, bacteria are responsible for the excretion of growth-promoting factors, such as vitamins (e.g. biotin, thiamine and cobalamine) and siderophores (important chelating agents for microalgal growth under iron-deficient conditions) (Subashchandrabose et al., 2011). In the study performed by De-Bashan et al. (2004), co-cultivation of *Azospirillum brasilense* with *C. vulgaris* and *C. sorokiniana* in alginate beads enhanced microalgal growth and improved nitrogen and phosphorus removal from a municipal wastewater used as culture medium. Apart from being effective in nutrients removal, these systems can further improve current wastewater treatment processes because (de Godos et al., 2009): (i) the costs associated to the oxygenation of activated sludge tanks can be significantly reduced; and (ii) the greenhouse effects associated to wastewater treatment plants can be considered negligible, since the CO₂ released by bacteria is converted into organic matter by microalgae.

Table 2.8 presents an overview of different microalgal-bacterial consortia that have been effectively applied in nitrogen, phosphorus and organic matter removal from different wastewater sources. Taking into account the CO₂/O₂ exchanges observed in these consortia and the associated beneficial effects, the “activated algae” concept has emerged as an attractive strategy for nutrients removal from wastewaters, combining the secondary and tertiary treatment steps in a single process (McGriff and McKinney, 1972). These systems have been firstly proposed by Oswald et al. (1957) using HRAPs, paddlewheel mixed, shallow, raceway-type ponds that are completely oxygenated (Cai et al., 2013; Craggs et al., 2013; Park and Craggs, 2011; Park et al., 2011; Rawat et al., 2011). Since this first report, HRAPs have been successfully applied around the world, being able to treat different wastewater streams, such as agricultural, domestic and industrial (Craggs et al., 2013; Park and Craggs, 2010, 2011; Rawat et al., 2011). In these reactors, microalgae live together with heterotrophic bacteria, promoting an effective removal of biochemical oxygen demand (BOD), COD and nutrients (Craggs et al., 2013; Rawat et al., 2011). When treating a domestic wastewater using a HRAP, Park and Craggs (2011) have reported NH₄-N and PO₄-P removal efficiencies of about 90 and 70%, respectively. Besides the advantages already reported for HRAPs, these systems present some limitations regarding: (i) light penetration and mixing, which are responsible for an imbalance between bacterial respiration and oxygen production by microalgae; (ii) the presence of protozoa and predatory zooplankton; and (iii) the necessity of a further harvesting step to separate small microalgae from the treated effluent. To overcome problems related to light penetration, mixing and presence of predators, several studies have proposed wastewater treatment using microalgal-

Table 2.8. Application of microalgal-bacterial consortia in nitrogen, phosphorus and carbon removal from different wastewaters and respective removal efficiencies

Microorganisms	Waste stream	System and operation mode	Removal time (d)	Nitrogen		Phosphorus		Carbon		References
				S _i (mg L ⁻¹)	R (%)	S _i (mg L ⁻¹)	R (%)	S _i (mg L ⁻¹)	R (%)	
Centrate wastewater native algal-bacterial consortium	Primary-treated domestic wastewater	Immobilized system (biofilm reactor), continuous mode, V=31 L	10	91 TN	70	7 PO ₄ -P	85	181 TOC	90	(Posadas et al., 2013)
Centrate wastewater native algal-bacterial consortium	Synthetic domestic wastewater	Closed suspended system, batch mode, V=1.9 L	7	50 NH ₄ -N	99.8	7 PO ₄ -P	99.8	300 TOC	100	(Alcántara et al., 2015b)
<i>Chlorella sorokiniana</i> and activated sludge native bacteria	Primary-treated piggyery wastewater	Immobilized system (biofilm reactor), continuous mode, V=7.5 L	7	656 NH ₄ -N	94-100	117 PO ₄ -P	70-90	1247 TOC	45	(de Godos et al., 2009)
Green algae and activated sludge native bacteria	Primary-treated municipal wastewater	Closed suspended system, semi-continuous mode, V=4 l	0.67	44.3 TN	61.2	1.4 PO ₄ -P	30.2-56.8	n.a.	n.a.	(Van Den Hende et al., 201
<i>Chlorella vulgaris</i> and brewery wastewater native microalgal-bacterial consortia	Brewery industry wastewater	Closed suspended system, batch mode, V=15 L	20	n.a.	n.a.	n.a.	n.a.	2172-3846 COD	13-15	(Raposo et al., 2010)
<i>Chlorella vulgaris</i> and municipal wastewater native bacteria	Primary-treated municipal wastewater	Closed suspended system, batch mode, V=1.5 L	8-20	17-207 NH ₄ -N	30.9-100	1.4-19.6 TP	65-98	n.a.	n.a.	(He et al., 2013)
Municipal wastewater native microalgae and bacteria	Primary-treated municipal wastewater	Open suspended system, batch mode, V=14 L	8-14	50.1 TN	93.7-95.8	8.8 PO ₄ -P		380 COD	91.2-96.2	(Su et al., 2012b)
Primary-treated wastewater native algal-bacterial	Primary-treated municipal wastewater	Closed suspended system, batch mode, V=14 L	8	14.6-18.9 NH ₄ -N	100	3.8-4.9 PO ₄ -P	54.5-72.6	36.9-63.6 TOC 103-191 COD	75.2 98	(Su et al., 2011)
<i>Scenedesmus</i> sp. and anaerobic sludge native bacteria	Starch wastewater	Closed suspended system, batch mode, V=0.06 L	5	30-50 NH ₄ -N	88.7	≈54 PO ₄ -P	80.1	1800-10000 COD	80.5	(Ren et al., 2015)

S_i - initial concentration (in mg L⁻¹); R - removal efficiency (in %); V - working volume; NO₃-N - nitrate-nitrogen; NH₄-N - ammonium-nitrogen; TN - total nitrogen; PO₄-P - phosphate-phosphorus; TP - total phosphorus; TOC - total organic carbon; COD - chemical oxygen demand; n.a. - not applicable.

bacterial consortia in closed PBRs. When growing a microalgal-bacterial consortium composed by *C. vulgaris* and primary-treated municipal wastewater native bacteria in tubular PBRs processing a primary-treated municipal wastewater ($\text{NH}_4\text{-N}$ and TP concentrations of 17-207 and 1.4-19.5 mg L^{-1} , respectively), He et al. (2013) have demonstrated removal efficiencies ranging between 30.9 and 100% for nitrogen and between 65 and 98% for phosphorus. Alcántara et al. (2015a) have used a closed tank to evaluate the performance of a microalgal consortium from a HRAP treating diluted vinasse and activated sludge native bacteria in nitrogen and organic matter removal from a synthetic wastewater containing 120 mg L^{-1} of $\text{NH}_4\text{-N}$ and 200 mg L^{-1} of total organic carbon (TOC). These authors have reported $\text{NH}_4\text{-N}$ removal efficiencies ranging between 75 and 96% and TOC removal efficiencies ranging between 86 and 90%. To avoid the requirements for further harvesting of microalgal biomass, some studies have reported the use of these consortia in immobilized growth systems. Posadas et al. (2013) have used a biofilm reactor to promote primary-treated domestic wastewater treatment (TN , $\text{PO}_4\text{-P}$ and TOC concentrations of 91, 7 and 181 mg L^{-1} , respectively) by a centrate wastewater native microalgal-bacterial consortium. This study has revealed TN , $\text{PO}_4\text{-P}$ and TOC removal efficiencies of 70, 85 and 90%, respectively. When growing *C. sorokiniana* and activated sludge native bacteria in a tubular biofilm PBR treating primary-treated piggery wastewater containing 656 mg L^{-1} of $\text{NH}_4\text{-N}$, 117 mg L^{-1} of $\text{PO}_4\text{-P}$ and 1247 mg L^{-1} of TOC, de Godos et al. (2009) have reported the following removal efficiencies: 94-100% for $\text{NH}_4\text{-N}$, 70-90% for $\text{PO}_4\text{-P}$ and 45% for TOC. Immobilization in solid carriers has also been reported in the literature. De-Bashan et al. (2004) have used an immobilized culture of *Chlorella* sp. and *A. brasilense* in alginate beads to treat a municipal wastewater with the following composition (in mg L^{-1}): 0.1-4.3 $\text{NH}_4\text{-N}$, 4-5.2 $\text{NO}_3\text{-N}$ and 4.1 $\text{PO}_4\text{-P}$. This study has resulted in an effective removal of nitrogen (both $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$) and phosphorus, with removal efficiencies ranging between 92 and 100%. To reduce time and costs associated to microalgal harvesting, some authors have also described the use of artificial consortia consisting of flocculating microorganisms. Van Den Hende et al. (2011) have reported the use of microalgal-bacterial flocs, mainly composed by *Chlorella* sp., *Pediastrum* sp., *Phormidium* sp., *Scenedesmus* sp. and activated sludge native bacteria, to treat a primary-treated municipal wastewater. The results obtained in this study have shown that these flocs were able to remove 61.2% of TN and 30.2-56.8% of $\text{PO}_4\text{-P}$. Similarly, Su et al. (2011) have grown a settleable microalgal-bacterial consortium in a primary-treated municipal wastewater, reporting a complete removal of $\text{NH}_4\text{-N}$, $\text{PO}_4\text{-P}$ removal efficiencies ranging between 54.5 and 72.6% and TOC

and COD removal efficiencies of 75.2 and 98%, respectively. To further improve the efficiency of these systems in nutrients removal, some authors have studied the effect of culturing conditions, such as light, nutrients concentration, pH and microalgal:bacterial ratio (Alcántara et al., 2015b; Liang et al., 2013; Medina and Neis, 2007; Su et al., 2012b). Liang et al. (2013) have studied the effect of pH in the performance of a microalgal-bacterium consortium composed by *C. vulgaris* and *Bacillus licheniformis* in nitrogen and phosphorus removal from a synthetic medium, reaching $\text{NH}_4\text{-N}$ removal efficiencies of 78% and TP removal efficiencies of 92%. When evaluating the effect of algae:sludge ratios on nutrients removal from a primary-treated municipal wastewater, Su et al. (2012b) have reported TN removal efficiencies ranging between 93.7 and 95.8%, $\text{PO}_4\text{-P}$ removal efficiencies ranging between 64.0 and 93.5% and COD removal efficiencies ranging between 91.2 and 96.2%.

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3. Materials and methods

3.1. Culture media

All the experiments regarding monocultures and microalgal consortia were performed in Organization for Economic Co-operation and Development (OECD) test medium (OECD, 2011) with the following composition (per litre): 15 mg NH_4Cl , 12 mg $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 18 mg $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 15 mg $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.6 mg KH_2PO_4 , 0.08 mg $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 0.1 mg $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$, 0.185 mg H_3BO_3 , 0.415 mg $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 3 μg ZnCl_2 , 1.5 μg $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 0.01 μg $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, 7 μg $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ and 50 mg NaHCO_3 ($\text{pH}=7.25\pm0.62$). Since this growth medium presents low concentrations of nitrogen and phosphorus, concentrations of these elements were increased to simulate the concentrations commonly present in a secondary-treated effluent. Accordingly, NaNO_3 and KH_2PO_4 were supplied at 250 and 45 mg L^{-1} (Wang et al., 2010), respectively. Nitrate was used as nitrogen source because this is the most thermodynamically stable form of inorganic nitrogen (Barsanti and Gualtieri, 2006) and also because it is the most abundant nitrogen form in the tertiary treatment step of wastewater treatment plants, where microalgae can play an important remediation role (Wang et al., 2010).

The experiments regarding microalgal-bacterial consortia required an organic carbon source to support bacterial growth. Accordingly, these experiments were performed in a synthetic medium simulating a secondary-treated effluent with the following composition (per litre): 300 mg $\text{C}_6\text{H}_{12}\text{O}_6$, 250 mg NaNO_3 , 10 mg $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 45 mg KH_2PO_4 , 1 mg $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.46 mg $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.05 mg $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and 0.06 mg $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$ ($\text{pH}=6.39\pm0.46$).

Both media were sterilized by autoclaving at 121 °C for 15 min.

3.2. Microorganisms and stock solutions preparation

The microorganisms used in this study were the microalgae *C. vulgaris* CCAP 211/11B and *P. subcapitata* CCAP 278/4 and the cyanobacteria *S. salina* LEGE 06079 and *M. aeruginosa* LEGE 91344. Both microalgae were obtained from Culture Collection of Algae and Protozoa (CCAP, UK), whereas the cyanobacteria were obtained from the Laboratory of Ecotoxicology, Genomic and Evolution of the Centre of Marine and Environmental Research of the University of Porto (CIIMAR, Portugal). Selection of these microorganisms was based on the following factors (Chinnasamy et al., 2009; McLarnon-Riches et al., 1998; Philippis and Vincenzini, 1998; Wahlen et al., 2011): (i) these microorganisms can be easily grown in laboratory cultures; and (ii) several authors have reported the use of these microorganisms in a wide variety of biotechnological applications, such as CO₂ capture, wastewater treatment, biofuels production and synthesis of bioactive compounds. Stock solutions of these microorganisms were prepared in OECD test medium (described in Section 3.1). The cells were incubated for seven days in 500-mL flasks at room temperature, under continuous fluorescent light with an irradiance of 120 $\mu\text{E m}^{-2} \text{ s}^{-1}$ at the surface of the flasks. Agitation was promoted by bubbling atmospheric air (filtered through 0.22- μm cellulose acetate membranes, Orange Scientific, Belgium) at the bottom of the flasks. After this period, cells were harvested through centrifugation at 2,900 g for 15 min (in an Eppendorf 5810 R centrifuge, Eppendorf, Germany) and appropriate dilutions in OECD test medium (described in Section 3.1) were performed to obtain an inoculum concentration of approximately 1.0×10^6 cells mL^{-1} .

For the microalgal-bacterial experiments, the bacteria selected to integrate the consortia were isolated from the secondary treatment step of a MWTP located in Rabada (Santo Tirso, Portugal), in May 2014. Activated sludge native bacteria were selected to constitute these consortia because these microorganisms were already adapted to the environmental conditions typically found in a MWTP. Among the microorganisms present in the samples, only three of them were able to grow on the synthetic medium described in Section 3.1. Accordingly, these microorganisms were isolated and identified (STABVIDA, Portugal) through 16S rRNA gene sequencing using four different primers: 27F, 518F, 800R and 1492R. Identification of these isolates has distinguished three rod-shaped Gram-negative bacteria belonging to the family Enterobacteriaceae (phylum Proteobacteria): *Enterobacter asburiae*, *Klebsiella* sp. and *Raoultella ornithinolytica*, formerly known as *Klebsiella*

ornithinolytica. These microorganisms are commonly found in water, soil, plants, sewage and others. After the isolation and identification steps, the isolates were grown in 250-mL Erlenmeyer flasks using Luria Broth as culture medium. Cells were incubated overnight at 30 °C with orbital agitation (150 rpm). After the incubation period, cells were harvested through centrifugation at 2,900 g for 15 min (in an Eppendorf 5810 R centrifuge, Eppendorf, Germany) and appropriate dilutions in the synthetic medium described in Section 3.1 were performed to obtain an inoculum concentration of approximately 1.0×10^8 CFU mL⁻¹ (CFU, colony forming units).

3.3. Experimental setup and cultivation conditions

Taking into account the different strategies covered by this study (Section 1.2), different experimental setups and cultivation conditions were adopted. The following sections describe in detail the experimental setup and cultivation conditions used in the different steps of this work.

3.3.1. Cultivation conditions using microalgal monocultures

To analyse the effect of light supply on microalgal monocultures (results presented in Section 4.1), batch experiments of *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* were performed in 500-mL flasks (VWR, Portugal) with a working volume of 400 mL (Figure 3.1-A). Cells were cultivated in OECD test medium (described in Section 3.1) for 12 days in the following conditions: (i) initial cell concentration of approximately 1.0×10^6 cells mL⁻¹, which corresponds to a biomass (cell dry weight - DW) concentration of about 0.05-0.08 g DW L⁻¹; (ii) initial pH was set at 7; (iii) room temperature (approximately 24.6 ± 1.6 °C); and (iv) continuous aeration with the injection of atmospheric air at the bottom of the flasks. The assays were carried out under different light irradiance values: 36, 60, 120 and 180 $\mu\text{E m}^{-2} \text{s}^{-1}$. Several research studies have applied similar light irradiance values for microalgal growth (Ho et al., 2012; Ho et al., 2010a; Ryu et al., 2009). For each irradiance value, different light cycles were evaluated: 10:14, 14:10, and 24:0 (light:dark ratio). The light:dark ratio of 24:0 was used because it promotes continuous photoautotrophic growth. To reduce production costs in terms of light requirements, the light:dark ratios of 10:14 and 14:10 were applied to simulate the number of light hours during winter and summer time, respectively. Taking into account the light irradiances and light:dark ratios evaluated in this

study, the corresponding average daily light irradiances are summarized in Table 3.1. For each condition, two independent experiments were performed.

To evaluate the combined effect of light supply and temperature on microalgal monocultures (results presented in Section 4.2), batch experiments of *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* were performed in 500-mL flasks (VWR, Portugal) with a working volume of 400 mL (Figure 3.1-B). Cells were cultivated in OECD test medium (described in Section 3.1) for 12 days in the following conditions: (i) initial cell concentration of approximately 1.0×10^6 cells mL⁻¹, which corresponds to a biomass concentration of about 0.05-0.08 g DW L⁻¹; (ii) initial pH was set at 7; (iii) continuous aeration with the injection of atmospheric air at the bottom of the flasks. The assays were carried out under different temperatures (15, 25 and 35 °C) and incident light irradiance values: 36 and 180 $\mu\text{E m}^{-2} \text{s}^{-1}$. The temperatures of 15, 25 and 35 °C were selected to simulate average temperatures observed in cold, warm and tropical regions, respectively. Light irradiance values were selected to observe the effect of low and high irradiance levels. For each temperature and irradiance value, different light cycles were evaluated: 10:14, 14:10, and 24:0 (light:dark ratio). Selection of these light:dark ratios was based on the factors already reported. Taking into account the light irradiances and light:dark ratios evaluated in this study, the corresponding average daily light irradiances are summarized in Table 3.1. For each condition, two independent experiments were performed.

Table 3.1. Average daily light irradiances evaluated in this study considering light:dark ratios and light irradiance values applied to the studied cultures

Light:dark ratio (h:h)	Light irradiance ($\mu\text{E m}^{-2} \text{s}^{-1}$)	Average daily light irradiance ($\mu\text{E m}^{-2} \text{s}^{-1}$)
10:14	36	15
	60	25
	120	50
	180	75
14:10	36	21
	60	35
	120	70
	180	105
24:0	36	36
	60	60
	120	120
	180	180

To evaluate the effect of CO₂ concentration in the air stream on microalgal monocultures (results presented in Section 4.3), batch experiments of *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* were performed in 500-mL flasks (VWR, Portugal) with a working volume of 400 mL (Figure 3.1-C). Cells were cultivated in OECD test medium (described in Section 3.1) for 7 days in the following conditions: (i) initial cell concentration of approximately 1.0×10^6 cells mL⁻¹, which corresponds to a biomass concentration of about 0.05-0.08 g DW L⁻¹; (ii) initial pH was set at 7; (iii) room temperature (approximately 23.4 ± 2.1 °C); (iv) continuous light supply at an average daily light irradiance of 164 ± 9 $\mu\text{E m}^{-2} \text{s}^{-1}$. Additionally, the cultures were continuously fed with an air stream containing atmospheric air and CO₂-enriched air (with CO₂ concentrations of 3, 5, 7, 9 and

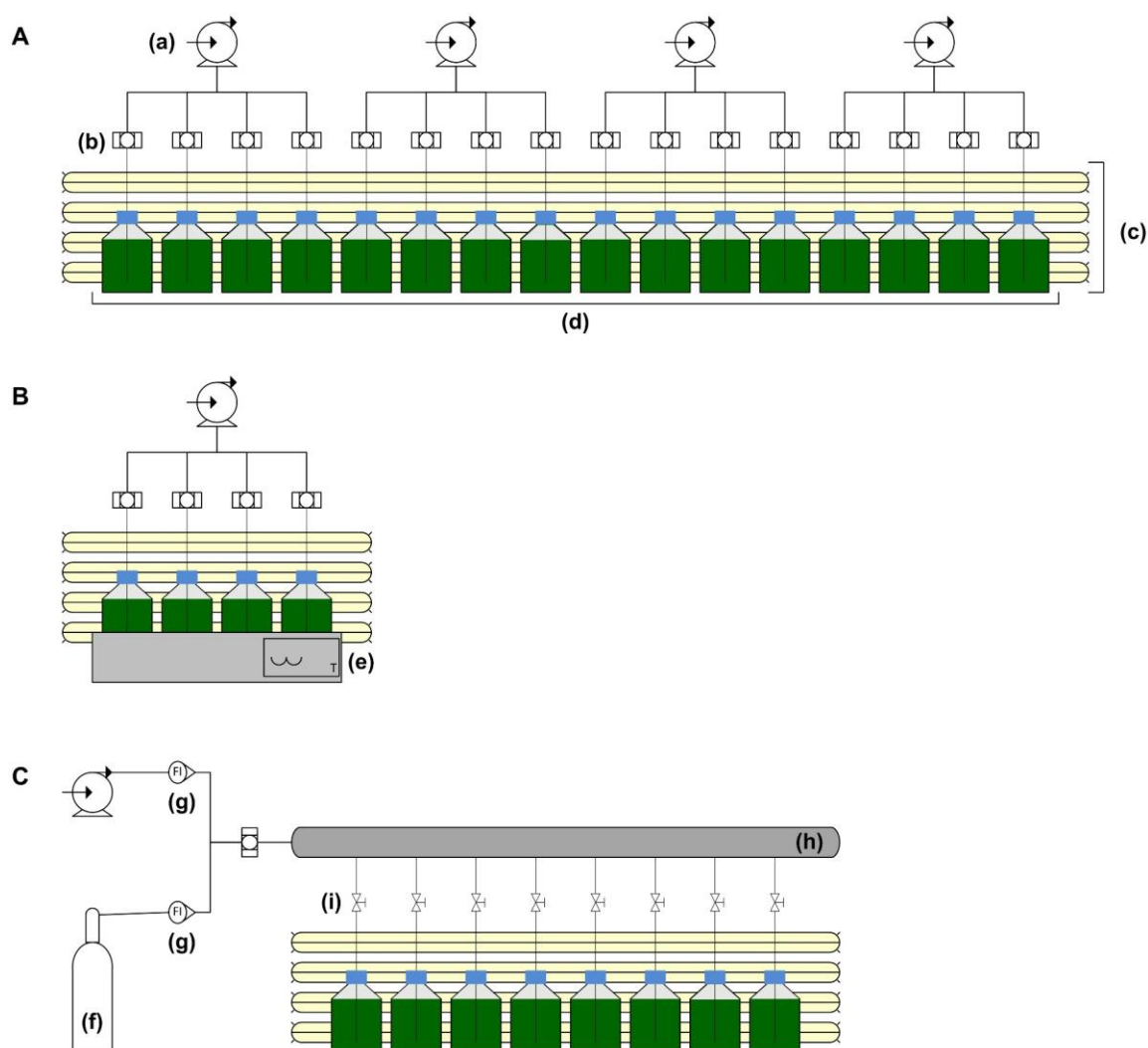


Figure 3.1. Schematic representation of the experimental setup used for the evaluation of different cultivation conditions in microalgal monocultures: light supply (A), light supply and temperature (B) and CO₂ concentration in the air stream (C). (a) air pump; (b) filter; (c) fluorescent lamps; (d) cultivation flasks; (e) thermostatic bath; (f) CO₂ bottle; (g) flow meters; (h) mixing column; (i) valves.

10% v/v) at 0.5 vvm (volume of gas per working volume per minute). These concentrations were selected because optimal CO₂ concentrations described for microalgae typically range between 5 and 10% (v/v). Although these microorganisms can tolerate higher CO₂ concentrations, several authors have reported maximum growth rates for CO₂ concentrations within this range (Hirata et al., 1996a; Hirata et al., 1996b; Maeda et al., 1995; Nakano et al., 1996). These concentrations were obtained by mixing atmospheric air with CO₂ at different proportions using two Key Instruments mass flow meters (RS Components, Spain), as it is shown in Figure 3.1-C. Before injection at the bottom of the flasks, the air stream was filtered through 0.22- μ m cellulose acetate membranes (Orange Scientific, Belgium). For each condition, two independent experiments were performed.

3.3.2. Cultivation conditions using microalgal and microalgal-bacterial consortia

To study microalgal consortia (results presented in Section 4.4), batch experiments with single and dual-species cultures of *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* were performed in 500-mL flasks (VWR, Portugal) with a working volume of 400 mL (Figure 3.2-A). Combination of these microorganisms has resulted in six different consortia: *C. vulgaris* + *P. subcapitata*, *C. vulgaris* + *S. salina*, *C. vulgaris* + *M. aeruginosa*, *P. subcapitata* + *S. salina*, *P. subcapitata* + *M. aeruginosa* and *S. salina* + *M. aeruginosa*. Single cultures were performed at the same time as control. Cells were cultivated in OECD test medium (described in Section 3.1) for 7 days in the following conditions: (i) initial cell concentration of approximately 1.0×10^6 cells mL⁻¹ (in co-cultures, initial cell concentration was approximately 2.0×10^6 cells mL⁻¹); (ii) initial pH was set at 7; (iii) room temperature (approximately 24.2 ± 1.5 °C); (iv) continuous light supply at an average daily light irradiance of 165 ± 10 μ E m⁻² s⁻¹; and (v) continuous aeration with the injection of atmospheric air at the bottom of the flasks. For each condition, four independent experiments were performed.

To study microalgal-bacterial consortia (results presented in Section 4.5), batch experiments with single and dual-species microalgal-bacterial consortia were performed in 500-mL flasks (VWR, Portugal) with a working volume of 400 mL (Figure 3.2-B). In these experiments, *C. vulgaris* was the photosynthetic microorganism selected to integrate these consortia because it presents high ability for nutrients removal, being widely used in wastewater treatment processes (Wang et al., 2010). Co-cultivation of *C. vulgaris* with the bacterial isolates described in Section 3.2 has resulted in three different consortia: CE - *C. vulgaris* +

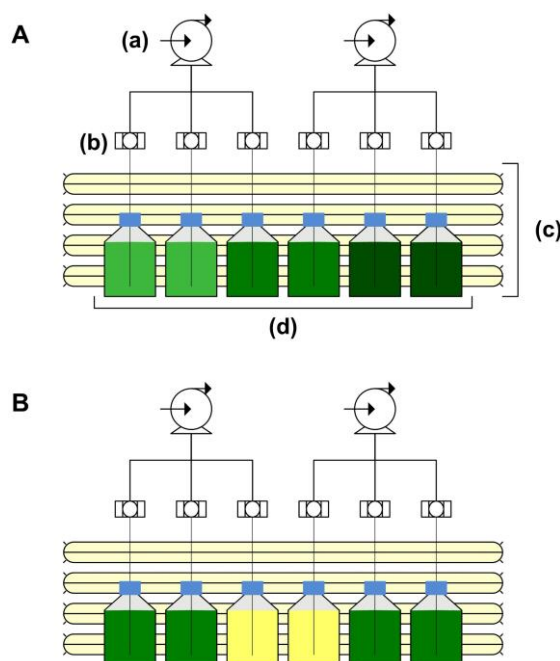


Figure 3.2. Schematic representation of the experimental setup used for the evaluation of different consortia: microalgal consortia (A) and microalgal-bacterial consortia (B). (a) air pump; (b) filter; (c) fluorescent lamps; (d) cultivation flasks.

E. asburiae, CK - *C. vulgaris* + *Klebsiella* sp. and CR - *C. vulgaris* + *R. ornithinolytica*. Single cultures of *C. vulgaris* and the bacterial isolates were performed at the same time as control. Cells were cultivated in the synthetic medium described in Section 3.1 for 7 days in the following conditions: (i) initial cell concentration of approximately 1.0×10^6 cells mL⁻¹ (for *C. vulgaris*) and 1.0×10^8 CFU mL⁻¹ (for the bacterial isolates); (ii) initial pH was set at 7; (iii) room temperature (approximately 22.3 ± 0.7 °C); (iv) continuous light supply at an average daily light irradiance of 162 ± 8 $\mu\text{E m}^{-2} \text{s}^{-1}$; and (v) continuous aeration with the injection of atmospheric air at the bottom of the flasks. For each condition, four independent experiments were performed.

3.3.3. Cultivation conditions in a 60-L open raceway pond

Batch experiments of the most effective microorganisms and/or consortia in terms of biomass production, CO₂ uptake and nutrients removal (single *C. vulgaris* culture and SC and CE consortia) were performed in an open raceway pond with a working volume of 60 L (results presented in Section 4.6). As for the batch experiments conducted in the 500-mL flasks, the culture medium used for the single *C. vulgaris* culture and for the SC consortium was the OECD test medium, whereas the culture medium used in the experiments with the

CE consortium was the synthetic medium, both of them described in Section 3.1. The raceway pond (schematically represented in Figure 3.3) presented a length of 88.9 cm and a width of 14.0 cm and was made of poly(methyl methacrylate) (PMMA). Operation of the reactor was performed at a constant depth of 25.8 cm, performing a total surface area of 0.14 m². Mixing was promoted by a 12-blade paddlewheel with a diameter of 30.0 cm and agitation was provided by a RZR 2021 mechanical stirrer (Heidolph Instruments, Germany). Cells were cultivated for 12 days in the following conditions: (i) initial cell concentrations similar to those used in the 500-mL flasks' experiments (see Sections 3.3.1 and 3.3.2); (ii) initial pH was set at 7; (iii) room temperature (approximately 18.9±1.0 °C); (iv) continuous light supply at an average daily light irradiance of 23±2 µE m⁻² s⁻¹ at the surface of the liquid. For each microorganism/consortium, two independent experiments were performed.

To evaluate which was the most suitable velocity to promote the complete agitation of the culture broth, three fluid velocities were evaluated prior to the experiments with *C. vulgaris* and the SC and CE consortia: 0.10, 0.15 and 0.20 m s⁻¹. Selection of these values was based on the capacities of the mechanical stirrer used and on the fluid velocities required to avoid the development of dead or stagnant zones, regions where little flow is observed. In these regions, cells' accumulation may occur, leading to the development of anaerobic conditions where anaerobic bacteria can proliferate and to the reduction of the pond volume, thus decreasing the residence time and biomass productivities (Hadiyanto et al., 2013). According to Sompech et al. (2012), regions of the reactor presenting fluid velocities inferior to 0.1 m s⁻¹ are considered dead zones. Additionally, Hadiyanto et al. (2013) have reported

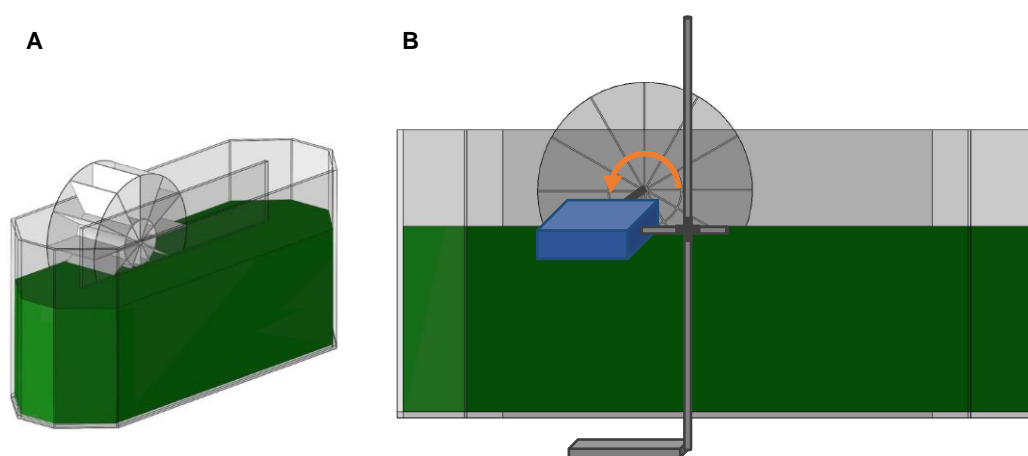


Figure 3.3. Schematic representation of the experimental setup used for the study of the most promising microorganisms and/or consortia in a pilot-scale unit: general view (A) and experimental setup including the mechanical stirrer used to promote the rotation of the paddlewheel (B).

that a good mixing requires fluid velocities ranging between 0.1 and 0.3 m s⁻¹. Taking into account the fluid flow displaced by the blades of the paddlewheel and the cross-sectional area of the fluid, rotational speeds required to achieve the studied fluid velocities were 26, 39 and 52 rotations per minute (rpm), respectively. These experiments were performed only for the single *C. vulgaris* culture, being the experimental conditions the same as those previously reported. Since a good mixing of the culture was obtained with the lowest rotational speed and once the other studied velocities have not strongly influenced microalgal growth, all the experiments were conducted at 26 rpm.

Finally, since the light supplied to the studied cultures has shown to be limiting for microalgal growth and once these conditions were not representative of those typically found in real environmental conditions, these experiments were reproduced in outdoor conditions in three different periods, so that different average daily light irradiances and weather conditions could be evaluated. As for the study of different rotational speeds, these experiments were conducted only for the single *C. vulgaris* culture, and all other conditions were maintained constant: (i) initial cell concentration of approximately 1.0×10^6 cells mL⁻¹, which corresponds to a biomass concentration of about 0.2 g DW L⁻¹; (ii) initial pH was set at 7; (iii) room temperature; and (iv) agitation was promoted through a paddlewheel rotating at 26 rpm. With these experiments, average daily light irradiances evaluated corresponded to 102, 153 and 204 $\mu\text{E m}^{-2} \text{s}^{-1}$. Average daily light irradiances were determined through hourly measurements of light irradiance (with the exception of the dark periods) during the cultivation time.

3.4. Microalgal growth monitoring

Microalgal growth was daily monitored through (i) optical density (OD) measurements; (ii) cell counting using an optical microscope; or (iii) cell dry weight determinations. For this, samples were collected in duplicates at 24-h intervals. OD measurements were performed by measuring absorbance at 750 nm in a V-1200 spectrophotometer (VWR, Portugal), according to the method proposed by Pegallapati and Nirmalakhandan (2013). Cell concentration (N_M , in cells mL⁻¹), was determined using a Neubauer counting chamber (Marienfeld, Germany), under a Leica DM LB (Leica Microsystems, Germany) microscope. Biomass concentration in terms of ash free dry weight (X , in mg DW L⁻¹) was determined by drying 10 mL of the collected samples for 24 h at 105 °C in previously dried and weighed

porcelain crucibles, until a constant weight was obtained. The dried biomass was then oxidized at 550 °C for 2 h in a muffle and re-weighed. The mass loss obtained after biomass oxidation divided by the samples' volume gives the ash free dry weight biomass concentration. A linear regression was established between OD and cell and biomass concentrations (Figure I.1, Annex I). Table 3.2 presents the respective equations.

In the studies regarding microalgal and microalgal-bacterial consortia, microalgal growth of single and dual-species cultures was evaluated through cell counting at 24-h intervals and cell dry weight determinations in the first and last day of culturing. On the other hand, bacterial growth was determined through CFU enumeration (N_B , in CFU mL⁻¹) at 2-h intervals for 8 h and then at 24, 48, 72, 96, 120, 144 and 168 h. For this, the necessary dilutions were prepared and plated on Plate Count Agar (VWR, Portugal) plates using the motion drop method (Reed and Reed, 1948) and plates were incubated at 30 °C for 16 h.

3.5. Daily measurements

For all the performed experiments, light irradiance, pH, temperature and dissolved oxygen concentrations were daily monitored. Light irradiance was measured using an ISO-TECH 1335 (RS Components, UK) light meter, while pH was measured using a pH212 (Hanna Instruments, Germany) microprocessor-based pH meter. Temperature and dissolved oxygen

Table 3.2. Calibration curves of OD and cell and biomass concentrations for the studied microorganisms

Microorganisms	OD (750 nm) = $m \cdot N$ or $X + b$	R^2	DL	QL
OD (750 nm) vs N (cells mL⁻¹)				
<i>C. vulgaris</i>	$y = ((3.92 \pm 0.06) \times 10^{-8}) \cdot x + ((1.34 \pm 0.60) \times 10^{-2})$	0.997	4.57×10^5	1.52×10^6
<i>P. subcapitata</i>	$y = ((5.45 \pm 0.08) \times 10^{-8}) \cdot x - ((4.33 \pm 1.12) \times 10^{-3})$	0.997	6.18×10^5	2.06×10^6
<i>S. salina</i>	$y = ((3.96 \pm 0.05) \times 10^{-8}) \cdot x + ((2.20 \pm 0.73) \times 10^{-2})$	0.999	5.53×10^5	1.84×10^6
<i>M. aeruginosa</i>	$y = ((6.21 \pm 0.32) \times 10^{-8}) \cdot x - ((4.36 \pm 3.10) \times 10^{-2})$	0.982	1.50×10^6	5.00×10^6
OD (750 nm) vs X (mg DW L⁻¹)				
<i>C. vulgaris</i>	$y = ((1.80 \pm 0.03) \times 10^{-3}) \cdot x + ((4.28 \pm 2.72) \times 10^{-2})$	0.998	45.4	151
<i>P. subcapitata</i>	$y = (2.61 \pm 0.08) \times 10^{-3} \cdot x + ((6.92 \pm 4.20) \times 10^{-2})$	0.995	48.2	161
<i>S. salina</i>	$y = (2.32 \pm 0.16) \times 10^{-3} \cdot x - ((1.63 \pm 0.68) \times 10^{-1})$	0.972	88.3	294
<i>M. aeruginosa</i>	$y = (2.04 \pm 0.10) \times 10^{-3} \cdot x + ((2.72 \pm 4.49) \times 10^{-2})$	0.986	66.1	220

OD - optical density measured at 750 nm; m - slope of the calibration curves; N - cell concentration (in cells mL⁻¹); X - biomass concentration (in mg DW L⁻¹); b - intercept; R^2 - coefficient of determination; DL - detection limit (in cells mL⁻¹ or mg DW L⁻¹); QL - quantification limit (in cells mL⁻¹ or mg DW L⁻¹).

concentration were measured using an Oxi 340i (WTW, Germany) oxygen sensor.

3.6. Kinetic growth parameters

Cell or biomass concentration values were used to determine specific growth rates (μ , in d^{-1}) and average biomass productivities (P , in $\text{mg DW L}^{-1} \text{d}^{-1}$). Specific growth rates were determined according to Equation 3.1:

$$\mu = \frac{\ln X_2 - \ln X_1}{t_2 - t_1} \quad \text{or} \quad \mu = \frac{\ln N_2 - \ln N_1}{t_2 - t_1} \quad (3.1)$$

where X_2 and X_1 correspond to biomass concentrations (in mg DW L^{-1}) and N_2 and N_1 correspond to cell concentrations (in cell mL^{-1} or CFU mL^{-1}) at times t_2 and t_1 (in d), the end and beginning of the exponential growth phase, respectively. Average biomass productivities were calculated from the variation in biomass concentration within the cultivation time, as shown in Equation 3.2 (Feng et al., 2012; Jacob-Lopes et al., 2009):

$$P = \frac{X_f - X_i}{t_f - t_i} \quad (3.2)$$

where X_f and X_i correspond to biomass concentrations (in mg DW L^{-1}) at times t_f and t_i (in d), the end and beginning of cultivation time, respectively.

3.7. Mathematical modelling of microalgal growth in response to different cultivation conditions

To determine the optimal growth conditions (average daily light irradiance, temperature and CO_2 concentration in the air stream) for the studied microorganisms, two kinetic growth models were developed.

The behaviour of specific growth rates for increasing average daily light irradiance values was described according to the model proposed by Steele (1977):

$$\mu = \frac{\mu_{\max} \cdot I}{I_{\text{opt}}} \cdot e^{\left(1 - \frac{I}{I_{\text{opt}}}\right)} \quad (3.3)$$

where μ_{max} corresponds to the maximum specific growth rate (in d^{-1}) achieved by the studied microorganisms, I denotes average daily light irradiance (in $\mu E\ m^{-2}\ s^{-1}$) and I_{opt} corresponds to the optimal value of average daily light irradiance (in $\mu E\ m^{-2}\ s^{-1}$) for microalgal growth.

On the other hand, the behaviour of specific growth rates for different temperatures was assumed to follow a skewed normal distribution, as reported by Dauta et al. (1990):

$$\mu = \mu_{max} \cdot e^{-\frac{(T-T_{opt})^2}{2\sigma^2}} \quad (3.4)$$

where T is the temperature (in $^{\circ}C$), T_{opt} is the optimal temperature (in $^{\circ}C$) for microalgal growth and σ is the standard deviation associated to the optimal temperature (in $^{\circ}C$).

Equations 3.3 and 3.4 were used to establish a two-dimensional model, resulting in the following expression:

$$\mu = \frac{\mu_{max} \cdot I}{I_{opt}} \cdot e^{\left(1 - \frac{I}{I_{opt}}\right)} \cdot e^{-\frac{(T-T_{opt})^2}{2\sigma^2}} \quad (3.5)$$

This expression was linearized (Equation 3.6) and the parameters μ_{max} , I_{opt} , T_{opt} and σ were determined by minimizing the sum of squared residuals using the Solver supplement of Microsoft Excel 2013.

$$\ln \mu = \ln \mu_{max} + \ln \frac{I}{I_{opt}} + 1 - \frac{I}{I_{opt}} - \frac{(T - T_{opt})^2}{2\sigma^2} \quad (3.6)$$

Development of this model was based on specific growth rates determined for each of the studied microorganisms when grown under different light and temperature conditions. These data were collected from the results obtained in study and from other results obtained from the literature, as it is possible to see in Table I.1 (Annex I).

The behaviour of specific growth rates with increasing CO_2 concentrations in the air stream was described according to the model proposed by Rosso et al. (1993):

$$\mu = \mu_{max} \cdot \frac{(C - a)(C - b)^2}{(C_{opt} - b)[(C_{opt} - b)(C - C_{opt}) - (C_{opt} - a)(C_{opt} + b - 2C)]} \quad (3.7)$$

where C denotes the concentration of CO₂ in the air stream (in % v/v), a and b are model parameters and C_{opt} is the optimal CO₂ concentration in the air stream (in % v/v). The parameters μ_{max} , a , b and C_{opt} were also determined by minimizing the sum of squared residuals using the Solver supplement of Microsoft Excel 2013.

The quality of both model fits was evaluated by calculating the coefficient of determination (R^2) and the root mean squared error (RMSE):

$$R^2 = 1 - \frac{\sum_{i=1}^n (z - \hat{z})^2}{\sum_{i=1}^n (z - \bar{z})^2} \quad (3.8)$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^n (z - \hat{z})^2}{n}} \quad (3.9)$$

where z denotes the experimental values, \hat{z} the predicted values by the model, \bar{z} the average of the experimental values and n the data size.

3.8. Elemental analysis of biomass and CO₂ uptake rate

In the last day of culturing, 10-mL samples were collected, washed twice with distilled water and freeze-dried in a Labconco FreeZone Plus 2.5 freeze dryer (VWR, Portugal). The carbon content of microalgal biomass was determined using a Flash EA1112 CHNS analyser (Thermo Finnigan CE Instruments, Italy) equipped with a gas chromatography column and a thermal conductivity detector. Average CO₂ uptake rates (R_C , in mg CO₂ L⁻¹ d⁻¹) of the studied microorganisms were calculated based on the relationship between microalgal carbon content and average biomass productivities and assuming that all the CO₂ assimilated by the studied microorganisms was incorporated in the biomass (de Moraes and Costa, 2007b):

$$R_C = \alpha_C \cdot P \cdot \frac{M_{CO_2}}{M_C} \quad (3.10)$$

where α_C is the carbon content of microalgal biomass (in % w/w) determined through elemental analysis, and M_{CO_2} and M_C correspond to the molecular weights (in g mol⁻¹) of CO₂ and carbon, respectively.

3.9. Nutrients concentration

Nutrients removal was evaluated by quantification of $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$ in the culture medium. In the experiments performed with microalgal-bacterial consortia, soluble COD in the culture medium was also determined. In the experiments regarding the effect of light supply and light supply and temperature, samples were collected in the first and last day of culturing. In the other experiments, samples were collected on a daily basis.

To quantify nitrogen concentration, the collected samples were centrifuged at 16,500 g for 10 min (in an Eppendorf 5418 centrifuge, Eppendorf, Germany) and the supernatants were stored at $-20\text{ }^\circ\text{C}$ until being analysed. $\text{NO}_3\text{-N}$ concentration was then determined according to Standard Methods (APHA, 1999). Firstly, the supernatants were thawed, diluted approximately 20 times in distilled water and filtered using $0.22\text{-}\mu\text{m}$ cellulose acetate membranes (Orange Scientific, Belgium). After filtration, absorbance of the samples was measured at 220 nm using a quartz cuvette in a T80 UV/VIS spectrophotometer (PG Instruments, UK). Distilled water previously filtered was used as blank. The calibration curve (Figure I.2, Appendix I) was determined by preparing NaNO_3 standards with concentrations ranging from 5 to 500 mg L^{-1} and submitting them to the same procedure as the analysed samples. Samples were analysed in triplicates.

To quantify phosphorus concentration, the collected samples were centrifuged and the supernatants were stored at $-20\text{ }^\circ\text{C}$ until being analysed (as it was described for nitrogen). Then $\text{PO}_4\text{-P}$ quantification was performed by measuring absorbance at 820 nm of a phosphomolybdate complex formed by reaction of inorganic phosphate with ammonium molybdate, as proposed by Lee et al. (2009). In this method, $60\text{ }\mu\text{L}$ of each of the thawed supernatants was pipetted into a well of a 96-well microtiter plate and then $140\text{ }\mu\text{L}$ of the reaction mix was added. The reaction mix was prepared by adding 1 part of reagent 1 (10% w/v ascorbic acid) to 6 parts of reagent 2 (0.42% w/v $(\text{NH}_4)_2\text{MoO}_4\cdot 4\text{H}_2\text{O}$ in 1 N H_2SO_4). The microtiter plate was incubated at $37\text{ }^\circ\text{C}$ for 1 h and the absorbance was measured at 820 nm in a SynergyTM HT 96-well microplate reader (Biotek Instruments, Inc., USA). The blank was measured by repeating the procedure using distilled water. To determine the calibration curve (Figure I.3, Appendix I), standards of KH_2PO_4 with concentrations ranging from 1 to 60 mg L^{-1} were submitted to the same procedure. Samples were analysed in triplicates.

Measurements of soluble COD were performed using the closed reflux titrimetric method according to Standard Methods (APHA, 1999). The samples were refluxed in a strong acidic solution with a known excess of potassium dichromate ($K_2Cr_2O_7$). After digestion, the amount of consumed $K_2Cr_2O_7$ was determined by titration of the unreduced $K_2Cr_2O_7$ with ferrous ammonium sulphate (FAS) and the organic matter was calculated in terms of oxygen equivalent. For this, the digestion vessels were filled with 2.5 mL of the collected samples, 1.5 mL of standard $K_2Cr_2O_7$ solution 0.01667 M (prepared by dissolving approximately 4.903 g of $K_2Cr_2O_7$ in 500 mL of distilled water, followed by the addition of 167 mL of H_2SO_4 and 33.3 g of $HgSO_4$ and dilution to 1 L) and 3.5 mL of sulphuric acid reagent (prepared by the addition of 5.5 g $AgSO_4$ to 500 mL H_2SO_4). The vessels were then placed in the block heater and digestion was performed at 150 °C for 2 h. After this period the vessels were cooled to room temperature and the contents were transferred to different beakers for titration assays using a 0.10 M FAS solution (prepared by dissolving 39.2 g of $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in distilled water, followed by the addition of 20 mL of H_2SO_4 and dilution to 1 L) as titrant and ferroin (Panreac, Spain) as indicator. The end point was detected by a change in colour from blue-green to red. The blank was performed by repeating this procedure using distilled water. Organic matter in terms of oxygen equivalent was determined according to Equation 3.11:

$$COD = \frac{(V_{FAS;B} - V_{FAS;S}) \cdot M_{FAS} \cdot 8000}{V_S} \quad (3.11)$$

where $V_{FAS;B}$ is the volume of FAS used in the titration of the blank (in mL), $V_{FAS;S}$ is the volume of FAS used in the titration of the samples (in mL), M is the molecular weight of FAS and V_S is the sample's volume (in mL).

3.10. Nutrients removal kinetics

In the experiments where nutrients concentrations were determined in the first and last day of culturing, average removal rates (R_S , in $mg\ S\ L^{-1}\ d^{-1}$) and nutrients removal efficiencies (R , in %) were determined. Average removal rates were calculated according to Equation 3.12:

$$R_S = \frac{S_i - S_f}{t_f - t_i} \quad (3.12)$$

where S_i and S_f correspond to nutrients concentrations (in mg S L⁻¹) at times t_i and t_f (in days), respectively. Nutrients removal efficiencies were determined according to Equation 3.13:

$$\%R = \frac{S_i - S_f}{S_i} \times 100 \quad (3.13)$$

Values corresponding to the time-course evolution of nutrients concentration ($S(t)$, in mg S L⁻¹) were used to determine uptake rates (k , in d⁻¹). These determinations were performed by fitting the experimental data to the modified Gompertz model (Zwietering et al., 1990). This model has already been applied to describe microalgal (Çelekli et al., 2008; Çelekli et al., 2009; Lacerda et al., 2011) and bacterial (Zwietering et al., 1990) growth and was here simplified to determine nutrients uptake kinetics, as represented in Equation 3.14:

$$S(t) = S_i + (S_f - S_i) \cdot \exp(-\exp[k \cdot (\lambda - t) + 1]) \quad (3.14)$$

where λ is the lag time (in days). The kinetic parameters, k and λ , were determined by minimizing the sum of squared residuals using the Solver supplement of Microsoft Excel 2013. The quality of the model fits was evaluated by calculating the performance indexes R^2 and RMSE, as described in Equations 3.8 and 3.9, respectively.

3.11. Nitrogen and phosphorus mass fractions in microalgal biomass

For each nutrient a mass balance was written and the mass fractions (α_S , in mg S mg⁻¹ DW) of nitrogen and phosphorus incorporated into microalgal biomass were determined. This mass balance was determined according to Equation 3.15 (Lee, 1992):

$$\frac{dS}{dt} = -\alpha_S \cdot \frac{dX}{dt} \quad (3.15)$$

By integrating Equation 3.15 over the cultivation time, Equation 3.16 was obtained:

$$(S_i - S_f) = \alpha_S \cdot (X_f - X_i) \quad (3.16)$$

With these data, the combined effect of average daily light irradiance and temperature on nitrogen and phosphorus mass fractions was evaluated. For this, a multiple linear regression analysis was performed according to the model described in Equation 3.17 (Bezerra et al., 2008):

$$y = \beta_0 + \sum_{i=1}^k \beta_i x_i + \sum_{i=1}^k \beta_{ii} x_i^2 + \sum_{1 \leq i \leq j}^k \beta_{ij} x_i x_j + \varepsilon \quad (3.17)$$

where y is the dependent variable k is the number of variables, β_0 is the constant term, β_i represents the coefficients of the linear parameters, x_i represents the variables, β_{ii} represents the coefficients of the quadratic parameter, β_{ij} represents the coefficients of the interaction parameters and ε is the residual associated to the experiments.

To transform each real value into coordinates with dimensionless values, real values were standardized according to Equation 3.18:

$$z_i = \left(\frac{x_i - \bar{x}}{s} \right) \quad (3.18)$$

where z_i is the standard variable, \bar{x} is the mean of x_i values and s is the standard deviation.

The significance of the regression coefficients was evaluated through the calculation of their confidence interval. The parameter β_i is valid if:

$$|\beta_i| > \frac{t_{n-k-1}^{\alpha/2} \sigma}{\sqrt{S_{xx} x_i}} \quad (3.19)$$

where t is the Student t distribution, n is the number of points, α is the significance level and S_{xx} is the sum of squares related to x_i given by:

$$\sum_{j=1}^n (x_{ij} - \bar{x}_i)^2. \quad (3.20)$$

β_i coefficients considered statistically significant to describe the behaviour of nitrogen and phosphorus mass fractions in response to different light and temperature conditions were determined according to the expression:

$$\alpha = \beta_0 + \beta_1 T + \beta_2 I + \beta_3 T^2 + \beta_4 I^2 + \beta_5 T I \quad (3.21)$$

where T is temperature (in °C) and I is average daily light irradiance (in $\mu\text{E m}^{-2} \text{s}^{-1}$).

3.12. Zeta potential measurements and cell size distribution

Microalgal biomass grown in the 60-L open raceway pond was evaluated in terms of surface charge and cell size distribution. Surface charge was determined through zeta potential measurements. For this, microalgal suspensions were centrifuged at 2,900 g for 15 min (in an Eppendorf 5810 R centrifuge, Eppendorf, Germany), washed twice and resuspended in MilliQ water (Millipore, Billerica, MA, USA) to obtain a final concentration of about 5.0×10^6 cells mL⁻¹. Zeta potential was measured using a ZetaSizer Nano ZS (Malvern Instruments, Worcestershire, UK). All the determinations were performed in a clear disposable zeta cell at approximately 25 °C. Mean values of each studied suspension were obtained by at least triplicate measurements of three independent experiments. Cell size distribution was determined in a Beckman Coulter LS 230 (Beckman, Germany) particle size analyser through laser diffraction. Using this method, cell size was determined in terms of volume and number distribution. Samples from two independent experiments were analysed in duplicates.

3.13. Statistical analysis

The statistical significance of the main results was evaluated using the Student's paired *t*-test to investigate whether the differences between the studied conditions could be considered statistically significant. This analysis was performed using the statistical software SPSS 22.0 (SPSS Inc., Chicago, USA). Statistical tests were carried out at a significance level of 0.05.

3.14. Techno-economic assessment of a microalgal production unit

The construction of a high rate pond facility in Sines, Portugal is proposed in this study. Sines is a municipality from the district of Setubal, which is located in the Alentejo Litoral region. Total area of this municipality is approximately 203 km² and in 2011, its population density was 70 inhabitants km⁻², corresponding to a total of 14,210 inhabitants (PORDATA, 2013).

As it was referred in Section 2.3.1, microalgal culturing in open ponds strongly depends on environmental factors, such as temperature, solar light irradiation and evaporation rates.

Therefore, selection of an adequate site for the installation of an algal facility should take into account these parameters. The average annual temperature observed in the region of Sines is around 17.2 °C (monthly minimum, maximum and average temperatures are presented in Figure 3.4-A). Horizontal light irradiation in Sines municipality (Figure 3.4-B) presents an annual average of 5.21 kWh m⁻² d⁻¹ (JRC, 2013). Water evaporative losses in open ponds are very common. Evaporation rate depends on different factors, such as (Rodrigues, 2009): (i) water temperature in the air-water surface; (ii) air-water surface area; and (iii) air temperature. Evaporation rates in lagoons from the south of Portugal were determined by Rodrigues (2009). In this study, the author has demonstrated average evaporation rates of 7.5 cm month⁻¹.

Selection of this local site was based on these environmental factors. However, other characteristics were considered: (i) the flat topography of this region, which avoids the need for land preparation before open pond construction; (ii) the presence of a thermoelectric power plant in this area, which can supply the facility with the required CO₂ and other utilities, such as steam; (iii) the presence of a biodiesel production plant able to generate energy from raw materials, such as oils and animal fats, with a production capacity of 27 kt yr⁻¹; (iv) the closeness to the coast, so that seawater can be easily used if required; and (v) the availability of sufficient domestic wastewater to feed the algal facility (the wastewater load at a typical Portuguese wastewater treatment plant is about 1,000 m³ h⁻¹ for a population equivalent of 170,000 inhabitants and the number of inhabitants of Setubal district is approximately 867,000 (PORDATA, 2013)).

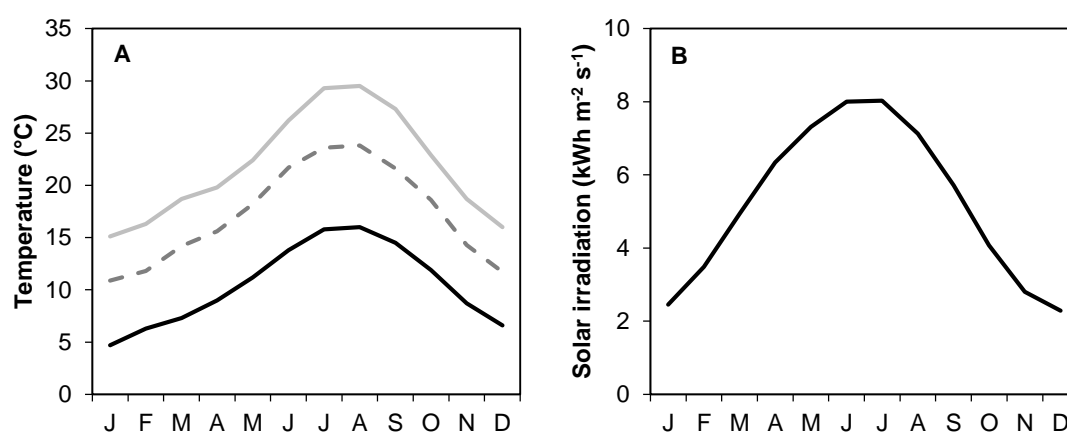


Figure 3.4. Minimum (black line), maximum (grey line) and average (dashed line) temperature (A) and horizontal solar irradiation (B) observed per month in Sines municipality, Portugal (Chazarra et al., 2001; JRC, 2013).

3.14.1. Process flowsheet and scenarios description

The overall process is schematically represented in Figure 3.5. As open pond systems are more commonly used for commercial-scale applications (Borowitzka, 1999; Jiménez et al., 2003; Tredici and Materassi, 1992) and lower investment and operational costs (important advantage in bioenergy production) are required, they are proposed in this study for *C. vulgaris* growth using domestic wastewater as culture medium (S_{WW}). *C. vulgaris* is a fast-growing microalga widely applied in wastewater treatment processes and biofuels production, due to its high biomass productivities and lipid contents and to their high ability for nutrients removal and resistance to contaminations (Chisti, 2007; Lardon et al., 2009; Wang et al., 2010). Additionally, the potential of this microalga for biomass production, CO₂ capture and nutrients removal has been demonstrated in this study (in Sections 4.1-4.6). As it was referred in Section 2.4.2, nitrogen and phosphorus concentrations in domestic wastewaters typically range between 25-55 and 7-12 mg L⁻¹, respectively. In this study, nitrogen and phosphorus concentrations in the domestic wastewater were assumed to be 50 and 10 mg L⁻¹, respectively (the same range of concentrations as those used in this study for culture medium preparation – Section 3.1). To enhance biomass productivities, CO₂ can be provided to cultures using flue gases from a thermoelectric power plant and some refinery processes (e.g., steam methane reforming). Microalgal biomass (S_{02} - output stream of open ponds) is then harvested by autoflocculation in a clarifier (S_{03}) followed by centrifugation (S_{04}) to a concentration of about 20% (w/w). To avoid the use of chemicals, cell disruption procedure suggested was the continuous pulsed electric field (PEF) tested for lipids extraction from *C. vulgaris* by Flisar et al. (2014). The extracted lipids (S_{05}) will be sold to the biodiesel plant located nearby. After a dehydration step, the remaining biomass (S_{06}) is then forwarded to the anaerobic digestion process. The output streams of this process are the following: (i) biogas that is burned in the combined heat and power (CHP) generation unit (S_{07}); (ii) fertilizer, considered a product of the process (S_{08}); and (iii) wastewater that is recycled to the open ponds (S_{AD}).

In this study, seven scenarios were considered, characterized by different efficiencies in some of the most important steps of the microalgal facility (Table 3.3). In the scenarios 1 (base scenario), 2 and 3, the effect of photosynthetic efficiency (2, 1 and 3%, respectively) was analysed. These values have already been determined for open ponds. Photosynthetic efficiencies achieved in open systems lie between one tenth and one third of the “theoretical”

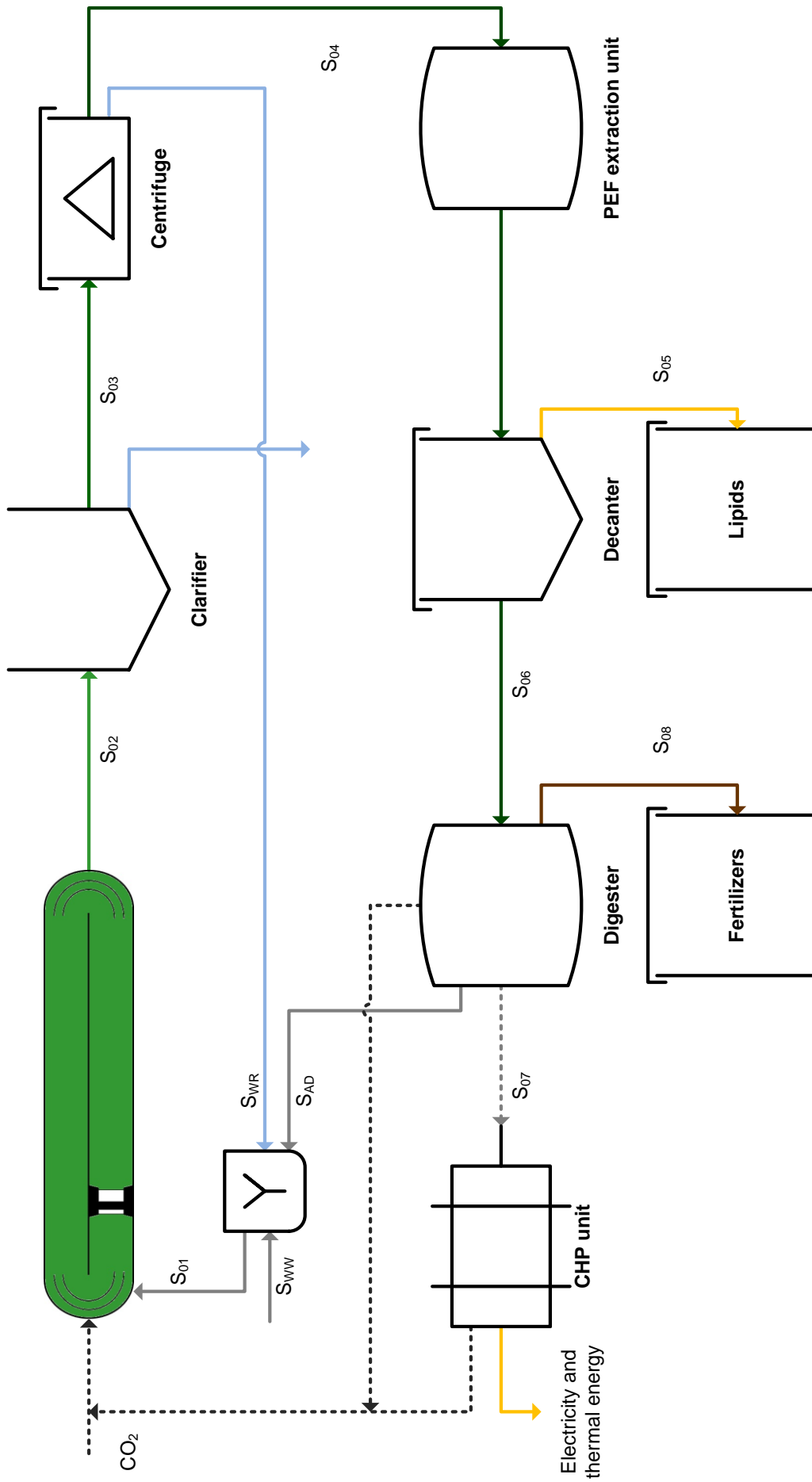


Figure 3.5. Process diagram. CHP - combined heat and power; PEF - pulsed electric field.

Table 3.3. Characterization of the seven scenarios (Sc) evaluated in this study

Scenarios' assumptions	Sc1	Sc2	Sc3	Sc4	Sc5	Sc6	Sc7
Photosynthetic efficiency (%)	2	1	3	2	2	2	2
Biomass productivity ($\text{g m}^{-2} \text{d}^{-1}$)	16.4	8.2	24.6	16.4	16.4	16.4	16.4
Lipids extraction efficiency (%)	75	75	75	60	90	75	75
Anaerobic digestion efficiency (%)	45	45	45	45	45	30	60

value of 10%, due to several losses (Jiménez et al., 2003; Norsker et al., 2011; Williams and Laurens, 2010): (i) inactive photon absorption; (ii) reflection; (iii) respiration; (iv) light saturation; and (v) photo-inhibition. In the scenarios 1, 4 and 5, the efficiency of lipids extraction (75, 60 and 90%, respectively) was assessed. PEF extraction efficiencies between 60 and 90% have already been reported in the literature (Flisar et al., 2014; Joannes et al., 2015; Show et al., 2015). Since lipids extraction efficiency is easier to control than cell lipid content, this value was assumed to be constant (25%). In the scenarios 1, 6 and 7, the efficiency of anaerobic digestion (45, 30 and 60%, respectively) was compared, since different studies have reported anaerobic digestion efficiencies within this range of values (Kinnunen et al., 2014; Tran et al., 2014).

3.14.2. Mass balance

Briefly, the process comprises four different steps (Figure 3.5): (i) microalgal growth; (ii) microalgal harvesting; (iii) combined cell disruption and lipids extraction; and (iv) anaerobic digestion followed by electricity production. For each process unit, mass balances were determined. Additionally, the net CO_2 balance was determined, taking into account the CO_2 streams involved in the process. In the cultivation step, CO_2 is fed into microalgal cultures. On the other hand, anaerobic digestion and electricity production in the CHP generation unit release CO_2 .

3.14.3. Energy balance

In this process, energy is mainly required in the following steps: microalgal cultivation, microalgal harvesting and cell disruption and lipids extraction. The energy balance performed to the microalgal facility allowed the evaluation of the energetic performance for

each of the studied scenarios. Accordingly, the energy returned on energy invested (EROEI) was determined as described in Equation 3.22:

$$EROEI = \frac{\text{Energy produced in the microalgal facility}}{\text{Total energy required}} \quad (3.22)$$

3.14.4. Economic assessment

The economic analysis of an industrial process should take into account the investment capital, also known as fixed capital, as well as annual production costs and annual revenues. With these parameters, it is possible to determine the economic viability of the project.

Fixed capital is the total investment cost needed to create the facility. It includes the equipment acquisition and installation costs, piping and electrical costs and also the costs of buildings, yard improvements, service facilities and land. Acquisition costs were determined for almost all the equipment required in this process. These costs were defined according to values already reported in the literature for different years and were normalized to 2015 cost basis using the Chemical Engineering Plant Cost Index, *PCI*, according to Equation 3.23:

$$\text{Cost} = \text{Base cost} \cdot \frac{PCI}{PCI_{base}} \quad (3.23)$$

The correct estimation of total capital investment should also include cost factors associated to direct and indirect costs. In the direct costs, typical factors that should be considered are: (i) installation costs; (ii) instrumentation and control; (iii) piping; (iv) electrical equipment and materials; (v) buildings; (vi) yard improvements; and (vii) service facilities. Indirect costs comprise engineering and supervision, construction expenses, contractor's fee and contingency (Peters and Timmerhaus, 1991). These factors typically represent a fraction of the total purchase costs.

To evaluate the economic viability of a project, it is also necessary to determine the annual production costs. These costs include variable and fixed costs. Variable costs oscillate according to productivity rates. In this study, these costs comprise raw materials, miscellaneous materials, utilities, such as electricity and steam, costs associated to the pre-concentration step with NaOH and to PEF extraction and shipping and packaging. On the other hand, fixed costs are constant and do not oscillate with productivity rates. In these costs

are included maintenance, operating labour, laboratory costs, supervision, plant overheads, insurance, local taxes and royalties. Normally, estimation of these costs is done by attributing a percentage of fixed capital or other variables to each of the referred parameters (Sinnott and Towler, 2009).

The viability of a project can be evaluated through the determination of net present value (NPV) and internal rate of return (IRR). NPV is the sum of present values of the individual cash-flows (revenues minus costs):

$$NPV = \sum_{i=0}^n \frac{CF_i}{(1+r)^i} \quad (3.24)$$

where CF_i corresponds to the cash flow in the year i and r is the interest rate. When NPV is positive, the viability of the project is ensured because it implies that net income is higher than costs. On the other hand, IRR is the interest rate that makes NPV of all cash flows (both positive and negative) from a particular investment equal to zero. In other words, IRR is the minimum interest value for which there is no income, but there are no other costs. Therefore, a project is economically feasible when this value is higher than the interest rate, so that revenues are higher than costs (Mithá, 2004).

To evaluate the time required to pay the investment, the payback time was determined according to Equation 3.25:

$$Payback\ time = \frac{Fixed\ capital}{Revenues - Annual\ production\ costs} \quad (3.25)$$

4. Results and discussion

4.1. Microalgal monocultures: the effect of light supply

4.1.1. Microalgal growth

Phototrophic cultivation of microalgae strongly depends on light energy. The growth of different microalgal strains under different light irradiance values and with different light cycles (different average daily light irradiances) has shown that these factors have a great influence on kinetic growth parameters. Figure II.1 (Annex II) shows the growth curves obtained for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* grown under the studied light conditions. Typical growth curves obtained when growing microalgae in batch conditions are very similar to those observed for bacterial growth, which consist of six different growth stages (Barsanti and Gualtieri, 2006; Monod, 1949): (i) adaptation or lag phase; (ii) acceleration phase; (iii) exponential or log phase; (iv) retardation phase; (v) stationary phase; and (vi) declining phase. In the growth curves presented in Figure II.1, it is possible to distinguish exponential and retardation phases. However, for *S. salina* and *M. aeruginosa* cultures performed with the lowest light irradiance values (36 and 60 $\mu\text{E m}^{-2} \text{s}^{-1}$) and the lowest light period (average daily light irradiances of 15 and 25 $\mu\text{E m}^{-2} \text{s}^{-1}$, respectively), it was possible to observe an adaptation phase of one day. For the other cultures the exponential growth phase started before completing the first day of culturing. Regarding the other growth phases, almost all cultures presented an exponential growth phase with the same duration, reaching the retardation phase on the seventh day of culturing. Similar behaviour was observed by Jacob-Lopes et al. (2009), when the effect of light cycles on *Aphanothece microscopica Nageli* cultures was analysed. The higher

adaptation phases observed in the lowest light conditions suggest that the studied microorganisms are better adapted to higher light supplies. Regarding the influence of light supply on kinetic growth parameters, Figure 4.1 shows the evolution of specific growth rates with increasing light supply for each of the studied microorganisms and Table 4.1 presents maximum biomass concentrations and average biomass productivities achieved.

Values obtained for specific growth rates have shown a minimum of $0.214 \pm 0.030 \text{ d}^{-1}$ for *S. salina* grown under an irradiance of $36 \mu\text{E m}^{-2} \text{ s}^{-1}$ and a light:dark ratio of 10:14 (average daily light irradiance of $15 \mu\text{E m}^{-2} \text{ s}^{-1}$), which was not statistically different ($p=0.44$) from the one obtained for *P. subcapitata* grown in the same conditions ($0.230 \pm 0.024 \text{ d}^{-1}$). Maximum values of $1.19 \pm 0.04 \text{ d}^{-1}$ were achieved by *C. vulgaris* grown under an irradiance of $180 \mu\text{E m}^{-2} \text{ s}^{-1}$ and a 24-h light period (average daily light irradiance of $180 \mu\text{E m}^{-2} \text{ s}^{-1}$), which was not statistically different from the values obtained for *P. subcapitata*

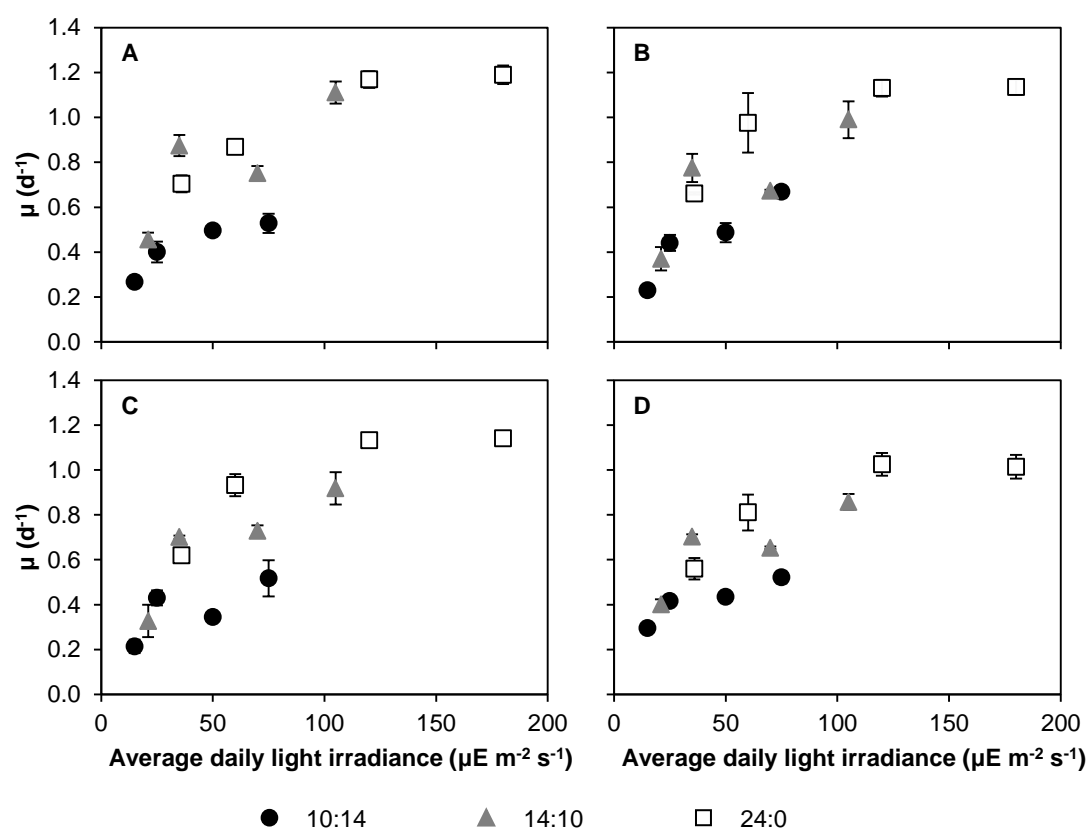


Figure 4.1. Specific growth rates (μ , in d^{-1}) determined for *C. vulgaris* (A), *P. subcapitata* (B), *S. salina* (C) and *M. aeruginosa* (D) grown under different light conditions. Average daily light irradiances were obtained by submitting microalgal cultures to different light irradiances ($36, 60, 120$ and $180 \mu\text{E m}^{-2} \text{ s}^{-1}$) and different light:dark ratios (10:14, 14:10 and 24:0). Error bars correspond to the standard deviation of the mean determined for two independent experiments.

($1.13 \pm 0.03 \text{ d}^{-1}$; $p=0.08$) and *S. salina* ($1.14 \pm 0.03 \text{ d}^{-1}$ $p=0.10$). Similar specific growth rates between *C. vulgaris* and *P. subcapitata* have already been reported (Pires et al., 2013b). Comparing the effect of light irradiance and light:dark ratio on specific growth rates, Figure 4.1 shows that an increase in light irradiance and in light exposure time (average daily light irradiance) contributes to higher specific growth rates in all studied microorganisms. Apart from a few exceptions, a statistically significant ($p<0.05$) increase in specific growth rates was observed for higher light irradiance values and higher light periods. These results are consistent with previous studies that reported a positive correlation between specific growth rates and light irradiance and period for different microalgae (Janssen et al., 1999; Sorokin and Krauss, 1958). Regarding maximum biomass concentrations and average biomass productivities (Table 4.1), a similar behaviour was observed. In general, higher light irradiance levels and light periods (average daily light irradiances) led to an increase in maximum biomass concentrations achieved and average biomass productivities. The highest value of maximum biomass concentration, $(1.35 \pm 0.13) \times 10^3 \text{ mg DW L}^{-1}$, was achieved by *C. vulgaris* under an irradiance value of $180 \mu\text{E m}^{-2} \text{ s}^{-1}$ and a 24-h light period (average daily light irradiance of $180 \mu\text{E m}^{-2} \text{ s}^{-1}$). Statistically lower values, $798 \pm 36 \text{ mg DW L}^{-1}$ ($p<0.01$), were obtained for *P. subcapitata* grown under the same conditions. Maximum biomass concentrations of $(1.26 \pm 0.06) \times 10^3$ and $(1.17 \pm 0.06) \times 10^3 \text{ mg DW L}^{-1}$ were achieved by *S. salina* and *M. aeruginosa* when grown in the same light conditions. However, these values were not statistically different ($p>0.05$) from the one determined for *C. vulgaris*. In the case of average biomass productivities, the lowest value, $21.5 \pm 2.3 \text{ mg DW L}^{-1} \text{ d}^{-1}$, was achieved for *P. subcapitata* under the lowest light supply (average daily light irradiance of $15 \mu\text{E m}^{-2} \text{ s}^{-1}$). On the other hand, the highest average biomass productivity value, $133 \pm 13 \text{ mg DW L}^{-1} \text{ d}^{-1}$, was achieved by *C. vulgaris* grown with a light irradiance of $180 \mu\text{E m}^{-2} \text{ s}^{-1}$ and a light:dark ratio of 24:0 (average daily light irradiance of $180 \mu\text{E m}^{-2} \text{ s}^{-1}$). *S. salina* and *M. aeruginosa* showed a similar behaviour in terms of average biomass productivity. The highest values achieved were 108 ± 5 and $107 \pm 5 \text{ mg DW L}^{-1} \text{ d}^{-1}$ for *S. salina* and *M. aeruginosa*, respectively, under the highest light irradiance value and with continuous light supply (average daily light irradiance of $180 \mu\text{E m}^{-2} \text{ s}^{-1}$). These values were statistically higher ($p<0.05$) than the highest average biomass productivity achieved by *P. subcapitata*: $74.5 \pm 3.4 \text{ mg DW L}^{-1} \text{ d}^{-1}$.

These results suggest that the studied microorganisms behave similarly when light irradiance and light exposure time is increased. However, the low productivity values achieved for

Table 4.1. Maximum biomass concentrations (X_{\max} , in mg DW L⁻¹) and average biomass productivities (P, in mg DW L⁻¹ d⁻¹) determined for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* grown under different light conditions

Average daily light irradiance ($\mu\text{E m}^{-2} \text{s}^{-1}$)	<i>C. vulgaris</i>		<i>P. subcapitata</i>		<i>S. salina</i>		<i>M. aeruginosa</i>	
	X_{\max} (mg DW L ⁻¹)	P (mg DW L ⁻¹ d ⁻¹)	X_{\max} (mg DW L ⁻¹)	P (mg DW L ⁻¹ d ⁻¹)	X_{\max} (mg DW L ⁻¹)	P (mg DW L ⁻¹ d ⁻¹)	X_{\max} (mg DW L ⁻¹)	P (mg DW L ⁻¹ d ⁻¹)
15	414±13	37.4±1.2	233±25	21.5±2.3	426±24	29.5±2.1	406±16	34.6±1.4
21	517±11	46.8±1.0	249±13	22.9±1.3	481±19	34.2±1.4	484±7	41.6±0.7
25	604±46	55.3±4.2	377±36	35.2±3.5	599±4	46.0±0.4	588±11	51.7±0.9
35	833±57	77.1±5.4	505±77	47.2±7.0	767±15	61.7±1.3	798±38	71.2±3.6
36	828±23	76.6±2.2	426±15	39.9±1.5	738±16	59.2±1.5	742±4	66.4±0.5
50	696±19	63.8±1.8	462±14	43.1±1.3	629±4	48.7±0.3	621±9	54.7±0.8
60	972±80	90.0±7.5	631±112	58.9±10.6	(1.09±0.04)×10 ³	92.0±4.0	(1.07±0.10)×10 ³	97.3±10.1
70	715±43	65.6±4.1	439±6	40.7±0.5	674±10	52.9±0.9	614±62	53.7±5.9
75	771±11	71.2±0.9	488±13	45.8±1.3	719±39	57.3±3.6	767±17	68.5±1.7
105	(1.08±0.14)×10 ³	100±13	696±7	64.9±0.8	914±30	75.4±2.9	991±7	89.5±0.7
120	(1.09±0.02)×10 ³	101±2	666±4	62.3±0.4	943±7	78.5±0.6	989±3	89.5±0.6
180	(1.35±0.13)×10 ³	133±13	798±36	74.5±3.4	(1.26±0.06)×10 ³	108±5	(1.17±0.06)×10 ³	107±5

Values are presented as the mean ± standard deviation of two independent experiments.

P. subcapitata, indicate that application of this microalga may be limited when the aim is to maximize biomass productivities at atmospheric CO₂ concentrations.

4.1.2. CO₂ uptake rates

Table 4.2 presents the mass fractions of carbon present in microalgal biomass determined through elemental analysis and CO₂ uptake rates determined using these values and average biomass productivities achieved in each of the studied conditions. Regarding carbon contents present in microalgal biomass, Table 4.2 shows that these values were not strongly influenced by an increase in light supply. Average carbon content determined for microalgal biomass in the studied conditions was 44.20±1.14% (w/w), which is in accordance with carbon contents reported for *S. obliquus* and *Chlorella pyrenoidosa* (approximately 50% w/w) in the study performed by Tang et al. (2011). On the other hand, Table 4.2 evidences that, in general, an increase in light supply resulted in an increase in CO₂ uptake rates. An increase in biomass productivities and in CO₂ uptake rates with increasing light supply has already been described (Richardson et al., 1983; Rubio et al., 2003). In fact, at light irradiance values below the light saturation point, photosynthetic rate is directly proportionally to light irradiance, resulting in an increase in biomass productivities and in CO₂ uptake. For irradiance values above the light saturation point, a photooxidation process occurs, damaging the photosystems and inhibiting photosynthesis and microalgal growth (Pulz, 2001; Suh and Lee, 2003). Similar results were observed in the studies performed by Jacob-Lopes et al. (2009) and Pires et al. (2013b). A maximum value of 195±19 mg CO₂ L⁻¹ d⁻¹ was obtained for *C. vulgaris* grown with a light irradiance of 180 µE m⁻² s⁻¹ and with a light:dark ratio of 24:0 (average daily light irradiance of 180 µE m⁻² s⁻¹). Similar CO₂ uptake rates are expected for both cyanobacteria grown in the same light conditions, since no statistically differences were observed on biomass productivities achieved by these microorganisms under an average daily light irradiance of 180 µE m⁻² s⁻¹. However, CO₂ uptake rate determined for *P. subcapitata* in the same culturing conditions was statistically lower ($p=0.02$): 129±6 mg CO₂ L⁻¹ d⁻¹.

The evolution of dissolved oxygen concentration and pH in culture medium can also give information about CO₂ uptake and hence, photosynthetic activity. Figures II.2 and II.3 from Annex II present, respectively, the daily evolution of dissolved oxygen concentration and pH in the culture medium for all studied conditions. Daily variation of dissolved oxygen

Table 4.2. Carbon contents (α_C , in % w/w) and CO₂ uptake rates (R_C , in mg CO₂ L⁻¹ d⁻¹) determined for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* grown under different light conditions

Average daily light irradiance ($\mu\text{E m}^{-2} \text{s}^{-1}$)	<i>C. vulgaris</i>		<i>P. subcapitata</i>		<i>S. salina</i>		<i>M. aeruginosa</i>	
	α_C (% w/w)	R_C (mg CO ₂ L ⁻¹ d ⁻¹)	α_C (% w/w)	R_C (mg CO ₂ L ⁻¹ d ⁻¹)	α_C (% w/w)	R_C (mg CO ₂ L ⁻¹ d ⁻¹)	α_C (% w/w)	R_C (mg CO ₂ L ⁻¹ d ⁻¹)
15	44.50	60.9±2.0	45.56	35.9±3.8	44.98	48.7±3.5	45.42	57.6±2.4
21	45.60	78.1±1.7	44.75	37.6±2.2	45.51	57.1±2.3	45.16	68.9±1.2
25	44.96	91.2±7.0	44.60	57.5±5.7	44.59	75.1±0.6	44.85	84.9±1.6
35	44.51	126±9	45.33	78.5±11.7	44.96	102±2	43.88	115±6
36	44.73	125±4	45.21	66.1±2.4	44.73	97.0±2.4	44.55	108±1
50	43.73	102±3	43.56	68.8±2.1	43.57	77.8±0.5	43.48	87.2±1.3
60	44.19	146±12	46.25	99.8±18.0	43.89	148±6	43.16	154±16
70	43.98	106±7	43.33	64.7±0.7	43.78	84.8±1.5	43.26	85.2±9.4
75	44.54	116±1	44.72	75.0±2.1	43.76	91.9±5.8	43.76	110±3
105	43.60	160±21	45.41	108±1	43.19	119±5	43.12	141±1
120	42.58	157±2	43.61	99.6±0.7	41.89	121±1	42.41	139±1
180	42.54	195±19	47.35	129±6	42.96	169±8	41.50	162±8

Values are presented as the mean ± standard deviation of two independent experiments.

concentration may be related to photosynthetic activity of microalgae, since photosynthetic microorganisms convert CO_2 into O_2 , according to Equation 2.1. Therefore, an increase in dissolved oxygen concentration in the culture medium can be associated to an increase in the photosynthetic activity (Kliphuis et al., 2011; Sánchez Mirón et al., 1999; Ugwu et al., 2007). Furthermore, all studied cultures experienced a significant increase after 24 h of culturing, meaning that photosynthetic activity in the first days of culturing was higher. In the following days, dissolved oxygen concentration remained approximately constant, especially after the seventh day of culturing, which can be associated to the reaching of the retardation phase (as shown in Figure II.2, Annex II), where specific growth rate becomes null and therefore, there is no increase in photosynthetic activity and in dissolved oxygen concentration. However, for cultures supplied with higher light irradiance values and for longer periods (average daily light irradiances of 60, 120 and $180 \mu\text{E m}^{-2} \text{s}^{-1}$), a decrease in dissolved oxygen concentration in the last days of culturing was observed, tending to reach its initial value. This decrease may be associated to a decrease in photosynthetic activity, mainly due to nutrients limitation. In fact, determination of nutrients (nitrogen and phosphorus) concentration in the first and last day of culturing has shown a significant reduction in $\text{NO}_3\text{-N}$ concentrations in the referred light conditions, as it is described in Section 4.1.3. When nutrients concentration is a limiting growth factor, microalgae tend to cease photosynthetic reactions, reaching the stationary growth phase and thus lowering dissolved oxygen concentration towards the equilibrium concentration.

On the other hand, pH values are related to the equilibrium established between CO_2 bubbled in the atmospheric air stream and HCO_3^- present in the culture medium, as described in Section 2.3.3. Photosynthetic CO_2 uptake by microalgae leads to an equilibrium change. Then, the equilibrium shifts to counteract the decrease of CO_2 concentration in the medium: some HCO_3^- will form CO_2 and OH^- , in order to maintain the equilibrium, and some CO_3^{2-} will take up a proton and form HCO_3^- ($\text{H}_2\text{O} + \text{CO}_3^{2-} \rightarrow \text{HCO}_3^- + \text{OH}^-$) to compensate for the decrease in $[\text{HCO}_3^-]$ (Uusitalo, 1996). These changes in the equilibrium lead to the release of OH^- ions and result in a new equilibrium at an increased pH. On the other hand, if microalgae uptake HCO_3^- , the equilibrium is altered and a new one is achieved by either removal of H^+ or addition of OH^- ions, increasing the overall pH of the culture medium. Therefore, analysis of Figure II.3 (Annex II) suggests that increasing pH values for all the studied cultures during the first four days is related to an increase in carbon uptake and hence, in photosynthetic activity. Additionally, the higher pH values achieved for higher light

supplies confirm the positive effect of light supply on biomass productivities and suggest higher CO₂ uptake rates in these conditions. As well as for dissolved oxygen concentrations, it is possible to observe a period where pH values remain constant or present a slightly decrease, which can be associated to a break in carbon uptake from microalgae. In this period, starting at seventh day of culturing, it was observed the beginning of retardation phase, which explains why carbon uptake was ceased and why pH values remain approximately the same. For cultures where higher light conditions were supplied, there was even a decrease in pH values, which can be attributed to nutrients limitation.

These results have shown that microalgal culturing can be effective in CO₂ capture from the atmosphere, which may reduce costs associated with CO₂ supply. Apart from *P. subcapitata*, all studied microorganisms seem to be effective in CO₂ capture due to their high biomass productivities, being promising alternatives for large-scale production.

4.1.3. Nutrients removal

In this study nitrogen and phosphorus concentrations were determined in the first and last day of culturing to evaluate the removal efficiencies of these nutrients under the studied conditions. Average removal rates and removal efficiencies are presented in Table 4.3.

Concerning nitrogen removal, when the lowest irradiance values (36 and 60 $\mu\text{E m}^{-2} \text{s}^{-1}$) and the lowest light period (10:14) were applied (average daily light irradiances of 15 and 25 $\mu\text{E m}^{-2} \text{s}^{-1}$), all studied microorganisms have shown removal efficiencies lower than the values established by EU legislation: removal efficiencies in these conditions were not higher than 66.4% (average removal rate of approximately $1.79 \pm 0.01 \text{ mg N L}^{-1} \text{d}^{-1}$). However, when higher average daily light irradiances were applied, removal efficiencies higher than 70% were obtained in all cultures except in those of *P. subcapitata* grown under the following conditions: light irradiance of 180 $\mu\text{E m}^{-2} \text{s}^{-1}$ and light:dark ratio of 10:14 (average daily light irradiance of 75 $\mu\text{E m}^{-2} \text{s}^{-1}$) and light irradiance of 60 $\mu\text{E m}^{-2} \text{s}^{-1}$ and light:dark ratio of 14:10 (average daily light irradiance of 35 $\mu\text{E m}^{-2} \text{s}^{-1}$). Additionally, when grown under continuous light supply, all studied microorganisms have shown removal efficiencies of about 100%. The same result was observed for all microorganisms grown under a 14:10 light:dark ratio and light irradiances of 120 and 180 $\mu\text{E m}^{-2} \text{s}^{-1}$ (average daily light irradiances of 70 and 105 $\mu\text{E m}^{-2} \text{s}^{-1}$, respectively). These results have shown that higher

Table 4.3. Average nutrients removal rates (R_s , in $\text{mg S L}^{-1} \text{ d}^{-1}$) and removal efficiencies (R , in %) determined for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* grown under different light conditions

Nutrients	Average daily light irradiance ($\mu\text{E m}^{-2} \text{ s}^{-1}$)	<i>C. vulgaris</i>		<i>P. subcapitata</i>		<i>S. salina</i>		<i>M. aeruginosa</i>	
		R_s ($\text{mg S L}^{-1} \text{ d}^{-1}$)	R (%)	R_s ($\text{mg S L}^{-1} \text{ d}^{-1}$)	R (%)	R_s ($\text{mg S L}^{-1} \text{ d}^{-1}$)	R (%)	R_s ($\text{mg S L}^{-1} \text{ d}^{-1}$)	R (%)
Nitrogen	15	1.08±0.03	42.3±1.6	1.07±0.21	43.5±0.7	1.27±0.02	48.5±0.7	1.42±0.04	53.6±1.7
	21	1.69±0.16	75.6±5.8	1.24±0.04	74.4±2.9	1.86±0.06	96.1±0.9	1.82±0.03	98.8±1.4
	25	1.50±0.03	53.6±1.0	1.43±0.05	52.1±1.7	1.30±0.08	46.9±3.6	1.79±0.01	66.4±0.6
	35	2.34±0.01	84.9±0.1	1.28±0.25	57.8±5.0	1.88±0.76	97.1±4.7	2.69±0.18	95.1±3.9
	36	2.43±0.38	97.1±1.7	2.62±0.08	88.0±2.7	2.83±0.16	92.5±1.0	2.89±0.07	97.3±1.1
	50	2.03±0.11	76.4±4.0	2.03±0.01	76.7±0.1	2.27±0.14	87.1±4.9	2.38±0.09	90.2±3.1
	60	2.34±0.01	84.9±0.1	1.28±0.25	57.8±5.0	1.88±0.76	97.1±4.7	2.69±0.18	95.1±3.9
	70	1.68±0.33	99.0±1.2	1.91±0.01	100±0	1.38±0.80	100±0	1.92±0.04	100±0
	75	2.40±0.05	86.2±1.7	1.97±0.02	68.9±0.8	2.45±0.02	86.1±0.6	2.59±0.03	89.8±0.4
	105	2.78±0.06	98.0±2.0	2.16±0.54	97.7±2.5	2.54±0.20	98.6±0.4	2.43±0.33	98.0±0.6
Phosphorus	120	2.60±0.02	94.6±1.0	2.84±0.06	93.3±2.3	2.95±0.04	95.5±0.5	2.75±0.07	95.2±0.6
	180	2.43±0.40	99.0±1.3	2.37±0.18	100±0	1.97±0.19	99.1±0.7	2.53±0.21	100±0
	15	0.149±0.035	16.9±3.4	0.268±0.115	17.5±7.9	0.157±0.007	17.3±0.6	0.109±0.081	13.4±8.8
	21	0.258±0.019	29.3±1.6	0.223±0.057	24.0±9.6	0.222±0.034	35.4±3.4	0.279±0.081	22.8±6.4
	25	0.168±0.002	20.2±0.5	0.215±0.070	23.0±6.7	0.203±0.086	18.0±1.7	0.137±0.058	15.1±5.7
	35	0.306±0.008	30.7±0.6	0.403±0.022	40.9±2.3	0.231±0.037	23.6±3.8	0.367±0.010	39.8±1.9
	36	0.279±0.092	29.3±7.4	0.259±0.056	34.2±4.9	0.316±0.034	35.4±3.4	0.255±0.068	29.7±6.0
	50	0.166±0.056	18.7±5.4	0.182±0.023	19.8±1.7	0.083±0.078	9.84±9.12	0.205±0.009	22.7±2.1
	60	0.287±0.012	35.0±0.5	0.321±0.018	34.2±1.8	0.321±0.018	34.7±3.7	0.338±0.030	36.4±3.1
	70	0.206±0.072	32.1±1.2	0.291±0.029	34.4±3.1	0.212±0.008	25.7±0.8	0.351±0.026	38.6±2.6
	75	0.240±0.191	18.3±9.0	0.235±0.018	27.0±2.0	0.231±0.064	33.9±0.6	0.218±0.050	26.3±5.7
	105	0.240±0.074	31.4±4.0	0.279±0.020	32.7±2.0	0.345±0.035	32.0±4.8	0.231±0.039	25.8±4.4
	120	0.388±0.052	42.7±3.6	0.489±0.058	51.3±4.4	0.998±0.093	37.9±7.1	0.332±0.031	36.2±2.8
	180	0.588±0.029	67.6±7.1	0.393±0.070	51.2±4.8	0.348±0.028	36.6±4.3	0.357±0.074	41.1±9.2

Values are presented as the mean ± standard deviation of two independent experiments.

light supplies favour nitrogen removal and that, in general, the studied microorganisms can be effectively applied in nitrogen removal. High nitrogen removal percentages have also been described in the literature. In the study performed by Xin et al. (2010), *Scenedesmus* sp. was able to remove 90.4% of $\text{NO}_3\text{-N}$ after 13 days of cultivation with an initial $\text{NO}_3\text{-N}$ concentration of approximately 15 mg N L^{-1} , a light irradiance of $25 \mu\text{E m}^{-2} \text{ s}^{-1}$ and a light:dark ratio of 14:10. A TN removal efficiency of 82.70% was obtained for *Chlorella zofingiensis* when cultured in a piggery effluent (with a TN concentration of 148 mg N L^{-1}) under constant light irradiance of $230 \mu\text{E m}^{-2} \text{ s}^{-1}$ (Zhu et al., 2013).

Regarding phosphorus uptake, removal efficiencies were far from satisfactory, as the minimum percentage of reduction established by EU legislation (80%) was not achieved. However, it is possible to state that increasing light irradiance values and light period resulted in higher phosphorus removal rates. In this study, all microorganisms have demonstrated a similar behaviour in terms of phosphorus uptake. However, the highest phosphorus removal, $67.6 \pm 7.1\%$ ($0.588 \pm 0.029 \text{ mg P L}^{-1} \text{ d}^{-1}$), was achieved by *C. vulgaris* grown under continuous light supply with an irradiance of $180 \mu\text{E m}^{-2} \text{ s}^{-1}$ (average daily light irradiance of $180 \mu\text{E m}^{-2} \text{ s}^{-1}$). This value was statistically higher ($p < 0.05$) than the highest removal efficiencies achieved by the other studied microorganisms. However, phosphorus removal efficiencies obtained in this study were lower than those referred in the literature. $\text{PO}_4\text{-P}$ removal percentages close to 100% were obtained for *Scenedesmus* sp. and *C. zofingiensis* in the studies performed by Xin et al. (2010) and Zhu et al. (2013), respectively.

The effect of light irradiance on nitrogen and phosphorus removal has already been described by Silva-Benavides and Torzillo (2012). In this study, the authors have reported that an increase in light irradiance from 20 to $60 \mu\text{E m}^{-2} \text{ s}^{-1}$ resulted in a more efficient removal of both nitrogen and phosphorus in batch cultures of *C. vulgaris* and *Planktothrix isothrix*, which is in accordance with the results demonstrated in this study. The discrepancy between nitrogen and phosphorus removal efficiencies obtained in this study suggests a nitrogen-limitation to microalgal growth in the cases of higher reduction percentages. According to the study performed by Bhola et al. (2011), *C. vulgaris* reached its maximum concentration for $\text{NO}_3\text{-N}$ concentrations of approximately 824 mg N L^{-1} . In this study, nitrogen was supplied at a concentration of about 41.2 mg N L^{-1} . As this value is lower than the one reported by Bhola et al. (2011), nitrogen limitation might have occurred in this study.

Furthermore, nitrogen limitations in wastewaters are very common, since low ratios between nitrogen and phosphorus, about 5:1, suggest a limitation of this nutrient to microalgal growth (Larsdotter, 2006). As the ratio between these nutrients in this study was approximately 9:1, it is possible to state that nitrogen was supplied in concentrations that can limit microalgal growth. To confirm the hypothesis of nitrogen limitation, higher nitrogen concentrations should be supplied to microalgal cultures.

4.1.4. Cell composition

The effect of light supply on cell composition was determined through the mass balances determined for nitrogen and phosphorus. Table 4.4 presents nitrogen and phosphorus mass fractions determined in microalgal biomass under the studied light conditions. Analysis of data present in Table 4.4 suggests that both nitrogen and phosphorus mass fractions were not strongly influenced by average daily light irradiance, since no statistical difference ($p>0.05$) was observed between the studied light irradiance values. However, these values can be related to the nitrogen limitation phenomenon discussed in Section 4.1.3, since average nitrogen and phosphorus mass fractions determined in microalgal biomass were lower than those reported in the typical composition of microalgal biomass ($\text{CO}_{0.48}\text{H}_{1.83}\text{N}_{0.11}\text{P}_{0.01}$): 6.59 and 1.33% (w/w), respectively (Chisti, 2007). Average nitrogen mass fraction was $3.75\pm0.92\%$ (w/w), whereas average phosphorus mass fraction was $0.448\pm0.153\%$ (w/w). The low nitrogen and phosphorus mass fractions determined in this study (approximately half of those reported in the above described molecular formula) suggest that the amounts of nitrogen and/or phosphorus supplied were not enough to satisfy microalgal requirements, thus confirming the hypothesis of nutrients limitation. The achievement of low nitrogen mass fractions in nitrogen-limited conditions has already been reported by Richardson et al. (1969). Additionally, it is possible to see from Table 4.4 that the lowest nitrogen mass fractions were obtained for cultures grown with the highest light supply (average daily light irradiance of $180\ \mu\text{E m}^{-2}\text{ s}^{-1}$). Under these conditions, nitrogen mass fractions determined for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* were 1.95 ± 0.24 , 3.19 ± 0.17 , 1.98 ± 0.11 and $2.42\pm0.12\%$ (w/w), respectively. For these light conditions, and provided that nitrogen was supplied in non-limiting concentrations, higher nitrogen mass fractions would be expected in microalgal biomass.

Table 4.4. Mass fractions of nitrogen (α_N , in % w/w) and phosphorus (α_P , in % w/w) incorporated in the biomass of *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* obtained through the mass balance performed for each nutrient

Average daily light irradiance ($\mu\text{E m}^{-2} \text{s}^{-1}$)	<i>C. vulgaris</i>		<i>P. subcapitata</i>		<i>S. salina</i>		<i>M. aeruginosa</i>	
	α_N (% w/w)	α_P (% w/w)	α_N (% w/w)	α_P (% w/w)	α_N (% w/w)	α_P (% w/w)	α_N (% w/w)	α_P (% w/w)
15	2.88±0.13	0.399±0.094	4.99±1.09	0.568±0.278	4.31±0.31	0.530±0.045	4.11±0.21	0.316±0.233
21	3.61±0.36	0.552±0.042	5.40±0.37	0.947±0.441	5.42±0.28	0.647±0.103	4.37±0.11	0.511±0.135
25	2.71±0.22	0.303±0.023	4.07±0.43	0.611±0.207	2.82±0.18	0.347±0.026	3.47±0.07	0.306±0.123
35	3.01±0.21	0.398±0.029	3.44±0.57	0.489±0.107	4.07±0.44	0.685±0.130	3.77±0.32	0.515±0.030
36	3.45±0.23	0.365±0.121	6.57±0.31	0.488±0.345	4.79±0.29	0.534±0.060	4.36±0.11	0.384±0.102
50	3.19±0.20	0.260±0.088	4.71±0.15	0.422±0.054	4.66±0.29	0.170±0.159	4.34±0.17	0.374±0.018
60	3.22±0.29	0.318±0.030	3.39±1.02	0.546±0.103	3.97±0.87	0.348±0.052	3.22±0.57	0.348±0.048
70	2.99±0.19	0.525±0.189	4.69±0.05	0.715±0.072	3.92±0.07	0.401±0.017	3.58±0.40	0.654±0.087
75	3.37±0.08	0.245±0.138	4.30±0.13	0.513±0.043	4.27±0.27	0.533±0.041	3.78±0.10	0.318±0.074
105	2.77±0.38	0.289±0.050	4.03±0.27	0.430±0.032	3.59±0.14	0.364±0.056	2.72±0.37	0.258±0.044
120	2.58±0.05	0.385±0.052	4.56±0.11	0.785±0.093	3.76±0.06	0.440±0.090	3.07±0.08	0.364±0.031
180	1.95±0.24	0.441±0.049	3.19±0.17	0.528±0.097	1.98±0.11	0.296±0.037	2.42±0.12	0.334±0.071

Values are presented as the mean ± standard deviation of two independent experiments.

4.2. Microalgal monocultures: the combined effect of light supply and temperature

4.2.1. Microalgal growth

When growing autotrophically, microalgae strongly depend on light supply and temperature (Barsanti and Gualtieri, 2006; Hu, 2004a). These environmental factors influence growth dynamics, including the specific growth rates, maximum biomass concentrations and average biomass productivities. Figure III.1 (Annex III) shows the growth curves obtained for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* under the studied light and temperature conditions. Analysis of Figure III.1 (Annex III) clearly shows that the growth of the studied microorganisms was favoured by increased light supplies and moderate temperatures (approximately 25 °C). This was observed by the significant increase in biomass concentrations within the cultivation time under these conditions and by the almost negligible adaptation phase observed in these cultures. Regarding the influence of light and temperature on kinetic growth parameters, Figure 4.2 shows the effect of average daily light irradiance and temperature on specific growth rates of *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa*. Maximum biomass concentrations achieved and average biomass productivities determined under these conditions are shown in Table 4.5. Specific growth rates determined for the studied microorganisms ranged from $0.0188 \pm 0.0033 \text{ d}^{-1}$ (for *P. subcapitata* grown at 35 °C with an average daily light irradiance of $15 \mu\text{E m}^{-2} \text{ s}^{-1}$) to $1.19 \pm 0.04 \text{ d}^{-1}$ (for *C. vulgaris* grown at 25 °C with an average daily light irradiance of $180 \mu\text{E m}^{-2} \text{ s}^{-1}$). Regarding light supply, an increase in average daily light irradiance resulted in statistically higher ($p < 0.05$) specific growth rates. Several studies have already reported the increase of specific growth rates with increasing light supplies (Sánchez et al., 1996; Schlesinger et al., 1981; Sorokin and Krauss, 1958). A positive relationship between specific growth rates and average daily light irradiance is not surprising, since microalgal growth is mainly autotrophic, requiring light as the major energy source. These results indicate that higher light supplies favoured the photosynthetic activity of the studied microorganisms, which was confirmed by the increase observed in average pH of the studied cultures: from 8.12 ± 0.29 (at $15 \mu\text{E m}^{-2} \text{ s}^{-1}$) to 8.76 ± 1.03 (at $180 \mu\text{E m}^{-2} \text{ s}^{-1}$). Figure III.2 (Annex III) presents the time-course evolution of pH in the culture medium for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* cultures grown under the studied light and temperature conditions. The increase in pH of the culture medium is related to an increase in carbon

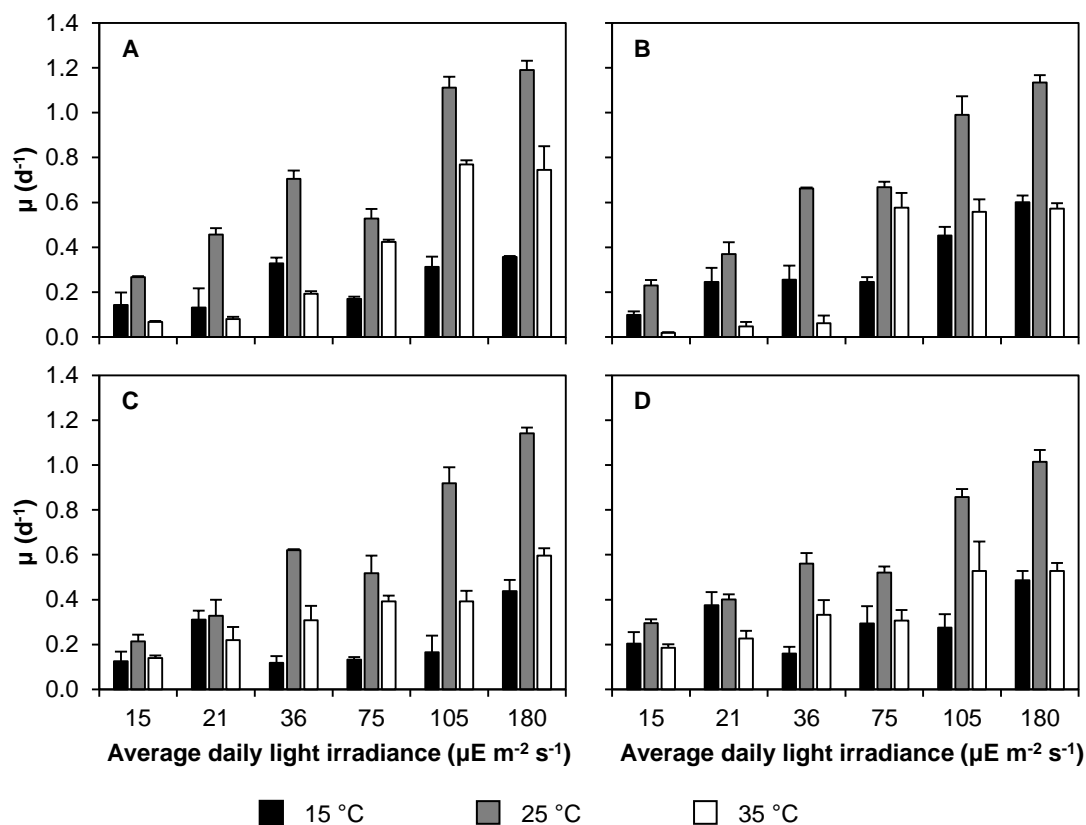


Figure 4.2. Specific growth rates (μ , in d^{-1}) determined for *C. vulgaris* (A), *P. subcapitata* (B), *S. salina* (C) and *M. aeruginosa* (D) grown under different light and temperature conditions. Average daily light irradiances were obtained by submitting microalgal cultures to different light irradiances (36 and 180 $\mu\text{E m}^{-2} \text{s}^{-1}$) and different light:dark ratios (10:14, 14:10 and 24:0). Error bars correspond to the standard deviation of the mean determined for two independent experiments.

uptake by microalgae or cyanobacteria and, hence, in photosynthetic activity (Uusitalo, 1996). Culturing temperature also contributed to considerable changes in the specific growth rates of the studied microorganisms. Specific growth rates determined at 25 °C were statistically higher ($p < 0.01$) than those determined at 15 and 35 °C. However, no statistical differences ($p = 0.09$) were observed between specific growth rates determined at 15 and 35 °C. These results indicate that the growth of the studied microorganisms in response to different temperatures may follow a normal distribution function, being the optimal culturing temperature approximately 25 °C. Evidence that the optimal temperature for autotrophic microalgal growth is near 25 °C was also given by the increase observed in pH (Figure III.2, Annex III) and dissolved oxygen concentration (Figure III.3, Annex III) at this temperature: for cultures performed at 15, 25 and 35 °C average pH of the culture medium was 8.32 ± 0.43 , 8.91 ± 0.91 and 8.09 ± 0.82 , respectively, whereas average dissolved oxygen concentration was 3.8 ± 1.1 , 6.5 ± 0.4 and $4.8 \pm 1.0 \text{ mg O}_2 \text{ L}^{-1}$, respectively. A similar behaviour was observed

by James et al. (1989) when evaluating the effect of temperature on the growth and fatty acid and amino acid composition of two microalgae belonging to the genera *Chlorella* and *Nannochloropsis*. For temperatures ranging from 15 to 35 °C, an increase in specific growth rates was observed until 25 °C, while for higher temperatures specific growth rates started decreasing. Similarly, when evaluating the optimum temperature and salinity conditions for the growth of *Chlorella ellipsoidea* and *N. oculata*, Cho et al. (2007) have demonstrated that keeping a constant salinity of 10, an increase in temperatures from 15 to 25 °C results in increased specific growth rates and, when temperature is increased to 30 °C, specific growth rates tend to decrease. Average specific growth rates determined for *C. pyrenoidosa* grown under a temperature range of 10 to 35 °C also increased until the temperature of 25 °C, starting decreasing when culturing temperature was set at 30 and 35 °C (Yang et al., 2010).

The influence of light supply and temperature on maximum biomass concentrations and average biomass productivities was similar to the one observed for specific growth rates (Table 4.5). In this study, maximum biomass concentration values ranged from 3.94 ± 0.49 (determined for *P. subcapitata* grown at 35 °C with an average daily light irradiance of $15 \mu\text{E m}^{-2} \text{s}^{-1}$) to $(1.35 \pm 0.13) \times 10^3 \text{ mg DW L}^{-1}$ (determined for *C. vulgaris* grown at 25 °C with an average daily light irradiance of $180 \mu\text{E m}^{-2} \text{s}^{-1}$). Minimum and maximum average biomass productivities were determined for the same microorganisms in the same conditions: 0.370 ± 0.046 (for *P. subcapitata* grown at 35 °C with an average daily light irradiance of $15 \mu\text{E m}^{-2} \text{s}^{-1}$) and $133 \pm 13 \text{ mg DW L}^{-1} \text{d}^{-1}$ (for *C. vulgaris* grown at 25 °C with an average daily light irradiance of $180 \mu\text{E m}^{-2} \text{s}^{-1}$), respectively. As for specific growth rates, an increase in average daily light irradiance from 15 to $180 \mu\text{E m}^{-2} \text{s}^{-1}$ resulted in statistically higher ($p < 0.05$) maximum biomass concentrations and average biomass productivities. Ugwu et al. (2007) have demonstrated that an increase in light irradiance resulted in an increase in biomass productivities, when growing *C. sorokiniana* with average daily light irradiances ranging from 100 to $250 \mu\text{E m}^{-2} \text{s}^{-1}$. Regarding the effect of temperature, statistically higher ($p < 0.05$) maximum biomass concentrations and average biomass productivities were determined for cultures grown at 25 °C. In the case of cultures grown at 15 and 35 °C, no statistical difference ($p > 0.05$) was observed in both maximum biomass concentrations and average biomass productivities. Han et al. (2013) have found that cultivation of *C. pyrenoidosa* at 22, 30 and 36 °C resulted in biomass productivities of 120 ± 2 , 141 ± 1 and $125 \pm 2 \text{ mg L}^{-1} \text{d}^{-1}$, respectively.

Table 4.5. Maximum biomass concentrations (X_{\max} , in mg DW L⁻¹) and average biomass productivities (P, in mg DW L⁻¹ d⁻¹) determined for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* grown under different light and temperature conditions

T (°C)	Average daily light irradiance ($\mu\text{E m}^{-2} \text{s}^{-1}$)	<i>C. vulgaris</i> X_{\max} (mg DW L ⁻¹)	<i>P. subcapitata</i> P (mg DW L ⁻¹ d ⁻¹)	<i>P. subcapitata</i> X_{\max} (mg DW L ⁻¹)	<i>P. subcapitata</i> P (mg DW L ⁻¹ d ⁻¹)	<i>S. salina</i> X_{\max} (mg DW L ⁻¹)	<i>S. salina</i> P (mg DW L ⁻¹ d ⁻¹)	<i>M. aeruginosa</i> X_{\max} (mg DW L ⁻¹)	<i>M. aeruginosa</i> P (mg DW L ⁻¹ d ⁻¹)
15	15	73.9±4.5	4.77±1.12	49.7±13.1	4.29±1.32	167±1	5.69±0.15	72.6±1.0	5.40±0.32
	21	107±19	8.53±1.83	70.8±4	6.10±0.66	173±12	6.47±1.21	109±20	9.08±1.81
	36	194±52	16.9±4.8	107±25	9.81±2.07	242±13	12.2±1.1	189±29	16.1±2.5
	75	331±46	27.2±4.3	113±3	9.61±0.29	349±11	21.7±1.4	211±11	17.9±1.0
	105	293±20	24.5±2.1	134±5	12.0±0.4	363±20	24.0±1.7	290±7	25.8±0.7
	180	588±71	50.4±6.6	459±27	41.8±2.7	501±33	35.7±3.2	458±7	41.0±0.5
25	15	414±13	37.4±1.2	234±25	21.5±2.3	426±24	29.5±2.1	406±16	34.6±1.4
	21	517±11	46.8±1.0	249±13	22.9±1.3	481±19	34.2±1.4	484±7	41.6±0.7
	36	828±23	76.6±2.2	426±15	39.9±1.5	738±16	59.2±1.5	742±3	66.4±0.5
	75	771±11	71.2±0.9	488±13	45.8±1.3	719±39	57.3±3.6	767±17	68.5±1.7
	105	(1.08±0.14)×10 ³	100±13	697±7	64.9±0.8	914±30	75.4±2.9	991±7	89.5±0.7
	180	(1.35±0.13)×10 ³	133±13	798±36	74.5±3.4	(1.26±0.06)×10 ³	108±5	(1.17±0.06)×10 ³	107±5
35	15	93.4±6.5	5.23±0.71	3.94±0.49	0.370±0.046	172±1	5.11±0.16	71.7±2.5	4.14±0.26
	21	108±2	7.04±0.26	12.7±1.1	1.20±0.10	228±16	10.1±1.5	131±17	9.41±1.53
	36	152±10	11.0±1.1	15.9±2.5	0.692±0.321	260±25	13.4±2.6	177±8	14.1±0.8
	75	396±29	34.7±2.6	190±5	17.1±0.4	309±7	17.4±0.7	220±26	17.5±2.4
	105	527±28	51.5±2.8	366±24	33.8±2.2	461±12	31.7±1.1	391±7	37.5±0.7
	180	518±58	47.3±5.6	290±19	27.0±1.8	436±20	30.5±1.8	371±26	32.1±2.4

Values are presented as the mean ± standard deviation of two independent experiments.

Comparing kinetic growth parameters determined for the studied microorganisms, it was possible to observe that *C. vulgaris* achieved the highest specific growth rate, maximum biomass concentration and average biomass productivity when cultured at 25 °C under an average daily light irradiance of 180 $\mu\text{E m}^{-2} \text{s}^{-1}$. In the same culturing conditions, specific growth rates determined for *P. subcapitata* and *S. salina* were not statistically different ($p>0.05$) from the one determined for *C. vulgaris*. In the case of *M. aeruginosa*, specific growth rate determined in these conditions was statistically lower ($p<0.05$). Regarding maximum biomass concentrations and average biomass productivities, values determined for *S. salina* and *M. aeruginosa* were not statistically different ($p>0.05$) from those determined for *C. vulgaris*. However, statistically lower ($p<0.05$) values were determined for *P. subcapitata*.

4.2.2. CO_2 uptake rates

The effect of light supply and temperature on carbon contents of microalgal biomass and CO_2 uptake rates is shown in Table 4.6. From Table 4.6 it is possible to observe that carbon contents in microalgal biomass were not strongly influenced by the studied operational conditions, being the average carbon mass fraction approximately $44.99 \pm 2.18\%$ (w/w). This value was not statistically different ($p=0.08$) from the one reported for the studied microorganisms grown under different average daily light irradiances (Section 4.1.2). However, data from Table 4.6 evidences a slight decrease in carbon mass fractions when cultivation conditions approximate optimal growth conditions. For example, average carbon mass fractions determined at 25 °C were lower than carbon mass fractions determined at 15 and 35 °C. The increase in carbon contents when microalgae are cultured in suboptimal conditions means that the amount of carbon required to produce a cell at the same growth rate under these conditions is higher than the one required when microalgae are submitted to optimal growth conditions (Hu, 2004a). Regarding CO_2 uptake rates, values determined for microalgae grown with different average daily light irradiances and temperatures have shown a similar behaviour to the one described for average biomass productivities: (i) an increase in average daily light irradiance resulted in increased CO_2 uptake rates; and (ii) cultivation at 15, 25 and 35 °C resulted in statistically higher ($p<0.01$) CO_2 uptake rates in cultures performed at 25 °C. As it was described for average biomass productivities, minimum CO_2 uptake rate ($0.664 \pm 0.083 \text{ mg CO}_2 \text{ L}^{-1} \text{ d}^{-1}$) was determined for *P. subcapitata*.

Table 4.6. Carbon contents (α_C , in % w/w) and CO_2 uptake rates (R_C , in $\text{mg CO}_2 \text{ L}^{-1} \text{ d}^{-1}$) determined for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* grown under different light and temperature conditions

T (°C)	Average daily light irradiance ($\mu\text{E m}^{-2} \text{ s}^{-1}$)	<i>C. vulgaris</i>			<i>P. subcapitata</i>			<i>S. salina</i>			<i>M. aeruginosa</i>		
		α_C (% w/w)	R_C ($\text{mg CO}_2 \text{ L}^{-1} \text{ d}^{-1}$)	α_C (% w/w)	α_C (% w/w)	R_C ($\text{mg CO}_2 \text{ L}^{-1} \text{ d}^{-1}$)	α_C (% w/w)	α_C (% w/w)	R_C ($\text{mg CO}_2 \text{ L}^{-1} \text{ d}^{-1}$)	α_C (% w/w)	α_C (% w/w)	R_C ($\text{mg CO}_2 \text{ L}^{-1} \text{ d}^{-1}$)	R_C ($\text{mg CO}_2 \text{ L}^{-1} \text{ d}^{-1}$)
15	15	46.53	8.14±1.91	49.47	49.47	7.77±2.39	46.47	45.97	9.69±0.26	45.97	45.97	9.10±0.55	9.10±0.55
	21	45.24	14.1±3.0	49.38	49.38	11.0±1.2	45.66	45.05	10.8±2.0	45.05	45.05	15.0±3.0	15.0±3.0
	36	45.11	28.0±7.9	48.98	48.98	17.6±3.7	44.78	44.51	20.1±1.8	44.51	44.51	26.3±4.1	26.3±4.1
	75	43.84	43.7±7.0	50.43	50.43	17.8±0.5	42.94	41.69	34.2±2.2	41.69	41.69	27.4±1.6	27.4±1.6
	105	43.36	38.9±3.4	50.71	50.71	22.2±0.7	43.43	42.86	38.1±2.8	42.86	42.86	40.6±1.0	40.6±1.0
	180	43.16	79.8±10.5	50.44	50.44	83.3±1.7	42.55	42.43	55.6±5.0	42.43	42.43	63.7±0.7	63.7±0.7
25	15	44.50	60.9±2.0	45.56	45.56	35.9±3.8	44.98	45.42	48.7±3.5	45.42	45.42	57.6±2.4	57.6±2.4
	21	45.60	78.1±1.7	44.75	44.75	37.6±2.2	45.51	45.16	57.1±2.3	45.16	45.16	68.9±1.2	68.9±1.2
	36	44.73	125±4	45.21	45.21	66.1±2.4	44.73	44.55	97.0±2.4	44.55	44.55	108±1	108±1
	75	44.54	116±1	44.72	44.72	75.0±2.1	43.76	43.76	91.9±5.8	43.76	43.76	110±2	110±2
	105	43.60	160±21	45.41	45.41	108±1	43.19	43.12	119±5	43.12	43.12	141±1	141±1
	180	42.54	195±19	47.35	47.35	129±6	42.96	41.50	169±8	41.50	41.50	162±8	162±8
35	15	47.18	9.09±1.22	48.96	48.96	0.664±0.083	45.08	44.48	8.44±0.26	44.48	44.48	6.43±0.41	6.43±0.41
	21	44.84	11.6±0.4	50.50	50.50	2.21±0.19	45.04	43.72	16.7±2.4	43.72	43.72	14.6±2.4	14.6±2.4
	36	44.97	18.1±1.8	48.47	48.47	1.23±0.57	45.38	45.02	22.3±4.3	45.02	45.02	22.0±1.3	22.0±1.3
	75	44.60	56.7±4.3	45.17	45.17	28.3±0.7	44.45	44.64	28.3±1.2	44.64	44.64	27.2±3.7	27.2±3.7
	105	43.67	82.3±4.4	44.04	44.04	54.5±3.6	44.32	43.69	51.5±1.8	43.69	43.69	58.3±1.1	58.3±1.1
	180	42.61	73.9±8.8	42.47	42.47	41.9±2.8	42.67	41.40	47.7±2.9	41.40	41.40	49.9±3.7	49.9±3.7

Values are presented as the mean ± standard deviation of two independent experiments.

grown at 35 °C with an average daily light irradiance of 15 $\mu\text{E m}^{-2} \text{s}^{-1}$. On the other hand, maximum CO_2 uptake rate ($195 \pm 19 \text{ mg CO}_2 \text{ L}^{-1} \text{d}^{-1}$) was determined for *C. vulgaris* grown at 25 °C with an average daily light irradiance of 180 $\mu\text{E m}^{-2} \text{s}^{-1}$. The increase of CO_2 uptake rates with increased light supplies has already been discussed in Section 4.1.2. Regarding the effect of different temperatures on CO_2 uptake rates, the obtained results suggest that optimal growth conditions result in increased CO_2 uptake from the atmosphere, which is in agreement with an increase in the photosynthetic activity of the studied microorganisms.

4.2.3. Nutrients removal

In this study nitrogen and phosphorus concentrations were determined in the first and last day of culturing to evaluate the removal efficiencies of these nutrients under the studied conditions. Average removal rates and removal efficiencies obtained for nitrogen and phosphorus are presented in Tables 4.7 and 4.8, respectively.

Regarding nitrogen removal (Table 4.7), maximum average removal rate, $2.89 \pm 0.07 \text{ mg N L}^{-1} \text{d}^{-1}$, was determined for *M. aeruginosa* grown at 25 °C, with an average daily light irradiance of 36 $\mu\text{E m}^{-2} \text{s}^{-1}$. On the other hand, maximum nitrogen removal efficiency achieved was 100% (for *C. vulgaris*, *P. subcapitata* and *M. aeruginosa* grown at 25 °C with an average daily light irradiance of 180 $\mu\text{E m}^{-2} \text{s}^{-1}$). The influence of light supply and temperature in these variables was very similar. In the case of average daily light irradiance, higher values resulted in statistically higher ($p < 0.05$) removal rates and removal efficiencies. In the study performed by Hu et al. (2000), $\text{NO}_3\text{-N}$ uptake rates determined for *Synechococcus* sp. grown in nitrate-contaminated groundwater increased proportionally to increasing average daily light irradiance up to 100 $\mu\text{E m}^{-2} \text{s}^{-1}$. Regarding the effects of temperature, microalgal growth at 25 °C resulted in nitrogen removal rates and removal efficiencies statistically higher ($p < 0.05$) than those determined at 15 and 35 °C. Nitrogen removal rates and removal efficiencies determined for the extreme temperatures were not statistically different ($p = 0.15$). Talbot and De la Noüe (1993) have demonstrated that cultivation of *P. bohnneri* in a secondary effluent from an activated sludge treatment plant at 30 °C for three days resulted in an effective removal of $\text{NH}_4\text{-N}$, whereas the same culture performed at 10 °C resulted in modest $\text{NH}_4\text{-N}$ removal.

Table 4.7. Average nitrogen removal rates (R_N , in $\text{mg N L}^{-1} \text{d}^{-1}$) and removal efficiencies (R , in %) determined for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* grown under different light and temperature conditions

T (°C)	Average daily light irradiance ($\mu\text{E m}^{-2} \text{s}^{-1}$)	<i>C. vulgaris</i>			<i>P. subcapitata</i>			<i>S. salina</i>			<i>M. aeruginosa</i>		
		R_N ($\text{mg N L}^{-1} \text{d}^{-1}$)	R (%)	R_N ($\text{mg N L}^{-1} \text{d}^{-1}$)	R_N ($\text{mg N L}^{-1} \text{d}^{-1}$)	R (%)	R_N ($\text{mg N L}^{-1} \text{d}^{-1}$)	R_N ($\text{mg N L}^{-1} \text{d}^{-1}$)	R (%)	R_N ($\text{mg N L}^{-1} \text{d}^{-1}$)	R_N ($\text{mg N L}^{-1} \text{d}^{-1}$)	R (%)	R (%)
15	15	0.658±0.277	36.8±9.6	0.115±0.061	7.55±3.62	0.278±0.199	8.98±6.55	0.497±0.151	16.5±4.7	0.497±0.151	0.497±0.151	16.5±4.7	
	21	0.561±0.035	37.9±1.7	0.221±0.098	16.5±7.1	0.723±0.161	25.3±6.0	0.827±0.250	27.1±5.8	0.827±0.250	0.827±0.250	27.1±5.8	
	36	1.67±0.69	78.9±6.0	0.472±0.100	28.3±5.8	0.816±0.141	30.0±5.8	1.21±0.15	40.2±4.9	1.21±0.15	1.21±0.15	40.2±4.9	
	75	0.759±0.225	24.8±9.0	0.713±0.474	25.3±13.2	1.45±0.33	45.7±13.8	1.17±0.12	41.1±3.2	1.17±0.12	1.17±0.12	41.1±3.2	
	105	2.11±0.07	77.2±5.6	1.69±0.54	50.5±10.0	2.32±0.31	68.3±5.0	1.87±0.28	69.8±3.3	1.87±0.28	1.87±0.28	69.8±3.3	
	180	2.56±0.49	93.4±9.8	2.36±0.25	79.1±4.2	2.33±0.27	75.0±13.1	2.58±0.34	85.3±6.3	2.58±0.34	2.58±0.34	85.3±6.3	
25	15	1.08±0.03	42.3±1.6	1.07±0.21	43.5±8.3	1.27±0.02	48.5±0.7	1.42±0.04	53.6±1.7	1.42±0.04	1.42±0.04	53.6±1.7	
	21	1.69±0.116	75.6±5.8	1.24±0.04	74.4±2.9	1.86±0.06	96.1±0.9	1.82±0.03	98.8±1.4	1.82±0.03	1.82±0.03	98.8±1.4	
	36	2.43±0.38	97.1±1.7	2.62±0.08	88.0±2.7	2.83±0.16	92.5±1.0	2.89±0.07	97.3±1.1	2.89±0.07	2.89±0.07	97.3±1.1	
	75	2.40±0.05	86.2±1.7	1.97±0.02	68.9±0.8	2.45±0.02	86.1±0.6	2.59±0.03	89.8±0.4	2.59±0.03	2.59±0.03	89.8±0.4	
	105	2.78±0.06	98.0±2.0	2.16±0.54	97.7±2.5	2.54±0.20	98.6±0.4	2.43±0.33	98.0±0.6	2.43±0.33	2.43±0.33	98.0±0.6	
	180	2.43±0.40	100±0	2.37±0.18	100±0	1.97±0.19	99.1±0.7	2.53±0.21	100±0	2.53±0.21	2.53±0.21	100±0	
35	15	0	0	0	0	0	0	0	0	0	0	0	
	21	0.131±0.039	6.68±1.93	0	0	0	0	0.0115±0.0006	0.0510±0.0141	0.0115±0.0006	0.0115±0.0006	0.0510±0.0141	
	36	0.482±0.292	16.3±8.2	0.0442±0.0071	1.37±0.75	0.330±0.081	15.1±3.0	0.0874±0.0360	4.00±1.55	0.0874±0.0360	0.0874±0.0360	4.00±1.55	
	75	0.959±0.558	37.0±21.3	0.804±0.246	30.9±9.2	2.22±0.87	58.7±9.5	1.47±0.11	53.5±2.4	1.47±0.11	1.47±0.11	53.5±2.4	
	105	1.60±0.12	63.4±4.8	1.75±0.07	70.6±2.7	1.29±0.01	61.4±0.6	1.85±0.06	73.5±1.6	1.85±0.06	1.85±0.06	73.5±1.6	
	180	2.41±0.04	88.6±1.5	1.95±0.05	78.1±1.6	1.25±0.12	63.8±1.9	2.14±0.02	91.1±0.6	2.14±0.02	2.14±0.02	91.1±0.6	

Values are presented as the mean ± standard deviation of two independent experiments.

Table 4.8. Average phosphorus removal rates (R_P , in $\text{mg P L}^{-1} \text{d}^{-1}$) and removal efficiencies (R , in %) determined for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* grown under different light and temperature conditions

T (°C)	Average daily light irradiance ($\mu\text{E m}^{-2} \text{s}^{-1}$)	<i>C. vulgaris</i>			<i>P. subcapitata</i>			<i>S. salina</i>			<i>M. aeruginosa</i>		
		R_P ($\text{mg P L}^{-1} \text{d}^{-1}$)	R (%)	R_P ($\text{mg P L}^{-1} \text{d}^{-1}$)	R_P ($\text{mg P L}^{-1} \text{d}^{-1}$)	R (%)	R_P ($\text{mg P L}^{-1} \text{d}^{-1}$)	R_P ($\text{mg P L}^{-1} \text{d}^{-1}$)	R (%)	R_P ($\text{mg P L}^{-1} \text{d}^{-1}$)	R_P ($\text{mg P L}^{-1} \text{d}^{-1}$)	R (%)	R (%)
15	15	0.110±0.013	13.5±1.6	0.0505±0.0154	0.0505±0.0154	6.18±1.74	0.0171±0.0092	0.0171±0.0092	1.97±1.09	0.00104±0.00040	0.00104±0.00040	1.13±0.03	
	21	0.0934±0.0607	11.8±7.2	0.220±0.044	0.220±0.044	26.2±4.3	0.107±0.026	0.107±0.026	10.9±2.5	0.120±0.060	0.120±0.060	12.4±5.7	
	36	0.265±0.037	32.7±4.5	0.158±0.087	0.158±0.087	20.6±12.2	0.126±0.047	0.126±0.047	13.4±5.1	0.182±0.067	0.182±0.067	18.3±6.2	
	75	0.275±0.025	29.5±3.0	0.0751±0.0061	0.0751±0.0061	9.47±0.67	0.386±0.089	0.386±0.089	44.6±9.5	0.416±0.031	0.416±0.031	26.3±2.1	
	105	0.255±0.130	29.1±12.3	0.157±0.068	0.157±0.068	20.1±9.7	0.215±0.034	0.215±0.034	20.9±4.4	0.389±0.050	0.389±0.050	37.8±0.9	
	180	0.387±0.010	44.2±1.0	0.252±0.073	0.252±0.073	27.5±6.0	0.275±0.008	0.275±0.008	29.1±1.0	0.255±0.027	0.255±0.027	21.4±3.1	
25	15	0.149±0.035	16.9±3.4	0.268±0.115	0.268±0.115	17.5±7.9	0.157±0.007	0.157±0.007	17.3±0.6	0.109±0.081	0.109±0.081	13.4±8.8	
	21	0.258±0.019	29.3±1.6	0.223±0.057	0.223±0.057	24.0±9.6	0.222±0.034	0.222±0.034	23.9±3.0	0.279±0.081	0.279±0.081	28.8±6.6	
	36	0.279±0.092	29.3±7.4	0.259±0.056	0.259±0.056	34.2±4.9	0.316±0.034	0.316±0.034	35.4±3.4	0.255±0.068	0.255±0.068	29.7±6.0	
	75	0.240±0.191	24.9±18.4	0.235±0.018	0.235±0.018	27.0±2.0	0.231±0.064	0.231±0.064	33.9±0.6	0.218±0.050	0.218±0.050	26.3±5.7	
	105	0.240±0.074	31.5±4.0	0.279±0.020	0.279±0.020	32.7±2.0	0.345±0.035	0.345±0.035	32.0±4.8	0.231±0.039	0.231±0.039	25.8±2.1	
	180	0.588±0.029	67.6±7.1	0.393±0.070	0.393±0.070	51.2±4.8	0.348±0.018	0.348±0.018	36.7±4.3	0.357±0.074	0.357±0.074	41.1±9.2	
35	15	0.0767±0.0300	7.76±2.60	0.0785±0.0109	0.0785±0.0109	7.89±0.67	0.0642±0.0495	0.0642±0.0495	6.67±4.98	0.0631±0.0490	0.0631±0.0490	6.56±4.90	
	21	0.160±0.017	16.4±3.0	0.143±0.026	0.143±0.026	14.6±3.5	0.167±0.029	0.167±0.029	16.8±4.1	0.137±0.027	0.137±0.027	13.1±3.4	
	36	0.171±0.047	16.8±3.9	0.184±0.070	0.184±0.070	17.5±5.6	0.188±0.066	0.188±0.066	17.9±5.4	0.157±0.060	0.157±0.060	15.0±5.1	
	75	0.895±0.015	21.0±1.7	0.0968±0.0213	0.0968±0.0213	9.84±2.07	0.378±0.006	0.378±0.006	42.9±0.8	0.282±0.030	0.282±0.030	26.1±2.5	
	105	0.316±0.021	33.3±2.0	0.241±0.020	0.241±0.020	26.6±2.2	0.194±0.036	0.194±0.036	21.0±4.6	0.352±0.027	0.352±0.027	36.0±2.5	
	180	0.278±0.063	38.3±14.1	0.440±0.067	0.440±0.067	38.7±4.3	0.210±0.046	0.210±0.046	22.7±4.3	0.543±0.072	0.543±0.072	54.2±3.2	

Values are presented as the mean ± standard deviation of two independent experiments.

In the case of phosphorus removal, maximum average removal rate, $0.588 \pm 0.029 \text{ mg P L}^{-1} \text{ d}^{-1}$, was determined for *C. vulgaris* grown at 25 °C with an average daily light irradiance of $180 \mu\text{E m}^{-2} \text{ s}^{-1}$. Phosphorus removal efficiencies ranged from 1.13 ± 0.03 (for *M. aeruginosa* grown at 15 °C, under the lowest average daily light irradiance) to $67.6 \pm 7.1\%$ (for *C. vulgaris* grown at 25 °C with an average daily light irradiance of $180 \mu\text{E m}^{-2} \text{ s}^{-1}$). These values were lower than those determined for nitrate, indicating that phosphorus assimilation is slower than $\text{NO}_3\text{-N}$ assimilation. Different studies have already reported higher removal efficiencies for nitrogen than for phosphorus (Lee and Lee, 2001; Talbot and De la Noüe, 1993). The influence of light supply and temperature on phosphorus removal rates and removal efficiencies was similar to the one observed for nitrogen removal. In general, an increase in light supply resulted in increased phosphorus removal rates and removal efficiencies. Statistically higher ($p < 0.05$) removal rates and removal efficiencies were determined when light irradiance increased from 15 to $180 \mu\text{E m}^{-2} \text{ s}^{-1}$. In the study performed by Li et al. (2012), an increase in average daily light irradiance from 0 to $200 \mu\text{E m}^{-2} \text{ s}^{-1}$ increased TP removal efficiencies from 65.8 to 87.0% (for *C. kessleri*) and from 79.3 to 83.0% (for *C. protothecoides*). The effects of temperature on phosphorus removal demonstrated that, in general, higher removal rates and removal efficiencies were obtained for cultures grown at 25 °C. However, these values were not statistically different ($p > 0.05$) from those determined for the other studied temperatures.

These results have shown that the influence of light supply and temperature on nitrogen and phosphorus removal is similar to the one observed for specific growth rates, maximum biomass concentrations and average biomass productivities, paralleling photosynthetic activity. Regarding the performance of the studied microorganisms in nitrogen and phosphorus removal, average removal rates and removal efficiencies were not statistically different ($p > 0.05$). Additionally, it was observed that the majority of cultures grown at 25 °C, under the highest light supplies have effectively removed nitrogen. These results constitute important findings for the application of microalgal cultures in the tertiary treatment step of wastewater treatment plants.

4.2.4. Cell composition

The mass balance written for nitrogen and phosphorus allowed the determination of the mass fractions of these nutrients in microalgal biomass for each of the studied conditions (Table

4.9). Mass fractions of nitrogen and phosphorus were close to those reported in the typical composition of microalgal biomass described in Section 4.1.4. To have a better understanding about the effects of light and temperature on nitrogen and phosphorus contents on microalgal biomass, contour graphs relating these variables were obtained for the selected microorganisms (Figure 4.3). Additionally, these parameters were analysed through multiple linear regression (described in Section 3.11) to evaluate which parameters significantly influenced nitrogen and phosphorus mass fractions (Table III.1, Annex III). From these data, it is possible to conclude that the effect of light and temperature on the biochemical composition of microalgal biomass presented some differences between the studied microorganisms. These observations are in agreement with the study performed by Goldman (1977), who concluded that the relationship between nitrogen contents and temperature may be species-specific. Regarding nitrogen mass fractions, temperature appears as the most important factor influencing this parameter: (i) in the case of *C. vulgaris* and *S. salina*, an increase in temperature resulted in lower nitrogen mass fractions; (ii) in *P. subcapitata*, both light and temperature have not significantly influenced ($p>0.05$) nitrogen mass fractions; and (iii) in *M. aeruginosa*, an increase in light and temperature resulted in lower nitrogen mass fractions and, on the other hand, the simultaneous increase in both light and temperature resulted in higher nitrogen mass fractions. As for nitrogen mass fractions, phosphorus mass fractions were also mainly influenced by temperature: (i) in *C. vulgaris*, an increase in temperature resulted in a decrease of phosphorus mass fractions, with the minimum value reached at approximately 25 °C, and the simultaneous increase in both light and temperature results in lower phosphorus mass fractions; (ii) in *P. subcapitata*, phosphorus mass fractions had a similar behaviour to the one described for nitrogen mass fractions in *M. aeruginosa*; and (iii) in *S. salina* and *M. aeruginosa*, an increase in temperature resulted in a decrease of phosphorus mass fractions, with the minimum value reached at approximately 25 °C. These results indicate that environmental factors, such as light and temperature, not only affect the photosynthetic activity and biomass productivities, but also cell metabolism and, consequently, biochemical composition, as previously reported by Hu (2004a). The preponderance of temperature influence on nitrogen and phosphorus mass fractions behaviour suggests that these parameters were not strongly influenced by average daily light irradiance. Similar results have already been reported by Mortensen et al. (1988). In this study, nitrogen and phosphorus mass fractions determined for batch cultures of *Chaetoceros gracilis* grown with different light intensities at 28 °C were not statistically

Table 4.9. Mass fractions of nitrogen (α_N , in % w/w) and phosphorus (α_P , in % w/w) incorporated in the biomass of *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* obtained through the mass balance performed for each nutrient

T (°C)	Average daily light irradiance ($\mu\text{E m}^{-2} \text{s}^{-1}$)	<i>C. vulgaris</i>			<i>P. subcapitata</i>			<i>S. salina</i>			<i>M. aeruginosa</i>		
		α_N (% w/w)	α_P (% w/w)	α_N (% w/w)	α_P (% w/w)	α_N (% w/w)	α_P (% w/w)	α_N (% w/w)	α_P (% w/w)	α_N (% w/w)	α_P (% w/w)	α_N (% w/w)	α_P (% w/w)
15	15	14.2±6.9	2.39±0.64	2.78±1.72	1.22±0.54	5.05±2.62	0.311±0.167	9.50±2.95	0.181±0.013				
	21	6.80±1.57	1.13±0.78	3.74±1.71	3.72±0.85	11.6±3.4	1.70±0.53	9.41±3.44	1.36±0.74				
	36	10.2±5.2	1.61±0.52	4.98±1.51	1.66±0.99	6.89±1.35	1.06±0.41	7.72±1.56	1.16±0.47				
	75	2.88±0.98	1.05±0.20	7.67±3.10	0.807±0.070	6.89±1.63	1.84±0.20	6.75±0.79	2.40±0.23				
	105	8.92±0.84	1.08±0.56	14.6±4.7	1.36±0.59	10.0±1.5	0.927±0.162	7.48±1.14	1.56±0.20				
	180	5.24±1.23	0.793±0.109	5.83±0.73	0.623±0.185	6.75±1.00	0.797±0.077	6.50±0.86	0.643±0.069				
25	15	2.88±0.13	0.399±0.094	4.99±1.09	0.568±0.278	4.31±0.31	0.530±0.045	4.11±0.21	0.316±0.233				
	21	3.61±0.36	0.552±0.042	5.40±0.37	0.947±0.441	5.42±0.28	0.647±0.103	4.37±0.11	0.511±0.135				
	36	3.45±0.23	0.365±0.121	6.57±0.31	0.488±0.345	4.79±0.29	0.534±0.060	4.36±0.11	0.384±0.102				
	75	3.37±0.08	0.245±0.138	4.30±0.13	0.513±0.043	4.27±0.27	0.533±0.041	3.78±0.10	0.318±0.074				
	105	2.77±0.38	0.289±0.050	4.03±0.27	0.430±0.032	3.59±0.14	0.364±0.056	2.72±0.37	0.258±0.044				
	180	1.95±0.24	0.441±0.049	3.19±0.17	0.528±0.097	1.98±0.11	0.296±0.037	2.42±0.12	0.334±0.071				
35	15	n.a.	1.51±0.63	n.a.	21.9±4.1	n.a.	1.30±1.00	n.a.	1.58±0.83				
	21	1.92±0.61	2.35±0.35	n.a.	12.4±2.5	0.856±0.161	1.71±0.40	0.0127±0.0069	1.39±0.38				
	36	4.52±2.26	1.60±0.47	6.60±3.34	27.5±8.5	2.54±0.81	1.45±0.59	0.638±0.266	1.15±0.44				
	75	2.86±1.68	0.607±0.065	4.94±1.52	0.595±0.132	13.2±5.2	2.24±0.10	8.66±1.39	1.67±0.30				
	105	3.43±0.32	0.675±0.058	5.34±0.42	0.735±0.079	4.20±0.15	0.631±0.119	2.14±0.07	0.407±0.031				
	180	5.26±0.65	0.608±0.156	7.47±0.55	1.69±0.28	4.22±0.48	0.711±0.162	6.89±0.54	1.75±0.27				

Values are presented as the mean ± standard deviation of two independent experiments. n.a. - not applicable.

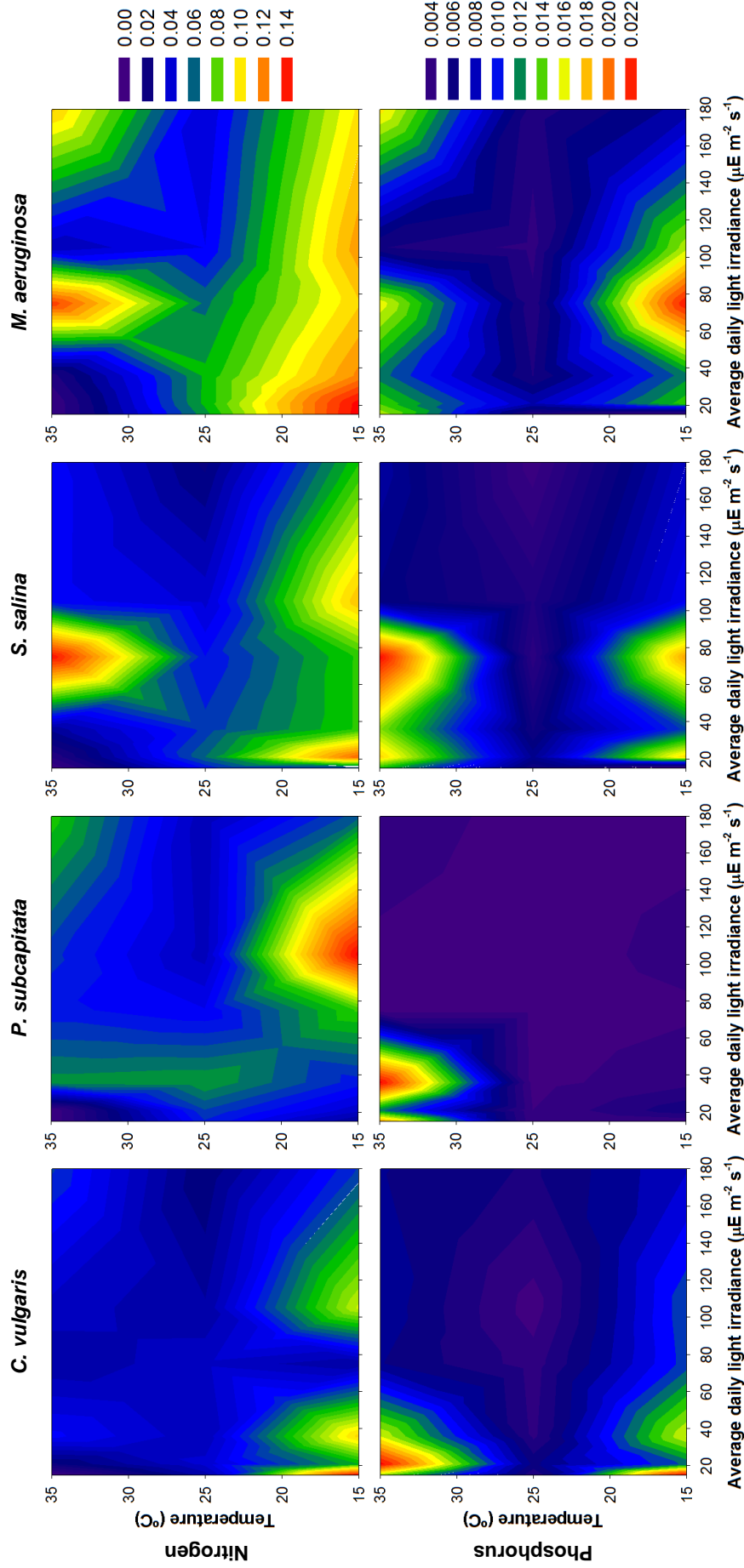


Figure 4.3. Influence of average daily light irradiance and temperature on nitrogen (α_N , in % w/w) and phosphorus (α_P , in % w/w) mass fractions incorporated in the biomass of *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa*.

different. The decrease of nitrogen and phosphorus mass fractions with increasing temperatures, which was common for the majority of the selected microorganisms has already been reported in the literature. In the study performed by Fu et al. (2007), an increase in temperature from 20 to 24 °C resulted in a decrease in nitrogen and phosphorus mass fractions in *Synechococcus* sp. The U-shape response observed for some microorganisms has also been described in the literature. According to Hu (2004a), at temperatures below and above the optimal growth temperature, microalgae and cyanobacteria require higher amounts of nutrients, such as nitrogen and phosphorus, to achieve the same growth rates as those reported for optimal temperatures. Accordingly, as it was already described for carbon mass fractions (Section 4.2.2), nitrogen and phosphorus mass fractions tend to be lower at the optimal growth temperature determined for this study: approximately 25 °C.

4.2.5. Optimal light and temperature conditions determined through mathematical modelling

Optimal growth conditions (average daily light irradiance and temperature) for the studied microorganisms were determined. For this, the model described by Equation 3.5 was applied and surface graphs (Figure 4.4) relating specific growth rates with average daily light irradiance and temperature were obtained. Analysis of Figure 4.4 shows that an increase in average daily light irradiance results in increased specific growth rates, with optimal average daily light irradiances varying according to the studied species. Regarding the effect of temperature on specific growth rates, Figure 4.4 evidences a similar behaviour between the studied microorganisms. When temperature increases from 15 to 35 °C, specific growth rates tend to increase until approximately 25 °C, where specific growth rates start decreasing, reaching values close to those observed at 15 °C.

Optimal average daily light irradiance and temperature determined through mathematical modelling for each microorganism are shown in Table 4.10. For determination of these parameters, it was assumed that maximum specific growth rates achieved by each microorganism could not be lower than the maximum specific growth rate determined for each microorganism: 1.30, 1.13, 1.14 and 1.02 d⁻¹ for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa*, respectively. Definition of this condition was based on the fact that each microorganism usually presents a maximum specific growth rate, which is obtained under optimal growth conditions (Bouterfas et al., 2002). From Table 4.10, it is possible to observe

that optimal temperatures determined for the studied microorganisms were very similar. T_{opt} values determined through mathematical modelling for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* were 25.4, 23.7, 26.4 and 25.6 °C, respectively. These values were slightly lower than optimal temperature determined for *C. vulgaris* growth in the study performed by Dauta et al. (1990). In this study, for a maximum specific growth rate of 1.30 d⁻¹, optimal temperature determined for *C. vulgaris* was 30 °C. However, other studies have reported optimal growth temperatures close to 25 °C. In the study performed by Claquin et al. (2008), average optimal temperature determined for eight species of marine microalgae (*Thalassiosira pseudonana*, *Skeletonema marinoi*, *Pseudo-nitzschia fraudulenta*, *Emiliana huxleyi*, *Isochrysis galbana*, *Isochrysis* aff. *galbana*, *Pavlova lutheri* and *Lepidodinium chlorophorum*) was 23.7±3.1 °C, corresponding to a maximum specific growth rate of

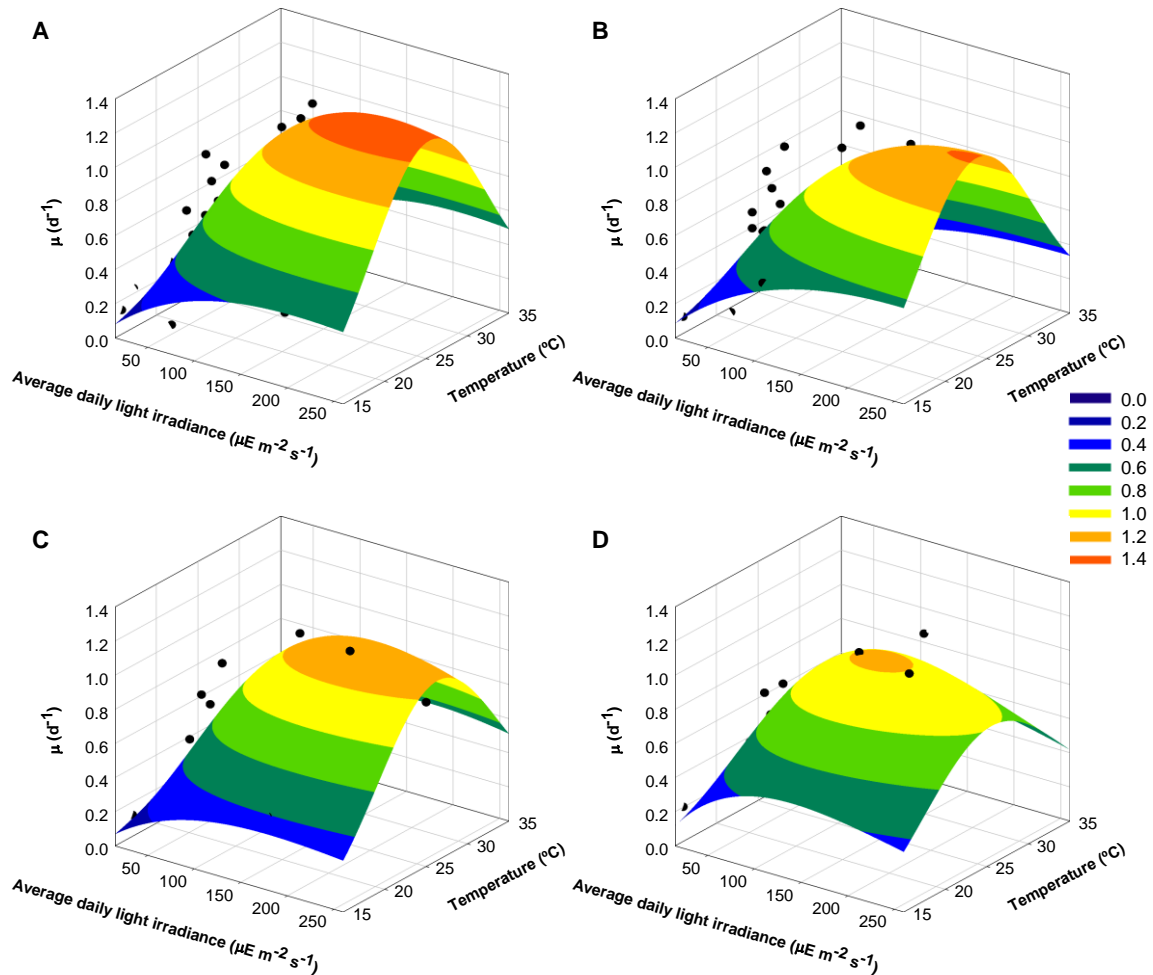


Figure 4.4. Influence of average daily light irradiance and temperature on specific growth rates (μ , in d⁻¹) of *C. vulgaris* (A), *P. subcapitata* (B), *S. salina* (C) and *M. aeruginosa* (D). The dots correspond to the experimental data. The surface graphs were obtained through mathematical modelling.

Table 4.10. Optimal growth conditions (average daily light irradiance and temperature) determined for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* through mathematical modelling

Model parameters	<i>C. vulgaris</i>	<i>P. subcapitata</i>	<i>S. salina</i>	<i>M. aeruginosa</i>
μ_{\max} (d ⁻¹)	1.30	1.21	1.14	1.02
I_{opt} (μE m ⁻² s ⁻¹)	208	258	178	140
T_{opt} (°C)	25.4	23.7	26.4	25.6
σ (°C)	7.0	7.0	7.2	8.2
RMSE (d ⁻¹)	0.29	0.20	0.32	0.26
n	29	27	18	18
Model validation				
RMSE (d ⁻¹)	0.39	0.28	0.26	0.18
n	9	9	6	6

Values were obtained through mathematical modelling. μ_{\max} - maximum specific growth rate (in d⁻¹); I_{opt} - optimal average daily light irradiance value for microalgal growth (in μE m⁻² s⁻¹); T_{opt} - optimal temperature for microalgal growth (in °C); σ - standard deviation associated to the optimal temperature (in °C); RMSE - root mean squared error (in d⁻¹); n - data size.

1.27±0.27 d⁻¹. Yang et al. (2010) have demonstrated that *C. vulgaris* can grow normally in the temperature range of 5 to 30 °C, being optimal growth temperature 25 °C. Through mathematical modelling, Aleya et al. (2011) have determined an optimal growth temperature for *Chlorella minutissima* of 28 °C, corresponding to a maximum specific growth rate of 0.7 d⁻¹. Regarding optimal average daily light irradiances determined using this model, Table 4.10 shows that I_{opt} values differ according to microalgal species, being 208, 258, 178 and 140 μE m⁻² s⁻¹ for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa*, respectively. Similar orders of magnitude have already been reported in the literature for several microalgae. Optimal average daily light irradiance values determined by Dauta et al. (1990) for *C. vulgaris*, *Fragilaria crotonensis*, *Staurostrum pingue* and *Synechocystis minima* ranged from 78 to 169 μE m⁻² s⁻¹. On the other hand, optimal average daily light irradiances determined for *S. minutum*, *C. microporum* and *C. subprotumidum* ranged from 250 to 263 μE m⁻² s⁻¹ (Bouterfas et al., 2002). However, optimal average daily light irradiance determined for *C. vulgaris* and *P. subcapitata* surpassed the range of values assessed in this study, meaning that optimal growth of these microalgae is expected to occur for an average daily light irradiance of 208 and 258 μE m⁻² s⁻¹, respectively. Although these results were not validated experimentally, it is possible to propose that the established models can be correctly applied to describe the response of specific growth rates of the studied microorganisms to light and temperature. In fact, optimal light and temperature conditions determined are in accordance with the ones already reported in the literature. Additionally,

the low *RMSE* values determined (ranging from 0.20 to 0.32 d⁻¹) indicate that these models correctly fit to the experimental data. Nevertheless, the current models were validated by evaluating the *RMSE* values obtained between specific growth rates determined by these models and a validation data set composed by specific growth rates determined in different light and temperature conditions (Table III.2, Annex III). With the current models, *RMSE* values determined for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* were 0.29, 0.20, 0.32 and 0.26 d⁻¹, respectively. On the other hand, *RMSE* values determined through application of this model to data obtained from other studies (validation data set) was 0.39, 0.28, 0.26 and 0.18 d⁻¹, respectively. These results indicate that the developed model can be correctly applied to the studied microorganisms grown under light and temperature conditions within the range of those reported in this study. Additionally, in this study specific mathematical models were determined for different microalgal species. Determination of an adequate model that describes microalgal growth in relation to light supply and temperature may result in several savings, especially in the optimization of cultivation conditions.

4.3. Microalgal monocultures: the effect of CO₂ concentration in the air stream

4.3.1. Microalgal growth

The study of different CO₂ concentrations in the air stream has shown that this parameter strongly influences microalgal growth. Figure IV.1 (Annex IV) presents the growth curves obtained for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* grown in air streams containing different CO₂ concentrations. Although increasing CO₂ concentrations have influenced the kinetic growth parameters of the selected microorganisms, similar growth curves were obtained. Figure IV.1 (Annex IV) shows that, with the exception of *C. vulgaris*, all studied cultures have followed: (i) an adaptation phase of approximately one day; (ii) an exponential growth phase until approximately the fourth day of culturing; and (iii) a retardation growth phase. In *C. vulgaris* cultures, the lack of an adaptation phase was observed and microalgal cells started growing exponentially from the beginning of the experiments. This exponential growth lasted until the fourth day of culturing, where cells entered the retardation growth phase. The lack of an adaptation phase for *C. vulgaris* cultures, especially in those aerated with higher CO₂ concentrations is not surprising, since microorganisms from the genus *Chlorella* are well known for their tolerance to high CO₂

levels (Keffer and Kleinheinz, 2002). Additionally, Figure IV.1 (Annex IV) shows that the growth of the selected microorganisms at atmospheric CO₂ concentrations was slower than in the other studied conditions, indicating that in cultures aerated with atmospheric air, carbon limitation might occur. The kinetic growth parameters determined for the selected microorganisms are shown in Table 4.11. Regarding specific growth rates, these values ranged from 0.660 ± 0.016 (for *P. subcapitata* grown with 10% v/v CO₂) to 1.50 d^{-1} (for *M. aeruginosa* grown with 5 and 7% v/v CO₂). For the range of CO₂ concentrations used in this study, a similar behaviour was observed for the selected microorganisms: specific growth rate values have increased until the CO₂ concentration of 5% (v/v), followed by a decrease observed for higher CO₂ concentrations. Similar results were obtained by de Moraes and Costa (2007a) for *Spirulina* sp. and *S. obliquus* subjected to CO₂ concentrations of 0, 6 and 12% (v/v): in these conditions, specific growth rates determined for *Spirulina* sp. were 0.33, 0.44 and 0.33 d^{-1} , whereas specific growth rates determined for *S. obliquus* were 0.15, 0.22 and 0.22 d^{-1} . The same behaviour was demonstrated by the same authors for the microalga *C. kessleri*. When grown with CO₂ concentrations of 0.04, 6, 12 and 18% (v/v), specific growth rates determined for this microalga were 0.26, 0.27, 0.27 and 0.20 d^{-1} , respectively (de Moraes and Costa, 2007b). Later, Chiu et al. (2008) have determined specific growth rates of 0.230, 0.492 and 0.127 d^{-1} for *Chlorella* sp. cultures fed with a gas stream containing 0.04, 2 and 5% (v/v) CO₂. The decrease observed in specific growth rates determined for cultures fed with CO₂ concentrations higher than 5% (v/v) may be related to some changes in the photosynthetic characteristics of the selected microorganisms. In fact, it has already been reported that, when grown under high CO₂ concentrations (5% v/v or more), some microalgae display lower affinity to CO₂, higher photosynthetic sensitivity to O₂ and lower activity of carbonic anhydrase, the enzyme responsible for photosynthetic utilization of inorganic carbon (Xia and Gao, 2005; Yang and Gao, 2003). Comparing specific growth rates determined for the selected microorganisms, Table 4.11 shows that increasing CO₂ concentrations until 5% (v/v) has strongly influenced the growth of the selected microorganisms, resulting in an increase in specific growth rates. Maximum biomass concentrations achieved in the last day of culturing ranged from 383 ± 94 (for *M. aeruginosa* grown with non-enriched air) to $(1.15 \pm 0.04) \times 10^3 \text{ mg DW L}^{-1}$ (for *S. salina* grown with 7% v/v CO₂). Values in the same order of magnitude were obtained for *Chlorella* sp. grown with CO₂ concentrations ranging from 0.04 to 15% (v/v) (Fulke et al., 2010) and for *S. obliquus* and *C. pyrenoidosa* fed with a gas stream containing 0.04 to 50% (v/v) CO₂

Table 4.11. Specific growth rates (μ , in d^{-1}), maximum biomass concentrations (X_{max} , in mg DW L^{-1}) and average biomass productivities (P , in $\text{mg DW L}^{-1} \text{d}^{-1}$) determined for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* grown with different CO_2 concentrations in the air stream

Parameters	% CO_2 (v/v)	<i>C. vulgaris</i>	<i>P. subcapitata</i>	<i>S. salina</i>	<i>M. aeruginosa</i>
μ (d^{-1})	Air	0.834 \pm 0.060	0.693 \pm 0.056	0.831 \pm 0.101	0.902 \pm 0.020
	3	1.22 \pm 0.04	0.885 \pm 0.014	1.20 \pm 0.02	1.31 \pm 0.06
	5	1.36 \pm 0.06	0.891 \pm 0.013	1.34 \pm 0.07	1.50 \pm 0.12
	7	1.30 \pm 0.04	0.819 \pm 0.034	1.33 \pm 0.02	1.50 \pm 0.04
	9	1.02 \pm 0.06	0.789 \pm 0.035	0.984 \pm 0.060	1.09 \pm 0.13
	10	0.899 \pm 0.046	0.660 \pm 0.016	0.814 \pm 0.028	1.01 \pm 0.01
X_{max} (mg DW L^{-1})	Air	472 \pm 26	483 \pm 41	456 \pm 40	383 \pm 94
	3	884 \pm 25	788 \pm 64	996 \pm 21	837 \pm 27
	5	819 \pm 10	647 \pm 154	920 \pm 38	885 \pm 74
	7	(1.11 \pm 0.07) $\times 10^3$	660 \pm 37	(1.15 \pm 0.04) $\times 10^3$	(1.01 \pm 0.04) $\times 10^3$
	9	(1.03 \pm 0.06) $\times 10^3$	903 \pm 64	973 \pm 86	839 \pm 180
	10	(1.01 \pm 0.06) $\times 10^3$	532 \pm 3	750 \pm 38	812 \pm 11
P ($\text{mg DW L}^{-1} \text{d}^{-1}$)	Air	66.0 \pm 4.0	72.2 \pm 6.5	68.0 \pm 6.6	57.5 \pm 14.1
	3	131 \pm 4	118 \pm 9	149 \pm 3	127 \pm 4
	5	122 \pm 2	98.3 \pm 22.6	139 \pm 5	136 \pm 11
	7	164 \pm 10	100 \pm 5	173 \pm 9	154 \pm 6
	9	150 \pm 8	129 \pm 9	136 \pm 16	118 \pm 28
	10	148 \pm 9	78.3 \pm 0.2	108 \pm 5	119 \pm 2

Values are presented as the mean \pm standard deviation of two independent experiments.

(Tang et al., 2011). The behaviour of this parameter has shown to be similar to the one determined for specific growth rates. For all studied microorganisms, the lowest biomass concentrations were achieved in cultures fed with CO₂ at atmospheric concentrations. For higher CO₂ levels, an increase in maximum biomass concentrations achieved was observed, followed by a decrease determined for cultures grown with 10% (v/v) CO₂ in the air stream. Average biomass productivities determined in these conditions have shown a similar behaviour, with the minimum value (57.5 ± 14.1 mg DW L⁻¹ d⁻¹) determined for *M. aeruginosa* grown with non-enriched air and the maximum one (173 ± 9 mg DW L⁻¹ d⁻¹) determined for *S. salina* grown with 7% (v/v) CO₂. Higher specific growth rates, maximum biomass concentrations and average biomass productivities determined in cultures fed with CO₂-enriched aeration have already been reported and may be related to an increased availability of CO₂ to microalgal cells, one of the main precursors of photosynthetic reactions (Picardo et al., 2013).

4.3.2. CO₂ uptake rates

Elemental analysis of microalgal biomass has shown that different CO₂ concentrations have not strongly influenced the carbon content of the studied microorganisms (Table 4.12), being average carbon contents $42.82 \pm 2.22\%$ (w/w). These results are in accordance with the results obtained by Tang et al. (2011). Assuming that all the CO₂ assimilated by microalgae was incorporated in the biomass, carbon contents, as well as average biomass productivities, were used to determine CO₂ uptake rates. These values (Table 4.12) have shown a minimum of 90.1 ± 22.0 mg CO₂ L⁻¹ d⁻¹ (determined for *M. aeruginosa* grown with non-enriched air) and a maximum of 264 ± 14 mg CO₂ L⁻¹ d⁻¹ (determined for *S. salina* grown with 7% v/v CO₂). Except from a few exceptions, these values were in the same order of magnitude as those reported by Tang et al. (2011) for the microalgae *S. obliquus* and *C. pyrenoidosa*, which ranged between 105 ± 6 and 288 ± 4 mg CO₂ L⁻¹ d⁻¹. The influence of increasing CO₂ concentrations on CO₂ uptake rate was similar to the one observed for the kinetic growth parameters: for all studied microorganisms, the lowest CO₂ uptake rates were achieved in cultures fed with CO₂ at atmospheric concentrations. For higher CO₂ levels, an increase in CO₂ uptake rates was observed, followed by a decrease determined for cultures grown with 10% (v/v) CO₂ in the air stream. These results have shown that the selected microorganisms can be effective in CO₂ capture, especially when cultured with enriched air streams. Taking into account the time-course evolution of pH in the studied cultures (Figure IV.2, Annex

Table 4.12. Carbon contents (α_c , in % w/w) and CO₂ uptake rates (R_c , in mg CO₂ L⁻¹ d⁻¹) determined for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* grown with different CO₂ concentrations in the air stream

% CO ₂ (v/v)	<i>C. vulgaris</i>			<i>P. subcapitata</i>			<i>S. salina</i>			<i>M. aeruginosa</i>		
	α_c (% w/w)	R_c (mg CO ₂ L ⁻¹ d ⁻¹)	α_c (% w/w)	α_c (% w/w)	R_c (mg CO ₂ L ⁻¹ d ⁻¹)	α_c (% w/w)	α_c (% w/w)	R_c (mg CO ₂ L ⁻¹ d ⁻¹)	α_c (% w/w)	R_c (mg CO ₂ L ⁻¹ d ⁻¹)	α_c (% w/w)	R_c (mg CO ₂ L ⁻¹ d ⁻¹)
Air	43.36	105±6	46.22	43.04	122±11	43.04	42.75	107±10	42.75	90.1±22.0	42.75	90.1±22.0
3	41.78	200±6	45.91	42.32	199±16	42.32	42.20	232±5	42.20	197±6	42.20	197±6
5	41.64	187±3	45.67	42.21	164±38	42.21	42.68	216±8	42.68	212±17	42.68	212±17
7	42.44	255±16	42.84	41.56	157±9	41.56	42.29	264±14	42.29	239±9	42.29	239±9
9	42.83	235±13	41.94	43.00	199±13	43.00	40.50	214±25	40.50	175±41	40.50	175±41
10	49.09	237±16	37.19	42.19	107±1	42.19	42.05	168±8	42.05	183±2	42.05	183±2

Values are presented as the mean ± standard deviation of two independent experiments.

IV), it is possible to infer on the mechanisms involved in CO₂ uptake in the studied conditions. According to Picardo et al. (2013), when the pH is in the range of 6.0 to 9.0, bicarbonate (HCO₃⁻) is the most common form of inorganic carbon present in solution, which enables the external carbonic anhydrase and promotes active transport of this carbon source into microalgal cells. On the other hand, for lower pH values (ranging between 5.0 and 7.0), CO₂ uptake occurs through diffusion, being the active transport suppressed. Therefore, in cultures aerated with only atmospheric air, the mechanism involved in CO₂ uptake may be active transport, due to the high pH values observed in these cultures (between 6.96±0.01 and 10.8±0.1). In cultures aerated with CO₂-enriched air, the presence of CO₂ resulted in a decrease in pH values, meaning that CO₂ uptake in these conditions may be performed through diffusion.

4.3.3. Nutrients removal

To evaluate the potential of the studied cultures in nitrogen and phosphorus uptake, concentrations of these nutrients within the cultivation time were determined and nutrients uptake kinetics were obtained. To infer about the suitability of these cultures for application in the tertiary treatment step of wastewater treatment, nutrients removal efficiencies and nutrients concentrations achieved in the last day of culturing were compared with target values defined by EU legislation. Figure 4.5 shows the time-course evolution of nitrogen and phosphorus concentrations for the studied cultures and Table 4.13 summarizes nutrients uptake kinetics obtained for the selected microorganisms grown with different CO₂ concentrations in the air stream.

Analysis of Figure 4.5 shows that the selected microorganisms have effectively removed nitrogen from the culture medium for all studied conditions. At the end of the experiments, nitrogen concentrations ranged between 0 and 8.58±1.28 mg N L⁻¹, values below the limits defined by EU legislation. Additionally, as it is possible to see from Table 4.13, nitrogen removal efficiencies ranged between 73.9±3.7 and 100%, indicating that the minimum percentage of reduction was also achieved. Analysing the influence of CO₂ concentrations on nitrogen removal, Figure 4.5 shows that in cultures fed with only atmospheric air, nitrogen concentrations achieved at the end of cultivation times were higher than those achieved in cultures fed with enriched air streams. In fact, Table 4.13 shows that, in general, nitrogen removal efficiencies obtained for cultures fed with non-enriched air streams were

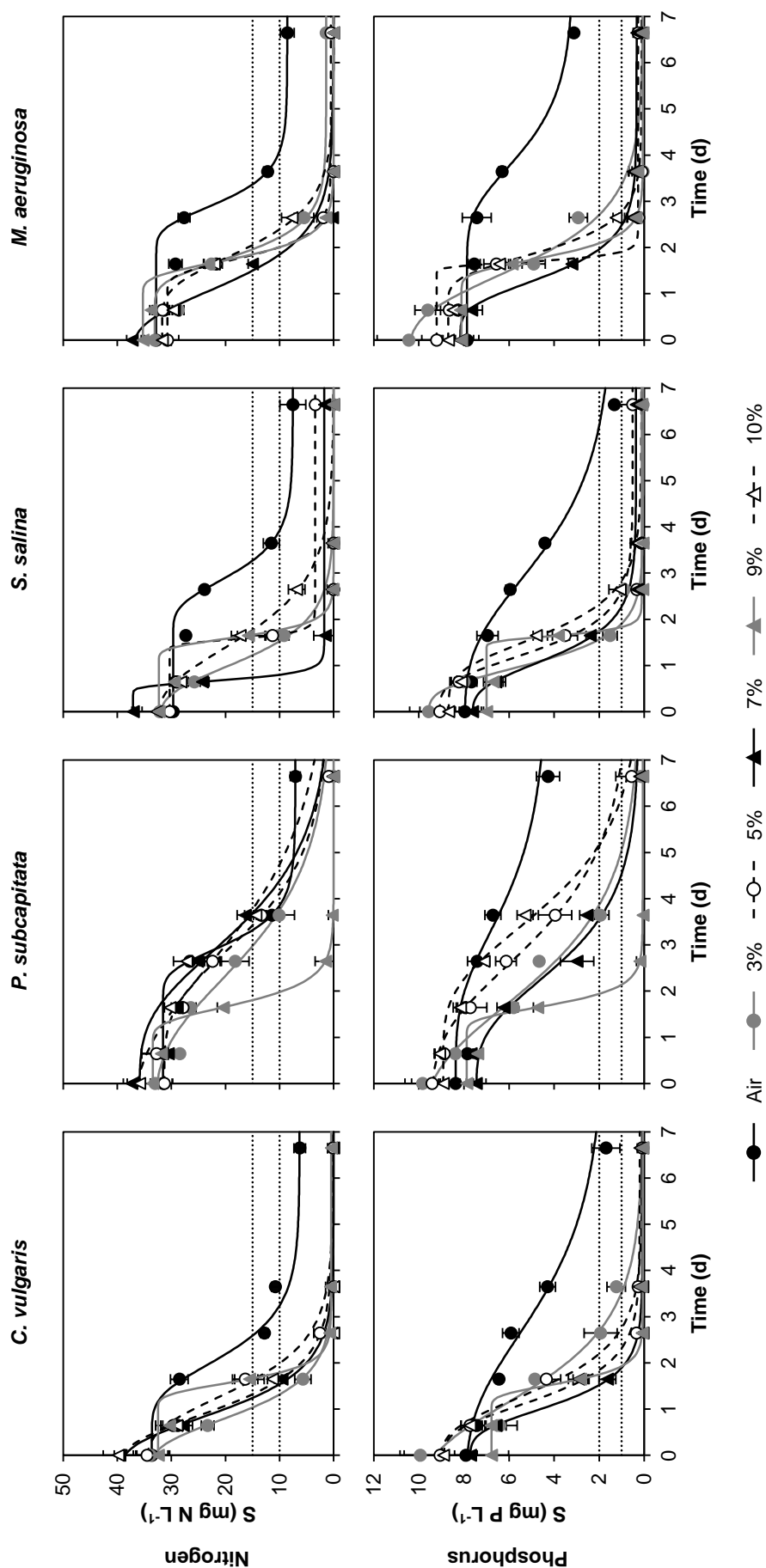


Figure 4.5. Time-course evolution of nitrogen and phosphorus concentration in the culture medium obtained for cultures of *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* grown with different CO₂ concentrations in the air stream. Error bars correspond to the standard deviation of the mean determined for two independent experiments. The lines represent the model fit of the modified Gompertz model to the experimental data. The horizontal dashed lines correspond to the limits for nutrients concentration in discharged effluents imposed by EU legislation.

lower than those of cultures grown with CO₂-enriched air. Lizzul et al. (2014) have demonstrated that cultivation of *C. sorokiniana* in Bold's Basal medium with atmospheric air and with an air stream containing 12% (v/v) CO₂ has resulted in TN removal efficiencies of 23 and 70%, respectively. Regarding phosphorus removal, lower removal efficiencies were also obtained in cultures aerated with non-enriched air. In these conditions, phosphorus removal efficiencies ranged between 49.0±4.3 and 83.5±0.3% and only *S. salina* was able to reach the first limit imposed by EU legislation (2 mg P L⁻¹) and the minimum percentage of reduction. For cultures grown with CO₂-enriched air streams, the limits established by EU legislation were achieved and phosphorus removal efficiencies have significantly increased, ranging from 93.9±7.6 to 99.5±0.1% (Table 4.13). An almost complete removal of TP was also observed by Ge et al. (2011) when growing the microalga *B. braunii* with CO₂ concentrations of 2, 5, 10 and 20% (v/v). These results are in agreement with the maximum biomass concentrations and average biomass productivities determined, which were lower for cultures performed with the lowest CO₂ concentration.

Looking at the curves defined by the modified Gompertz model (Figure 4.5), which accurately fit experimental data, it is possible to conclude that this mathematical model correctly describes nitrogen and phosphorus removal in the studied conditions. Also, coefficients of determination obtained for the predicted values were very close to one ($R^2 \geq 0.952$) and RMSE values were low (RMSE ≤ 2.6 mg S L⁻¹), confirming the suitability of this model to the experimental data.

Regarding nitrogen uptake kinetics, lag time values obtained for cultures fed with non-enriched air were higher than those of cultures grown with CO₂-enriched air. In the case of nitrogen uptake rates, values determined for *C. vulgaris* and *P. subcapitata* were not strongly dependent on the CO₂ concentration used. For these microorganisms, maximum uptake rates determined were 1.83±0.11 d⁻¹ (for *C. vulgaris* grown with an air stream containing 3% v/v CO₂) and 2.11±0.13 d⁻¹ (for *P. subcapitata* grown with a non-enriched air stream). On the other hand, different CO₂ concentrations in the air stream have strongly influenced nitrogen uptake rates determined for *S. salina* and *M. aeruginosa*. Nitrogen uptake rates determined for *S. salina* ranged between 1.16±0.23 and 10.4±0.9 d⁻¹, whereas the same values determined for *M. aeruginosa* ranged between 1.43±0.05 and 3.26±0.03 d⁻¹. For both microorganisms, maximum uptake rates were determined when CO₂ concentration in the air stream was set at 5% (v/v). These results are in agreement with specific growth rates

Table 4.13. Nitrogen and phosphorus removal efficiencies (R, in %) and kinetic parameters of nitrogen and phosphorus uptake (λ , in d, and k, in d⁻¹) determined for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* grown with different CO₂ concentrations in the air stream

Microorganisms	% CO ₂ (v/v)	Nitrogen				Phosphorus					
		R (%)	λ (d)	k (d ⁻¹)	R ²	RMSE (mg N L ⁻¹)	R (%)	λ (d)	k (d ⁻¹)	R ²	RMSE (mg P L ⁻¹)
<i>C. vulgaris</i>	Air	81.4±3.1	1.27±0.03	1.48±0.25	0.953	2.3	78.3±8.4	0.869±0.017	0.593±0.016	0.977	0.3
	3	99.6±0.7	0.192±0.027	1.83±0.11	0.998	0.6	99.4±0.1	0	0.936±0.007	0.966	0.6
	5	99.6±0.7	0.522±0.032	1.42±0.09	0.992	1.3	99.0±0.9	0.523±0.003	1.45±0.08	0.986	0.4
	7	100±0	0.190±0.005	1.65±0.09	0.997	0.9	99.0±0.3	0.489±0.009	2.17±0.02	0.999	0.1
	9	98.7±2.1	0.690±0.011	1.53±0.05	0.987	1.6	99.1±0.2	0.991±0.011	2.47±0.04	0.999	0.1
	10	100±0	0.220±0.002	1.61±0.02	0.993	1.3	97.8±1.3	0.513±0.014	1.85±0.01	0.995	0.3
<i>P. subcapitata</i>	Air	77.6±2.3	2.40±0.19	2.11±0.13	0.983	1.3	49.0±4.3	1.77±0.05	0.677±0.006	0.952	0.3
	3	99.7±0.7	0.725±0.012	0.669±0.043	0.985	1.4	98.3±1.3	0.212±0.003	0.698±0.009	0.984	0.4
	5	97.1±4.4	1.60±0.01	0.809±0.008	0.992	1.0	93.9±7.6	1.01±0.01	0.664±0.003	0.991	0.3
	7	99.6±0.6	0.556±0.008	0.521±0.034	0.953	2.6	98.3±1.3	1.07±0.02	0.909±0.018	0.976	0.4
	9	100±0	1.05±0.04	1.79±0.11	0.989	1.5	99.1±0.2	0.926±0.009	1.56±0.02	0.976	0.5
	10	99.7±0.3	1.43±0.05	0.720±0.025	0.963	2.4	99.2±0.3	2.02±0.08	0.763±0.009	0.984	0.4
<i>S. salina</i>	Air	74.6±8.1	2.28±0.10	1.91±0.06	0.987	1.0	83.5±0.3	1.32±0.09	0.665±0.012	0.984	0.3
	3	100±0	0.364±0.012	1.71±0.06	0.996	0.8	99.4±0.1	0.462±0.003	2.25±0.11	0.995	0.3
	5	88.7±1.5	1.45±0.09	10.4±0.9	0.974	2.1	94.2±9.6	0.624±0.001	1.92±0.08	0.991	0.4
	7	95.3±4.5	0.540±0.020	8.96±0.06	0.997	0.8	95.1±1.2	0.490±0.015	0.868±0.032	0.997	0.2
	9	100±0	1.37±0.07	5.02±0.04	0.993	1.2	98.5±1.6	1.52±0.11	1.64±0.01	0.981	0.4
	10	100±0	0.474±0.046	1.16±0.23	0.994	1.0	98.6±1.1	0.858±0.072	1.63±0.08	0.997	0.2
<i>M. aeruginosa</i>	Air	73.9±3.7	2.40±0.10	2.26±0.11	0.973	1.6	59.1±0.1	2.73±0.09	1.04±0.06	0.980	0.2
	3	95.8±1.4	1.24±0.07	2.20±0.08	0.979	1.9	99.5±0.1	0.500±0.002	1.17±0.03	0.987	0.5
	5	98.2±2.2	1.40±0.05	3.26±0.03	0.999	0.4	97.0±1.9	1.46±0.03	4.26±0.15	0.996	0.3
	7	100±0	0.336±0.021	1.43±0.05	0.986	1.8	95.8±1.9	0.694±0.006	1.92±0.04	0.998	0.1
	9	100±0	1.36±0.11	3.24±0.01	0.998	0.6	99.3±0.1	1.24±0.04	1.86±0.06	0.983	0.5
	10	100±0	1.12±0.06	1.55±0.03	0.994	1.0	98.7±1.4	1.33±0.09	2.23±0.09	0.999	0.1

Values are presented as the mean ± standard deviation of two independent experiments. R² - coefficient of determination; RMSE - root mean squared error (in mg S L⁻¹).

determined, which reached their maximum for CO₂ concentrations of 5% (v/v). The relationship between nitrogen uptake rates and specific growth rates is not surprising, as nitrogen is one of the most important macronutrients for microalgal growth. Since NO₃-N was the only nitrogen source supplied to the studied cultures, nitrogen removal observed is a result of nitrate assimilation and reduction into NH₄-N.

As for nitrogen removal, in phosphorus uptake higher lag times were determined for cultures grown with only atmospheric air. Additionally, it was observed that phosphorus uptake rates determined for *C. vulgaris*, *P. subcapitata* and *S. salina* were not strongly influenced by CO₂ concentrations. On the other hand, phosphorus uptake rates determined for *M. aeruginosa* have followed a similar behaviour to the one observed for specific growth rates. For CO₂ concentrations up to 5% (v/v), phosphorus uptake rates have increased, followed by a decrease for CO₂ concentrations of 7 and 10% (v/v). These results are not surprising because phosphorus is also one of the most important macronutrients for microalgae, which means that higher phosphorus uptake rates are expected for cultures presenting higher specific growth rates. As it was reported for carbon uptake (Section 4.3.2), phosphorus removal mechanisms also depend on the pH of the culture medium. In this study, pH values of cultures aerated with only atmospheric air (Figure IV.2, Annex IV) have exceeded 8.0, indicating that both phosphate assimilation and precipitation have occurred in these conditions (see Section 2.4.2). On the other hand, in cultures supplied with higher CO₂ concentrations, pH values ranged between 5.40±0.01 and 7.67±0.12, indicating that phosphorus removal in these conditions occurred through bioassimilation.

4.3.4. Cell composition

Table 4.14 presents nitrogen and phosphorus mass fractions determined in microalgal biomass and Figure 4.6 shows the variation of C:N, C:P and N:P molar ratios with the CO₂ concentrations used in the air streams. Since these values were calculated assuming that all nitrogen and phosphorus removed from the culture medium were incorporated into the biomass and once phosphorus removal in cultures performed with non-enriched air streams was due to precipitation, C:P and N:P values in these conditions were not determined.

Average C:N, C:P and N:P molar ratios determined in this study were 12±3, 115±30 and 9±2, being these values in agreement with the typical molecular formula described for microalgae (C:N, C:P and N:P molar ratios reported by this formula are 9, 100 and 11,

respectively) (Chisti, 2007). Analysis of Figure 4.6 shows that the influence of CO₂ concentrations on biomass composition is different for each of the studied species, meaning that this behaviour may be species-specific. In fact, several authors have already reported that the influence of different CO₂ concentrations on cell composition of microalgal biomass depends on the species used (Burkhardt et al., 1999; Giordano and Ratti, 2013). However, for all studied microorganisms, similar trends can be observed between N:P and C:P ratios with increasing CO₂ concentrations. A similar behaviour was described by Burkhardt et al. (1999) when culturing the diatoms *Phaeodactylum tricornutum*, *Skeletonema costatum*, *Asterionella glacialis*, *Thalassiosira weissflogii*, *Thalassiosira punctigera* and *Coscinodiscus wailesii* and the dinoflagellate *Scrippsiella trochoidea* under different CO₂ concentrations. Comparing the behaviour of C:N and C:P ratios with increasing CO₂ concentrations, Figure 4.6-A shows that, in general, an increase in CO₂ concentration results in increased C:N and C:P molar ratios in the microalga *C. vulgaris*. This behaviour was close to the one demonstrated for *A. glacialis* in the study performed by Burkhardt et al. (1999). Taking into account that the carbon content in *C. vulgaris* was not strongly influenced by the CO₂ concentration used (Table 4.12), the increase observed in C:N ratios can be associated to nitrogen limitation. In *P. subcapitata* (Figure 4.6-B), however, it is possible to see that increased CO₂ concentrations have not strongly influenced C:N and C:P molar ratios, being these values approximately constant. These results may be related to the lower

Table 4.14. Mass fractions of nitrogen (α_N , in % w/w) and phosphorus (α_P , in % w/w) incorporated in the biomass of *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* obtained through the mass balance performed for each nutrient

Mass fractions	%CO ₂ (v/v)	<i>C. vulgaris</i>	<i>P. subcapitata</i>	<i>S. salina</i>	<i>M. aeruginosa</i>
α_N (% w/w)	Air	6.23±0.16	5.10±0.09	4.90±0.06	6.34±0.04
	3	3.89±0.35	4.19±0.06	3.24±0.01	3.75±0.04
	5	4.22±0.01	4.66±0.06	2.90±0.02	3.35±0.10
	7	3.62±0.05	5.59±0.06	3.07±0.05	3.64±0.05
	9	3.22±0.14	3.89±0.06	3.58±0.08	4.49±0.05
	10	4.01±0.01	6.88±0.01	4.55±0.07	4.02±0.01
α_P (% w/w)	Air	n.a.	n.a.	n.a.	n.a.
	3	1.14±0.02	1.23±0.02	0.958±0.041	1.23±0.02
	5	1.10±0.01	1.36±0.04	0.925±0.017	0.992±0.006
	7	0.700±0.057	1.09±0.06	0.630±0.049	0.763±0.026
	9	0.675±0.018	0.908±0.065	0.764±0.010	1.02±0.03
	10	0.885±0.011	1.70±0.07	1.19±0.01	1.09±0.01

Values are presented as the mean ± standard deviation of two independent experiments. n.a. - not applicable.

dependence on CO₂ concentrations demonstrated for *P. subcapitata* specific growth rates. In the study performed by Burkhardt et al. (1999), specific growth rates determined for *P. tricornutum* were not affected by changes in CO₂, which has resulted in a similar behaviour between C:N and C:P molar ratios. Elemental molar ratios determined for *S. salina* and *M. aeruginosa* (Figure 4.6-C and 4.6-D, respectively) have shown similar trends: (i) C:N and C:P have increased for CO₂ concentrations up to 5% (v/v); and (ii) for higher CO₂ concentrations C:N and C:P started decreasing. The same response to increasing CO₂ concentrations was reported for elemental ratios of *T. punctigera* (Burkhardt et al., 1999). In these conditions, the lowest C:N and C:P molar ratios were determined for the lowest CO₂ concentration. The increase followed by a decrease observed in C:N molar ratios can be associated to nitrogen limitation in cultures where higher C:N molar ratios were determined and is related to the kinetic growth parameters determined in this study. Looking at Figure 4.6-C and 4.6-D, higher C:N ratios and higher nitrogen limitations have occurred in cultures grown with 5% (v/v) CO₂ (those providing the highest specific growth rates).

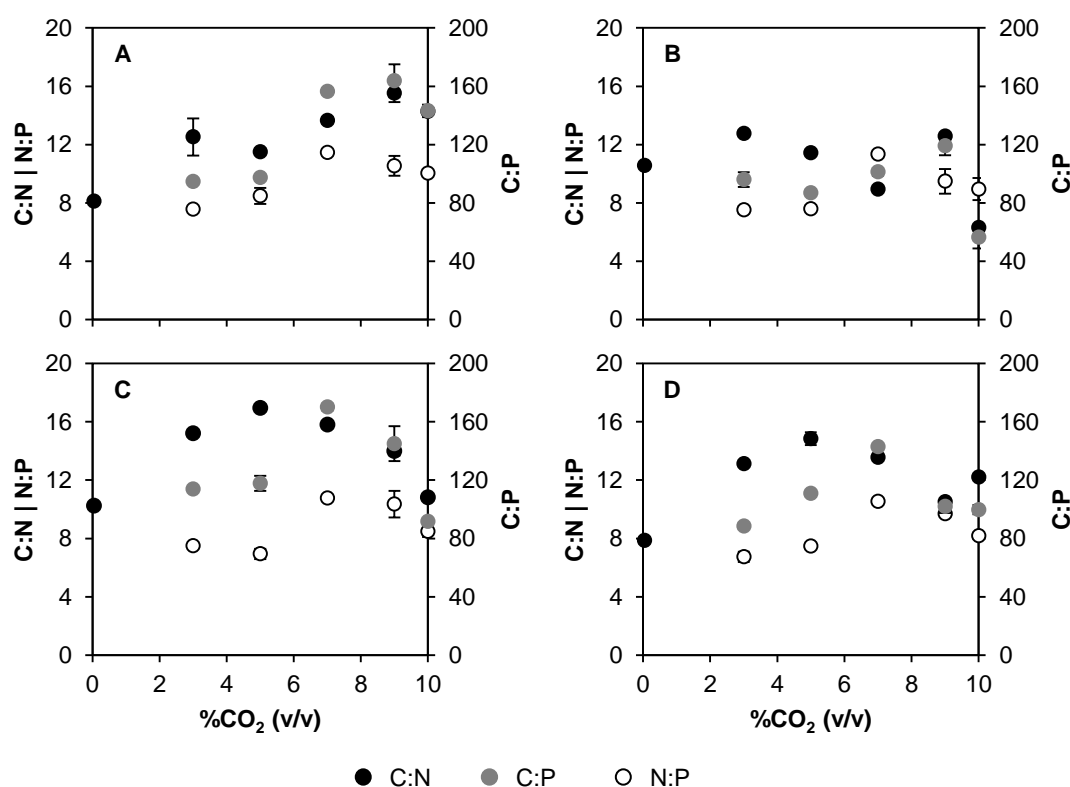


Figure 4.6. C:N, C:P and N:P molar ratios determined after the cultivation time for *C. vulgaris* (A), *P. subcapitata* (B), *S. salina* (C) and *M. aeruginosa* (D) grown with different CO₂ concentrations in the air stream. Error bars correspond to the standard deviation of the mean determined for two independent experiments. C:P and N:P ratios were not determined for cultures performed with non-enriched air streams since in these cultures phosphorus removal was due to precipitation.

Lower C:N molar ratios determined for cultures grown with non-enriched air streams (in the case of *C. vulgaris*, *S. salina* and *M. aeruginosa*) also suggest that these conditions might be limiting to microalgal growth, due to carbon limitation.

4.3.5. Optimal CO₂ concentration determined through mathematical modelling

Specific growth rates determined for each microorganism under different CO₂ concentrations were used to establish mathematical models (Figure 4.7) able to describe the influence of different CO₂ concentrations on this kinetic growth parameter. The closeness of the model fits represented in Figure 4.7, as well as the R² and RMSE values determined for each model fit (Table 4.15), indicate that this mathematical approach correctly describes the behaviour of specific growth rates of the selected microorganisms grown with CO₂ concentrations ranging from approximately 0.04 to 10% (v/v). From the developed models, it was also possible to determine optimal CO₂ concentrations and maximum specific growth rates that can be achieved by the selected microorganisms (Table 4.15). Regarding maximum specific growth rates, these values ranged between 0.892 and 1.50 d⁻¹. The lowest maximum specific growth rate was determined for *P. subcapitata*. These results are in agreement with those reported in Section 4.3.1, where lower specific growth rates were achieved by *P. subcapitata*. Optimal CO₂ concentrations determined through mathematical modelling were 5.35, 4.87, 5.55 and 5.62% (v/v) for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa*, respectively. Optimal CO₂ concentrations of approximately 5% (v/v) have already been reported for several microalgae. When growing *C. vulgaris* with CO₂ concentrations of 0.04, 5 and 15% (v/v), Yun et al. (1997) have observed maximum growth for cultures fed with 5% (v/v) CO₂. Similarly, Ono and Cuello (2003) have reported that although the microalga *Euglena gracilis* could grow in CO₂ concentrations ranging between 5 and 45% (v/v), best growth was achieved at 5% (v/v). In the study performed by de Moraes and Costa (2007a) with CO₂ concentrations of 0, 6 and 12% (v/v), both *Spirulina* sp. and *S. obliquus* have shown best growth at 6% (v/v).

These results suggest that the selected microorganisms can grow well under CO₂ concentrations ranging from those commonly present in the atmosphere up to 10% (v/v). However, the best results were achieved for CO₂ concentrations of 5% (v/v). Accordingly, these findings can be useful for the selection of microalgal species able to grow in this range of CO₂ concentrations.

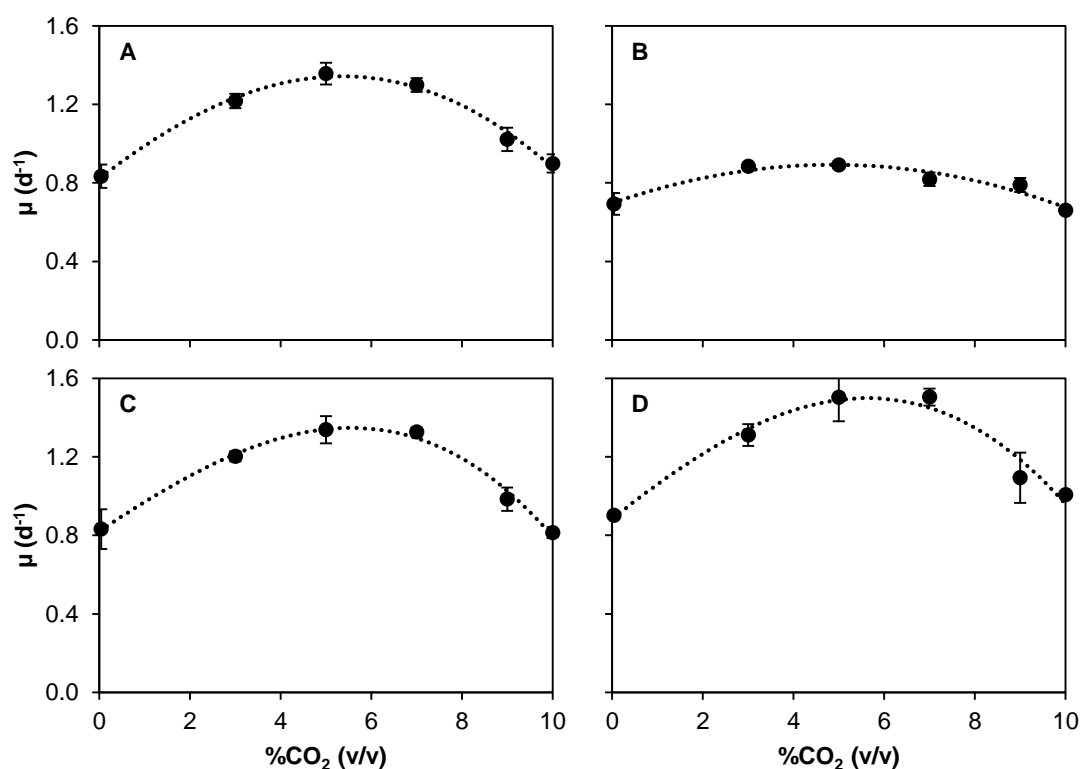


Figure 4.7. Influence of different CO₂ concentrations on specific growth rates (μ , in d⁻¹) of *C. vulgaris* (A), *P. subcapitata* (B), *S. salina* (C) and *M. aeruginosa* (D). The dots correspond to the experimental data and error bars correspond to the standard deviation of the mean determined for two independent experiments. The dashed lines were obtained through mathematical modelling.

Table 4.15. Optimal CO₂ concentrations in the air stream determined for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* through mathematical modelling

Parameters	<i>C. vulgaris</i>	<i>P. subcapitata</i>	<i>S. salina</i>	<i>M. aeruginosa</i>
μ_{\max} (d ⁻¹)	1.34	0.892	1.35	1.50
C_{opt} (% v/v)	5.35	4.87	5.55	5.62
a	-5.32	-5.53	-7.25	-6.36
b	13.2	15.3	12.2	12.7
R ²	0.989	0.926	0.988	0.955
RMSE (d ⁻¹)	0.02	0.024	0.02	0.05
n	6	6	6	6

Values were obtained through mathematical modelling. μ_{\max} - maximum specific growth rate (in d⁻¹); C_{opt} - optimal CO₂ concentration in the air stream for microalgal growth (in % v/v); a and b - model parameters; R² - coefficient of determination; RMSE - root mean squared error (in d⁻¹); n - data size.

4.4. Microalgal consortia

As reported in Section 3.3.2, several consortia (*C. vulgaris* + *P. subcapitata*, *C. vulgaris* + *S. salina*, *C. vulgaris* + *M. aeruginosa*, *P. subcapitata* + *S. salina*, *P. subcapitata* + *M. aeruginosa* and *S. salina* + *M. aeruginosa*) were evaluated in terms of biomass production, CO₂ capture and nutrients removal. These experiments have shown that these processes were favoured in the consortia containing *S. salina* (from now on SC for *S. salina* + *C. vulgaris*, SP for *S. salina* + *P. subcapitata* and SM for *S. salina* + *M. aeruginosa*). The following sections present in detail the results obtained for these specific consortia. The overall results obtained for all studied consortia are presented in Annex V.

4.4.1. Microalgal growth

Co-cultivation of *C. vulgaris*, *P. subcapitata* and *M. aeruginosa* with *S. salina* has shown significant influence on microalgal growth. Figure 4.8 presents the growth curves obtained for single cultures of the studied microorganisms (Figure 4.8-A) and for dual-species cultures containing *S. salina* (Figure 4.8-B). In these growth curves, it is possible to distinguish the adaptation phase, which lasted for approximately one day for all studied cultures, and the exponential growth phase. Regarding single cultures, the studied microalgae have shown a similar growth behaviour, reaching similar maximum biomass concentrations (approximately $(21.7 \pm 3.3) \times 10^6$ cells mL⁻¹). However, looking at the growth curves obtained for the three studied consortia, it is possible to see a significant increase ($p < 0.05$) in maximum biomass concentrations achieved in the SC and SM consortia (approximately $(41.3 \pm 3.3) \times 10^6$ cells mL⁻¹). The SP consortium has shown a similar behaviour to the one observed for single cultures. Higher biomass concentrations achieved in the SC and SM consortia suggest a symbiotic relationship between these microorganisms (Reyna-Martínez et al., 2014).

To evaluate the potential of the three studied consortia in biomass production, kinetic growth parameters, such as specific growth rates and average biomass productivities, were determined (Table 4.16). In this study, specific growth rates ranged from 0.643 ± 0.044 to 0.950 ± 0.107 d⁻¹. From Table 4.16 it is possible to observe that similar values ($p > 0.05$) were obtained for the single cultures of *S. salina*, *P. subcapitata* and *M. aeruginosa* and for the consortia containing these microorganisms. On the other hand, statistically higher ($p < 0.05$)

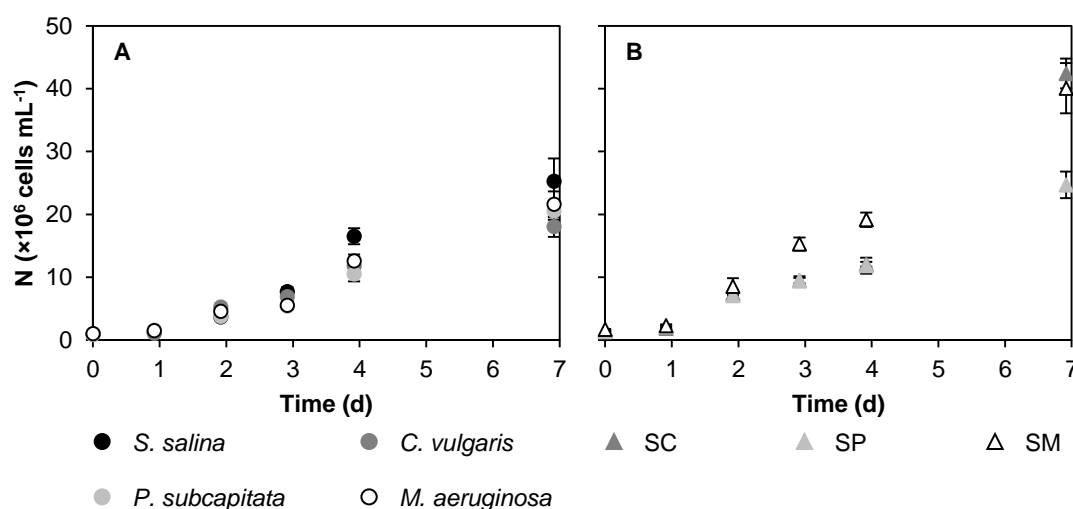


Figure 4.8. Growth curves obtained for the studied microorganisms when grown in single (A) and dual-species (B) cultures containing *S. salina*. Error bars correspond to the standard deviation of the mean determined for four independent experiments.

Table 4.16. Specific growth rates (μ , in d^{-1}), maximum cell concentrations (N_{\max} , in cell mL^{-1}) and average biomass productivities (P , in $\text{mg DW L}^{-1} \text{d}^{-1}$) determined for single and dual-species cultures containing *S. salina*

Microorganisms	μ (d^{-1})	N_{\max} ($\times 10^6$ cells mL^{-1})	P (mg DW $\text{L}^{-1} \text{d}^{-1}$)
<i>S. salina</i>	0.690 ± 0.028	25.3 ± 3.6	60.2 ± 9.4
<i>C. vulgaris</i>	0.950 ± 0.107	18.0 ± 1.6	47.0 ± 3.1
<i>P. subcapitata</i>	0.665 ± 0.060	20.5 ± 1.4	41.2 ± 9.2
<i>M. aeruginosa</i>	0.702 ± 0.047	21.6 ± 2.1	49.9 ± 3.1
<i>S. salina</i> + <i>C. vulgaris</i> (SC)	0.811 ± 0.045	42.4 ± 2.4	96.9 ± 8.2
<i>S. salina</i> + <i>P. subcapitata</i> (SP)	0.643 ± 0.044	24.7 ± 2.1	63.6 ± 4.1
<i>S. salina</i> + <i>M. aeruginosa</i> (SM)	0.696 ± 0.018	40.1 ± 4.0	83.9 ± 10.2

Values are presented as the mean \pm standard deviation of four independent experiments.

specific growth rates were determined for the single and dual-species cultures of *C. vulgaris*. Comparing the values determined for single and dual-species cultures, it was observed that dual-species cultures have not favoured doubling times: specific growth rates were close to those of the microorganisms composing each studied consortia. On the other hand, co-cultivation of *S. salina* with the other studied microorganisms has strongly influenced average biomass productivities and maximum biomass concentrations achieved. Average biomass productivities values achieved by single cultures ranged from 41.2 ± 9.2 to 60.2 ± 9.4 mg DW $\text{L}^{-1} \text{d}^{-1}$, whereas for dual-species cultures, these values ranged from 63.6 ± 4.1 to 96.9 ± 8.2 mg DW $\text{L}^{-1} \text{d}^{-1}$. Values determined for the SC and SM consortia were statistically higher ($p < 0.05$) than those determined for each individual microorganism. The

SP consortium has not resulted in higher average biomass productivities ($63.6 \pm 4.1 \text{ mg DW L}^{-1} \text{ d}^{-1}$) than those achieved in single cultures ($p > 0.05$). These results indicate that the SC and SM consortia enhanced average biomass productivities, which might be related to a symbiotic relationship between these microorganisms. Johnson and Admassu (2013) have reported that the co-culture of less productive strains with others presenting higher average biomass productivities results in denser cultures, reaching average biomass productivities close to those of the most productive strains. Accordingly, *C. vulgaris* and *M. aeruginosa* can be effectively used in co-culture with *S. salina*, improving average biomass productivities.

4.4.2. CO₂ uptake rates

Table 4.17 presents carbon mass fractions incorporated in the biomass resulting from single and dual-species cultures and CO₂ uptake rates determined assuming that all CO₂ assimilated by the studied cultures was incorporated into microalgal biomass. Analysis of Table 4.17 shows that carbon contents present in microalgal biomass resulting from single and dual-species cultures was not statistically different ($p = 0.99$). Average carbon contents determined in single cultures was $44.26 \pm 2.18 \%$ (w/w), whereas in dual-species cultures, this value was $44.23 \pm 2.99 \%$ (w/w). Although single and dual-species cultures have resulted in similar carbon contents, CO₂ uptake rates determined for dual-species cultures were statistically higher ($p = 0.03$) than those of single cultures. These results are not surprising because average biomass productivities determined in dual-species cultures were also statistically higher than those of single cultures (Section 4.4.1). Comparing the three consortia, SC consortium was the most effective in CO₂ uptake, reaching CO₂ uptake rates of

Table 4.17. Carbon contents (α_c , in % w/w) and CO₂ uptake rates (R_c , in mg CO₂ L⁻¹ d⁻¹) determined for single and dual-species cultures containing *S. salina*

Microorganisms	α_c (% w/w)	R_c (mg CO ₂ L ⁻¹ d ⁻¹)
<i>S. salina</i>	42.51	93.8 \pm 14.7
<i>C. vulgaris</i>	44.31	76.3 \pm 5.0
<i>P. subcapitata</i>	47.32	71.4 \pm 16.0
<i>M. aeruginosa</i>	42.90	78.4 \pm 4.8
<i>S. salina</i> + <i>C. vulgaris</i> (SC)	42.38	150 \pm 13
<i>S. salina</i> + <i>P. subcapitata</i> (SP)	47.68	111 \pm 7
<i>S. salina</i> + <i>M. aeruginosa</i> (SM)	42.64	131 \pm 16

Values are presented as the mean \pm standard deviation of four independent experiments.

150±13 mg CO₂ L⁻¹ d⁻¹. Higher CO₂ uptake rates were reported for a mixture of *Chlorella* sp. and *Scenedesmus* sp. in the study performed by Koreivienė et al. (2014). In this study, cultivation of this consortium for three weeks in BG-11 medium and in different concentrations of a primary-treated municipal wastewater has resulted in a CO₂ uptake of up to 1.37 g CO₂ L⁻¹ d⁻¹.

4.4.3. Nutrients removal

Nitrogen and phosphorus concentrations were determined within the cultivation time to evaluate the potential of the studied cultures in nutrients removal. Figure 4.9 shows the time-course evolution of nitrogen (A and B) and phosphorus (C and D) concentrations for single (A and C) and dual-species cultures (B and D).

Nitrogen uptake was higher in dual-species cultures than in single cultures. In single cultures, only *C. vulgaris* and *P. subcapitata* were able to reach the limits established by EU legislation. In these cultures, the upper limit (15 mg N L⁻¹) was reached between the fourth and fifth day of culturing. On the other hand, *S. salina* and *M. aeruginosa* were not able to reach the limits imposed by legislation within the cultivation time: nitrogen concentrations in the last day of culturing were 15.6±0.7 and 18.5±0.2 mg N L⁻¹, respectively. Co-cultivation of *S. salina* with the other studied microorganisms has effectively improved nitrogen uptake, as the three studied consortia have reached the limit imposed by EU legislation. However, these cultures have shown different uptake profiles: the SP and SM consortia have reached this limit between the second and third day of culturing, whereas the SC consortium has only reached the same value after the fourth day of culturing. Although this last consortium seems to have lower uptake rates, the lowest nitrogen concentration (5.27±0.60 mg N L⁻¹) was achieved by this culture. This value was not statistically different from the one obtained for the SP consortium (7.31±1.36 mg N L⁻¹, *p*=0.05), but it was statistically lower than the one obtained for the SM consortium (6.01±0.30 mg N L⁻¹, *p*=0.03). In addition to the limit concentration for discharged effluents, load reduction percentages or removal efficiencies were also determined (Table 4.18). Nitrogen removal efficiencies determined for single cultures ranged from 49.8±3.7 to 70.6±4.1%. In the study performed by Li et al. (2011b) TN removal efficiencies of 89% were reported for *Chlorella* sp., with the majority of the removal occurring in the first four days of culturing. Taking into account the minimum percentage reduction established by EU legislation, only *C. vulgaris*

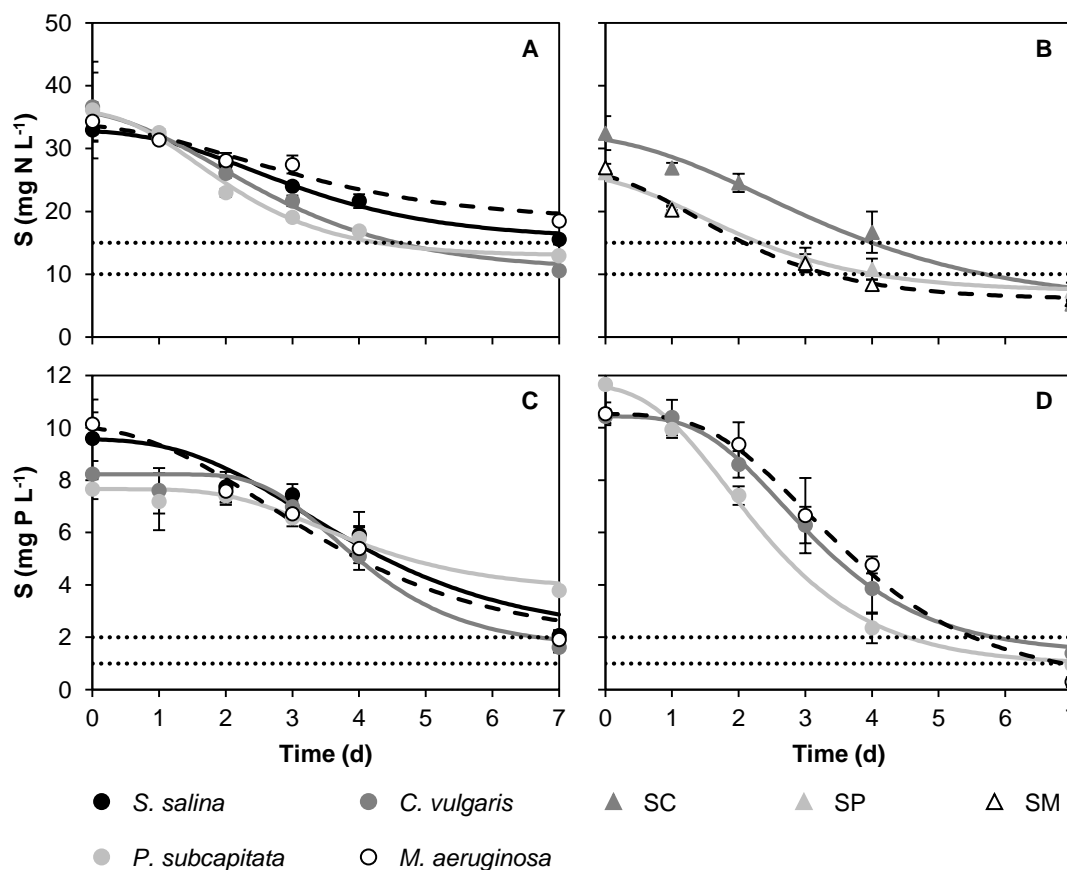


Figure 4.9. Time-course evolution of nitrogen (A and B) and phosphorus (C and D) concentration in the culture medium obtained for single (A and C) and dual-species (B and D) cultures containing *S. salina*. Error bars correspond to the standard deviation of the mean determined for four independent experiments. The lines represent the model fit of the modified Gompertz model to the experimental data. The horizontal dashed lines correspond to the limits for nutrients concentration in discharged effluents imposed by EU legislation.

achieved the minimum value of 70%. However, this value was not statistically different ($p=0.11$) from the one obtained for *P. subcapitata* ($62.9\pm9.3\%$). Removal efficiencies determined for both cyanobacteria were, in turn, statistically lower ($p<0.05$). Regarding the three studied consortia, they achieved reduction percentages that exceeded the 70% defined by legislation. The consortium presenting the highest removal efficiency was the SC ($84.5\pm2.3\%$), followed by the SM ($77.7\pm0.9\%$) and SP ($72.0\pm5.3\%$) consortia. In general, nitrogen removal efficiencies determined for the co-cultures were statistically higher ($p<0.05$) than those determined for each individual microorganism, except in the SP consortium, where nitrogen removal efficiency was statistically higher than the one determined for the single *S. salina* culture ($p<0.01$), but it was not statistically different from the value determined for *P. subcapitata* ($p=0.09$). In the study performed by Chinnasamy et al. (2010), a microalgal consortium comprising both native unicellular and filamentous

strains was able to remove 99.8% of $\text{NO}_3\text{-N}$ after 72 h of culturing in sterilized carpet industry effluent. The co-culture of *C. vulgaris* with the cyanobacterium *P. isothrix* under shaken conditions and with a light irradiance of $60 \mu\text{mol m}^{-2} \text{s}^{-1}$ has shown that the highest removal of $\text{NH}_4\text{-N}$ was achieved by the microalga ($56.03 \pm 0.81 \text{ mg N L}^{-1}$), followed by the co-culture ($52.44 \pm 0.78 \text{ mg N L}^{-1}$) and the cyanobacterium ($48.17 \pm 0.15 \text{ mg N L}^{-1}$) (Silva-Benavides and Torzillo, 2012). Additionally, in a study performed by Renuka et al. (2013), three different microalgal consortia (MC1 – *Calothrix* sp., *Lyngbya* sp., *Ulothrix* sp. and *Chlorella* sp.; MC2 – *Phormidium* sp., *Limnothrix* sp., *Anabaena* sp., *Westiellopsis* sp., *Fischerella* sp. and *Spirogyra* sp.; and MC3 – *Chlorella* sp., *Scenedesmus* sp., *Chlorococcum* sp. and *Chroococcus* sp.) were able to remove about 90% of $\text{NO}_3\text{-N}$ from sewage wastewater on the tenth day. Nitrogen uptake rates were also evaluated in this study. Looking at the profiles of nutrients removal, it is clear that nutrients uptake by the studied microorganisms goes through an adaptation phase, also known as lag phase, followed by an exponential decrease and a stationary phase. Therefore, the modified Gompertz model was fitted to the experimental data to determine nutrients removal kinetic parameters. The curved lines present in Figure 4.9 represent the model fit of the modified Gompertz model to the experimental data. With this model fit, lag times and nutrients uptake rates were determined (Table 4.18). Additionally, the quality of the model fit was evaluated through analysis of the parameters R^2 and RMSE. Analysis of these performance parameters ($R^2 \geq 0.965$ and $\text{RMSE} \leq 1.5 \text{ mg N L}^{-1}$) indicates that the Gompertz model correctly describes nitrogen uptake by the studied cultures. Looking at lag time values, it is possible to conclude that the studied cultures presented a short lag time, starting nitrogen uptake before completing the first day of culturing. The highest lag times were observed for single cultures, ranging from 0.285 ± 0.004 to 0.800 ± 0.034 d. On the other hand, lag times estimated for the three studied consortia ranged from 0.0348 ± 0.0008 to 0.349 ± 0.021 d. An immediate assimilation of $\text{NO}_3\text{-N}$ was also observed by the three consortia developed by Renuka et al. (2013). Regarding nitrogen uptake rates, the values estimated through the modified Gompertz model were similar for both single and dual-species cultures. Values determined for each individual microorganism ranged from 0.527 ± 0.064 to $0.874 \pm 0.011 \text{ d}^{-1}$, whereas the same values determined for the three studied consortia ranged from 0.497 ± 0.053 to $0.772 \pm 0.017 \text{ d}^{-1}$. Ruiz et al. (2013) reported $\text{NO}_3\text{-N}$ removal rates of $1.5 \pm 0.3 \text{ d}^{-1}$ for *C. vulgaris* when cultured in commercial synthetic Combo medium. However, lower removal rates were obtained in the study performed by Wang et al. (2014). TN removal rates of 0.16 d^{-1} were determined for

Table 4.18. Nitrogen and phosphorus removal efficiencies (R, in %) and kinetic parameters of nitrogen and phosphorus uptake (λ , in d, and k, in d⁻¹) determined for single and dual-species cultures containing *S. salina*

Nutrients	Microorganisms	R (%)	λ (d)	k (d ⁻¹)	R ²	RMSE (mg S L ⁻¹)
Nitrogen	<i>S. salina</i>	52.3±2.2	0.800±0.034	0.628±0.010	0.991	0.6
	<i>C. vulgaris</i>	70.6±4.1	0.313±0.012	0.629±0.043	0.992	0.7
	<i>P. subcapitata</i>	62.9±9.3	0.427±0.006	0.874±0.011	0.988	0.9
	<i>M. aeruginosa</i>	49.8±3.7	0.285±0.004	0.527±0.064	0.965	0.9
	<i>S. salina</i> + <i>C. vulgaris</i> (SC)	84.5±2.3	0.349±0.021	0.497±0.053	0.969	1.5
	<i>S. salina</i> + <i>P. subcapitata</i> (SP)	72.0±5.3	0.0355±0.0012	0.722±0.027	0.989	0.7
	<i>S. salina</i> + <i>M. aeruginosa</i> (SM)	77.7±0.9	0.0348±0.0008	0.772±0.017	0.990	0.7
Phosphorus	<i>S. salina</i>	77.0±3.6	1.35±0.10	0.561±0.037	0.954	0.5
	<i>C. vulgaris</i>	80.2±2.8	2.53±0.07	0.922±0.030	0.982	0.3
	<i>P. subcapitata</i>	50.4±2.5	2.01±0.09	0.729±0.026	0.970	0.2
	<i>M. aeruginosa</i>	81.3±3.0	0.726±0.008	0.537±0.043	0.972	0.4
	<i>S. salina</i> + <i>C. vulgaris</i> (SC)	85.9±2.7	1.43±0.11	0.827±0.008	0.998	0.1
	<i>S. salina</i> + <i>P. subcapitata</i> (SP)	91.8±1.3	0.632±0.012	0.836±0.009	0.998	0.2
	<i>S. salina</i> + <i>M. aeruginosa</i> (SM)	97.2±1.9	1.57±0.08	0.684±0.013	0.990	0.3

Values are presented as the mean ± standard deviation of four independent experiments. R² - coefficient of determination; RMSE - root mean squared error (in mg S L⁻¹).

the freshwater microalgae *Chlorella* sp. and *Micractinium* sp. grown in primary effluent wastewater. When cultured in high strength wastewater (a mixture of anaerobic digestion centrate and primary effluent), TN removal rates determined for *Chlorella* sp. and *Micractinium* sp. were even lower (0.06 and 0.05 d⁻¹, respectively). These results suggest that nutrients uptake rates are influenced by the composition of the applied culture medium (Wang et al., 2014). In single cultures, *C. vulgaris* and *P. subcapitata* showed, at the same time, the shortest lag times and the highest uptake rates, explaining the lowest nitrogen concentrations achieved by these cultures in the last day of culturing. Likewise, the SP and SM consortia presented the lowest lag time values and the highest uptake rates, explaining the achievement of the concentration value established by EU legislation after the second day of culturing.

Phosphorus uptake by single and dual-species cultures (Figure 4.9-C and 4.9-D, respectively) was similar to nitrogen uptake: phosphorus concentrations achieved by the studied consortia in the last day of culturing were lower than those achieved by single cultures. Among single cultures, only *C. vulgaris* and *M. aeruginosa* were able to achieve the first limit defined by EU legislation (2 mg P L⁻¹). Achievement of these values (1.62±0.21 mg P L⁻¹ for *C. vulgaris* and 1.90±0.33 mg P L⁻¹ for *M. aeruginosa*) was observed only at the end of cultivation time. Single cultures of *S. salina* and *P. subcapitata*, in turn, were not able to reach the limits established by legislation, even at the end of the experiments. However, all studied consortia were able to effectively remove phosphorus from the culture medium, reaching the limits imposed by EU legislation. In these cultures, the SP consortium has reached this value after the fourth day of culturing whereas the SC and SM consortia have reached this limit after the fifth day of culturing. At the end of cultivation time, the lowest phosphorus concentration was achieved by the SM consortium (0.289±0.188 mg P L⁻¹), followed by the SP (0.940±0.098 mg P L⁻¹) and SC (1.38±0.26 mg P L⁻¹) consortia. Values of phosphorus removal efficiencies (Table 4.18) evidence a better performance of the studied consortia over each individual culture. Values determined for single cultures ranged from 50.4±2.5 to 81.3±3.0%, while for dual-species cultures ranged from 85.9±2.7 to 97.2±1.9%. As it is possible to see from Table 4.18, only the single cultures of *C. vulgaris* and *M. aeruginosa* were able to reach the minimum reduction percentage of 80% established by EU legislation. However, these values were not statistically different ($p>0.05$) from phosphorus removal efficiency determined for *S. salina* (77.0±3.6%). PO₄-P removal percentages close to 100% were obtained for the microalgae

Scenedesmus sp. and *C. zoofingensis* in the studies performed by Xin et al. (2010) and Zhu et al. (2013), respectively. In dual-species cultures, all studied combinations resulted in a reduction percentage higher than 80%. The highest removal efficiency was observed for the SM consortium ($97.2 \pm 1.9\%$), followed by the SP ($91.8 \pm 1.3\%$) and SC ($85.9 \pm 2.7\%$) consortia. Additionally, it was observed that phosphorus removal efficiencies determined for these cultures were statistically higher ($p < 0.05$) than those determined for each individual microorganism. Chinnasamy et al. (2010) have reported $\text{PO}_4\text{-P}$ removal efficiencies of 96.6% using a microalgal consortia grown in carpet industry effluent. The co-culture of *C. vulgaris* with *P. isothrix* under shaken conditions and with a light irradiance of $60 \mu\text{mol m}^{-2} \text{s}^{-1}$ has shown that the highest removal of TP was achieved by the co-culture ($7.55 \pm 0.05 \text{ mg P L}^{-1}$), followed by the single cultures of the microalga ($7.52 \pm 0.14 \text{ mg P L}^{-1}$) and the cyanobacterium ($7.50 \pm 0.10 \text{ mg P L}^{-1}$). Renuka et al. (2013) have also successfully applied three microalgal consortia in the treatment of primary-treated sewage effluent, achieving $\text{PO}_4\text{-P}$ removal efficiencies between 87 and 90% after the second day of culturing. As well as for nitrogen, phosphorus uptake rates were determined by fitting the modified Gompertz model to the experimental data. The model fit of the modified Gompertz model to phosphorus concentration is represented by the curved lines present in Figure 4.9-C and 4.9-D. Table 4.18 presents values of lag time and uptake rate given by this model. As it is possible to see from the performance indexes ($R^2 \geq 0.954$ and $\text{RMSE} \leq 0.5 \text{ mg P L}^{-1}$), the modified Gompertz model is also able to describe the behaviour of the studied cultures regarding phosphorus uptake. Looking at lag time values determined, it is possible to conclude that nitrogen assimilation was faster than phosphorus assimilation (higher lag time values were determined for this nutrient). In the study performed by Ruiz et al. (2011), $\text{PO}_4\text{-P}$ uptake was slower than $\text{NO}_3\text{-N}$ (considering removal curves obtained for the experiments working with nitrogen and phosphorus concentrations similar to those used in this study). Comparing single and dual-species cultures, longer lag times were determined for those single: lag time values determined for single cultures ranged from 0.726 ± 0.008 to 2.53 ± 0.07 d, whereas lag times estimated for the three studied consortia ranged from 0.632 ± 0.012 to 1.57 ± 0.08 d. The decrease in lag times when culturing these microorganisms as co-cultures was also observed in the case of nitrogen uptake and may be explained by competition for the same nutrients. In dual-species cultures, where cultured microorganisms were competing for the same nutrients, both microorganisms readily started nutrients uptake. Since these nutrients (nitrogen and phosphorus) are required by microalgae for macromolecular biosynthesis, an increase in nutrients uptake may be related to the increased

biomass productivities reported for the studied consortia. Regarding phosphorus uptake rates, estimated values were higher for the consortia than for each individual microorganism, except in the consortium SC, where uptake rate ($0.827 \pm 0.008 \text{ d}^{-1}$) was higher than the uptake rate determined for the single *S. salina* culture ($0.561 \pm 0.037 \text{ d}^{-1}$), but was lower than that of the single *C. vulgaris* culture ($0.922 \pm 0.030 \text{ d}^{-1}$). Phosphorus removal rates determined for single cultures ranged from 0.537 ± 0.043 to $0.922 \pm 0.030 \text{ d}^{-1}$, whereas removal rates determined for the studied consortia ranged from 0.684 ± 0.013 to $0.836 \pm 0.009 \text{ d}^{-1}$. Higher $\text{PO}_4\text{-P}$ removal rates were determined in the study performed by Ruiz et al. (2013) for *C. vulgaris* using different synthetic media and wastewaters: $\text{PO}_4\text{-P}$ removal rates determined ranged from 2.0 ± 0.2 to $8.7 \pm 1.2 \text{ d}^{-1}$. However, in the study performed by Wang et al. (2014), lower uptake rates were determined: when cultured in primary effluent wastewater, *Chlorella* sp. and *Micractinium* sp. achieved $\text{PO}_4\text{-P}$ uptake rates of 0.17 and 0.19 d^{-1} , respectively, whereas in sludge centrate, $\text{PO}_4\text{-P}$ uptake rates were 0.32 and 0.27 d^{-1} , respectively. As for nitrogen removal, it can be proposed that nutrient uptake rates strongly depend on the composition of the used culture medium. The longer lag times, as well as the lower uptake rates determined for single cultures explain the difficulties of these cultures in achieving the limits established by legislation. On the other hand, lower lag time values and higher phosphorus uptake rates determined for dual-species cultures resulted in higher phosphorus uptake, with the achievement of the limits imposed by legislation, for the consortium SP, after the fourth day of culturing and, for the SC and SM consortia, after the fifth day of culturing.

4.5. Microalgal-bacterial consortia

4.5.1. Microalgal and bacterial growth

Figure 4.10 shows the growth curves obtained for *C. vulgaris* and for the three isolates when grown in single cultures (Figure 4.10-A, 4.10-C and 4.10-E) and in the three studied consortia (Figure 4.10-B, 4.10-D and 4.10-F). Kinetic growth parameters, such as specific growth rates, maximum cell concentrations and average biomass productivities, are shown in Table 4.19. Regarding the bacterial isolates, data from Figure 4.10 evidence the typical growth phases under batch conditions. Cultures of *E. asburiae* (Figure 4.10-A and 4.10-B) have experienced an exponential growth phase of approximately 24 h. At this stage the

bacterial concentration started decreasing until the end of cultivation time. Comparing the behaviour of this isolate in single cultures and in the CE consortium, maximum cell concentration achieved in single cultures ($(11.9 \pm 1.4) \times 10^8$ CFU mL⁻¹) was statistically higher ($p=0.01$) than maximum cell concentration achieved in the consortium ($(8.70 \pm 0.17) \times 10^8$ CFU mL⁻¹). Regarding *Klebsiella* sp. growth curves (Figure 4.10-C and 4.10-D), exponential growth phase also lasted for approximately 24 h in both cultures. After

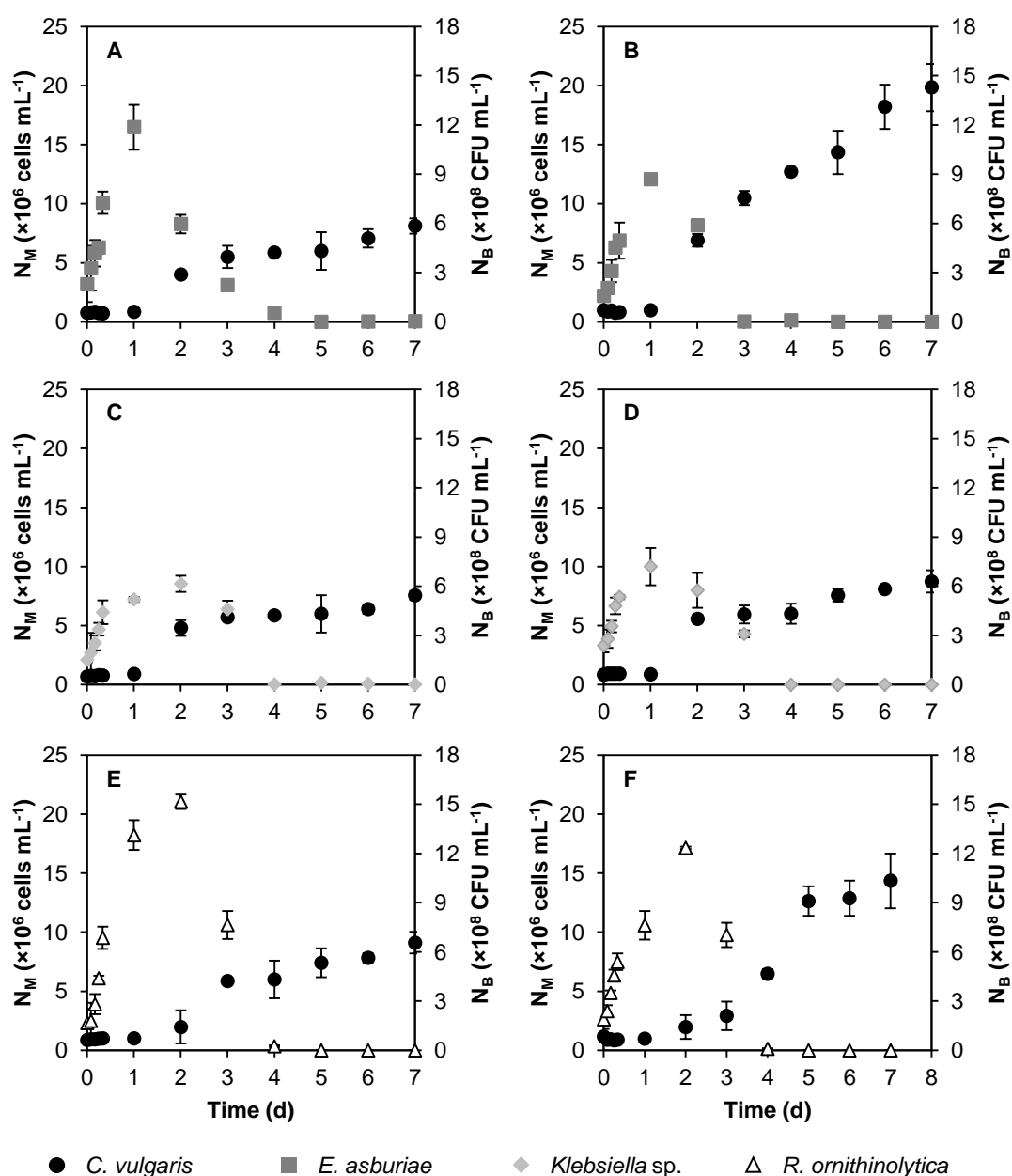


Figure 4.10. Growth curves obtained for *C. vulgaris* and for the bacterial isolates when grown in single (A, C and E) and dual-species (B, D and F) cultures containing *C. vulgaris*. Error bars correspond to the standard deviation of the mean determined for four independent experiments.

this time cell concentration of single cultures was kept approximately constant (stationary growth phase) until the third day of culturing, where cell concentration started decreasing. In the CK consortium, an absence of the stationary growth phase was observed and cell concentration started decreasing after the first day of culturing. *R. ornithinolytica* growth curves (Figure 4.10-E and 4.10-F) have shown an exponential growth phase of approximately one day, followed by a retardation phase until the second day of culturing and then a declining phase until the end of cultivation time. As it was observed for *E. asburiae*, maximum cell concentration achieved by *R. ornithinolytica* in single cultures ($(15.1 \pm 0.4) \times 10^8$ CFU mL⁻¹) was statistically higher ($p=0.04$) than the one achieved in the CR consortium ($(12.4 \pm 0.1) \times 10^8$ CFU mL⁻¹). The short duration of the exponential growth phase observed for the bacteria is related to their higher specific growth rates, when compared to those of microalgae. Additionally, since these studies were performed in batch, the end of this growth phase is associated to the decrease observed in soluble COD (Figure 4.11-E and 4.11-F). Lower cell concentrations achieved in the CE and CR consortia, as well as the lack of a stationary growth phase until the third day of culturing for *Klebsiella* sp. grown in the consortium, may be related to the competition for the organic carbon source with *C. vulgaris*. Although microalgal growth is mainly autotrophic, when both organic and inorganic carbon sources are supplied, microalgae perform both photosynthesis and oxidative assimilation (Cuellar-Bermudez et al., 2015; Ogawa and Aiba, 1981). This assumption can be confirmed through the decrease observed in soluble COD in the single *C. vulgaris* culture (Figure 4.11-E). Regarding microalgal growth, CE and CR consortia have shown a great influence on *C. vulgaris* growth kinetics. Figure 4.10 also shows the growth curves obtained for *C. vulgaris* grown in single cultures and in the consortia. Analysis of these growth curves shows that, in all conditions, *C. vulgaris* experienced an adaptation phase that lasted approximately one day, followed by the exponential growth phase. Duration of this growth phase was strongly influenced by the presence of the co-cultivated microorganisms: (i) in the single *C. vulgaris* culture (Figure 4.10-A, 4.10-C and 4.10-E), this growth phase lasted for two days; (ii) in the CE consortium (Figure 4.10-B) it lasted until the fourth day of culturing, followed by a linear growth until the end of cultivation time; (iii) in the CK consortium (Figure 4.10-D) it lasted for two days; and (iv) in the CR consortium (Figure 4.10-F) it lasted for three days.

Concerning the kinetic growth parameters (Table 4.19), specific growth rates determined for the single *C. vulgaris* culture (0.910 ± 0.026 d⁻¹) were not statistically different ($p>0.05$) from

those determined for this microalga in the CE ($1.19 \pm 0.15 \text{ d}^{-1}$), CK ($0.959 \pm 0.117 \text{ d}^{-1}$) and CR ($0.929 \pm 0.034 \text{ d}^{-1}$) consortia. On the other hand, co-cultivation of *C. vulgaris* with *E. asburiae* and *R. ornithinolytica* has resulted in an increased microalgal cell concentration. Maximum cell concentrations achieved in single cultures ($(8.27 \pm 0.79) \times 10^6 \text{ cells mL}^{-1}$) were statistically lower ($p < 0.05$) than those determined for the CE ($(19.8 \pm 2.0) \times 10^6 \text{ cells mL}^{-1}$) and CR ($(14.4 \pm 2.3) \times 10^6 \text{ cells mL}^{-1}$) consortia. Higher cell concentrations achieved in the CE consortium were not surprising since bacteria from the genus *Enterobacter* are known for their role as plant growth-promoting bacteria (Bashan et al., 1993). Maximum cell concentration achieved in the CK consortium ($(8.74 \pm 0.94) \times 10^6 \text{ cells mL}^{-1}$) was not statistically higher ($p = 0.18$) than the one determined for single *C. vulgaris* cultures. Statistically higher ($p < 0.05$) average biomass productivities were also determined for the CE and CR consortia: 35.9 ± 1.8 and $31.4 \pm 3.0 \text{ mg DW L}^{-1} \text{ d}^{-1}$, respectively. On the other hand, average biomass productivities determined for the single *C. vulgaris* culture and for the CK consortium were not statistically different ($p = 0.57$).

These results suggest that co-cultivation of *C. vulgaris* with *E. asburiae* and *R. ornithinolytica* improved microalgal growth. The improvement in *C. vulgaris* growth is probably related to metabolic cooperation established between the microalga and the co-cultured bacterium. Previous studies have concluded that the growth of *C. vulgaris* can be promoted by the presence of plant growth-promoting bacteria, such as *A. brasilense*,

Table 4.19. Specific growth rates (μ , in d^{-1}), maximum cell concentrations ($N_{M,\max}$, in cell mL^{-1} , or $N_{B,\max}$, in CFU mL^{-1}) and average biomass productivities (P , in $\text{mg DW L}^{-1} \text{ d}^{-1}$) determined for the studied microorganisms when grown in single and dual-species cultures containing *C. vulgaris*

Microorganisms	μ (d^{-1})	$N_{M,\max}$ ($\times 10^6 \text{ cells mL}^{-1}$)	$N_{B,\max}$ ($\times 10^8 \text{ CFU mL}^{-1}$)	P ($\text{mg DW L}^{-1} \text{ d}^{-1}$)
<i>C. vulgaris</i>	0.910 ± 0.026	8.27 ± 0.79	n.a.	21.4 ± 4.0
<i>E. asburiae</i>	n.d.	n.a.	11.9 ± 1.4	n.d.
<i>Klebsiella</i> sp.	n.d.	n.a.	6.15 ± 0.49	n.d.
<i>R. ornithinolytica</i>	n.d.	n.a.	15.1 ± 0.4	n.d.
<i>C. vulgaris</i> + <i>E. asburiae</i> (CE)	1.19 ± 0.15	19.8 ± 2.0	8.70 ± 0.17	35.9 ± 1.8
<i>C. vulgaris</i> + <i>Klebsiella</i> sp. (CK)	0.959 ± 0.117	8.74 ± 0.94	7.20 ± 1.13	25.0 ± 1.2
<i>C. vulgaris</i> + <i>R. ornithinolytica</i> (CR)	0.929 ± 0.034	14.4 ± 2.3	12.4 ± 0.1	31.4 ± 3.0

Values are presented as the mean \pm standard deviation of four independent experiments. n.a. - not applicable; n.d. - not determined.

Rhizobium sp. and *B. licheniformis* (De-Bashan et al., 2004; Kim et al., 2014; Liang et al., 2013). Additionally, in the study performed by Park et al. (2008), co-inoculation of *C. ellipsoidea* with eight bacterial strains isolated from a long-term culture of this microalga resulted in microalgal growth increase of up to three times compared to *C. ellipsoidea* alone.

4.5.2. CO₂ uptake rates

Table 4.20 presents carbon contents determined for the studied microorganisms when grown in single and dual-species cultures containing *C. vulgaris*. In all microalgal cultures (single *C. vulgaris* culture and CE, CK and CR consortia) average carbon content determined was $48.63 \pm 1.91\%$ (w/w). This value is very close to the 50% (w/w) reported in the study performed by Tang et al. (2011) for *C. pyrenoidosa*. On the other hand, average carbon content determined for the bacterial isolates (grown in single cultures) was $45.32 \pm 0.28\%$ (w/w). Similar orders of magnitude were determined for different native aquatic and cultured bacteria in the study performed by Fagerbakke et al. (1996): $60.19 \pm 6.57\%$ (w/w). With carbon contents determined for microalgal cultures and assuming that organic carbon assimilation by *C. vulgaris* (through mixotrophy) was almost negligible, CO₂ uptake rates were determined. CO₂ uptake rate determined for the single *C. vulgaris* culture was 45.6 ± 7.4 mg CO₂ L⁻¹ d⁻¹. Similar values ($p > 0.05$) were determined for the CK (45.6 ± 2.2 mg CO₂ L⁻¹ d⁻¹) and CR (52.9 ± 5.1 mg CO₂ L⁻¹ d⁻¹) consortia. However, CO₂ uptake rate determined for the CE consortium was statistically higher ($p = 0.02$): 63.8 ± 3.2 mg CO₂ L⁻¹ d⁻¹, which might be related to the highest average biomass productivities determined for this consortium.

Table 4.20. Carbon contents (α_c , in % w/w) and CO₂ uptake rates (R_c , in mg CO₂ L⁻¹ d⁻¹) determined for the studied microorganisms when grown in single and dual-species cultures containing *C. vulgaris*

Microorganisms	α_c (% w/w)	R_c (mg CO ₂ L ⁻¹ d ⁻¹)
<i>C. vulgaris</i>	50.28	45.6 ± 7.4
<i>E. asburiae</i>	45.62	n.d.
<i>Klebsiella</i> sp.	45.07	n.d.
<i>R. ornithinolytica</i>	45.27	n.d.
<i>C. vulgaris</i> + <i>E. asburiae</i> (CE)	48.48	63.8 ± 3.2
<i>C. vulgaris</i> + <i>Klebsiella</i> sp. (CK)	49.75	45.6 ± 2.2
<i>C. vulgaris</i> + <i>R. ornithinolytica</i> (CR)	45.99	52.9 ± 5.1

Values are presented as the mean \pm standard deviation of four independent experiments. n.d. - not determined.

4.5.3. Nutrients removal

Figure 4.11 shows the time-course evolution of nitrogen (Figure 4.11-A and 4.11-B), phosphorus (Figure 4.11-C and 4.11-D) and COD (Figure 4.11-E and 4.11-F) present in the synthetic medium for single cultures of *C. vulgaris* and the bacterial isolates (Figure 4.11-A, 4.11-C and 4.11-E) and for the studied consortia (Figure 4.11-B, 4.11-D and 4.11-F).

From Figure 4.11-A, it is possible to see that single *C. vulgaris* cultures were able to effectively remove nitrogen from the synthetic medium, reaching concentrations below the limits imposed by EU legislation at the end of cultivation time ($0.495 \pm 0.231 \text{ mg N L}^{-1}$). Regarding the bacterial isolates, Figure 4.11-A also shows the ability of *Klebsiella* sp. and *R. ornithinolytica* to assimilate $\text{NO}_3\text{-N}$, being *R. ornithinolytica* faster than *Klebsiella* sp. On the other hand, *E. asburiae* was not able to remove nitrogen from the synthetic medium. At the end of cultivation time, nitrogen concentration present in the culture medium resulting from the single *Klebsiella* sp. culture was $17.1 \pm 0.3 \text{ mg N L}^{-1}$, whereas nitrogen concentration in the one resulting from the single *R. ornithinolytica* culture was almost negligible. $\text{NO}_3\text{-N}$ removal by bacteria was thought to be an anaerobic process conducted by denitrifying bacteria. However, the presence of oxygen in the synthetic medium within the cultivation time (Figure VI.1, Annex VI) suggests another nitrogen removal mechanism. In fact, several studies have reported the ability of some bacteria, especially from the genus *Klebsiella*, to assimilate $\text{NO}_3\text{-N}$ (Zhou et al., 2007). In this process, nitrate is consecutively reduced into nitrite and into ammonia, which is then incorporated into carbon skeletons (Piñar et al., 1997; Piñar et al., 1998; Zhou et al., 2007). The studied consortia have also shown a great ability for nitrogen removal, reaching concentrations close to 0 mg N L^{-1} at the end of the experiments. Since the synthetic medium used in this study did not present ammonia in its composition, it is thought that the mechanism involved in nitrogen removal by *C. vulgaris* was assimilation followed by reduction into ammonia. Although both single and dual-species cultures of *C. vulgaris* have achieved the limits for nitrogen concentration in discharged effluents, it is possible to see that nitrogen uptake in the studied consortia was faster than in the single *C. vulgaris* culture. Load reduction percentages or removal efficiencies were also determined for the studied cultures (Table 4.21). Nitrogen removal efficiencies determined in this study ranged from 0 to 100%. Taking into account the minimum percentage reduction established by EU legislation, 70%, this value was achieved by the single *R. ornithinolytica* and *C. vulgaris* cultures and by all studied consortia. In a

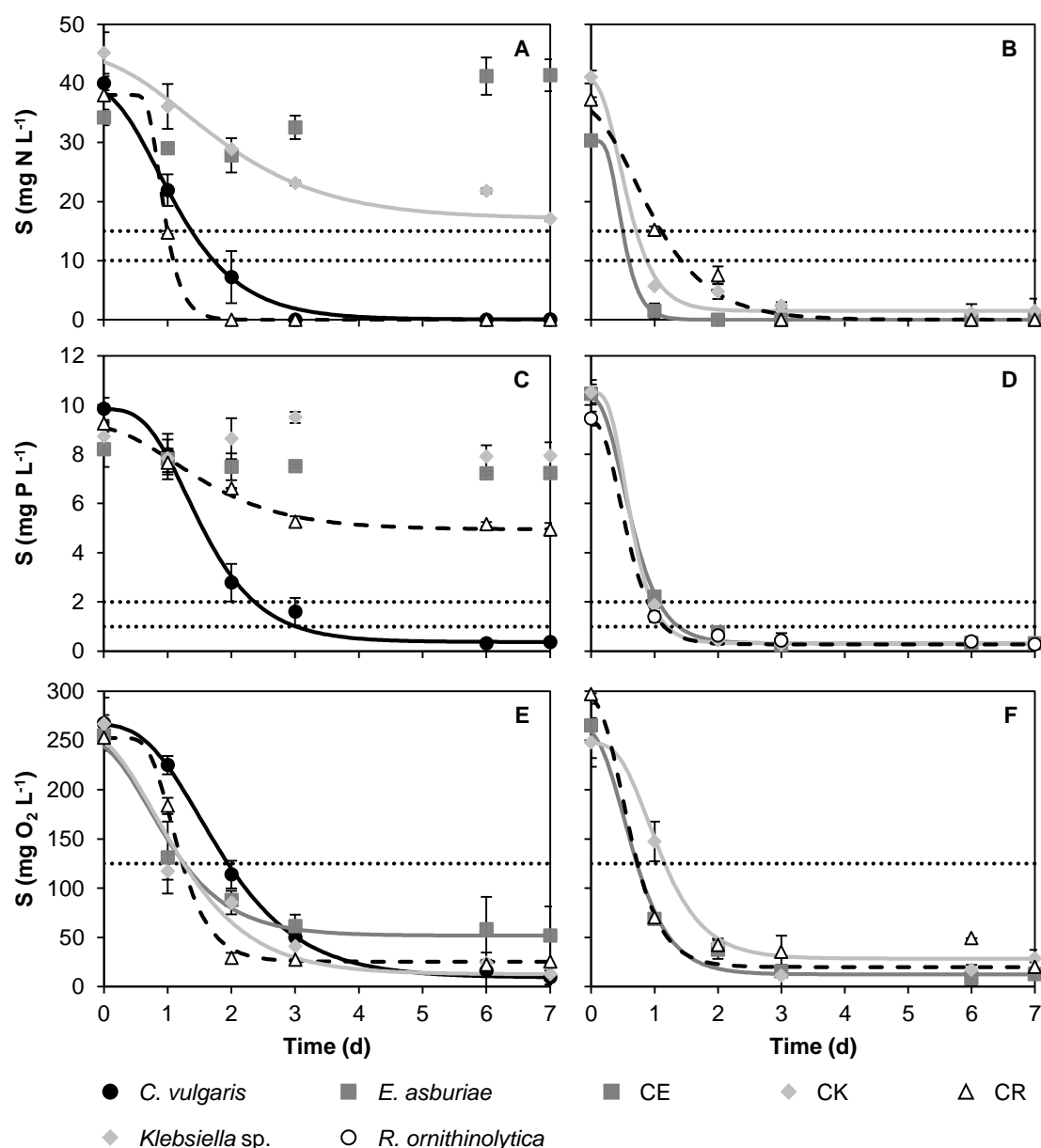


Figure 4.11. Time-course evolution of nitrogen (A and B), phosphorus (C and D) and soluble COD (E and F) concentration in the culture medium obtained for the studied microorganisms when grown in single (A, C and E) and dual-species (B, D and F) cultures containing *C. vulgaris*. Error bars correspond to the standard deviation of the mean determined for four independent experiments. The lines represent the model fit of the modified Gompertz model to the experimental data. The horizontal dashed lines correspond to the limits for nutrients concentration in discharged effluents imposed by EU legislation.

previous study conducted by Wang et al. (2010), a NO₃-N removal of 62.5% was obtained when growing *Chlorella* sp. in a wastewater collected from the secondary settling tank of a MWTP located in Minnesota (USA). Looking at nutrients removal profiles (Figure 4.11), nutrients uptake by *C. vulgaris* and the bacterial isolates grown in single cultures and in the developed consortia goes through an adaptation phase, also known as lag phase, followed by an exponential decrease and a stationary phase. Therefore, the modified Gompertz model

was fitted to the experimental data to determine the kinetic parameters associated to nutrients uptake. The curved lines present in Figure 4.11 represent the model fits of the modified Gompertz model to the experimental data. Analysis of the performance parameters R^2 and RMSE (Table 4.21) indicates that the Gompertz model correctly describes nutrients (nitrogen, phosphorus and organic carbon) uptake by the studied microorganisms and consortia: $R^2 \geq 0.974$ and low RMSE values. Looking at lag time values determined for nitrogen uptake, it is possible to conclude that single *C. vulgaris*, *Klebsiella* sp. and *R. ornithinolytica* cultures, as well as the studied consortia, presented a short lag time, starting nitrogen uptake before completing the first day of culturing. Regarding nitrogen uptake rates, the values obtained through the modified Gompertz model ranged from 0.831 ± 0.122 to $5.61 \pm 0.43 \text{ d}^{-1}$. The lowest value was determined for the single *Klebsiella* sp. culture. However, this value was not statistically different from those determined for the single *C. vulgaris* culture ($1.42 \pm 0.23 \text{ d}^{-1}$; $p=0.12$) and for the CR consortium ($1.58 \pm 0.16 \text{ d}^{-1}$; $p=0.08$). Statistically higher values were determined for the single *R. ornithinolytica* culture ($5.48 \pm 0.16 \text{ d}^{-1}$; $p<0.01$) and for the CE and CK consortia: $5.61 \pm 0.43 \text{ d}^{-1}$ ($p=0.02$) and $3.32 \pm 0.02 \text{ d}^{-1}$ ($p<0.01$), respectively. Lower $\text{NO}_3\text{-N}$ removal rates ($1.5 \pm 0.3 \text{ d}^{-1}$) were reported for *C. vulgaris* grown in commercial synthetic Combo medium (Ruiz et al., 2013). These results have shown that the CE and CK consortia have favoured nitrogen uptake, resulting in increased nitrogen uptake rates and in a reduction in the time required for the achievement of the EU legislation limits for discharged effluents (Table 4.21). When grown in single cultures, the first limit imposed by EU legislation was reduced to, at least, half of the value determined for the single *C. vulgaris* culture. When grown in single cultures, the first limit imposed by legislation (15 mg N L^{-1}) was reached after 33.1 h of culturing, whereas the same value was reached after 11.9 and 17.2 h in the CE and CK consortia, respectively. Although nitrogen concentrations after the second day of culturing were almost negligible, microalgal growth in the CE and CR consortia proceeds after the third day. These results may be due to the uncoupled microalgal growth. According to the Droop (1968) model, which assumes that microalgal growth depends on the intracellular carbon, nitrogen and phosphorus quotas instead of the extracellular available nutrients, microalgal growth after nutrients depletion (uncoupled growth) is possible to occur.

Regarding phosphorus uptake (Figure 4.11-C and 4.11-D), *C. vulgaris* single cultures and consortia were able to effectively remove this nutrient from the synthetic medium, reaching

Table 4.21. Nitrogen, phosphorus and COD removal efficiencies (R , in %), kinetic parameters of nitrogen, phosphorus and soluble COD uptake (λ , in d, and k , in d^{-1}) and time necessary to reach the limits established by EU legislation (t_1 and t_2 , in h) determined for the studied microorganisms when grown in single and dual-species cultures containing *C. vulgaris*

Nutrients	Microorganisms	R (%)	λ (d)	k (d^{-1})	t_1 (h)	t_2 (h)	R^2	RMSE ($mg\ S\ L^{-1}$)
Nitrogen	<i>C. vulgaris</i>	99.8±0.3	0.135±0.032	1.42±0.23	33.1	41.5	0.991	1.0
	<i>E. asburiae</i>	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	<i>Klebsiella</i> sp.	49.9±1.2	0.0851±0.0203	0.831±0.122	n.a.	n.a.	0.984	1.9
	<i>R. ornithinolytica</i>	99.5±0.9	0.688±0.013	5.48±0.16	9.62	11.7	1.00	0.1
	<i>C. vulgaris</i> + <i>E. asburiae</i> (CE)	100±0	0.250±0.084	5.61±0.43	11.9	14.2	1.00	0.1
	<i>C. vulgaris</i> + <i>Klebsiella</i> sp. (CK)	97.0±4.8	0.146±0.009	3.32±0.02	17.2	21.1	0.992	1.5
	<i>C. vulgaris</i> + <i>R. ornithinolytica</i> (CR)	100±0	0.0549±0.0359	1.58±0.16	26.5	34.1	0.989	1.7
Phosphorus	<i>C. vulgaris</i>	95.9±0.6	0.650±0.030	1.56±0.21	56.8	72.8	0.997	0.3
	<i>E. asburiae</i>	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	<i>Klebsiella</i> sp.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	<i>R. ornithinolytica</i>	43.1±4.7	0.170±0.021	1.07±0.01	n.a.	n.a.	0.992	0.2
	<i>C. vulgaris</i> + <i>E. asburiae</i> (CE)	96.2±1.4	0.179±0.033	3.01±0.28	25.5	32.9	0.999	0.2
	<i>C. vulgaris</i> + <i>Klebsiella</i> sp. (CK)	95.6±2.2	0.281±0.031	4.02±1.27	22.9	26.6	1.00	0.1
	<i>C. vulgaris</i> + <i>R. ornithinolytica</i> (CR)	95.8±2.1	0.179±0.010	3.60±0.15	23.9	27.8	0.998	0.2
COD	<i>C. vulgaris</i>	91.0±8.3	0.637±0.152	1.16±0.64	46.8	n.a.	0.999	3.4
	<i>E. asburiae</i>	85.3±5.1	0.0427±0.0183	1.49±0.53	30.0	n.a.	0.988	9.8
	<i>Klebsiella</i> sp.	95.2±0.9	0	0.954±0.066	30.5	n.a.	0.983	12.7
	<i>R. ornithinolytica</i>	87.9±4.4	0	1.23±0.11	29.9	n.a.	0.974	19.0
	<i>C. vulgaris</i> + <i>E. asburiae</i> (CE)	95.0±0.7	0.116±0.054	2.53±0.52	17.4	n.a.	0.993	9.1
	<i>C. vulgaris</i> + <i>Klebsiella</i> sp. (CK)	85.6±3.8	0.431±0.204	2.12±0.40	27.8	n.a.	0.991	9.9
	<i>C. vulgaris</i> + <i>R. ornithinolytica</i> (CR)	89.4±2.9	0.153±0.007	3.02±0.02	17.4	n.a.	0.982	15.7

Values are presented as the mean ± standard deviation of four independent experiments. R^2 - coefficient of determination; RMSE - root mean squared error (in $mg\ S\ L^{-1}$); n.a. - not applicable.

the limits defined by EU legislation. Since the pH of the synthetic medium in these cultures (Figure VI.2, Annex VI) has not exceeded 8, except in the last days of culturing where no measurable quantity of phosphorus was detected, phosphate precipitation is not expected to occur. Accordingly, it can be proposed that the mechanism involved in phosphorus removal was assimilation. Among the bacterial isolates, only *R. ornithinolytica* was able to uptake phosphorus, however, with modest efficiencies ($43.1 \pm 4.7\%$). In this culture, phosphorus concentration decreased from 9.25 ± 0.06 to 5.26 ± 0.22 mg P L⁻¹ (in the third day of culturing), being constant until the end of the experiments. The ceasing of phosphorus uptake at the third day is apparently related to the declining growth phase experienced by *R. ornithinolytica* after the second day of culturing. The assimilation and storage of phosphate in the form of polyphosphates has already been reported for several microorganisms, known as polyphosphate accumulating microorganisms (Mino et al., 1998; Zafiriadis et al., 2012). However, the low removal efficiencies demonstrated by the bacterial isolates may be related to the low ability of activated sludge for phosphorus uptake (Su et al., 2012b). Comparing *C. vulgaris* cultures, it is clear that phosphorus uptake by the three studied consortia was faster than phosphorus uptake determined for single *C. vulgaris* cultures. Regarding phosphorus removal efficiencies, values determined for all *C. vulgaris* cultures were not statistically different ($p > 0.05$), ranging from 95.6 ± 2.2 to $96.2 \pm 1.4\%$. In these conditions, the minimum reduction percentage of 80% established by EU legislation was achieved. Similar removal percentages were obtained by Wang et al. (2010) when culturing *Chlorella* sp. in a wastewater collected before and after primary settling: 83.2 and 90.6%, respectively. As well as for nitrogen, phosphorus uptake rates were determined by fitting the modified Gompertz model to the experimental data. Table 4.21 presents values of lag time, uptake rates and time required to achieve the limits for phosphorus concentration in discharged effluents established by EU legislation. Similar lag time values ($p > 0.05$) were determined for both nitrogen and phosphorus uptake, indicating that both nutrients were readily assimilated (lag time values lower than one day). Comparing single *C. vulgaris* cultures with the studied consortia, a statistically higher lag time ($p < 0.05$) was determined for single *C. vulgaris* cultures (0.650 ± 0.030 d). In the CE, CK and CR consortia, lag time values were 0.179 ± 0.033 , 0.281 ± 0.031 and 0.179 ± 0.010 d, respectively. In the case of phosphorus uptake rates, a statistically lower value ($p < 0.05$) was determined for the single *C. vulgaris* culture: 1.56 ± 0.21 d⁻¹. When cultured with *E. asburiae*, *Klebsiella* sp. and *R. ornithinolytica*, phosphorus uptake rates increased to 3.01 ± 0.28 , 4.02 ± 1.27 and 3.60 ± 0.15 d⁻¹, respectively. Phosphorus uptake rates in the same order of magnitude were

obtained for *C. vulgaris* in the study performed by Ruiz et al. (2013): when growing *C. vulgaris* in different synthetic media and different wastewaters, phosphorus removal rates ranged from 2.0 ± 0.2 to 8.7 ± 1.2 d⁻¹. The reduction of lag times, as well as the increase in phosphorus uptake rates determined for dual-species cultures, have contributed to a decrease in the time required for the achievement of EU legislation limits. In single *C. vulgaris* cultures the time required for the achievement of the first limit established for phosphorus was 56.8 h, whereas in the CE, CK and CR consortia this value decreased to 25.5, 22.9 and 23.9 h, respectively. The almost complete depletion of phosphorus after the second day of culturing and the increase in microalgal cell concentration in the CE and CR consortia reinforces the idea that uncoupled microalgal growth might have occurred, as described by Droop (1968). The increase in phosphorus uptake rates observed between single *C. vulgaris* cultures and the CK consortium, as well as the absence of effect of *Klebsiella* sp. on microalgal growth, suggests that in this consortium luxury uptake of phosphorus might have occurred. According to Powell et al. (2008, 2009), microalgae can adopt this assimilation mechanism, which consists in the uptake of nutrients for storage within the biomass rather than biomass production.

In terms of organic matter present in the synthetic medium, all studied cultures have effectively removed soluble COD (Figure 4.11-E and 4.11-F), reaching the limit defined by EU legislation (125 mg O₂ L⁻¹). From Figure 4.11-E, it is possible to see that soluble COD present in the synthetic medium corresponding to the bacterial isolates reached very low concentrations at the third day of culturing, which is in accordance with the decrease in biomass concentrations observed in single cultures of these bacteria (Figure 4.10-A, 4.10-C and 4.10-E). COD removal in the single *C. vulgaris* culture (Figure 4.11-E) indicates that microalgal growth in these conditions was mixotrophic. Additionally, it is possible to see from Figure 4.11-E and 4.11-F that the supplied organic carbon source was almost depleted within the first three days in the studied consortia and within the first five days in the single *C. vulgaris* culture. A complete COD removal was not observed in this study probably because of the detection limit of the analytical method used for COD determinations. These results indicate that after the third and fifth days, the concentration of organic carbon was very low. However, the low concentrations of organic carbon have not limited microalgal growth, especially in the CE and CR consortia, where exponential growth phase lasted for longer periods of time (Figure 4.10-B and 4.10-F). One possible reason for these results is the ability of microalgae to perform photosynthesis, using CO₂ (supplied in the air stream

bubbled into the cultures) as carbon source (Su et al., 2011). At the end of the experiments, similar COD removal percentages (Table 4.21) were obtained in all studied cultures, ranging from 85.3 ± 5.1 to $95.0 \pm 0.7\%$. Similar removal percentages (approximately 90%) were obtained in the study performed by Li et al. (2011b) when culturing *Chlorella* sp. in a domestic wastewater. Similarly, a COD removal percentage of 98% was obtained by Su et al. (2011) when culturing a wastewater-born algal-bacterial culture in a domestic wastewater. High removal efficiencies determined for the studied cultures may be related to the high ability of the selected microorganisms to use organic carbon, which was reflected in the low lag time values obtained through the modified Gompertz model (Table 4.21). However, it was observed that the studied consortia have reached the limit imposed by EU legislation before the single cultures of the studied microorganisms. This observation was confirmed by the COD uptake rates obtained through the modified Gompertz model (Table 4.21). According to these data, COD uptake rates determined for single cultures were not statistically different ($p > 0.05$), ranging from 0.954 ± 0.066 to $1.49 \pm 0.53 \text{ d}^{-1}$. On the other hand, statistically higher ($p < 0.05$) values were determined for the CE, CK and CR consortia, ranging from 2.12 ± 0.40 to $3.02 \pm 0.02 \text{ d}^{-1}$. Therefore, the time required for the achievement of EU legislation limits was significantly reduced in the studied consortia. Comparing *C. vulgaris* cultures, this value decreased from 46.8 h (in single cultures) to 17.4, 27.8 and 17.4 h (in the CE, CK and CR consortia, respectively). Higher uptake rates determined for the studied consortia suggest a better performance of mixed cultures in COD removal. These results are in accordance with the study performed by Su et al. (2012b), which showed that COD removal efficiencies of single algal cultures ($66.0 \pm 6.0\%$) were lower than those determined for different algae:sludge ratios (91.2 ± 1.7 - $96.2 \pm 1.1\%$). The lower ability of single-cultured microalgae for COD removal may be related to the absence of heterotrophic bacteria to enhance organic carbon degradation (Su et al., 2012b).

In general, nutrients uptake rates determined for the developed consortia were higher than those determined for the single cultures used as control, evidencing the synergistic effect of microalgal-bacterial consortia. These results suggest that the studied consortia improve nutrients removal kinetics, which is in agreement with the determined kinetic growth parameters. Microalgae require high amounts of nitrogen and phosphorus for proteins, nucleic acids and phospholipids synthesis (Silva-Benavides and Torzillo, 2012), meaning that an increase in microalgal growth may result in an increased assimilation of both nitrogen and phosphorus. The improvement of nutrients uptake kinetics in the studied consortia may

be related to the direct or indirect effect of the presence of bacterial isolates in the studied consortia. On the one hand, nutrients uptake rates may be higher due to the direct uptake promoted by the co-cultured isolates. This is the case of nitrogen uptake in the CK and CR consortia, phosphorus uptake in the CR consortium and COD uptake in the three studied consortia. On the other hand, the presence of bacterial isolates in the studied consortia may have resulted in the release of CO₂ and other metabolites to the culture medium, positively affecting microalgal growth. The excretion of CO₂ to the culture medium is considered one of the most important factors contributing to the success of microalgal-bacterial cultures, especially in wastewater treatment processes (Su et al., 2011, 2012). Additionally, it has already been reported that bacteria supply microalgae with organic growth factors and vitamins that enhance microalgal growth (Bordel et al., 2009; Natrah et al., 2014; Unnithan et al., 2014).

4.6. Cultivation in a 60-L open raceway pond

4.6.1. Selection of the appropriate fluid velocity

As it was referred in Section 3.3.3, *C. vulgaris* growth under different fluid velocities was evaluated to determine the most suitable fluid velocity to provide a good mixing of the culture broth. This study has shown that all studied velocities were able to promote a good mixing of the culture broth, since there was no evidence of cells' sedimentation at the bottom of the raceway pond. These results are in accordance with the fluid velocities required to promote a good mixing reported by Hadiyanto et al. (2013): between 0.1 and 0.3 m s⁻¹. Taking into account these observations, biomass productivities and nutrients removal within the cultivation time were evaluated to determine whether the studied velocities could influence these parameters. In terms of biomass concentrations achieved, it can be seen from Figure 4.12 that the study of different fluid velocities has not strongly influenced *C. vulgaris* growth. Figure 4.12 shows a similar pattern between the three growth curves obtained, with no statistical differences ($p>0.05$) between biomass concentrations achieved within the cultivation time. Furthermore, kinetic growth parameters, such as specific growth rates, maximum biomass concentrations and average biomass productivities, determined for the different fluid velocities (Table 4.22) were not statistically different ($p>0.05$). Regarding specific growth rates, values obtained for cultures grown with fluid velocities of 0.10, 0.15

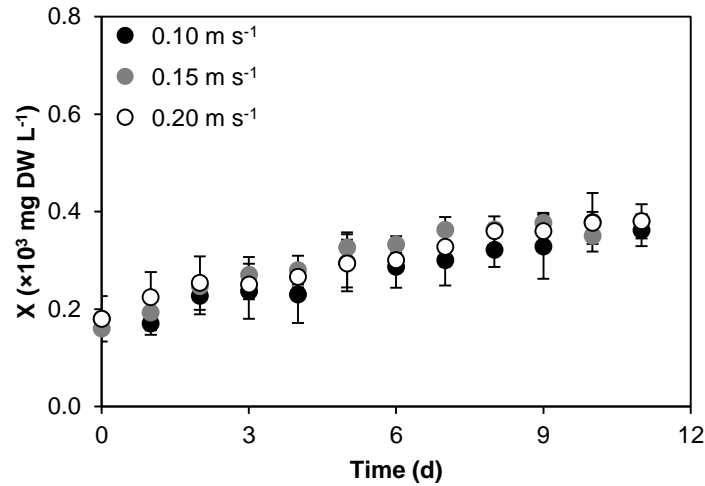


Figure 4.12. Growth curves obtained for *C. vulgaris* grown in the raceway pond under different fluid velocities. Error bars correspond to the standard deviation of the mean determined for two independent experiments.

Table 4.22. Specific growth rates (μ , in d^{-1}), maximum biomass concentrations (X_{\max} , in mg DW L^{-1}) and average biomass productivities (P , in $\text{mg DW L}^{-1} \text{d}^{-1}$) determined for *C. vulgaris* grown in the raceway pond under different fluid velocities

Fluid velocity (m s^{-1})	μ (d^{-1})	X_{\max} (mg DW L^{-1})	P ($\text{mg DW L}^{-1} \text{d}^{-1}$)
0.10	0.313 ± 0.044	378 ± 60	18.0 ± 2.0
0.15	0.289 ± 0.030	380 ± 14	20.0 ± 1.3
0.20	0.298 ± 0.033	380 ± 35	18.2 ± 3.2

Values are presented as the mean \pm standard deviation of two independent experiments.

and 0.20 m s^{-1} were 0.313 ± 0.044 , 0.289 ± 0.030 and $0.298 \pm 0.033 \text{ d}^{-1}$, respectively. On the other hand, maximum biomass concentrations achieved ranged between 378 ± 60 and $380 \pm 35 \text{ mg DW L}^{-1}$ and average biomass productivities ranged between 18.0 ± 2.0 and $20.0 \pm 1.3 \text{ mg DW L}^{-1} \text{d}^{-1}$. These results indicate that increasing fluid velocities from 0.10 to 0.20 m s^{-1} in this raceway pond has not contributed to an increase in *C. vulgaris* growth. Additionally, it is possible to see from Figure 4.12 and Table 4.22 that microalgal growth was not favoured in these conditions, when comparing with the results obtained for *C. vulgaris* monocultures grown in the 500-mL flasks (Sections 4.1-4.3). For example, specific growth rates obtained in the raceway pond were about 70% lower than maximum specific growth rates reported in Sections 4.1-4.3 (approximately 1.3 d^{-1}). One possible reason for the low biomass productivities and specific growth rates obtained in these experiments might be light limitation. These experiments were performed in indoor conditions, using a light source able to provide an average daily light irradiance of

$23 \pm 2 \mu\text{E m}^{-2} \text{s}^{-1}$ at the surface of the liquid. Taking into account the optimal average daily light irradiance determined for *C. vulgaris* in Section 4.2.5 ($208 \mu\text{E m}^{-2} \text{s}^{-1}$), the average daily light irradiance used in these experiments can be considered limiting for microalgal growth. Additionally, these cultures presented an increased light path (25.8 cm) when compared to the one observed in the 500-mL flasks (approximately 8.6 cm), meaning that cells located at the bottom of the raceway pond may not get enough light to perform photosynthesis. According to Janssen (2016) and Chen et al. (2016), light penetration in microalgal cultures decreases as the culture depth increases, being lower as the cultures get denser.

A similar behaviour was observed in terms of nutrients uptake from the culture medium. Time-course evolution of nitrogen and phosphorus concentration in the culture medium (Figure 4.13) evidences a slight decrease in both nitrogen and phosphorus concentrations. After the cultivation time, nitrogen concentration decreased from 34.7 ± 1.9 to $25.8 \pm 1.8 \text{ mg N L}^{-1}$, whereas phosphorus concentration decreased from 8.45 ± 0.46 to $6.59 \pm 0.27 \text{ mg P L}^{-1}$. These results indicate that the studied conditions have not favoured nitrogen and phosphorus uptake by *C. vulgaris* and might be related to the low photosynthetic activity demonstrated by this microalga in these conditions. Regarding the effect of fluid velocity on nutrients removal, no statistical difference ($p > 0.05$) was observed between the studied velocities.

As the range of fluid velocities studied have promoted a good mixing of the cultures (application of these fluid velocities has not resulted in microalgal sedimentation) and once

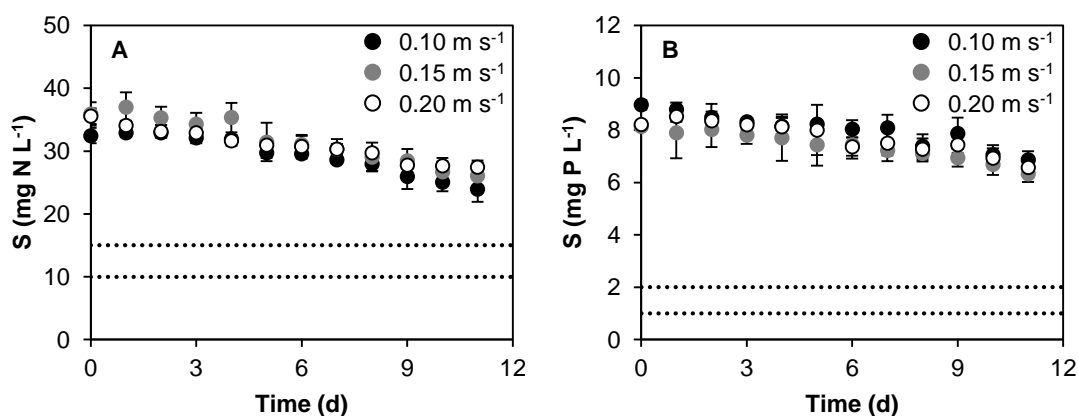


Figure 4.13. Time-course evolution of nitrogen (A) and phosphorus (B) concentration in the culture medium obtained for *C. vulgaris* grown in the raceway pond under different fluid velocities. Error bars correspond to the standard deviation of the mean determined for two independent experiments. The horizontal dashed lines correspond to the limits for nutrients concentration in discharged effluents imposed by EU legislation.

different velocities have resulted in a similar behaviour in terms of microalgal growth and nutrients uptake, the lowest fluid velocity (0.10 m s^{-1}) was selected for the further studies regarding the open raceway pond. This velocity was considered sufficient to promote a good mixing of the culture broth presenting, at the same time, reduced energetic costs.

4.6.2. Cultivation of the single *C. vulgaris* culture and the SC and CE consortia

Since the single *C. vulgaris* culture and the SC and CE consortia have demonstrated to be the most effective cultures in terms of biomass production, CO_2 capture and nutrients uptake in the 500-mL flasks (Sections 4.1-4.5), their behaviour at higher scale was assessed. Accordingly, cultures of these microorganism and consortia were performed in the 60-L open raceway pond described in Section 3.3.3.

Growth curves determined for these cultures are shown in Figure 4.14. Additionally, Table 4.23 presents the kinetic growth parameters (specific growth rates, maximum biomass concentrations and average biomass productivities). Looking at the growth curves present in Figure 4.14, it is possible to observe that the studied cultures have shown a similar growth behaviour, with biomass concentrations ranging from 218 ± 33 to $414 \pm 60 \text{ mg DW L}^{-1}$. However, it is possible to observe that the single *C. vulgaris* culture presented an increased adaptation phase, when compared to that of the SC and CE consortia: the single *C. vulgaris* culture presented an adaptation phase of approximately one day, whereas the SC and CE consortia started growing immediately after the beginning of the cultivation time. Although *C. vulgaris* growth was not significantly different from the one obtained in the SC and CE consortia, the increased lag phase observed in this culture suggests that the studied consortia can be more advantageous in microalgal biomass production than the single *C. vulgaris* culture, which is in accordance with the results obtained in Sections 4.4 and 4.5. As it was observed in the growth curves, the kinetic growth parameters determined for the single *C. vulgaris* culture and for the studied consortia were not statistically different ($p > 0.05$). Specific growth rates determined for the single *C. vulgaris* culture and for the SC and CE consortia were 0.313 ± 0.044 , 0.257 ± 0.031 and $0.266 \pm 0.049 \text{ d}^{-1}$, respectively. On the other hand, maximum biomass concentrations ranged from 378 ± 60 (obtained for the single *C. vulgaris* culture) to $585 \pm 7 \text{ mg DW L}^{-1}$ (obtained for the CE consortium). Although these values were not statistically different, maximum biomass concentrations determined for the SC and CE consortia were higher than those determined for the single *C. vulgaris* culture,

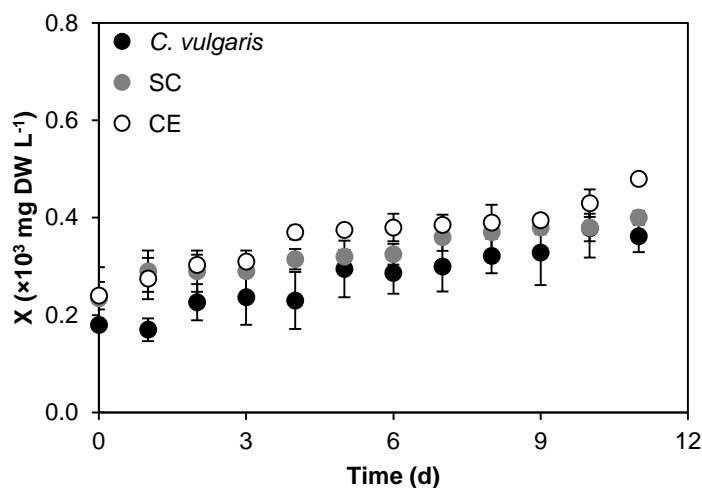


Figure 4.14. Growth curves obtained for the single *C. vulgaris* culture and for the SC and CE consortia grown in the raceway pond. Error bars correspond to the standard deviation of the mean determined for two independent experiments.

Table 4.23. Specific growth rates (μ , in d^{-1}), maximum biomass concentrations (X_{\max} , in mg DW L^{-1}) and average biomass productivities (P , in $\text{mg DW L}^{-1} \text{d}^{-1}$) determined for the single *C. vulgaris* culture and for the SC and CE consortia grown in the raceway pond

Microorganisms	μ (d^{-1})	X_{\max} (mg DW L^{-1})	P ($\text{mg DW L}^{-1} \text{d}^{-1}$)
<i>C. vulgaris</i>	0.313 ± 0.044	378 ± 60	18.0 ± 2.0
<i>S. salina</i> + <i>C. vulgaris</i> (SC)	0.257 ± 0.031	400 ± 14	15.0 ± 0.4
<i>C. vulgaris</i> + <i>E. asburiae</i> (CE)	0.266 ± 0.049	585 ± 7	21.8 ± 1.7

Values are presented as the mean \pm standard deviation of two independent experiments.

confirming that in the studied consortia cooperative interactions may occur, leading to increased biomass concentrations. Average biomass productivities determined for the studied cultures were also very similar, ranging from 15.0 ± 0.4 (value determined for the SC consortium) to $21.8 \pm 1.7 \text{ mg DW L}^{-1} \text{d}^{-1}$ (value determined for the CE consortium). The slight increase in biomass concentrations within the cultivation time observed in these cultures might be a result of light limitation, as it was reported in Section 4.6.1. In fact, all these cultures were performed with an average daily light irradiance of $23 \pm 2 \mu\text{E m}^{-2} \text{s}^{-1}$, which has demonstrated to be limiting for microalgal growth. Due to light limitation and to the low average biomass productivities achieved, CO_2 uptake rates (Table 4.24) determined for these cultures were also statistically ($p < 0.05$) lower than those obtained in the 500-mL flasks (Sections 4.4 and 4.5). In this study CO_2 uptake rates ranged between 23.5 ± 0.6 (value determined for the SC consortium) and $34.5 \pm 2.6 \text{ mg CO}_2 \text{ L}^{-1} \text{d}^{-1}$ (value determined for the CE consortium). On the other hand, CO_2 uptake rates determined in the 500-mL flasks

(Sections 4.4 and 4.5) ranged between 45.6 ± 7.4 (value determined for the single *C. vulgaris* culture) and 150 ± 13 mg CO₂ L⁻¹ d⁻¹ (value determined for the SC consortium).

Regarding nutrients removal in these cultures, Figure 4.15 presents the time-course evolution of nitrogen and phosphorus concentration in the culture medium. Analysis of Figure 4.15 also evidences the low photosynthetic activity of the cultures when grown in the raceway pond with an average daily light irradiance of 23 ± 2 $\mu\text{E m}^{-2} \text{s}^{-1}$. Under these conditions, only a slight decrease was observed in nitrogen and phosphorus concentrations, being the concentrations achieved in the last day of culturing above the limits defined by EU legislation. Nitrogen concentration decreased from about 33.9 ± 1.7 to 24.2 ± 3.0 mg N L⁻¹, whereas phosphorus concentration decreased from approximately 8.69 ± 0.56 to 6.12 ± 0.78 mg P L⁻¹. Comparing the single *C. vulgaris* culture with the SC and CE consortia, Figure 4.15 evidences a similar behaviour between the studied cultures. However, it is possible to observe that the CE consortium was more efficient in nutrients uptake from the culture medium, especially in the first days of culturing. For example, nitrogen concentration in the first day of culturing decreased from 33.4 ± 1.0 to 24.6 ± 0.7 mg N L⁻¹. Although the limits established by EU legislation were not achieved and the differences between the studied cultures could not be considered statistically significant ($p > 0.05$), these results are in agreement with those obtained in the 500-mL flasks (Section 4.5), where the CE consortium has shown to be more effective in terms of biomass production, CO₂ capture and nutrients uptake from the culture medium than the single *C. vulgaris* culture.

Since the results obtained in the raceway pond were far from those obtained in the 500-mL flasks, possibly due to light limitation, the single *C. vulgaris* culture was evaluated in the same raceway pond, but in outdoor conditions (where average daily light irradiance reaching the surface of the raceway pond was higher than the one achieved using artificial light).

Table 4.24. Carbon contents (α_c , in % w/w) and CO₂ uptake rates (R_c , in mg CO₂ L⁻¹ d⁻¹) determined for the single *C. vulgaris* culture and for the SC and CE consortia grown in the raceway pond

Microorganisms	α_c (% w/w)	R_c (mg CO ₂ L ⁻¹ d ⁻¹)
<i>C. vulgaris</i>	44.18	29.2 ± 3.3
<i>S. salina</i> + <i>C. vulgaris</i> (SC)	42.80	23.5 ± 0.6
<i>C. vulgaris</i> + <i>E. asburiae</i> (CE)	43.15	34.5 ± 2.6

Values are presented as the mean \pm standard deviation of two independent experiments.

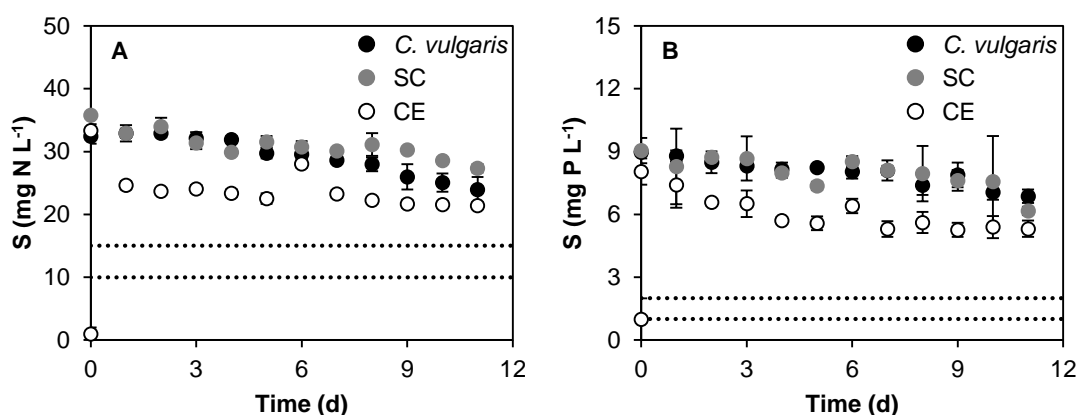


Figure 4.15. Time-course evolution of nitrogen (A) and phosphorus (B) concentration in the culture medium obtained for the single *C. vulgaris* culture and for the SC and CE consortia grown in the raceway pond. Error bars correspond to the standard deviation of the mean determined for two independent experiments. The horizontal dashed lines correspond to the limits for nutrients concentration in discharged effluents imposed by EU legislation.

4.6.3. Cultivation of *C. vulgaris* in outdoor conditions

C. vulgaris growth in outdoor conditions in different periods allowed the evaluation of different average daily light irradiances on microalgal growth, CO₂ capture and nutrients uptake. Since the experiments were performed between spring and summer 2016, temperature oscillations in this period were not considered significant. However, different light conditions were observed in this period, being average daily light irradiances 102, 153 and 204 $\mu\text{E m}^{-2} \text{s}^{-1}$. Therefore, these experiments were used to compare the results obtained in outdoor conditions with the one obtained in the laboratory with artificial light ($23 \pm 2 \mu\text{E m}^{-2} \text{s}^{-1}$).

Growth curves obtained in these conditions are shown in Figure 4.16. On the other hand, kinetic growth parameters are presented in Table 4.25. Analysis of Figure 4.16 shows that *C. vulgaris* growth was favoured by increased average daily light irradiances, as it was reported in Section 4.1.1. Comparing the different average daily light irradiances studied, the increase in microalgal growth was more pronounced in cultures supplied with average daily light irradiances of 153 and 204 $\mu\text{E m}^{-2} \text{s}^{-1}$. These results are in accordance with optimal light irradiances determined for *C. vulgaris* in Section 4.2.5 (208 $\mu\text{E m}^{-2} \text{s}^{-1}$). The kinetic growth parameters present in Table 4.25 also suggest an increased microalgal growth in cultures grown with higher average daily light irradiances. Regarding specific growth rates,

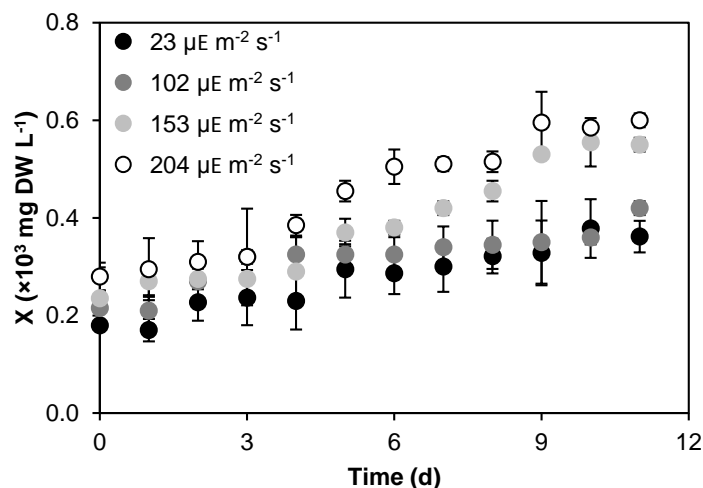


Figure 4.16. Growth curves obtained for *C. vulgaris* grown in the raceway pond under different average daily light irradiances. Error bars correspond to the standard deviation of the mean determined for two independent experiments.

Table 4.25. Specific growth rates (μ , in d^{-1}), maximum biomass concentrations (X_{max} , in mg DW L^{-1}) and average biomass productivities (P , in $\text{mg DW L}^{-1} \text{d}^{-1}$) determined for the single *C. vulgaris* culture grown in the raceway pond under different average daily light irradiances

Average daily light irradiance ($\mu\text{E m}^{-2} \text{s}^{-1}$)	μ (d^{-1})	X_{max} (mg DW L^{-1})	P ($\text{mg DW L}^{-1} \text{d}^{-1}$)
23	0.313 ± 0.044	378 ± 60	18.0 ± 2.0
102	0.438 ± 0.039	420 ± 14	18.6 ± 0.4
153	0.400 ± 0.027	555 ± 49	29.1 ± 1.9
204	0.526 ± 0.137	600 ± 14	29.1 ± 0.4

Values are presented as the mean \pm standard deviation of two independent experiments.

these values ranged between 0.313 ± 0.044 (for cultures grown with artificial light – $23 \pm 2 \mu\text{E m}^{-2} \text{s}^{-1}$) and $0.526 \pm 0.137 \text{ d}^{-1}$ (for cultures grown with an average daily light irradiance of $204 \mu\text{E m}^{-2} \text{s}^{-1}$). Regarding maximum biomass concentrations, an increase of approximately 37% was observed between cultures grown with 23 and $204 \mu\text{E m}^{-2} \text{s}^{-1}$. On the other hand, average biomass productivities ranged between 18.0 (for cultures grown with an average daily light irradiance of $23 \mu\text{E m}^{-2} \text{s}^{-1}$) and $29.1 \text{ mg DW L}^{-1} \text{d}^{-1}$ (for cultures grown with average daily light irradiances of 153 and $204 \mu\text{E m}^{-2} \text{s}^{-1}$).

CO_2 uptake rates determined in these conditions (Table 4.26) also evidence higher photosynthetic activity in cultures performed with higher light supplies. CO_2 uptake rates determined at $23 \mu\text{E m}^{-2} \text{s}^{-1}$ ($29.2 \pm 3.3 \text{ mg CO}_2 \text{ L}^{-1} \text{d}^{-1}$) almost doubled in cultures performed in outdoor conditions under the highest average daily light irradiance achieved

Table 4.26. Carbon contents (α_c , in % w/w) and CO₂ fixation rates (R_c , in mg CO₂ L⁻¹ d⁻¹) determined for the single *C. vulgaris* culture grown in the raceway pond under different average daily light irradiances

Average daily light irradiance ($\mu\text{E m}^{-2} \text{s}^{-1}$)	α_c (% w/w)	R_c (mg CO ₂ L ⁻¹ d ⁻¹)
23	44.18	29.2±3.3
102	41.69	28.5±0.6
153	43.39	46.3±3.1
204	43.67	46.6±0.6

Values are presented as the mean \pm standard deviation of two independent experiments.

(46.6±0.6 mg CO₂ L⁻¹ d⁻¹). However, these values were significantly lower than those obtained for *C. vulgaris* in the 500-mL flasks for average daily light irradiances ranging between 15 and 180 $\mu\text{E m}^{-2} \text{s}^{-1}$ (Section 4.1.2). Under these conditions, CO₂ uptake rates oscillated between 60.9±2.0 and 195±12 mg CO₂ L⁻¹ d⁻¹. The low CO₂ uptake rates determined in the open pond might be related to the low photosynthetic activity demonstrated by *C. vulgaris* in these conditions where light limitation might have occurred and also to difficulties in mass transfer. In this study, mass transfer was promoted exclusively by the rotation of the paddlewheel. However, several authors have suggested the use of CO₂ sumps at an adequate flow ratio to obtain a good mass transfer in raceway ponds (Benemann et al., 1987; Craggs et al., 2012; de Godos et al., 2014; Ketheesan and Nirmalakhandan, 2012; Mendoza et al., 2013).

In terms of nutrients uptake, Figure 4.17 presents the time-course evolution of nitrogen and phosphorus concentration in the culture medium for the different average daily light irradiances studied in the raceway pond. Analysis of Figure 4.17 evidences higher nutrients uptake in cultures grown with increased average daily light irradiances, which is in accordance with the results obtained in the 500-mL flasks (Sections 4.1 and 4.2) and to the higher photosynthetic activity observed in these conditions. In cultures performed under the lowest average daily light irradiance, nitrogen concentration decreased from 32.5±1.2 to 23.9±2.0 mg N L⁻¹, corresponding to a removal efficiency of 26.3±1.0%. In the same conditions, phosphorus concentration decreased from 9.0±0.2 to 6.9±0.3 mg P L⁻¹, which corresponds to a removal efficiency of 23.4±1.4%. The almost negligible removal efficiencies obtained in these conditions were not surprising since light limitation might have occurred. On the other hand, as average daily light irradiance increased, higher removal efficiencies were obtained for both nitrogen and phosphorus, with maximum removal efficiencies obtained in cultures performed with the highest average daily light irradiance

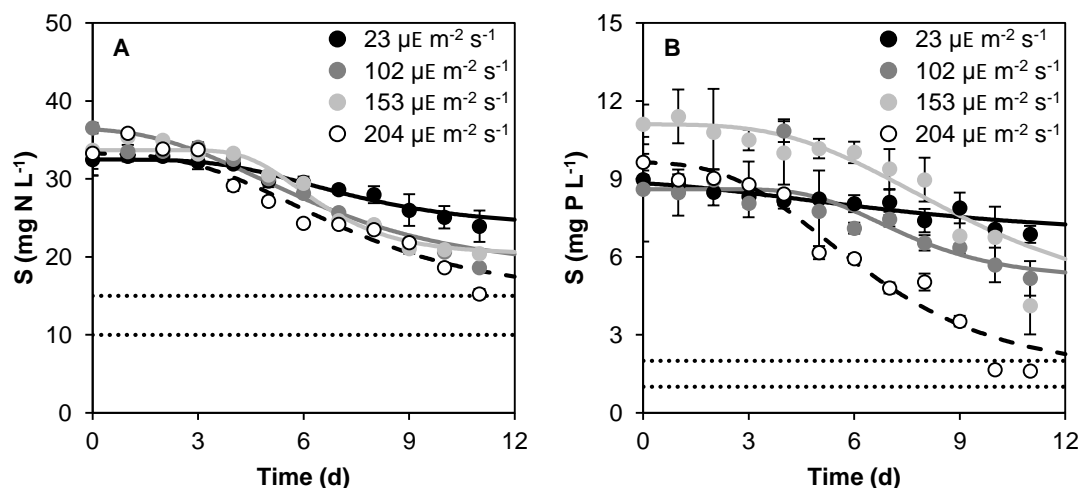


Figure 4.17. Time-course evolution of nitrogen (A) and phosphorus (B) concentration in the culture medium obtained for the single *C. vulgaris* culture grown in the raceway pond under different average daily light irradiances. Error bars correspond to the standard deviation of the mean determined for two independent experiments. The lines represent the model fit of the modified Gompertz model to the experimental data. The horizontal dashed lines correspond to the limits for nutrients concentration in discharged effluents imposed by EU legislation.

(54.2 ± 1.8 and $83.4 \pm 3.6\%$ for nitrogen and phosphorus, respectively). Table 4.27 presents nitrogen and phosphorus removal efficiencies, as well as kinetic parameters associated to nitrogen and phosphorus uptake. As it is possible to observe in both Figure 4.17 and Table 4.27, the limits established by EU legislation for nitrogen and phosphorus concentration in discharged effluents were not achieved, except in the case of phosphorus in cultures grown with the highest light supply ($204 \mu\text{E m}^{-2} \text{s}^{-1}$). Taking into account the kinetic parameters determined through the model fit of the modified Gompertz model to the experimental data, it is possible to observe increased lag times and lower uptake rates in these cultures, when compared to those obtained for *C. vulgaris* in Sections 4.3.3, 4.4.3 and 4.5.3 for cultures grown in the 500-mL flasks. In the open raceway pond, lag time values ranged between 0 and 4.69 ± 0.11 d, whereas nitrogen and phosphorus uptake rates ranged between 0.218 ± 0.014 and $0.659 \pm 0.012 \text{ d}^{-1}$. On the other hand, in 500-mL flasks (Section 4.3.3, for example), lag times ranged between 0.190 ± 0.005 and 2.40 ± 0.19 d and nutrients uptake rates ranged between 0.669 ± 0.043 and $10.4 \pm 0.9 \text{ d}^{-1}$. Regarding nutrients uptake mechanisms involved in these cultures, $\text{NO}_3\text{-N}$ uptake occurred through assimilation. Similarly, the evolution of pH and dissolved oxygen concentration in the culture medium (Figure VII.1, Annex VII) also suggests phosphorus assimilation (pH values within the cultivation time were not higher than 8 and dissolved oxygen concentrations were not very high).

Table 4.27. Nitrogen and phosphorus removal efficiencies (R , in %) and kinetic parameters of nitrogen and phosphorus uptake (λ , in d, and k , in d^{-1}) determined for the single *C. vulgaris* culture grown in the raceway pond under different average daily light irradiances

Nutrients	Average daily light irradiance ($\mu\text{E m}^{-2} \text{s}^{-1}$)	R (%)	λ (d)	k (d^{-1})	R^2	RMSE (mg S L^{-1})
Nitrogen	23	26.3 \pm 1.0	3.53 \pm 0.54	0.386 \pm 0.074	0.968	0.5
	102	49.0 \pm 2.4	1.67 \pm 0.87	0.319 \pm 0.090	0.961	1.0
	153	39.4 \pm 2.7	4.33 \pm 0.46	0.659 \pm 0.012	0.971	0.9
	204	54.2 \pm 1.8	2.68 \pm 0.02	0.327 \pm 0.021	0.932	1.4
Phosphorus	23	23.4 \pm 1.4	(2.01 \pm 0.45) $\times 10^{-3}$	0.218 \pm 0.014	0.837	0.2
	102	39.9 \pm 1.8	4.69 \pm 0.11	0.503 \pm 0.069	0.674	0.8
	153	62.8 \pm 2.3	4.07 \pm 0.20	0.280 \pm 0.029	0.913	0.4
	204	83.4 \pm 3.6	2.38 \pm 0.56	0.360 \pm 0.043	0.945	0.6

Values are presented as the mean \pm standard deviation of two independent experiments. R^2 - coefficient of determination; RMSE - root mean squared error (in mg S L^{-1}).

Although the limits imposed by legislation were not achieved using the open raceway pond, promising results were obtained in cultures grown with the highest light supply. These results constitute important findings for microalgal cultivation in outdoor conditions for wastewater treatment purposes, since the nitrogen and phosphorus concentrations achieved in the last day of culturing in cultures grown with an average daily light irradiance of $204 \mu\text{E m}^{-2} \text{s}^{-1}$ were very close to the limits imposed by EU legislation (15.2 ± 0.6 and $1.6\pm 0.1 \text{ mg S L}^{-1}$ for nitrogen and phosphorus, respectively). However, it should be noted that an adequate light supply should be provided to microalgal cultures. Alternatively a different configuration of the raceway pond should be adopted to reduce the light path and thus avoid light shading in the periphery zones of the raceway pond. Another solution might be the periodically harvesting of microalgal biomass with medium recirculation until the achievement of the EU legislation limits. The continuous harvesting of biomass will avoid shading effects of microalgae, thus improving light penetration and therefore the photosynthetic activity. Preliminary experiments have been performed in this direction. When evaluating the effect of different fluid velocities on microalgal growth (Section 4.6.1), flocs formation was observed at the lowest velocity. In fact, for lower fluid velocities, low net zeta potential values were observed, resulting in aggregates formation. Table 4.28 presents zeta potential values determined for *C. vulgaris* grown in the open pond under different fluid velocities, as well as particles' size and pH values. Analysis of Table 4.28 shows that zeta potential determined for *C. vulgaris* grown in the open raceway pond at different fluid velocities ranged between -24.2 ± 4.3 (value determined at 0.10 m s^{-1}) and -

35.1±4.0 mV (value determined at 0.20 m s⁻¹). Zeta potential measurements give information about the charge of cell surfaces: negative or positive values of zeta potential depend on the functional groups present on cell surfaces and also on the pH of the culture medium (Ozkan and Berberoglu, 2013). Functional groups commonly found on cell surfaces include hydroxyl (–OH), carboxyl (–COOH) and amine (–NH₂) (Ferreira et al., 2011; González-Fernández and Ballesteros, 2012; Ozkan and Berberoglu, 2013). Since these groups are ionizable, when cells are exposed to low pH values, functional groups are protonated and net surface charge becomes positive, and on the other hand, when cells are exposed to high pH values, functional groups are deprotonated and the resulting net surface charge is negative. At the point of zero charge (PZC), corresponding to an intermediate pH, some groups are protonated while others are deprotonated and the surface charge is neutralized (Hadjoudja et al., 2010; Stumm and Morgan, 2012). Negative values observed for *C. vulgaris* were expected, since the pH of the culture medium measured when the samples were collected was high (8.07±0.23) and PZC reported for algae is approximately pH 3 (Hadjoudja et al., 2010), indicating that functional groups on *C. vulgaris* surface were deprotonated. Additionally, net zeta potential values give information about suspensions' stability. When absolute value of zeta potential is high, repulsive forces prevail over van der Waals forces, and hence, particles/cells are stable in the dispersed form. On the other hand, for low net zeta potential values, van der Waals forces (usually attractive) are higher than repulsive ones and the stability of the suspension is affected, resulting in the formation of aggregates and further settling (de Schryver et al., 2008; Zita and Hermansson, 1994). Net zeta potential values determined for *C. vulgaris* were lower for the lowest fluid velocities tested, which is in agreement with the evidence of flocs formation in the lowest fluid velocity and to the higher particle size observed in these conditions. According to Table 4.28, particle size determined for *C. vulgaris* oscillated between 12.3±3.2 (value determined at 0.20 m s⁻¹) and 43.8±5.5 µm (value determined at 0.10 m s⁻¹), being these values statistically different

Table 4.28. Zeta potential (in mV) and average particle size (in µm) determined for *C. vulgaris* cells grown in the raceway pond under different fluid velocities

Fluid velocity (m s ⁻¹)	pH	Zeta potential (mV)	Cell size (µm)
0.10	8.25±0.01	-24.2±4.3	43.8±5.5
0.15	8.15±0.02	-29.2±5.2	33.0±9.9
0.20	7.81±0.41	-35.1±4.0	12.3±3.2

Values are presented as the mean ± standard deviation of two independent experiments.

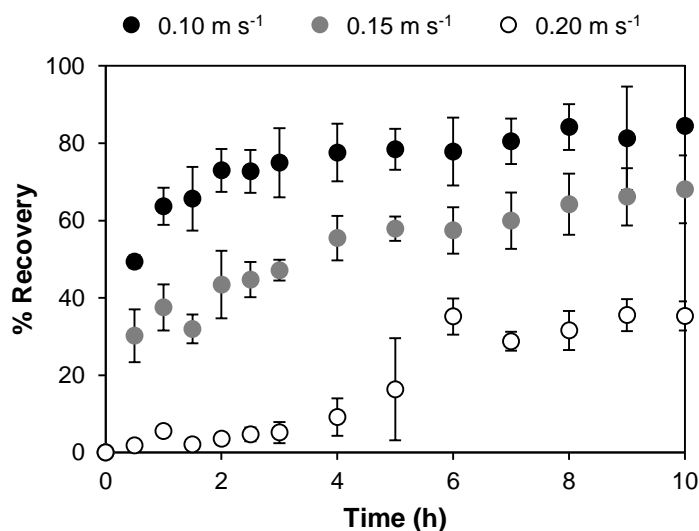


Figure 4.18. *C. vulgaris* removal percentages determined within the settling period after cultivation for 11 days in the open raceway pond under different fluid velocities. Error bars correspond to the standard deviation of the mean determined for two independent experiments.

($p < 0.01$). Aggregates formation in culture grown under the lowest fluid velocity can also be a result of the increased pH values observed in these cultures: 8.25 ± 0.01 against 8.15 ± 0.02 and 7.81 ± 0.41 determined for cultures grown under fluid velocities of 0.15 and 0.20 m s⁻¹, respectively. According to Vandamme et al. (2012), autoflocculation of microalgae can occur by increasing the pH of the culture medium. Taking into account this innate ability of *C. vulgaris* to form aggregates, sedimentation experiments were performed to evaluate the sedimentation kinetics of this microalga. The recovery efficiency of microalgal cells as a function of time is represented in Figure 4.18. Analysis of these data clearly evidences a faster sedimentation kinetics in cultures grown with the lowest fluid velocity (cultures presenting the lowest net zeta potential value and the highest particle size), with the complete microalgal settling occurring after 4 h of the beginning of the sedimentation experiments.

These results indicate that biomass harvesting with medium recirculation can be a viable alternative to improve microalgal growth in the raceway pond and consequent nutrient uptake, since a fast and economic harvesting of microalgal biomass can be achieved. Under these conditions microalgal harvesting can be obtained without the requirement of a more energy- and cost-intensive harvesting procedure and without the contamination of microalgal biomass (with chemical flocculants, for example).

4.7. Techno-economic assessment of a microalgal production unit for wastewater treatment and bioenergy production

Taking into account the biomass concentrations achieved by *C. vulgaris* grown in the open pond in outdoor conditions (Section 4.6.3), a TEA of a microalgal production unit was performed. This analysis aimed the economic evaluation of an integrated system combining microalgal production with CO₂ capture, nutrients removal from wastewaters and bioenergy production for the case of Portugal.

4.7.1. Mass balance

The overall process is schematically represented in Figure 3.5 and the mass balance to the different streams involved in the process is presented in Table 4.29. The following sections include a description of each step and all the considerations assumed to determine mass balances for each process unit.

The proposed algal facility consists of 25 similar high rate ponds with 0.3 m height, performing a total pond area of 100 ha. These open ponds may operate during diurnal periods, since photosynthetic growth does not occur at night. Therefore, this period may be used for shut down for cleaning and maintenance. Average biomass productivities were determined taking into account the operation time of open ponds, average horizontal light irradiance observed in Sines (Figure 3.4-B) and the assumed photosynthetic efficiencies (1 to 3%). Photosynthetic efficiencies ranging between approximately 1.5 and 4.5% were already reported for *C. vulgaris* (Hirayama et al., 1996). Accordingly, annual average biomass productivity determined for the base scenario in this region is approximately 16 g m⁻² d⁻¹, which is similar to the values determined by Doucha and Lívanský (1995) for *Chlorella* sp. grown in open ponds (25 g m⁻² d⁻¹). Considering annual average biomass productivities and the pond volume (3.0×10⁵ m³) and considering the average evaporation rate reported in Section 3.14 (0.075 m month⁻¹), the input stream of water and nutrients (S₀₁) required for microalgal growth on a daily basis corresponds to 3.5×10⁴ m³ d⁻¹ (in the base scenario). This water input is obtained from domestic wastewater (S_{WW}, 2.4×10⁴ m³ d⁻¹), from water recycling (S_{WR}, 1.1×10⁴ m³ day⁻¹) and from water resulting from the anaerobic digestion step (S_{AD}, 62 m³ d⁻¹). Flow rates of the recycling water (S_{WR}) can be regulated to avoid excessive dilution of the cultures in rainy days. Assuming that biomass concentration

Table 4.29. Mass balance to the flow streams involved in the process determined for each of the studied scenarios (Sc)

Streams	Sc1	Sc2	Sc3	Sc4	Sc5	Sc6	Sc7
S_{01} - water input in the cultivation step ($\times 10^4 \text{ m}^3 \text{ d}^{-1}$)	3.5	1.9	5.2	3.5	3.5	3.5	3.5
S_{02} - biomass flow rate after the cultivation step ^a ($\times 10^4 \text{ m}^3 \text{ d}^{-1}$)	3.3	1.6	4.9	3.3	3.3	3.3	3.3
S_{03} - biomass flow rate after the pre-concentration step ($\times 10^3 \text{ m}^3 \text{ d}^{-1}$)	8.2	4.1	12	8.2	8.2	8.2	8.2
S_{04} - biomass flow rate after the centrifugation step ($\text{m}^3 \text{ d}^{-1}$)	78	39	117	78	78	78	78
S_{05} - extracted lipids flow rate ($\text{m}^3 \text{ d}^{-1}$)	3.4	1.7	5.1	2.7	4.1	3.4	3.4
S_{06} - biomass flow rate after the lipids extraction step ($\text{m}^3 \text{ d}^{-1}$)	75	37	112	75	74	75	75
S_{07} - biogas flow rate after the anaerobic digestion step (t d^{-1})	6.6	3.3	10	6.9	6.3	4.4	8.9
S_{08} - flow rate of the residue produced in the anaerobic digestion step (t d^{-1})	7.0	3.5	11	7.3	6.6	8.9	5.1
S_{EV} - water flow rate required to compensate for evaporation ($\times 10^3 \text{ m}^3 \text{ d}^{-1}$)	2.5	2.5	2.5	2.5	2.5	2.5	2.5
S_{WW} - wastewater flow rate required to feed the culture ($\times 10^4 \text{ m}^3 \text{ d}^{-1}$)	2.4	0.62	5.2	2.4	2.4	2.4	2.4
S_{WR} - recycling water flow rate required to feed the culture ($\times 10^4 \text{ m}^3 \text{ d}^{-1}$)	1.1	1.3	0	1.1	1.1	1.1	1.1
S_{AD} - anaerobic digestion effluent flow rate required to feed the culture ($\text{m}^3 \text{ d}^{-1}$)	62	31	93	62	62	62	62

^a Biomass flow rate after the cultivation step was determined considering annual average biomass productivities and assuming a final biomass concentration of 0.5 g L^{-1} (Jiménez et al., 2003; Norsker et al., 2011).

achieved during the cultivation step is 0.5 g L^{-1} (value achieved in this study when culturing *C. vulgaris* in a raceway pond under solar light conditions, Section 4.6.3), biomass flow rate after the cultivation step (S_{02}) is, in the base scenario, $3.3 \times 10^4 \text{ m}^3 \text{ d}^{-1}$. Input and output streams of microalgal production in the studied scenarios are summarized in Table 4.29.

As it was previously referred, domestic wastewater will be used as culture medium. Wastewater will provide nutrients, such as nitrogen and phosphorus, to microalgae. On the other hand, CO_2 resulting from the thermoelectric power plant and refinery processes will be supplied to the cultures. Taking into account the annual average biomass productivities and the typical molecular formula described for microalgae, $\text{CO}_{0.48}\text{H}_{1.83}\text{N}_{0.11}\text{P}_{0.01}$ (Chisti, 2007), theoretical nitrogen, phosphorus and carbon removal rates were estimated. For these determinations, it was assumed that all nitrogen, phosphorus and carbon removed from the wastewater or from the flue gas were incorporated into microalgal biomass. Although the typical molecular formula of microalgal biomass was not determined for *C. vulgaris*, it has already been applied by several authors to determine C, N and P removal rates by microalgae

from the genus *Chlorella* (Gouveia, 2011; Wang and Lan, 2010). Considering the base scenario, nitrogen, phosphorus and carbon removal rates are 1.1, 0.22 and 8.4 g m⁻² d⁻¹. With these values, minimum concentrations of these nutrients required in the feed stream were determined. Accordingly, for the same scenario, minimum nitrogen and phosphorus concentrations in the feed stream are 31 and 6.2 mg L⁻¹, respectively, whereas CO₂ requirements correspond to 39 t d⁻¹. Table 4.30 presents average removal rates determined for nitrogen, phosphorus and carbon in the studied scenarios, as well as minimum required concentrations of these nutrients.

The proposed harvesting techniques for this study include a pre-concentration step through flocculation followed by centrifugation, where biomass concentration achieved was assumed to be 200 g L⁻¹ (Davis et al., 2011; Williams and Laurens, 2010). The use of a pre-concentration step aims the reduction of the flow rate to be processed in the centrifugation step, which may result in significant savings in terms of energy. In the pre-concentration step, flocculation may be induced by the addition of NaOH as flocculant. The amount of flocculant used was assumed to be 9 mg g⁻¹ of biomass, as reported by Vandamme (2013). In the last step, a harvesting efficiency of 95% was also assumed. Harvesting efficiencies higher than 94% were obtained in different studies, when applying centrifugation for the harvesting of microalgal biomass (Barros et al., 2015; Molina Grima et al., 2003). With this harvesting efficiency and the average biomass productivities, the flow rate of the output stream from the centrifugation step (S_{04}) corresponds to 78 m³ d⁻¹ (in the base scenario). Knowing the initial composition of the domestic wastewater, as well as elemental composition of microalgae and total biomass collected per harvesting, it is possible to determine effluent composition. Considering that all nitrogen and phosphorus removed from

Table 4.30. Average removal rates of nitrogen (R_N , in g m⁻² d⁻¹), phosphorus (R_P , in g m⁻² d⁻¹) and carbon (R_C , in g m⁻² d⁻¹) and minimum concentrations ($[N]$, $[P]$ and $[C]$, in mg L⁻¹ or t d⁻¹) required for microalgal cultivation in each of the studied scenarios (Sc)

Nutrients loads and removal rates	Sc1	Sc2	Sc3	Sc4	Sc5	Sc6	Sc7
R_N (g m ⁻² d ⁻¹)	1.1	0.54	1.6	1.1	1.1	1.1	1.1
$[N]$ (mg L ⁻¹)	31	15	46	31	31	31	31
R_P (g m ⁻² d ⁻¹)	0.22	0.11	0.33	0.22	0.22	0.22	0.22
$[P]$ (mg L ⁻¹)	6.2	3.1	9.2	6.2	6.2	6.2	6.2
R_C (g m ⁻² d ⁻¹)	8.4	4.2	13	8.4	8.4	8.4	8.4
$[C]$ (t d ⁻¹)	39	19	58	39	39	39	39

wastewater is incorporated into microalgal biomass, effluent composition in nitrogen and phosphorus (for the studied scenarios) range between 3.2-5.3 and 0.57-1.0 mg L⁻¹, respectively. Nitrogen and phosphorus concentrations in the resulting effluent are lower than the limits established by EU legislation for effluents' discharge (Directive 1991/271/EEC, 1991; Directive 1998/15/EC, 1998), which means that the proposed process promotes the efficient treatment of domestic wastewaters.

For cell disruption, PEF technology was proposed. It is a non-thermal method usually applied in food processing applications for inactivation of microbes, helping to maintain the food quality for human consumption (Guderjan et al., 2005; Taher et al., 2011). This technique uses short and high voltage pulses, which induce the non-thermal permeabilization of cell membranes and, in determined conditions, the complete disruption of cells into fragments. It is a rapid (treatment time is less than a second), flexible and energy-efficient method (heat is minimized) that avoids the use of organic solvents, usually toxic, thus not affecting the biochemical composition of microalgal biomass (Flisar et al., 2014; Guderjan et al., 2007; Guderjan et al., 2005). Regarding microalgal products, PEF is considered to have high potential for the extraction of different compounds, due to the low energy consumption, easy scale-up and low operational costs. This extraction method does not use any toxic extraction solvent (not requiring a solvent recovery step) and is highly effective when directly applied to wet feedstocks (Halim et al., 2012; Joannes et al., 2015; Taher et al., 2011). PEF has already been applied in lipids extraction from *C. vulgaris* (Flisar et al., 2014; Foltz, 2012; Luengo et al., 2014). It was considered a clean, cheap and quick extraction process, being a promising method for the production of biodiesel and pharmaceutical and dietary products. Considering the base scenario, presenting a PEF efficiency of 75% (Joannes et al., 2015), total microalgal oil extracted, with a density of 0.86 kg L⁻¹ (Schlagermann et al., 2012), corresponds to 3.4 m³ d⁻¹. For the other studied PEF efficiencies, 60 (scenario 4) and 90% (scenario 5), total lipids extracted are 2.7 and 5.1 m³ d⁻¹, respectively. For the base scenario, biodiesel production through transesterification of the extracted lipids results in a biodiesel productivity of 3.0 t d⁻¹ (3.47 m³ d⁻¹).

Biomass resulting from the oil extraction step (75 m³ d⁻¹ in the base scenario) is subjected to anaerobic digestion followed by electricity production. In this step different process efficiencies were evaluated (45, 30 and 60%). Taking into account the elemental

composition of microalgal biomass after lipids extraction and considering that CH₄ and CO₂ fractions in the biogas are 60 and 40% (v/v), respectively (typical composition of the biogas consists of 55-70% (v/v) CH₄ and 30-45% (v/v) CO₂ (Sialve et al., 2009)), resulting biogas stream in the base scenario has the following composition: 2.3 t d⁻¹ of CH₄ and 4.3 t d⁻¹ of CO₂. Additionally, a residue rich in nitrogen and phosphorus is produced (approximately 7.0 t d⁻¹ in the base scenario). Due to its high content in nitrogen, this residue can then be used as fertilizer.

Based on the results described in Section 4.3, the use of a CO₂ concentration of about 5% (v/v) was proposed, which has shown to be optimal for *C. vulgaris* growth. Taking into account the results obtained in this study, it is expected a slight decrease in the pH of the culture to about 6.5, which will not be harmful for microalgae. During this process different CO₂ streams are involved. Since CO₂ will be mainly supplied from the flue gas of a thermoelectric power plant working with natural gas, it is expected that this flue gas presents residual sulphite and nitrite concentrations. Accordingly, there is no need of a purification step prior to addition in the ponds. Considering a CO₂ uptake efficiency of 80%, net CO₂ balances were determined for the studied scenarios (Table 4.31). These results have shown negative values in all the scenarios (net CO₂ balances range between -30 and -10 t d⁻¹), which means that the proposed process is a net zero emission process able to efficiently uptake CO₂ from the flue gases of a thermoelectric power plant. Comparing the studied scenarios, it is possible to conclude that the most effective in CO₂ uptake is the scenario 3, the one assuming the highest photosynthetic efficiency (3%). In this scenario, annual CO₂ uptake corresponds to 1.1×10⁴ t.

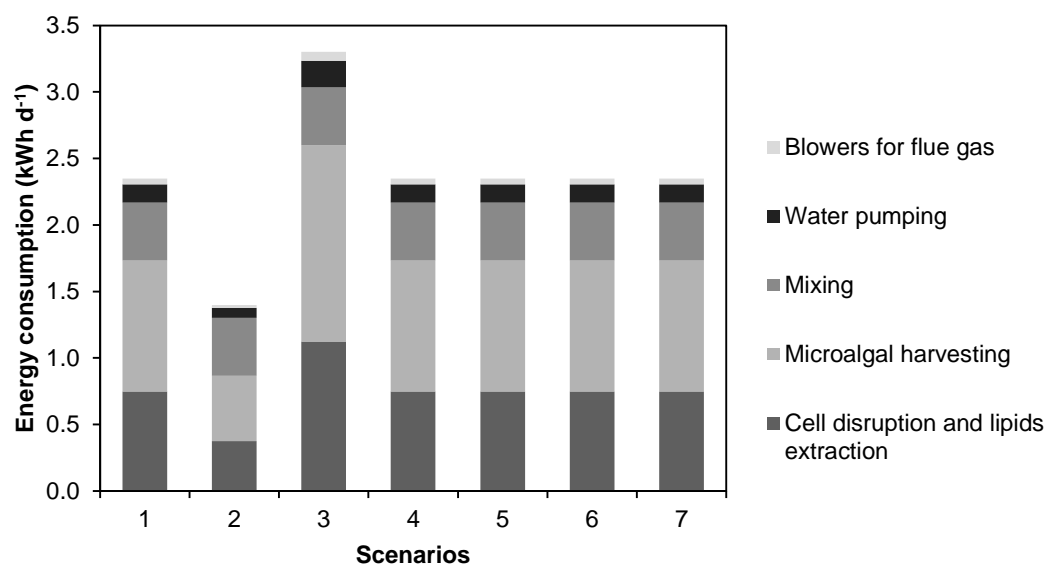
4.7.2. Energy balance

Figure 4.19 shows the electrical requirements determined for the microalgal facility in each of the studied scenarios. In the cultivation step, energy is required in three different stages: (i) mixing; (ii) water pumping; and (iii) blowers for flue gas. Considering a mixing velocity of 0.23 m s⁻¹ and all the head losses occurring in the open ponds (corresponding to head losses around the bends, 0.010 m, through the sumps, 0.026 m, and down the straightaways, 0.11 m), it is possible to determine the power required to overcome all these head losses. Assuming 25 similar open ponds operating at the same time, energy required daily can be determined. Therefore, assuming a 12:12 light:dark ratio, the total energy required for

Table 4.31. Net CO₂ balance determined for each of the studied scenarios (Sc)

CO ₂ streams	Sc1	Sc2	Sc3	Sc4	Sc5	Sc6	Sc7
CO ₂ required for microalgal growth (t d ⁻¹)	39	19	58	39	39	39	39
CO ₂ resulting from the anaerobic digestion step (t d ⁻¹)	4.3	2.1	6.4	4.5	4.1	2.9	5.7
CO ₂ resulting from CHP generation (t d ⁻¹)	6.4	3.2	9.7	6.7	6.1	4.3	8.6
Net CO₂ balance (t d⁻¹)	-20	-10	-30	-20	-21	-24	-17

CHP - Combined heat and power.

**Figure 4.19.** Electrical requirements determined for different steps of the microalgal facility in each of the studied scenarios.

mixing corresponds to 4.4×10^3 kWh d⁻¹. Energy needed in water pumping is calculated based on the flow rate of the input (S_{01}). Pump and motor efficiencies of 88 and 83% were assumed (Lundquist et al., 2010). Therefore, for the base scenario, a water input of 3.5×10^4 m³ d⁻¹ corresponds to an energy input of 1.4×10^3 kWh d⁻¹. Energy required for CO₂ distribution was determined based on the CO₂ requirements for each studied scenario (Table 4.31), assuming a CO₂ concentration in the flue gas of 5% (v/v). Additionally, air blower efficiency was considered to be 77% (The Engineering Toolbox, 2015). Accordingly, the energy consumption associated to the air blowers was 4.4×10^2 kWh d⁻¹ for the base scenario.

Energy consumption in biomass harvesting corresponds to the energy required for centrifugation. Accordingly, this value was determined taking into account the flow rate resulting from the pre-concentration step (S_{03}) and the specific energy consumption, 1.2 kWh m⁻³, already reported for microalgal harvesting through centrifugation (Tredici et

al., 2015). For the base scenario, the harvesting step presents a power consumption of $9.8 \times 10^3 \text{ kWh d}^{-1}$.

Regarding cell disruption and lipids extraction through the continuous PEF method, energy required was determined assuming the specific energy consumption reported by Flisar et al. (2014). According to the authors, the energy required to process 1 L of culture broth for 1 h is 14.4 kJ, which corresponds to 4.0 kWh m^{-3} . Taking into account the flow rates to be processed in this unit (S_{04}) for each scenario, energy consumption for this step was determined. For the base scenario, an energy input of $7.5 \times 10^3 \text{ kWh d}^{-1}$ is required.

Although energy is required in several processes, in the CHP generation unit there is an energetic output composed by electrical (40% of the total energy) and thermal energy (45% of the total energy) (Zamalloa et al., 2011). Assuming that the biogas produced by anaerobic digestion of microalgae presents a chemical composition similar to the one obtained from household waste, inferior and superior calorific power correspond to 6.0 and 6.6 kWh m^{-3} , respectively (Naskeo Environnement, 2009). With the flow rates resulting from the anaerobic digestion process (S_{07}) for each scenario and considering an average value between inferior and superior calorific power, values for electrical and thermal energy produced were determined. Considering the base scenario, total electrical and thermal energy produced is 1.4×10^4 and $1.6 \times 10^4 \text{ kWh d}^{-1}$, respectively.

Analysing the ratio between the energy produced by the microalgal facility (corresponding to the energy obtained from the extracted lipids and the one obtained in the CHP generation unit) and the total energy required, the EROEI was determined (Table 4.32). For all studied scenarios, EROEI was higher than one, which means that the studied scenarios are energetically efficient. With an EROEI of 3.0, the scenario 7, which assumes an anaerobic digestion efficiency of 60%, is the most efficient in terms of energy.

4.7.3. Economic assessment

This section presents a detailed economic analysis of the proposed system of wastewater treatment and energy production (electricity and biofuels) using microalgae.

Regarding fixed capital, acquisition costs were determined for almost all the equipment required in this process: high rate ponds, air blowers, clarifier, centrifuge, decanter, digester

Table 4.32. Net energy balance determined for each of the studied scenarios (Sc)

Energetic streams	Sc1	Sc2	Sc3	Sc4	Sc5	Sc6	Sc7
Energy required in microalgal cultivation ($\times 10^3$ kWh d ⁻¹)	6.2	5.3	7.0	6.2	6.2	6.2	6.2
Energy required in microalgal harvesting ($\times 10^3$ kWh d ⁻¹)	9.8	4.9	15	9.8	9.8	9.8	9.8
Energy required in cell disruption and lipids extraction ($\times 10^3$ kWh d ⁻¹)	7.5	3.7	11	7.5	7.5	7.5	7.5
Energy obtained from the extracted lipids ($\times 10^4$ kWh d ⁻¹)	3.0	1.5	4.6	2.4	3.6	3.0	3.0
Electrical energy produced in the CHP generation unit ($\times 10^4$ kWh d ⁻¹)	1.4	0.69	2.1	1.4	1.3	0.92	1.8
Thermal energy produced in the CHP generation unit ($\times 10^4$ kWh d ⁻¹)	1.6	0.77	2.3	1.6	1.5	1.0	2.1
EROEI	2.5	2.1	2.7	2.3	2.7	2.1	3.0

CHP - Combined heat and power; EROEI - energy returned on energy invested.

and CHP generation unit (Table 4.33). For the studied scenarios, total acquisition costs determined ranged between 5.7 and 6.3 million euros. This value was obtained assuming that the estimated costs account for 90% of total purchase costs. Including the cost factors associated to the direct and indirect costs in the calculus of fixed capital results in a total capital investment between 14.4 and 15.8 million euros (Table 4.34). For the studied scenarios, this value is mainly influenced by the expenses associated to the acquisition costs, followed by those associated to engineering and supervision, equipment installation, piping and service facilities.

Annual production costs are presented in detail in Table 4.35. In this case-study, it was assumed that raw materials costs were negligible because all the process requirements can be found in the local site of the facility: nutrients are supplied in the domestic wastewater that is daily fed into the algal ponds and CO₂ is obtained from flue gas emissions from the thermoelectric power plant located in Sines and from the anaerobic digestion and CHP generation processes. Regarding the utilities, electrical energy was considered the most important one. According to the energetic balance, total energy required oscillates between the studied scenarios, being 2.3×10^4 kWh d⁻¹ in the base scenario. Considering electricity costs of 0.10 € kWh⁻¹ (Peters and Timmerhaus, 1991), total annual costs for utilities are, for the base scenario, 857 thousand euros. Assuming NaOH requirements of 9 mg g⁻¹ of biomass and NaOH costs of 0.682 USD kg⁻¹ (OrbiChem, 2013), the costs associated to the pre-concentration step were determined. Production costs for PEF extraction were determined assuming lipids extraction costs of 10 € t⁻¹ (Pulsemaster, 2016). Operating labour costs were

Table 4.33. Total purchase costs (in k€) of the major equipment determined for each of the studied scenarios (Sc)

Equipments	Sc1	Sc2	Sc3	Sc4	Sc5	Sc6	Sc7
High rate ponds ^a	3,479	3,479	3,479	3,479	3,479	3,479	3,479
Air blowers ^b	123	62	185	123	123	123	123
Clarifier ^c	325	163	488	325	325	325	325
Centrifuge ^d	14	14	14	14	14	14	14
Decanter ^e	44	44	89	44	44	44	44
Digester and CHP generation unit ^f	1,399	1,399	1,399	1,399	1,399	1,399	1,399
Total	5,753	5,530	6,023	5,753	5,753	5,753	5,753
Total purchase costs	5,983	5,734	6,281	5,983	5,983	5,983	5,983

^a 34,000 USD per ha (2009), including the costs associated to paddlewheels and liners (Davis et al., 2011);

^b 2,500 € per 200 m³ h⁻¹ (2012) (Acién et al., 2012); ^c 948,000 USD per 23,200 m³ (2010) (Lundquist et al., 2010); ^d 4,500 USD per ha (1996) (Benemann and Oswald, 1996); ^e 45,000 € per 4 m³ h⁻¹ (2012) (Acién et al., 2012); ^f 10,000 USD per ha (1996) (Benemann and Oswald, 1996); CHP - combined heat and power.

Table 4.34. Estimation of the total capital (fixed capital) cost (in k€) for each of the studied scenarios (Sc)

Costs	Factor ^a	Sc1	Sc2	Sc3	Sc4	Sc5	Sc6	Sc7
Direct costs								
Total purchase costs	1.00	5,983	5,734	6,281	5,983	5,983	5,983	5,983
Purchased equipment installation	0.20	1,197	1,147	1,256	1,197	1,197	1,197	1,197
Instrumentation and control	0.15	897	860	942	897	897	897	897
Piping	0.20	1,197	1,147	1,256	1,197	1,197	1,197	1,197
Electrical	0.10	598	573	628	598	598	598	598
Buildings	0.15	897	860	942	897	897	897	897
Yard improvements	0.05	299	287	314	299	299	299	299
Service facilities	0.20	1,197	1,147	1,256	1,197	1,197	1,197	1,197
Indirect costs								
Engineering and supervision	0.30	1,795	1,720	1,884	1,795	1,795	1,795	1,795
Construction expenses	0.05	299	287	314	299	299	299	299
Contractor's fee	0.03	179	172	188	179	179	179	179
Contingency	0.08	479	459	502	479	479	479	479
Total capital cost		15,017	14,391	15,765	15,017	15,017	15,017	15,017

^a Fraction of the total purchased costs (Acién et al., 2012).

calculated assuming 10 operators with an average salary per month of 1000 €. The other parameters were determined basing on the percentages proposed by Sinnott and Towler (2009) and showed in Table 4.35. Resulting annual production costs are, for the base scenario, approximately 3.0 million euros.

Table 4.35. Estimation of the annual production costs (in k€) for each of the studied scenarios (Sc)

Costs	Sc1	Sc2	Sc3	Sc4	Sc5	Sc6	Sc7
Variable costs							
Raw materials	0	0	0	0	0	0	0
Miscellaneous materials ^a	75	74	77	75	75	75	75
Utilities	857	510	1,205	857	857	857	857
Pre-concentration with NaOH	35	17	52	35	35	35	35
PEF extraction	11	5	16	9	13	11	11
Shipping and packaging ^b	0	0	0	0	0	0	0
Fixed costs							
Maintenance ^c	751	738	766	751	751	751	751
Operating labour	120	120	120	120	120	120	120
Laboratory costs ^d	24	24	24	24	24	24	24
Supervision ^d	24	24	24	24	24	24	24
Plant overheads ^e	60	60	60	60	60	60	60
Insurance ^f	150	148	153	150	150	150	150
Local taxes ^g	300	295	306	300	300	300	300
Royalties ^f	150	148	153	150	150	150	150
Annual production costs	2,557	2,164	2,956	2,555	2,559	2,557	2,557

^a 10% of the maintenance costs; ^b usually negligible; ^c 5% of the total fixed capital; ^d 20% of the operating labour costs; ^e 50% of the operating labour costs; ^f 1% of the total fixed capital; ^g 2% of the total fixed capital; PEF - pulsed electric field.

Although the major aims of this process are the production of lipids and energy (both electrical and thermal), credits from wastewater treatment, CO₂ capture and from the production of a nitrogen-rich residue that can be used as fertilizer should also be considered.

Therefore, considering lipids sales of 1 € kg⁻¹, electricity and steam sales of 0.10 € kWh⁻¹ (Peters and Timmerhaus, 1991), a credit of 3.50 € kg⁻¹ of nitrogen removed and 2.40 € kg⁻¹ of phosphorus removed (Zamalloa et al., 2011), a credit of 30 € t⁻¹ of CO₂ captured (Declercq et al., 2011) and an income from fertilizers sales of 0.40 € kg⁻¹ (Acién et al., 2012), total annual revenues for the studied scenarios oscillate between 2.5 and 7.4 million euro (Figure 4.20), being the best scenario, the one assuming a photosynthetic efficiency of 3% (scenario 3), and the worst, the one considering a photosynthetic efficiency of 1% (scenario 2). The other studied scenarios have shown similar annual revenues of about 5.0 million euros.

Regarding economic viability, NPV and IRR were determined for the process here described, assuming a 10% interest rate and a 30-year bond to fund the facility construction (Table 4.36). According to these values, it is possible to state that the project is economically

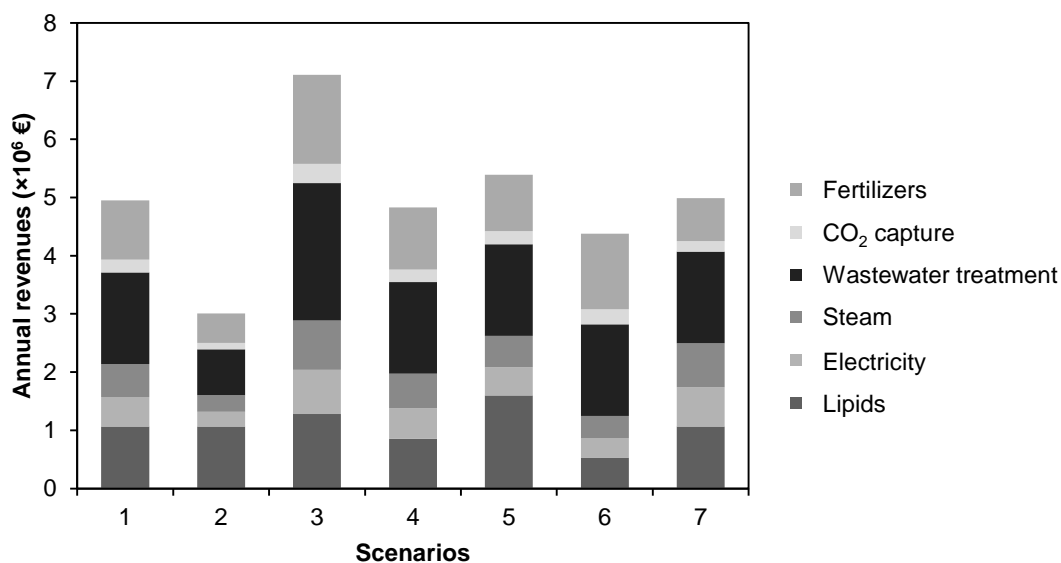


Figure 4.20. Annual revenues obtained in each of the studied scenarios.

Table 4.36. Economic viability of the proposed project for each of the studied scenarios (Sc)

Economic viability parameters	Sc1	Sc2	Sc3	Sc4	Sc5	Sc6	Sc7
Interest rate (%)	10	10	10	10	10	10	10
Lifetime (yr)	30	30	30	30	30	30	30
Net present value (k€)	5,287	-2,124	22,609	4,267	6,307	4,940	5,634
Internal rate of return (%)	14	n.a.	26	13	15	14	14
Payback time (yr)	8	n.a.	4	8	7	8	8

n.a. - Not applicable.

viable for all the studied scenarios, except in the scenario 2. According to Table 4.36, all studied scenarios except scenario 2 presented a positive NPV, ranging between 4.3 and 22.6 million euros. However, the best scenario is the third one, the one assuming a photosynthetic efficiency of 3%, since the IRR determined for this scenario (26%) is much higher than the assumed interest rate. Additionally, a payback time of about 4 years was determined for this scenario, whereas for the other studied scenarios payback times determined range between 7 and 8 years. These results indicate that at the end of the 30-year bond term, this project would be fully amortized and debt-free for all studied scenarios, except scenario 2.

Taking into account the NPV obtained in each of the studied scenarios, a sensitivity analysis was performed to evaluate which are the crucial conditions for an economically viable process (Figure 4.21). Analysis of Figure 4.21 shows that photosynthetic efficiency is the

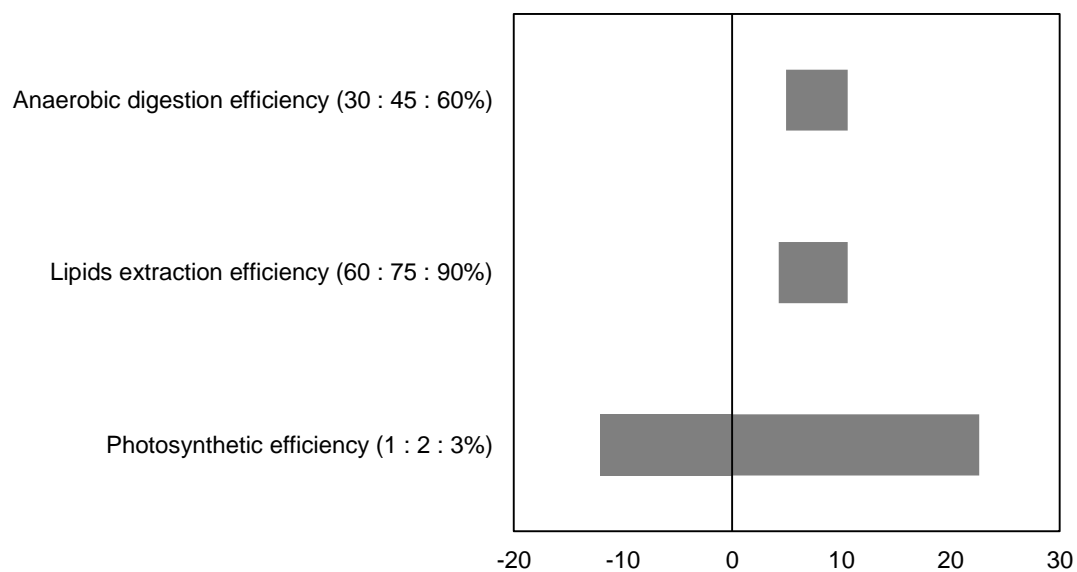


Figure 4.21. Sensitivity analysis of the process considering the different studied scenarios.

most important factor influencing NPV: for photosynthetic efficiencies ranging between 1 and 3%, NPV oscillates between -12.1 and 22.6 million euros.

5. Concluding remarks and future work

5.1. General conclusions

The aim of this work was to develop a microalgal production system able to achieve increased biomass productivities and effectively uptake CO₂ and nutrients from the culture medium. To achieve these goals, different microalgae and cyanobacteria were selected: *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa*. Additionally, different approaches were followed.

In a first approach, different cultivation parameters (light, temperature and CO₂ concentration in the air stream) were evaluated to determine optimal growth conditions for the selected microorganisms. Regarding the effect of light supply on microalgal cultures, higher light irradiance values and light periods resulted in higher average biomass productivities and CO₂ uptake rates. Furthermore, results have shown that *C. vulgaris*, *S. salina* and *M. aeruginosa* presented the highest average biomass productivities and CO₂ uptake rates: average biomass productivities determined for these microorganisms ranged between 107±5 and 133±13 mg DW L⁻¹ d⁻¹, whereas CO₂ uptake rates ranged between 162±8 and 195±19 mg CO₂ L⁻¹ d⁻¹. In terms of nitrogen removal efficiencies, all microalgal strains showed high removal efficiencies, close to 100%, especially when cultured under higher light irradiance values and light:dark ratios. Phosphorus removal increased with light irradiance and with light:dark ratio. The highest removal efficiency, 67.6±7.1%, was achieved by the microalga *C. vulgaris*. The study of the combined effect of light and temperature on microalgal growth, CO₂ capture and nutrients removal has shown that these processes are favoured by increased light supplies. Additionally, it was observed that the studied microorganisms presented higher photosynthetic activity (higher biomass

productivities, CO₂ uptake rates and nitrogen and phosphorus removal efficiencies) at 25 °C. Among the studied microorganisms, *C. vulgaris*, *S. salina* and *M. aeruginosa* have shown to be the most effective in biomass production. Development of a mathematical model able to describe the behaviour of specific growth rates in response to average daily light irradiance and temperature allowed the determination of optimal light and temperature conditions for the selected microorganisms. Regarding temperature, the optimal value determined was 25.3±1.1 °C. On the other hand, optimal average daily light irradiance varied with the species, being 208, 258, 178 and 140 µE m⁻² s⁻¹ for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa*, respectively. Another parameter evaluated in this work was CO₂ concentration in the air stream. For this, microalgal cultures were aerated with an air stream presenting CO₂ concentrations ranging from the one commonly present in atmospheric air (≈0.04% v/v) to 10% (v/v). The results have shown that *P. subcapitata* presented the lowest growth and nutrients removal kinetics, as well as the lowest CO₂ fixation rates. Regarding the other studied microorganisms, increasing CO₂ concentrations up to 5% (v/v) has resulted in increased specific growth rates, followed by a decrease observed for higher CO₂ concentrations. This behaviour was correctly described by a mathematical model developed in this study. With this model, optimal CO₂ concentrations were determined: 5.35, 4.87, 5.55 and 5.62% (v/v) for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa*, respectively. Average biomass productivities and CO₂ fixation rates have shown a similar behaviour to the one reported for specific growth rates, with minimum values determined for cultures grown with non-enriched air streams. Nitrogen and phosphorus removal efficiencies were also higher in cultures performed with CO₂-enriched air streams, reaching values very close to 100%.

In a second approach, different consortia were established to evaluate their potential in biomass production, CO₂ capture and nutrients removal. Microalgal and microalgal-bacterial consortia were evaluated. In the study regarding microalgal consortia, dual-species cultures constituted by the above referred microorganisms (*C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa*) were performed. This study has demonstrated that microalgal consortia constituted by *S. salina* have favoured microalgal growth, CO₂ uptake rates and nutrients uptake. Average biomass productivities determined for the consortia SC, SP and SM were 51, 35 and 41% higher than those determined for the single cultures of *C. vulgaris* (47.0±3.1 mg DW L⁻¹ d⁻¹), *P. subcapitata* (41.2±9.2 mg DW L⁻¹ d⁻¹) and *M. aeruginosa* (49.9±3.1 mg DW L⁻¹ d⁻¹), respectively. Higher CO₂ uptake rates were also obtained for the

referred consortia (between 111 ± 7 and 150 ± 13 mg CO₂ L⁻¹ d⁻¹). Additionally, the three studied consortia have effectively removed nitrogen and phosphorus from the culture medium, reaching the limit concentrations established by EU legislation. The study involving microalgal-bacterial consortia was performed using dual-species cultures of *C. vulgaris* and activated sludge native bacteria. This work has shown that consortia of *C. vulgaris* with selected bacterial isolates from a MWTP resulted in a synergistic relationship between these microorganisms, increasing biomass production and nutrients removal. After seven days of culturing, cell concentration of *C. vulgaris* in the CE and CR consortia was, respectively, 58 and 42% higher than the one determined for the single *C. vulgaris* culture: $(8.27\pm0.79)\times10^6$ cells mL⁻¹. The CE and CR consortia were also effective in CO₂ capture, reaching CO₂ uptake rates of 63.8 ± 3.2 and 52.9 ± 5.1 mg CO₂ L⁻¹ d⁻¹, respectively. Additionally, the three studied consortia have contributed to the increase observed in nutrients (nitrogen, phosphorus and organic carbon) uptake rates, resulting in a significant reduction in the time required for the achievement of EU legislation limits. In the studied consortia, the time required by the single *C. vulgaris* culture to achieve the established limits was reduced to, at least, half of its value. Therefore, it can be concluded that the studied consortia (CE, CK and CR) can be a promising alternative in wastewater polishing.

The scale-up of microalgal cultures was performed in a 60-L open raceway pond. The single *C. vulgaris* culture and the SC and CE consortia were cultured in indoor and outdoor conditions. The comparison between these three systems was performed indoor, under an average daily light irradiance of 23 ± 2 $\mu\text{E m}^{-2} \text{ s}^{-1}$. Biomass productivities and nutrients removal efficiencies were very low. However, *C. vulgaris* growth in this system, but with natural light conditions, has confirmed the potential of this microalga for biomass production, CO₂ uptake and nitrogen and phosphorus removal from the culture medium. Average biomass productivity determined for *C. vulgaris* grown with an average daily light irradiance of $204 \mu\text{E m}^{-2} \text{ s}^{-1}$ was 29.1 ± 0.4 mg DW L⁻¹ d⁻¹, whereas average biomass productivity determined for *C. vulgaris* grown indoor was 18.0 ± 2.0 mg DW L⁻¹ d⁻¹. Similarly, CO₂ uptake rates determined for this microalga with an average daily light irradiance of $204 \mu\text{E m}^{-2} \text{ s}^{-1}$ was 46.6 ± 0.6 mg CO₂ L⁻¹ d⁻¹, whereas the same value determined for *C. vulgaris* grown indoor was 29.2 ± 3.3 mg CO₂ L⁻¹ d⁻¹. Finally, *C. vulgaris* growth in outdoor conditions allowed the achievement of the limits established by EU legislation for nitrogen and phosphorus concentrations in discharged effluents.

Taking into account the preliminary results obtained using the open raceway pond in outdoor conditions and the results obtained in the experiments conducted at laboratory-scale, a TEA of microalgal biomass production with CO₂ capture and nutrients removal from wastewaters was performed. This assessment has resulted in the proposal of an economically viable process of microalgal production in Portugal concerning wastewater treatment, CO₂ emission saving and bioenergy production purposes. For this process, seven scenarios were considered, assuming different efficiencies in some of the most important steps of microalgal processing. From the considered scenarios, six were economically viable. From those, the one assuming a photosynthetic efficiency of 3%, a lipids extraction efficiency of 75% and an anaerobic digestion efficiency of 45% (scenario 3) was considered the most effective one in terms of: (i) CO₂ uptake (1.1×10^4 t yr⁻¹); (ii) energy production (annual energy produced was 1.6×10^7 kWh and annual lipids productivity was 1.9×10^3 m³); and (iii) economic viability (NPV of 22.6 million euros with an IRR of 26% and a payback time of 4 years). In addition, since this project assume the use of domestic wastewater as culture medium, this scenario is also effective in nitrogen and phosphorus removal, processing 1.9×10^7 m³ of wastewater per year.

In summary, this work proposes a microalgal production system for biomass production, CO₂ uptake and nutrients removal from the culture medium. From the obtained results, the best candidates for these applications include *C. vulgaris*, *S. salina* and *M. aeruginosa*. The microalgal consortium SC and the microalgal-bacterial consortium CE have also demonstrated to be attractive alternatives for these purposes. Taking into account the most appropriate cultivation conditions, the results have shown that (i) optimal average daily light irradiance for these microorganisms ranges between 140 and 208 $\mu\text{E m}^{-2} \text{ s}^{-1}$; optimal temperature corresponds to 25.3 ± 1.1 °C; and (iii) optimal CO₂ concentration in the air stream ranges between 5.35 and 5.62% (v/v). To increase the applicability of this system to a pilot-scale unit, an open raceway pond was selected because of its simplicity and reduced operational costs. Finally, these assumptions have resulted in the development of an economically viable project of microalgal biomass production for CO₂ capture, nutrients uptake and bioenergy production.

5.3. Future work

This study has demonstrated the huge potential of microalgae (in single and dual-species cultures) in CO₂ capture and nutrients removal from the culture medium. Optimal growth conditions (in terms of light supply, temperature and CO₂ concentration in the air stream) were determined at laboratory-scale. Additionally, microalgal and microalgal-bacterial consortia have been proposed as effective remediation systems. Although promising results were obtained in this study, their validation in a larger scale would be very important to confirm the suitability of the proposed systems in CO₂ capture and nutrients removal.

Regarding the use of the open raceway pond, a semi-continuous operation mode should be adopted to promote the harvesting of microalgal biomass and the recirculation of the culture medium, so that a complete nutrients removal can be achieved. More experiments in outdoor conditions and in different seasons of the year should be performed using both single cultures and the most effective consortia. These results would be interesting to evaluate if the proposed systems are robust enough to accomplish the remediation goals under real environmental conditions. Additionally, to validate the results obtained through mathematical modelling, it would be interesting to conduct the experiments in the open raceway reactor in the optimal growth conditions determined.

Besides the operation under real environmental conditions, the use of real liquid and gaseous effluents should be evaluated to infer about their toxicity to microalgal cultures and to optimize the operation mode, hydraulic residence times and the optimal cultivation conditions required for an effective uptake of CO₂ and nutrients.

Finally, different PBR designs should be simulated using mechanistic/hybrid models and Computational Fluid Dynamics (CFD) tools. These models can be very useful in the optimization of process conditions and operating strategies during process development. CFD has been frequently applied to study the flow dynamics, the concentration of species, temperature and pressure inside PBRs, providing the rapid determination of critical zones (regions that have adverse conditions for microalgal growth). Additionally, CFD tools should be used to study the light and gas distribution in PBRs, important challenges in PBR design.

References

- Acién, F., Fernández, J., Magán, J., Molina, E., 2012. Production cost of a real microalgae production plant and strategies to reduce it. *Biotechnology Advances* 30, 1344-1353.
- Alcántara, C., Domínguez, J.M., García, D., Blanco, S., Pérez, R., García-Encina, P.A., Muñoz, R., 2015a. Evaluation of wastewater treatment in a novel anoxic-aerobic algal-bacterial photobioreactor with biomass recycling through carbon and nitrogen mass balances. *Bioresource Technology* 191, 173-186.
- Alcántara, C., Fernández, C., García-Encina, P.A., Muñoz, R., 2015b. Mixotrophic metabolism of *Chlorella sorokiniana* and algal-bacterial consortia under extended dark-light periods and nutrient starvation. *Applied Microbiology and Biotechnology* 99, 2393-2404.
- Aleya, L., Dauta, A., Reynolds, C.S., 2011. Endogenous regulation of the growth-rate responses of a spring-dwelling strain of the freshwater alga, *Chlorella minutissima*, to light and temperature. *European Journal of Protistology* 47, 239-244.
- Allen, M.R., Frame, D.J., Huntingford, C., Jones, C.D., Lowe, J.A., Meinshausen, M., Meinshausen, N., 2009. Warming caused by cumulative carbon emissions towards the trillionth tonne. *Nature* 458, 1163-1166.
- Anjos, M., Fernandes, B.D., Vicente, A.A., Teixeira, J.A., Dragone, G., 2013. Optimization of CO₂ bio-mitigation by *Chlorella vulgaris*. *Bioresource Technology* 139, 149-154.
- APHA, 1999. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington DC, USA.
- Araújo, S.C., Garcia, V.M.T., 2005. Growth and biochemical composition of the diatom *Chaetoceros* cf. *wighamii* brightwell under different temperature, salinity and carbon dioxide levels. I. Protein, carbohydrates and lipids. *Aquaculture* 246, 405-412.
- Arbib, Z., Ruiz, J., Álvarez-Díaz, P., Garrido-Perez, C., Perales, J.A., 2014. Capability of different microalgae species for phytoremediation processes: wastewater tertiary treatment, CO₂ bio-fixation and low cost biofuels production. *Water Research* 49, 465-474.
- Aslan, S., Kapdan, I.K., 2006. Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. *Ecological Engineering* 28, 64-70.

- Barros, A.I., Gonçalves, A.L., Simões, M., Pires, J.C.M., 2015. Harvesting techniques applied to microalgae: a review. *Renewable and Sustainable Energy Reviews* 41, 1489-1500.
- Barsanti, L., Gualtieri, P., 2006. *Algae - anatomy, biochemistry and biotechnology*, 2nd ed. CRC Press, USA, pp. 162-168.
- Bashan, Y., Holguin, G., Lifshitz, R., 1993. Isolation and characterization of plant growth-promoting rhizobacteria, in: Glick, B.R., Thompson, J.E. (Eds.), *Methods in plant molecular biology and biotechnology*. CRC Press, Boca Raton, USA, pp. 331-345.
- Belkin, S., Boussiba, S., 1991. Resistance of *Spirulina platensis* to ammonia at high pH values. *Plant and Cell Physiology* 32, 953-958.
- Benemann, J.R., Oswald, W.J., 1996. Systems and economic analysis of microalgae ponds for conversion of CO₂ to biomass - final report. Department of Civil Engineering of the California University, Berkeley, USA.
- Benemann, J.R., Tillett, D.M., Weissman, J.C., 1987. Microalgae biotechnology. *Trends in Biotechnology* 5, 47-53.
- Bernard, O., Rémond, B., 2012. Validation of a simple model accounting for light and temperature effect on microalgal growth. *Bioresource Technology* 123, 520-527.
- Bezerra, M.A., Santelli, R.E., Oliveira, E.P., Villar, L.S., Escalera, L.A., 2008. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta* 76, 965-977.
- Bhola, V., Desikan, R., Santosh, S.K., Subburamu, K., Sanniyasi, E., Bux, F., 2011. Effects of parameters affecting biomass yield and thermal behaviour of *Chlorella vulgaris*. *Journal of Bioscience and Bioengineering* 111, 377-382.
- Boelee, N., Temmink, H., Janssen, M., Buisman, C., Wijffels, R., 2011. Nitrogen and phosphorus removal from municipal wastewater effluent using microalgal biofilms. *Water Research* 45, 5925-5933.
- Boonma, S., Chaiklangmuang, S., Chaiwongsar, S., Pekkoh, J., Pumas, C., Ungsethaphand, T., Tongsir, S., Peerapornpisal, Y., 2014. Enhanced carbon dioxide fixation and bio-oil production of a microalgal consortium. *CLEAN – Soil, Air, Water* 43, 761-766.

- Bordel, S., Guieysse, B., Muñoz, R., 2009. Mechanistic model for the reclamation of industrial wastewaters using algal-bacterial photobioreactors. *Environmental Science & Technology* 43, 3200-3207.
- Borowitzka, M.A., 1999. Commercial production of microalgae: ponds, tanks, and fermenters. *Progress in Industrial Microbiology* 35, 313-321.
- Bosca, C., Dauta, A., Marvalin, O., 1991. Intensive outdoor algal cultures: how mixing enhances the photosynthetic production rate. *Bioresource Technology* 38, 185-188.
- Bouterfas, R., Belkoura, M., Dauta, A., 2002. Light and temperature effects on the growth rate of three freshwater algae isolated from a eutrophic lake. *Hydrobiologia* 489, 207-217.
- Bouterfas, R., Belkoura, M., Dauta, A., 2006. The effects of irradiance and photoperiod on the growth rate of three freshwater green algae isolated from a eutrophic lake. *Limnetica* 25, 647-656.
- Brennan, L., Owende, P., 2010. Biofuels from microalgae - a review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable & Sustainable Energy Reviews* 14, 557-577.
- Burkhardt, S., Zondervan, I., Riebesell, U., 1999. Effect of CO₂ concentration on C:N:P ratio in marine phytoplankton: a species comparison. *Limnology and Oceanography* 44, 683-690.
- Cai, T., Park, S.Y., Li, Y., 2013. Nutrient recovery from wastewater streams by microalgae: status and prospects. *Renewable & Sustainable Energy Reviews* 19, 360-369.
- Caporgno, M.P., Taleb, A., Olkiewicz, M., Font, J., Pruvost, J., Legrand, J., Bengoa, C., 2015. Microalgae cultivation in urban wastewater: nutrient removal and biomass production for biodiesel and methane. *Algal Research* 10, 232-239.
- Çelekli, A., Balcı, M., Bozkurt, H., 2008. Modelling of *Scenedesmus obliquus*; function of nutrients with modified Gompertz model. *Bioresource Technology* 99, 8742-8747.
- Çelekli, A., Yavuzatmaca, M., Bozkurt, H., 2009. Modeling of biomass production by *Spirulina platensis* as function of phosphate concentrations and pH regimes. *Bioresource Technology* 100, 3625-3629.

- Cellamare, M., Rolland, A., Jacquet, S., 2010. Flow cytometry sorting of freshwater phytoplankton. *Journal of Applied Phycology* 22, 87-100.
- Cembella, A.D., 2003. Chemical ecology of eukaryotic microalgae in marine ecosystems. *Phycologia* 42, 420-447.
- Chazarra, A., Barceló, A.M., Pires, V., Cunha, S., Mendes, M., Neto, J., 2001. Iberian climate atlas - air temperature and precipitation (1971-2000). AEMET-IM - Agencia Estatal de Meteorología de España and Instituto de Meteorologia de Portugal, Madrid, Spain.
- Cheah, W.Y., Show, P.L., Chang, J.-S., Ling, T.C., Juan, J.C., 2015. Biosequestration of atmospheric CO₂ and flue gas-containing CO₂ by microalgae. *Bioresource Technology* 184, 190-201.
- Chen, Z., Zhang, X., Jiang, Z., Chen, X., He, H., Zhang, X., 2016. Light/dark cycle of microalgae cells in raceway ponds: effects of paddlewheel rotational speeds and baffles installation. *Bioresource Technology* 219, 387-391.
- Cheng, J., Huang, Y., Feng, J., Sun, J., Zhou, J., Cen, K., 2013. Improving CO₂ fixation efficiency by optimizing *Chlorella* PY-ZU1 culture conditions in sequential bioreactors. *Bioresource Technology* 144, 321-327.
- Cheng, L., Zhang, L., Chen, H., Gao, C., 2006. Carbon dioxide removal from air by microalgae cultured in a membrane-photobioreactor. *Separation and purification technology* 50, 324-329.
- Chiang, C.-L., Lee, C.-M., Chen, P.-C., 2011. Utilization of the cyanobacteria *Anabaena* sp. CH1 in biological carbon dioxide mitigation processes. *Bioresource Technology* 102, 5400-5405.
- Chinnasamy, S., Bhatnagar, A., Hunt, R.W., Das, K., 2010. Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. *Bioresource Technology* 101, 3097-3105.
- Chinnasamy, S., Ramakrishnan, B., Bhatnagar, A., Das, K.C., 2009. Biomass production potential of a wastewater alga *Chlorella vulgaris* ARC 1 under elevated levels of CO₂ and temperature. *International Journal of Molecular Sciences* 10, 518-532.
- Chisti, Y., 2007. Biodiesel from microalgae. *Biotechnology Advances* 25, 294-306.

- Chiu, S.-Y., Kao, C.-Y., Chen, C.-H., Kuan, T.-C., Ong, S.-C., Lin, C.-S., 2008. Reduction of CO₂ by a high-density culture of *Chlorella* sp. in a semicontinuous photobioreactor. *Bioresource Technology* 99, 3389-3396.
- Chiu, S.-Y., Kao, C.-Y., Huang, T.-T., Lin, C.-J., Ong, S.-C., Chen, C.-D., Chang, J.-S., Lin, C.-S., 2011. Microalgal biomass production and on-site bioremediation of carbon dioxide, nitrogen oxide and sulfur dioxide from flue gas using *Chlorella* sp. cultures. *Bioresource Technology* 102, 9135-9142.
- Chiu, S.-Y., Kao, C.-Y., Tsai, M.-T., Ong, S.-C., Chen, C.-H., Lin, C.-S., 2009. Lipid accumulation and CO₂ utilization of *Nannochloropsis oculata* in response to CO₂ aeration. *Bioresource Technology* 100, 833-838.
- Cho, S.H., Ji, S.-C., Hur, S.B., Bae, J., Park, I.-S., Song, Y.-C., 2007. Optimum temperature and salinity conditions for growth of green algae *Chlorella ellipsoidea* and *Nannochloris oculata*. *Fisheries Science* 73, 1050-1056.
- Chojnacka, K., Chojnacki, A., Górecka, H., 2004. Trace element removal by *Spirulina* sp. from copper smelter and refinery effluents. *Hydrometallurgy* 73, 147-153.
- Christenson, L., Sims, R., 2011. Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. *Biotechnology Advances* 29, 686-702.
- Claquin, P., Probert, I., Lefebvre, S., Veron, B., 2008. Effects of temperature on photosynthetic parameters and TEP production in eight species of marine microalgae. *Aquatic Microbial Ecology* 51, 1-11.
- Craggs, R., Sutherland, D., Campbell, H., 2012. Hectare-scale demonstration of high rate algal ponds for enhanced wastewater treatment and biofuel production. *Journal of Applied Phycology* 24, 329-337.
- Craggs, R.J., Lundquist, T.J., Benemann, J.R., 2013. Wastewater treatment and algal biofuel production, in: Borowitzka, M.A., Moheimani, N.R. (Eds.), *Algae for Biofuels and Energy*. Springer Netherlands, The Netherlands, pp. 153-163.
- Crofcheck, C.L., Xinyi, E., Shea, A.P., Monstross, M., Crocker, M., Andrews, R., 2012. Influence of media composition on the growth rate of *Chlorella vulgaris* and *Scenedesmus acutus* utilized for CO₂ mitigation. *Journal of Biochemical Technology* 4, 589-594.

- Cuellar-Bermudez, S.P., Garcia-Perez, J.S., Rittmann, B.E., Parra-Saldivar, R., 2015. Photosynthetic bioenergy utilizing CO₂: an approach on flue gases utilization for third generation biofuels. *Journal of Cleaner Production* 98, 53-65.
- Dauta, A., Devaux, J., Piquemal, F., Boumnich, L., 1990. Growth rate of four freshwater algae in relation to light and temperature. *Hydrobiologia* 207, 221-226.
- Davis, R., Aden, A., Pienkos, P.T., 2011. Techno-economic analysis of autotrophic microalgae for fuel production. *Applied Energy* 88, 3524-3531.
- Davison, I.R., 1991. Environmental effects on algal photosynthesis: temperature. *Journal of Phycology* 27, 2-8.
- De-Bashan, L.E., Bashan, Y., 2010. Immobilized microalgae for removing pollutants: review of practical aspects. *Bioresource Technology* 101, 1611-1627.
- De-Bashan, L.E., Hernandez, J.-P., Morey, T., Bashan, Y., 2004. Microalgae growth-promoting bacteria as “helpers” for microalgae: a novel approach for removing ammonium and phosphorus from municipal wastewater. *Water Research* 38, 466-474.
- de Godos, I., González, C., Becares, E., García-Encina, P.A., Muñoz, R., 2009. Simultaneous nutrients and carbon removal during pretreated swine slurry degradation in a tubular biofilm photobioreactor. *Applied Microbiology and Biotechnology* 82, 187-194.
- de Godos, I., Mendoza, J., Acien, F., Molina, E., Banks, C., Heaven, S., Rogalla, F., 2014. Evaluation of carbon dioxide mass transfer in raceway reactors for microalgae culture using flue gases. *Bioresource Technology* 153, 307-314.
- de Morais, M.G., Costa, J.A.V., 2007a. Biofixation of carbon dioxide by *Spirulina* sp. and *Scenedesmus obliquus* cultivated in a three-stage serial tubular photobioreactor. *Journal of Biotechnology* 129, 439-445.
- de Morais, M.G., Costa, J.A.V., 2007b. Carbon dioxide fixation by *Chlorella kessleri*, *C. vulgaris*, *Scenedesmus obliquus* and *Spirulina* sp. cultivated in flasks and vertical tubular photobioreactors. *Biotechnology Letters* 29, 1349-1352.
- de Morais, M.G., Costa, J.A.V., 2007c. Isolation and selection of microalgae from coal fired thermoelectric power plant for biofixation of carbon dioxide. *Energy Conversion and Management* 48, 2169-2173.

- de Schryver, P., Crab, R., Defoirdt, T., Boon, N., Verstraete, W., 2008. The basics of bio-flocs technology: the added value for aquaculture. *Aquaculture* 277, 125-137.
- Decision 406/2009/EC, 2009. Decision of the European Parliament and European Council of 23 April 2009 on the effort of Member States to reduce their greenhouse gas emissions to meet the Community's greenhouse gas emission reduction commitments up to 2020, *Official Journal of the European Union* L 140/136.
- Declercq, B., Delarue, E., D'haeseleer, W., 2011. Impact of the economic recession on the European power sector's CO₂ emissions. *Energy Policy* 39, 1677-1686.
- Degen, J., Uebele, A., Retze, A., Schmid-Staiger, U., Trösch, W., 2001. A novel airlift photobioreactor with baffles for improved light utilization through the flashing light effect. *Journal of Biotechnology* 92, 89-94.
- Directive 1991/271/EEC, 1991. Directive of the European Council of 21 May 1991 concerning urban waste-water treatment, *Official Journal of the European Union* L135, 40-52.
- Directive 1998/15/EC, 1998. Directive of the European Commission of 27 February 1998 amending Council Directive 91/271/EEC with respect to certain requirements established in Annex I thereof, *Official Journal of the European Union* L67, 29-30.
- Doucha, J., Lívanský, K., 1995. Novel outdoor thin-layer high density microalgal culture system: productivity and operational parameters. *Algological Studies* 76, 129-147.
- Doucha, J., Straka, F., Lívanský, K., 2005. Utilization of flue gas for cultivation of microalgae (*Chlorella* sp.) in an outdoor open thin-layer photobioreactor. *Journal of Applied Phycology* 17, 403-412.
- Droop, M.R., 1968. Vitamin B₁₂ and marine ecology. IV. The kinetics of uptake, growth and inhibition in *Monochrysis lutheri*. *Journal of the Marine Biological Association of the United Kingdom* 48, 689-733.
- EC. 2008. Communication from the European Commission - Energy efficiency: delivering the 20% target, European Commission. Brussels.
- Eriksen, N., 2008. The technology of microalgal culturing. *Biotechnology Letters* 30, 1525-1536.

- Eroglu, E., Smith, S.M., Raston, C.L., 2015. Application of various immobilization techniques for algal bioprocesses, in: Moheimani, N.R., McHenry, M.P., de Boer, K., Bahri, P.A. (Eds.), *Biomass and Biofuels from Microalgae*. Springer International Publishing, Switzerland.
- Fagerbakke, K.M., Heldal, M., Norland, S., 1996. Content of carbon, nitrogen, oxygen, sulfur and phosphorus in native aquatic and cultured bacteria. *Aquatic Microbial Ecology* 10, 15-27.
- Falkowski, P.G., Raven, J.A., 2007. *Aquatic photosynthesis*, 2nd ed. Princeton University Press, Princeton, USA, pp. 5-21.
- Feng, P., Deng, Z., Fan, L., Hu, Z., 2012. Lipid accumulation and growth characteristics of *Chlorella zofingiensis* under different nitrate and phosphate concentrations. *Journal of Bioscience and Bioengineering* 114, 405-410.
- Fergola, P., Cerasuolo, M., Pollio, A., Pinto, G., DellaGreca, M., 2007. Allelopathy and competition between *Chlorella vulgaris* and *Pseudokirchneriella subcapitata*: experiments and mathematical model. *Ecological Modelling* 208, 205-214.
- Ferreira, C., Pereira, A., Pereira, M., Melo, L., Simões, M., 2011. Physiological changes induced by the quaternary ammonium compound benzyldimethyldodecylammonium chloride on *Pseudomonas fluorescens*. *Journal of Antimicrobial Chemotherapy* 66, 1036-1043.
- Flisar, K., Meglic, S.H., Morelj, J., Golob, J., Miklavcic, D., 2014. Testing a prototype pulse generator for a continuous flow system and its use for *E. coli* inactivation and microalgae lipid extraction. *Bioelectrochemistry* 100, 44-51.
- Foess, G.W., Steinbrecher, P., Williams, K., Garrett, G.S., 1998. Cost and performance evaluation of BNR processes. *Florida Water Resources Journal* 1, 11-13.
- Foltz, G., 2012. Algae lysis with pulsed electric fields. Dissertation for obtention of the Master Degree in Electrical Engineering, California State Polytechnic University, San Luis Obispo, California.
- Fouilland, E., 2012. Biodiversity as a tool for waste phycoremediation and biomass production. *Reviews in Environmental Science and Bio/Technology* 11, 1-4.
- Fu, F.-X., Warner, M.E., Zhang, Y., Feng, Y., Hutchins, D.A., 2007. Effects of increased temperature and CO₂ on photosynthesis, growth, and elemental ratios in marine

- Synechococcus* and *Prochlorococcus* (cyanobacteria). *Journal of Phycology* 43, 485-496.
- Fukami, K., Nishijima, T., Ishida, Y., 1997. Stimulative and inhibitory effects of bacteria on the growth of microalgae. *Hydrobiologia* 358, 185-191.
- Fulazzaky, M.A., Abdullah, N.H., Yusoff, A.R.M., Paul, E., 2015. Conditioning the alternating aerobic-anoxic process to enhance the removal of inorganic nitrogen pollution from a municipal wastewater in France. *Journal of Cleaner Production* 100, 195-201.
- Fulke, A.B., Mudliar, S., Yadav, R., Shekh, A., Srinivasan, N., Ramanan, R., Krishnamurthi, K., Devi, S.S., Chakrabarti, T., 2010. Bio-mitigation of CO₂, calcite formation and simultaneous biodiesel precursors production using *Chlorella* sp. *Bioresource Technology* 101, 8473-8476.
- García-González, M., Moreno, J., Cañavate, J.P., Anguis, V., Prieto, A., Manzano, C., Florencio, F.J., Guerrero, M.G., 2003. Conditions for open-air outdoor culture of *Dunaliella salina* in southern Spain. *Journal of Applied Phycology* 15, 177-184.
- Ge, Y., Liu, J., Tian, G., 2011. Growth characteristics of *Botryococcus braunii* 765 under high CO₂ concentration in photobioreactor. *Bioresource Technology* 102, 130-134.
- Gibbins, J., Chalmers, H., 2008. Carbon capture and storage. *Energy Policy* 36, 4317-4322.
- Giordano, M., Ratti, S., 2013. The biomass quality of algae used for CO₂ sequestration is highly species-specific and may vary over time. *Journal of Applied Phycology* 25, 1431-1434.
- Glass, A.D.M., 1983. Regulation of Ion Transport. *Annual Reviews of Plant Physiology* 34, 311-326.
- Goldman, J.C., 1977. Temperature effects on phytoplankton growth in continuous culture. *Limnology and Oceanography* 22, 932-936.
- Gómez-Serrano, C., Morales-Amaral, M., Acién, F., Escudero, R., Fernández-Sevilla, J., Molina-Grima, E., 2015. Utilization of secondary-treated wastewater for the production of freshwater microalgae. *Applied Microbiology and Biotechnology* 99, 6931-6944.

- González-Fernández, C., Ballesteros, M., 2012. Microalgae autoflocculation: an alternative to high-energy consuming harvesting methods. *Journal of Applied Phycology* 25, 991-999.
- González-Fernández, C., Molinuevo-Salces, B., García-González, M.C., 2011. Nitrogen transformations under different conditions in open ponds by means of microalgae-bacteria consortium treating pig slurry. *Bioresource Technology* 102, 960-966.
- González, L.E., Cañizares, R.O., Baena, S., 1997. Efficiency of ammonia and phosphorus removal from a Colombian agroindustrial wastewater by the microalgae *Chlorella vulgaris* and *Scenedesmus dimorphus*. *Bioresource Technology* 60, 259-262.
- González López, C.V., Acién Fernández, F.G., Fernández Sevilla, J.M., Sánchez Fernández, J.F., Cerón García, M.C., Molina Grima, E., 2009. Utilization of the cyanobacteria *Anabaena* sp. ATCC 33047 in CO₂ removal processes. *Bioresource Technology* 100, 5904-5910.
- Gouveia, L. 2011. Microalgae as a feedstock for biofuels, Springer. Lisboa.
- Gouveia, L., Graça, S., Sousa, C., Ambrosano, L., Ribeiro, B., Botrel, E.P., Neto, P.C., Ferreira, A.F., Silva, C.M., 2016. Microalgae biomass production using wastewater: Treatment and costs: Scale-up considerations. *Algal Research* 16, 167-176.
- Grobbelaar, J.U., 2004. Algal nutrition - mineral nutrition, in: Richmond, A. (Ed.) *Handbook of microalgal culture: biotechnology and applied phycology*. Blackwell Science Ltd, Oxford, UK, pp. 3-19.
- Gross, E.M., 2003. Allelopathy of aquatic autotrophs. *Critical Reviews in Plant Sciences* 22, 313-339.
- Guderjan, M., Elez-Martínez, P., Knorr, D., 2007. Application of pulsed electric fields at oil yield and content of functional food ingredients at the production of rapeseed oil. *Innovative Food Science and Emerging Technologies* 8, 55-62.
- Guderjan, M., Töpfl, S., Angersbach, A., Knorr, D., 2005. Impact of pulsed electric field treatment on the recovery and quality of plant oils. *Journal of Food Engineering* 67, 281-287.
- Hadiyanto, H., Elmore, S., Van Gerven, T., Stankiewicz, A., 2013. Hydrodynamic evaluations in high rate algae pond (HRAP) design. *Chemical Engineering Journal* 217, 231-239.

- Hadjoudja, S., Deluchat, V., Baudu, M., 2010. Cell surface characterisation of *Microcystis aeruginosa* and *Chlorella vulgaris*. *Journal of Colloid and Interface Science* 342, 293-299.
- Halim, R., Danquah, M.K., Webley, P.A., 2012. Extraction of oil from microalgae for biodiesel production: a review. *Biotechnology Advances* 30, 709-732.
- Hameed, M., Ebrahim, O., 2007. Biotechnological potential uses of immobilized algae. *International Journal of Agriculture and Biology* 9, 183-192.
- Han, F., Wang, W., Li, Y., Shen, G., Wan, M., Wang, J., 2013. Changes of biomass, lipid content and fatty acids composition under a light–dark cyclic culture of *Chlorella pyrenoidosa* in response to different temperature. *Bioresource Technology* 132, 182-189.
- Hansen, P.J., 2002. Effect of high pH on the growth and survival of marine phytoplankton: implications for species succession. *Aquatic Microbial Ecology* 28, 279-288.
- He, P., Mao, B., Lü, F., Shao, L., Lee, D., Chang, J., 2013. The combined effect of bacteria and *Chlorella vulgaris* on the treatment of municipal wastewaters. *Bioresource Technology* 146, 562-568.
- He, S., Xue, G., 2010. Algal-based immobilization process to treat the effluent from a secondary wastewater treatment plant (WWTP). *Journal of Hazardous Materials* 178, 895-899.
- Hellebust, J.A., Ahmad, I., 1989. Regulation of nitrogen assimilation in green microalgae. *Biological Oceanography* 6, 241-255.
- Hernández, D., Riaño, B., Coca, M., García-González, M., 2013. Treatment of agro-industrial wastewater using microalgae-bacteria consortium combined with anaerobic digestion of the produced biomass. *Bioresource Technology* 135, 598-603.
- Hirata, S., Hayashitani, M., Taya, M., Tone, S., 1996a. Carbon dioxide fixation in batch culture of *Chlorella* sp. using a photobioreactor with a sunlight-collection device. *Journal of Fermentation and Bioengineering* 81, 470-472.
- Hirata, S., Taya, M., Tone, S., 1996b. Characterization of *Chlorella* cell cultures in batch and continuous operations under a photoautotrophic condition. *Journal of Chemical Engineering of Japan* 29, 953-959.

- Hirayama, S., Ueda, R., Sugata, K., 1996. Evaluation of active oxygen effect on photosynthesis of *Chlorella vulgaris*. *Free Radical Research* 25, 247-254.
- Ho, S.-H., Chen, C.-Y., Chang, J.-S., 2012. Effect of light intensity and nitrogen starvation on CO₂ fixation and lipid/carbohydrate production of an indigenous microalga *Scenedesmus obliquus* CNW-N. *Bioresource Technology* 113, 244-252.
- Ho, S.-H., Chen, C.-Y., Lee, D.-J., Chang, J.-S., 2011. Perspectives on microalgal CO₂-emission mitigation systems - a review. *Biotechnology Advances* 29, 189-198.
- Ho, S.-H., Chen, C.-Y., Yeh, K.-L., Chen, W.-M., Lin, C.-Y., Chang, J.-S., 2010a. Characterization of photosynthetic carbon dioxide fixation ability of indigenous *Scenedesmus obliquus* isolates. *Biochemical Engineering Journal* 53, 57-62.
- Ho, S.-H., Chen, W.-M., Chang, J.-S., 2010b. *Scenedesmus obliquus* CNW-N as a potential candidate for CO₂ mitigation and biodiesel production. *Bioresource Technology* 101, 8725-8730.
- Hoffmann, J.P., 1998. Wastewater treatment with suspended and nonsuspended algae. *Journal of Phycology* 34, 757-763.
- Hu, Q., 2004a. Environmental effects on cell composition, in: Richmond, A. (Ed.) *Handbook of microalgal culture: biotechnology and applied phycology*. Blackwell Science Ltd, Oxford, UK, pp. 83-94.
- Hu, Q., 2004b. Industrial production of microalgal cell-mass and secondary products – major industrial species, in: Richmond, A. (Ed.) *Handbook of microalgal culture: biotechnology and applied phycology*. Blackwell Science Ltd, Oxford, UK, pp. 268-271.
- Hu, Q., Westerhoff, P., Vermaas, W., 2000. Removal of nitrate from groundwater by cyanobacteria: quantitative assessment of factors influencing nitrate uptake. *Applied and Environmental Microbiology* 66, 133-139.
- Huang, X., Huang, Z., Wen, W., Yan, J., 2013. Effects of nitrogen supplementation of the culture medium on the growth, total lipid content and fatty acid profiles of three microalgae (*Tetraselmis subcordiformis*, *Nannochloropsis oculata* and *Pavlova viridis*). *Journal of Applied Phycology* 25, 129-137.

- Hulatt, C.J., Thomas, D.N., 2011. Productivity, carbon dioxide uptake and net energy return of microalgal bubble column photobioreactors. *Bioresource Technology* 102, 5775-5787.
- IEA, 2011. CO₂ emissions from fuel combustion. International Energy Agency, Paris, France.
- Ince, O., 1998. Performance of a two-phase anaerobic digestion system when treating dairy wastewater. *Water Research* 32, 2707-2713.
- Iwasaki, I., Kurano, N., Miyachi, S., 1996. Effects of high-CO₂ stress on photosystem II in a green alga, *Chlorococcum littorale*, which has a tolerance to high CO₂. *Journal of Photochemistry and Photobiology B: Biology* 36, 327-332.
- Jacob-Lopes, E., Scoparo, C.H.G., Lacerda, L.M.C.F., Franco, T.T., 2009. Effect of light cycles (night/day) on CO₂ fixation and biomass production by microalgae in photobioreactors. *Chemical Engineering and Processing: Process Intensification* 48, 306-310.
- Jagmann, N., Philipp, B., 2014. Reprint of design of synthetic microbial communities for biotechnological production processes. *Journal of Biotechnology* 192, 293-301.
- James, C., Al-Hinty, S., Salman, A., 1989. Growth and ω3 fatty acid and amino acid composition of microalgae under different temperature regimes. *Aquaculture* 77, 337-351.
- Janssen, M., 2016. Microalgal photosynthesis and growth in mass culture, in: Jack, L. (Ed.) *Advances in Chemical Engineering*. Academic Press, Massachusetts, USA, pp. 185-256.
- Janssen, M., Kuijpers, T.C., Veldhoen, B., Ternbach, M.B., Tramper, J., Mur, L.R., Wijffels, R.H., 1999. Specific growth rate of *Chlamydomonas reinhardtii* and *Chlorella sorokiniana* under medium duration light/dark cycles: 13–87 s. *Journal of Biotechnology* 70, 323-333.
- Jeyanayagam, S., 2005. True confessions of the biological nutrient removal process. *Florida Water Resources Journal* 1, 37-46.
- Jiménez, C., Cossío, B.R., Labella, D., Niell, F.X., 2003. The feasibility of industrial production of *Spirulina* (*Arthrospira*) in Southern Spain. *Aquaculture* 217, 179-190.

- Joannes, C., Sipaut, C., Dayou, J., Yasir, S., Mansa, R. 2015. Review paper on cell membrane electroporation of microalgae using electric field treatment method for microalgae lipid extraction. *9th CUTSE International Conference*, 3rd to 4th December 2014, Miri, Sarawak, Malaysia.
- John, R.P., Anisha, G.S., Nampoothiri, K.M., Pandey, A., 2011. Micro and macroalgal biomass: a renewable source for bioethanol. *Bioresource Technology* 102, 186-193.
- Johnson, K.R., Admassu, W., 2013. Mixed algae cultures for low cost environmental compensation in cultures grown for lipid production and wastewater remediation. *Journal of Chemical Technology and Biotechnology* 88, 992-998.
- JRC, 2013. Photovoltaic geographical information system - incident global irradiation, <http://re.jrc.ec.europa.eu/pvgis/apps4/pvest.php>, (accessed 20-01-2013).
- Judd, S., van den Broeke, L.J., Shurair, M., Kuti, Y., Znad, H., 2015. Algal remediation of CO₂ and nutrient discharges: a review. *Water Research* 87, 356-366.
- Keffer, J., Kleinheinz, G., 2002. Use of *Chlorella vulgaris* for CO₂ mitigation in a photobioreactor. *Journal of Industrial Microbiology and Biotechnology* 29, 275-280.
- Keith, D.W., 2009. Why capture CO₂ from the atmosphere? *Science* 325, 1654-1655.
- Kellam, S.J., Walker, J.M., 1989. Antibacterial activity from marine microalgae in laboratory culture. *British Phycological Journal* 24, 191-194.
- Ketheesan, B., Nirmalakhandan, N., 2012. Feasibility of microalgal cultivation in a pilot-scale airlift-driven raceway reactor. *Bioresource Technology* 108, 196-202.
- Kim, B.-H., Ramanan, R., Cho, D.-H., Oh, H.-M., Kim, H.-S., 2014. Role of *Rhizobium*, a plant growth promoting bacterium, in enhancing algal biomass through mutualistic interaction. *Biomass and Bioenergy* 69, 95-105.
- Kinnunen, V., Craggs, R., Rintala, J., 2014. Influence of temperature and pretreatments on the anaerobic digestion of wastewater grown microalgae in a laboratory-scale accumulating-volume reactor. *Water Research* 57, 247-257.
- Kliphuis, A.M., Martens, D.E., Janssen, M., Wijffels, R.H., 2011. Effect of O₂:CO₂ ratio on the primary metabolism of *Chlamydomonas reinhardtii*. *Biotechnology and Bioengineering* 108, 2390-2402.

- Kong, Q.-x., Li, L., Martinez, B., Chen, P., Ruan, R., 2009. Culture of microalgae *Chlamydomonas reinhardtii* in wastewater for biomass feedstock production. *Applied Biochemistry and Biotechnology* 160, 9-18.
- Koreivienė, J., Valčiukas, R., Karosienė, J., Baltrėnas, P., 2014. Testing of *Chlorella/Scenedesmus* microalgae consortia for remediation of wastewater, CO₂ mitigation and algae biomass feasibility for lipid production. *Journal of Environmental Engineering and Landscape Management* 22, 105-114.
- Kumar, A., Ergas, S., Yuan, X., Sahu, A., Zhang, Q., Dewulf, J., Malcata, F.X., Van Langenhove, H., 2010. Enhanced CO₂ fixation and biofuel production via microalgae: recent developments and future directions. *Trends in Biotechnology* 28, 371-380.
- Kumar, K., Banerjee, D., Das, D., 2014. Carbon dioxide sequestration from industrial flue gas by *Chlorella sorokiniana*. *Bioresource Technology* 152, 225-233.
- Lacerda, L.M.C.F., Queiroz, M.I., Furlan, L.T., Lauro, M.J., Modenesi, K., Jacob-Lopes, E., Franco, T.T., 2011. Improving refinery wastewater for microalgal biomass production and CO₂ biofixation: predictive modeling and simulation. *Journal of Petroleum Science and Engineering* 78, 679-686.
- Lam, M.K., Lee, K.T., Mohamed, A.R., 2012. Current status and challenges on microalgae-based carbon capture. *International Journal of Greenhouse Gas Control* 10, 456-469.
- Lardon, L., Hélias, A., Sialve, B., Steyer, J.-P., Bernard, O., 2009. Life-cycle assessment of biodiesel production from microalgae. *Environmental Science & Technology* 43, 6475-6481.
- Larsdotter, K., 2006. Wastewater treatment with microalgae - a literature review. *Vatten* 62, 31.
- Ledda, C., Idà, A., Allemand, D., Mariani, P., Adani, F., 2015. Production of wild *Chlorella* sp. cultivated in digested and membrane-pretreated swine manure derived from a full-scale operation plant. *Algal Research* 12, 68-73.
- Lee, B., Park, S.Y., Heo, Y.S., Yea, S.S., Kim, D.-E., 2009. Efficient colorimetric assay of RNA polymerase activity using inorganic pyrophosphatase and ammonium molybdate. *Bulletin of Korean Chemistry Society* 30, 2485-2488.

- Lee, J.M., 1992. Biochemical engineering, 1st ed. Prentice Hall, Englewood Cliffs, New Jersey, pp. 6.12-6.16.
- Lee, K., Lee, C.-G., 2001. Effect of light/dark cycles on wastewater treatments by microalgae. *Biotechnology and Bioprocess Engineering* 6, 194-199.
- Lee, Y.-K., 2001. Microalgal mass culture systems and methods: their limitation and potential. *Journal of Applied Phycology* 13, 307-315.
- Lee, Y.-K., 2004. Algal nutrition - heterotrophic carbon nutrition, in: Richmond, A. (Ed.) *Handbook of microalgal culture: biotechnology and applied phycology*. Blackwell Science Ltd, Oxford, UK, pp. 116-124.
- Lefebvre, S., Hussenot, J., Brossard, N., 1996. Water treatment of land-based fish farm effluents by outdoor culture of marine diatoms. *Journal of Applied Phycology* 8, 193-200.
- Leite, G.B., Abdelaziz, A.E., Hallenbeck, P.C., 2013. Algal biofuels: challenges and opportunities. *Bioresource Technology* 145, 134-141.
- Li, F.-F., Yang, Z.-H., Zeng, R., Yang, G., Chang, X., Yan, J.-B., Hou, Y.-L., 2011a. Microalgae capture of CO₂ from actual flue gas discharged from a combustion chamber. *Industrial & Engineering Chemistry Research* 50, 6496-6502.
- Li, Y., Chen, Y.-F., Chen, P., Min, M., Zhou, W., Martinez, B., Zhu, J., Ruan, R., 2011b. Characterization of a microalga *Chlorella* sp. well adapted to highly concentrated municipal wastewater for nutrient removal and biodiesel production. *Bioresource Technology* 102, 5138-5144.
- Li, Y., Horsman, M., Wang, B., Wu, N., Lan, C.Q., 2008. Effects of nitrogen sources on cell growth and lipid accumulation of green alga *Neochloris oleoabundans*. *Applied Microbiology and Biotechnology* 81, 629-636.
- Li, Y., Zhou, W., Hu, B., Min, M., Chen, P., Ruan, R.R., 2012. Effect of light intensity on algal biomass accumulation and biodiesel production for mixotrophic strains *Chlorella kessleri* and *Chlorella protothecoides* cultivated in highly concentrated municipal wastewater. *Biotechnology and Bioengineering* 109, 2222-2229.
- Liang, Z., Liu, Y., Ge, F., Xu, Y., Tao, N., Peng, F., Wong, M., 2013. Efficiency assessment and pH effect in removing nitrogen and phosphorus by algae-bacteria combined

- system of *Chlorella vulgaris* and *Bacillus licheniformis*. *Chemosphere* 92, 1383-1389.
- Lim, S.-L., Chu, W.-L., Phang, S.-M., 2010. Use of *Chlorella vulgaris* for bioremediation of textile wastewater. *Bioresource Technology* 101, 7314-7322.
- Lin, L., Chan, G., Jiang, B., Lan, C., 2007. Use of ammoniacal nitrogen tolerant microalgae in landfill leachate treatment. *Waste Management* 27, 1376-1382.
- Lin, Q., Lin, J., 2011. Effects of nitrogen source and concentration on biomass and oil production of a *Scenedesmus rubescens* like microalga. *Bioresource Technology* 102, 1615-1621.
- Litchman, E., Steiner, D., Bossard, P., 2003. Photosynthetic and growth responses of three freshwater algae to phosphorus limitation and daylength. *Freshwater Biology* 48, 2141-2148.
- Liu, J., Danneels, B., Vanormelingen, P., Vyverman, W., 2016. Nutrient removal from horticultural wastewater by benthic filamentous algae *Klebsormidium* sp., *Stigeoclonium* spp. and their communities: From laboratory flask to outdoor Algal Turf Scrubber (ATS). *Water Research* 92, 61-68.
- Lizzul, A., Hellier, P., Purton, S., Baganz, F., Ladommatos, N., Campos, L., 2014. Combined remediation and lipid production using *Chlorella sorokiniana* grown on wastewater and exhaust gases. *Bioresource Technology* 151, 12-18.
- Luengo, E., Condón-Abanto, S., Álvarez, I., Raso, J., 2014. Effect of pulsed electric field treatments on permeabilization and extraction of pigments from *Chlorella vulgaris*. *The Journal of Membrane Biology* 247, 1269-1277.
- Lundquist, T.J., Woertz, I.C., Quinn, N., Benemann, J.R., 2010. A realistic technology and engineering assessment of algae biofuel production. Energy Biosciences Institute, Berkeley, CA.
- Maeda, K., Owada, M., Kimura, N., Omata, K., Karube, I., 1995. CO₂ fixation from the flue gas on coal-fired thermal power plant by microalgae. *Energy Conversion and Management* 36, 717-720.
- Malhotra, S.K., Lee, G.F., Rohlich, G., 1964. Nutrient removal from secondary effluent by alum flocculation and lime precipitation. *International Journal of Air and Water Pollution* 8, 487-500.

- Mallick, N., 2002. Biotechnological potential of immobilized algae for wastewater N, P and metal removal: a review. *Biometals* 15, 377-390.
- Mandal, S.K., Singh, R.P., Patel, V., 2011. Isolation and characterization of exopolysaccharide secreted by a toxic dinoflagellate, *Amphidinium carterae* Hulburt 1957 and its probable role in harmful algal blooms (HABs). *Microbial Ecology* 62, 518-527.
- Martinez, M., Jimenez, J., El Yousfi, F., 1999. Influence of phosphorus concentration and temperature on growth and phosphorus uptake by the microalga *Scenedesmus obliquus*. *Bioresource Technology* 67, 233-240.
- McGinn, P.J., Dickinson, K.E., Bhatti, S., Frigon, J.-C., Guiot, S.R., O'Leary, S.J., 2011. Integration of microalgae cultivation with industrial waste remediation for biofuel and bioenergy production: opportunities and limitations. *Photosynthesis Research* 109, 231-247.
- McGriff, E.C., McKinney, R.E., 1972. The removal of nutrients and organics by activated algae. *Water Research* 6, 1155-1164.
- McLarnon-Riches, C.J., Rolph, C.E., Greenway, D.L., Robinson, P.K., 1998. Effects of environmental factors and metals on *Selenastrum capricornutum* lipids. *Phytochemistry* 49, 1241-1247.
- Medina, M., Neis, U., 2007. Symbiotic algal bacterial wastewater treatment: effect of food to microorganism ratio and hydraulic retention time on the process performance. *Water Science & Technology* 55, 165-171.
- Mendes, L.B.B., Vermelho, A.B., 2013. Allelopathy as a potential strategy to improve microalgae cultivation. *Biotechnology for Biofuels* 6, 152-165.
- Mendoza, J.L., Granados, M.R., de Godos, I., Ación, F.G., Molina, E., Heaven, S., Banks, C.J., 2013. Oxygen transfer and evolution in microalgal culture in open raceways. *Bioresource Technology* 137, 188-195.
- Mennaa, F.Z., Arbib, Z., Perales, J.A., 2015. Urban wastewater treatment by seven species of microalgae and an algal bloom: Biomass production, N and P removal kinetics and harvestability. *Water Research* 83, 42-51.

- Michel, C., Legendre, L., Therriault, J.-C., Demers, S., 1989. Photosynthetic responses of Arctic sea-ice microalgae to short-term temperature acclimation. *Polar Biology* 9, 437-442.
- Mino, T., Van Loosdrecht, M., Heijnen, J., 1998. Microbiology and biochemistry of the enhanced biological phosphate removal process. *Water Research* 32, 3193-3207.
- Mithá, O., 2004. *Análise de projectos de investimento*, 1st ed. Escolar Editora, Lisbon, Portugal.
- Molina Grima, E., Belarbi, E.H., Ación Fernández, F.G., Robles Medina, A., Chisti, Y., 2003. Recovery of microalgal biomass and metabolites: process options and economics. *Biotechnology Advances* 20, 491-515.
- Monod, J., 1949. The growth of bacterial cultures. *Annual Reviews in Microbiology* 3, 371-394.
- Moreira, D., Pires, J.C.M., 2016. Atmospheric CO₂ capture by algae: negative carbon dioxide emission path. *Bioresource Technology* 215, 371-379.
- Moreno-Garrido, I., 2008. Microalgae immobilization: current techniques and uses. *Bioresource Technology* 99, 3949-3964.
- Moriarty, D.J., 1997. The role of microorganisms in aquaculture ponds. *Aquaculture* 151, 333-349.
- Mortensen, S.H., Børsheim, K.Y., Rainuzzo, J., Knutsen, G., 1988. Fatty acid and elemental composition of the marine diatom *Chaetoceros gracilis* Schütt. Effects of silicate deprivation, temperature and light intensity. *Journal of Experimental Marine Biology and Ecology* 122, 173-185.
- Moss, R.H., Edmonds, J.A., Hibbard, K.A., Manning, M.R., Rose, S.K., Van Vuuren, D.P., Carter, T.R., Emori, S., Kainuma, M., Kram, T., 2010. The next generation of scenarios for climate change research and assessment. *Nature* 463, 747-756.
- Muñoz, R., Guieysse, B., 2006. Algal-bacterial processes for the treatment of hazardous contaminants: a review. *Water Research* 40, 2799-2815.
- Mustafa, E.-M., Phang, S.-M., Chu, W.-L., 2012. Use of an algal consortium of five algae in the treatment of landfill leachate using the high-rate algal pond system. *Journal of Applied Phycology* 24, 953-963.

- Mutanda, T., Ramesh, D., Karthikeyan, S., Kumari, S., Anandraj, A., Bux, F., 2011. Bioprospecting for hyper-lipid producing microalgal strains for sustainable biofuel production. *Bioresource Technology* 102, 57-70.
- Najdenski, H.M., Gigova, L.G., Iliev, I.I., Pilarski, P.S., Lukavský, J., Tsvetkova, I.V., Ninova, M.S., Kussovski, V.K., 2013. Antibacterial and antifungal activities of selected microalgae and cyanobacteria. *International Journal of Food Science & Technology* 48, 1533-1540.
- Nakano, Y., Miyatake, K., Okuno, H., Hamazaki, K., Takenaka, S., Honami, N., Kiyota, M., Aiga, I., Kondo, J. 1996. Growth of photosynthetic algae *Euglena* in high CO₂ conditions and its photosynthetic characteristics. *International Symposium on Plant Production in Closed Ecosystems*, Narita, Japan. pp. 49-54.
- Naskeo Environnement, 2009. Biogas renewable energy - the biogas composition, http://www.biogas-renewable-energy.info/biogas_composition.html, (accessed 22-12-2015).
- Natrah, F.M., Bossier, P., Sorgeloos, P., Yusoff, F.M., Defoirdt, T., 2014. Significance of microalgal-bacterial interactions for aquaculture. *Reviews in Aquaculture* 6, 48-61.
- Nayak, M., Karemore, A., Sen, R., 2016. Performance evaluation of microalgae for concomitant wastewater bioremediation, CO₂ biofixation and lipid biosynthesis for biodiesel application. *Algal Research* 16, 216-223.
- Neilson, A., Lewin, R., 1974. The uptake and utilization of organic carbon by algae: an essay in comparative biochemistry. *Phycologia* 13, 227-264.
- Norsker, N.-H., Barbosa, M.J., Vermuë, M.H., Wijffels, R.H., 2011. Microalgal production - a close look at the economics. *Biotechnology Advances* 29, 24-27.
- Norton, T.A., Melkonian, M., Andersen, R.A., 1996. Algal biodiversity. *Phycologia* 35, 308-326.
- O'Neill, B.C., Oppenheimer, M., 2002. Dangerous climate impacts and the Kyoto Protocol. *Science* 296, 1971-1972.
- OECD, 2011. Freshwater alga and cyanobacteria, growth inhibition test. Organisation for Economic Co-operation and Development Test Guideline 201.

- Ogawa, T., Aiba, S., 1981. Bioenergetic analysis of mixotrophic growth in *Chlorella vulgaris* and *Scenedesmus acutus*. *Biotechnology and Bioengineering* 23, 1121-1132.
- Ogbonna, J.C., Yoshizawa, H., Tanaka, H., 2000. Treatment of high strength organic wastewater by a mixed culture of photosynthetic microorganisms. *Journal of Applied Phycology* 12, 277-284.
- Olguín, E.J., 2012. Dual purpose microalgae-bacteria-based systems that treat wastewater and produce biodiesel and chemical products within a biorefinery. *Biotechnology Advances* 30, 1031-1046.
- Olguín, E.J., Galicia, S., Mercado, G., Pérez, T., 2003. Annual productivity of *Spirulina* (*Arthrospira*) and nutrient removal in a pig wastewater recycling process under tropical conditions. *Journal of Applied Phycology* 15, 249-257.
- Ono, E., Cuello, J.L. 2003. Selection of optimal microalgae species for CO₂ sequestration. *Proceedings of the 2nd Annual Conference on Carbon Sequestration*, Alexandria, Egypt. Citeseer. pp. 1-7.
- OrbiChem, 2013. Chemical market insight and foresight on a single page - caustic soda, http://www.orbichem.com/userfiles/CNF%20Samples/cas_13_11.pdf, (accessed 22-02-2016).
- Oswald, W., Gotaas, H., Golueke, C., Kellen, W., Gloyna, E., Hermann, E., 1957. Algae in waste treatment. *Sewage and Industrial Wastes* 29, 437-457.
- Ota, M., Kato, Y., Watanabe, H., Watanabe, M., Sato, Y., Smith, R.L., Inomata, H., 2009. Effect of inorganic carbon on photoautotrophic growth of microalga *Chlorococcum littorale*. *Biotechnology Progress* 25, 492-498.
- Ozkan, A., Berberoglu, H., 2013. Physico-chemical surface properties of microalgae. *Colloids and Surfaces B: Biointerfaces* 112, 287-293.
- Paerl, H., Pinckney, J., 1996. A mini-review of microbial consortia: their roles in aquatic production and biogeochemical cycling. *Microbial Ecology* 31, 225-247.
- Park, J., Craggs, R., 2010. Wastewater treatment and algal production in high rate algal ponds with carbon dioxide addition. *Water Science & Technology* 61, 633-639.

- Park, J., Craggs, R., 2011. Algal production in wastewater treatment high rate algal ponds for potential biofuel use. *Water Science & Technology* 63, 2403-2410.
- Park, J., Craggs, R., Shilton, A., 2011. Wastewater treatment high rate algal ponds for biofuel production. *Bioresource Technology* 102, 35-42.
- Park, Y., Je, K.-W., Lee, K., Jung, S.-E., Choi, T.-J., 2008. Growth promotion of *Chlorella ellipsoidea* by co-inoculation with *Brevundimonas* sp. isolated from the microalga. *Hydrobiologia* 598, 219-228.
- Parmar, A., Singh, N.K., Pandey, A., Gnansounou, E., Madamwar, D., 2011. Cyanobacteria and microalgae: a positive prospect for biofuels. *Bioresource Technology* 102, 10163-10172.
- Paul, S., Nicholas, A., Tseng, C., Borowitzka, M., 2013. Seaweed and microalgae. *Aquaculture*, Second edition 268-293.
- Pegallapati, A.K., Nirmalakhandan, N., 2013. Internally illuminated photobioreactor for algal cultivation under carbon dioxide-supplementation: performance evaluation. *Renewable Energy* 56, 129-135.
- Pereira, H., Barreira, L., Mozes, A., Florindo, C., Polo, C., Duarte, C.V., Custódio, L., Varela, J., 2011. Microplate-based high throughput screening procedure for the isolation of lipid-rich marine microalgae. *Biotechnology for Biofuels* 4, 61-72.
- Peters, M.S., Timmerhaus, K.D., 1991. Plant design and economics for chemical engineers, 4th ed. McGraw-Hill, New York, USA.
- Philippis, R., Vincenzini, M., 1998. Exocellular polysaccharides from cyanobacteria and their possible applications. *FEMS Microbiology Reviews* 22, 151-175.
- Piao, W., Kim, Y., Kim, H., Kim, M., Kim, C., 2015. Life cycle assessment and economic efficiency analysis of integrated management of wastewater treatment plants. *Journal of Cleaner Production* 113, 325-337.
- Picardo, M.C., Medeiros, J.L., Queiroz, F.A.O., Chaloub, R.M., 2013. Effects of CO₂ enrichment and nutrients supply intermittency on batch cultures of *Isochrysis galbana*. *Bioresource Technology* 143, 242-250.
- Pielke, R.A., 2009. An idealized assessment of the economics of air capture of carbon dioxide in mitigation policy. *Environmental Science & Policy* 12, 216-225.

- Piñar, G., Duque, E., Haidour, A., Oliva, J., Sánchez-Barbero, L., Calvo, V., Ramos, J.L., 1997. Removal of high concentrations of nitrate from industrial wastewaters by bacteria. *Applied and Environmental Microbiology* 63, 2071-2073.
- Piñar, G., Kovárová, K., Egli, T., Ramos, J.L., 1998. Influence of carbon source on nitrate removal by nitrate-tolerant *Klebsiella oxytoca* CECT 4460 in batch and chemostat cultures. *Applied and Environmental Microbiology* 64, 2970-2976.
- Pires, J.C.M., Alvim-Ferraz, M.C.M., Martins, F.G., Simões, M., 2012. Carbon dioxide capture from flue gases using microalgae: engineering aspects and biorefinery concept. *Renewable & Sustainable Energy Reviews* 16, 3043-3053.
- Pires, J.C.M., Alvim-Ferraz, M.C.M., Martins, F.G., Simões, M., 2013a. Wastewater treatment to enhance the economic viability of microalgae culture. *Environmental Science and Pollution Research* 20, 5096-5105.
- Pires, J.C.M., Gonçalves, A.L., Martins, F.G., Alvim-Ferraz, M.C.M., Simões, M., 2013b. Effect of light supply on CO₂ capture from atmosphere by *Chlorella vulgaris* and *Pseudokirchneriella subcapitata*. *Mitigation and Adaptation Strategies for Global Change* 19, 1109-1119.
- Pires, J.C.M., Gonçalves, A.L., Martins, F.G., Alvim-Ferraz, M.C.M., Simões, M., 2014. Effect of light supply on CO₂ capture from atmosphere by *Chlorella vulgaris* and *Pseudokirchneriella subcapitata*. *Mitigation and Adaptation Strategies for Global Change* 19, 1109-1117.
- Pires, J.C.M., Martins, F.G., Alvim-Ferraz, M.C.M., Simões, M., 2011. Recent developments on carbon capture and storage: an overview. *Chemical Engineering Research and Design* 89, 1446-1460.
- PORDATA, 2013. Densidade populacional segundo os Censos em Portugal, <http://www.pordata.pt/Portugal/Densidade+populacional+segundo+os+Censos-412>, (accessed 05-03-2013).
- Posadas, E., García-Encina, P.-A., Soltau, A., Domínguez, A., Díaz, I., Muñoz, R., 2013. Carbon and nutrient removal from centrates and domestic wastewater using algal-bacterial biofilm bioreactors. *Bioresource Technology* 139, 50-58.
- Posten, C., 2009. Design principles of photo-bioreactors for cultivation of microalgae. *Engineering in Life Sciences* 9, 165-177.

- Posten, C., Schaub, G., 2009. Microalgae and terrestrial biomass as source for fuels - a process view. *Journal of Biotechnology* 142, 64-69.
- Powell, N., Shilton, A., Chisti, Y., Pratt, S., 2009. Towards a luxury uptake process via microalgae - defining the polyphosphate dynamics. *Water Research* 43, 4207-4213.
- Powell, N., Shilton, A.N., Pratt, S., Chisti, Y., 2008. Factors influencing luxury uptake of phosphorus by microalgae in waste stabilization ponds. *Environmental Science & Technology* 42, 5958-5962.
- Pratt, R., Daniels, T., Eiler, J.J., Gunnison, J., Kumler, W., Oneto, J.F., Strait, L.A., Spoehr, H., Hardin, G., Milner, H., 1944. Chlorellin, an antibacterial substance from *Chlorella*. *American Association for the Advancement of Science* 99, 351-2.
- Pulsemaster, 2016. FAQ about pulsed electric field processing, <https://www.pulsemaster.us/pef-pulsemaster/faq>, (accessed 23-12-2015).
- Pulz, O., 2001. Photobioreactors: production systems for phototrophic microorganisms. *Applied Microbiology and Biotechnology* 57, 287-293.
- Pulz, O., Gross, W., 2004. Valuable products from biotechnology of microalgae. *Applied Microbiology and Biotechnology* 65, 635-648.
- Qiang, H., Richmond, A., 1996. Productivity and photosynthetic efficiency of *Spirulina platensis* as affected by light intensity, algal density and rate of mixing in a flat plate photobioreactor. *Journal of Applied Phycology* 8, 139-145.
- Qin, L., Wang, Z., Sun, Y., Shu, Q., Feng, P., Zhu, L., Xu, J., Yuan, Z., 2016. Microalgae consortia cultivation in dairy wastewater to improve the potential of nutrient removal and biodiesel feedstock production. *Environmental Science and Pollution Research* 23, 8379-8387.
- Queiroz, M.I., Lopes, E.J., Zepka, L.Q., Bastos, R.G., Goldbeck, R., 2007. The kinetics of the removal of nitrogen and organic matter from parboiled rice effluent by cyanobacteria in a stirred batch reactor. *Bioresource Technology* 98, 2163-2169.
- Ramanan, R., Kannan, K., Deshkar, A., Yadav, R., Chakrabarti, T., 2010. Enhanced algal CO₂ sequestration through calcite deposition by *Chlorella* sp. and *Spirulina platensis* in a mini-raceway pond. *Bioresource Technology* 101, 2616-2622.

- Ramanan, R., Kim, B.-H., Cho, D.-H., Oh, H.-M., Kim, H.-S., 2016. Algae-bacteria interactions: evolution, ecology and emerging applications. *Biotechnology Advances* 34, 14-29.
- Ramkrishnan, U., Bruno, B., Swaminathan, S., 2014. Sequestration of CO₂ by halotolerant algae. *Journal of Environmental Health Science and Engineering* 12, 81-87.
- Raposo, M., Oliveira, S.E., Castro, P.M., Bandarra, N.M., Morais, R.M., 2010. On the utilization of microalgae for brewery effluent treatment and possible applications of the produced biomass. *Journal of the Institute of Brewing* 116, 285-292.
- Rawat, I., Kumar, R., Mutanda, T., Bux, F., 2011. Dual role of microalgae: phycoremediation of domestic wastewater and biomass production for sustainable biofuels production. *Applied Energy* 88, 3411-3424.
- Reed, R.W., Reed, G.B., 1948. "Drop plate" method of counting viable bacteria. *Canadian Journal of Research* 26e, 317-326.
- Ren, H.-Y., Liu, B.-F., Kong, F., Zhao, L., Ren, N., 2015. Hydrogen and lipid production from starch wastewater by co-culture of anaerobic sludge and oleaginous microalgae with simultaneous COD, nitrogen and phosphorus removal. *Water Research* 85, 404-412.
- Renaud, S., Zhou, H., Parry, D., Thinh, L.-V., Woo, K., 1995. Effect of temperature on the growth, total lipid content and fatty acid composition of recently isolated tropical microalgae *Isochrysis* sp., *Nitzschia closterium*, *Nitzschia paleacea*, and commercial species *Isochrysis* sp. (clone T. ISO). *Journal of Applied Phycology* 7, 595-602.
- Renuka, N., Sood, A., Ratha, S.K., Prasanna, R., Ahluwalia, A.S., 2013. Evaluation of microalgal consortia for treatment of primary treated sewage effluent and biomass production. *Journal of Applied Phycology* 25, 1529-1537.
- Reyna-Martínez, R., Gomez-Flores, R., López-Chuken, U.J., González-González, R., Fernández-Delgadillo, S., Balderas-Rentería, I., 2014. Lipid production by pure and mixed cultures of *Chlorella pyrenoidosa* and *Rhodotorula mucilaginosa* isolated in Nuevo Leon, Mexico. *Applied Biochemistry and Biotechnology* 175, 354-359.
- Richardson, B., Orcutt, D.M., Schwertner, H.A., Martinez, C.L., Wickline, H.E., 1969. Effects of nitrogen limitation on the growth and composition of unicellular algae in continuous culture. *Applied Microbiology* 18, 245-250.

- Richardson, K., Beardall, J., Raven, J.A., 1983. Adaptation of unicellular algae to irradiance: an analysis of strategies. *New Phytologist* 93, 157-191.
- Richardson, T.L., Gibson, C.E., Heaney, S.I., 2000. Temperature, growth and seasonal succession of phytoplankton in Lake Baikal, Siberia. *Freshwater Biology* 44, 431-440.
- Richmond, A., 1988. *Spirulina*, in: Borowitzka, M.A., Borowitzka, L. (Eds.), *Micro-algal biotechnology*. Cambridge University Press, New York, USA, pp. 85-121.
- Richmond, A., Vonshak, A., 1978. *Spirulina* culture in Israel. *Archives in Hydrobiology* 11, 274-280.
- Robarts, R.D., Zohary, T., 1987. Temperature effects on photosynthetic capacity, respiration, and growth rates of bloom-forming cyanobacteria. *New Zealand Journal of Marine and Freshwater Research* 21, 391-399.
- Rodrigues, C.M.M., 2009. Cálculo da evaporação de albufeiras de grande regularização do sul de Portugal. Dissertation for obtention of the degree of Doctor of Philosophy, Universidade de Évora, Évora, Portugal.
- Rosso, L., Lobry, J.R., Flandrois, J.P., 1993. An unexpected correlation between cardinal temperatures of microbial growth highlighted by a new model. *Journal of Theoretical Biology* 162, 447-463.
- Rubio, F.C., Camacho, F.G., Sevilla, J.M.F., Chisti, Y., Grima, E.M., 2003. A mechanistic model of photosynthesis in microalgae. *Biotechnology and Bioengineering* 81, 459-473.
- Ruiz-Marin, A., Mendoza-Espinosa, L.G., Stephenson, T., 2010. Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater. *Bioresource Technology* 101, 58-64.
- Ruiz, J., Álvarez, P., Arbib, Z., Garrido, C., Barragán, J., Perales, J., 2011. Effect of nitrogen and phosphorus concentration on their removal kinetic in treated urban wastewater by *Chlorella vulgaris*. *International Journal of Phytoremediation* 13, 884-896.
- Ruiz, J., Arbib, Z., Álvarez-Díaz, P., Garrido-Pérez, C., Barragán, J., Perales, J., 2013. Photobiotreatment model (PhBT): a kinetic model for microalgae biomass growth and nutrient removal in wastewater. *Environmental Technology* 34, 979-991.

- Ryu, H.J., Oh, K.K., Kim, Y.S., 2009. Optimization of the influential factors for the improvement of CO₂ utilization efficiency and CO₂ mass transfer rate. *Journal of Industrial and Engineering Chemistry* 15, 471-475.
- Safonova, E., Kvitko, K., Iankevitch, M., Surgko, L., Afti, I., Reisser, W., 2004. Biotreatment of industrial wastewater by selected algal-bacterial consortia. *Engineering in Life Sciences* 4, 347-353.
- Samorì, G., Samorì, C., Guerrini, F., Pistocchi, R., 2013. Growth and nitrogen removal capacity of *Desmodesmus communis* and of a natural microalgae consortium in a batch culture system in view of urban wastewater treatment: Part I. *Water Research* 47, 791-801.
- Sánchez, J.G., Pérez, J.S., Camacho, F.G., Sevilla, J.F., Grima, E.M., 1996. Optimization of light and temperature for growing *Chlorella* sp. using response surface methodology. *Biotechnology Techniques* 10, 329-334.
- Sánchez Mirón, A., Contreras Gómez, A., García Camacho, F., Molina Grima, E., Chisti, Y., 1999. Comparative evaluation of compact photobioreactors for large-scale monoculture of microalgae. *Journal of Biotechnology* 70, 249-270.
- Sayre, R., 2010. Microalgae: the potential for carbon capture. *Bioscience* 60, 722-727.
- Schlagermann, P., Göttlicher, G., Dillschneider, R., Rosello-Sastre, R., Posten, C., 2012. Composition of algal oil and its potential as biofuel. *Journal of Combustion* 2012, 1-14.
- Schlesinger, D., Molot, L., Shuter, B., 1981. Specific growth rates of freshwater algae in relation to cell size and light intensity. *Canadian Journal of Fisheries and Aquatic Sciences* 38, 1052-1058.
- Shi, J., Podola, B., Melkonian, M., 2007. Removal of nitrogen and phosphorus from wastewater using microalgae immobilized on twin layers: an experimental study. *Journal of Applied Phycology* 19, 417-423.
- Show, K.-Y., Lee, D.-J., Tay, J.-H., Lee, T.-M., Chang, J.-S., 2015. Microalgal drying and cell disruption - recent advances. *Bioresource Technology* 184, 258-266.
- Sialve, B., Bernet, N., Bernard, O., 2009. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. *Biotechnology Advances* 27, 409-416.

- Silva-Benavides, A.M., Torzillo, G., 2012. Nitrogen and phosphorus removal through laboratory batch cultures of microalga *Chlorella vulgaris* and cyanobacterium *Planktothrix isothrix* grown as monoalgal and as co-cultures. *Journal of Applied Phycology* 24, 267-276.
- Singh, G., Thomas, P.B., 2012. Nutrient removal from membrane bioreactor permeate using microalgae and in a microalgae membrane photoreactor. *Bioresource Technology* 117, 80-85.
- Singh, S., Kate, B., Banerjee, U., 2005. Bioactive compounds from cyanobacteria and microalgae: an overview. *Critical Reviews in Biotechnology* 25, 73-95.
- Singh, U.B., Ahluwalia, A., 2013. Microalgae: a promising tool for carbon sequestration. *Mitigation and Adaptation Strategies for Global Climate Change* 18, 73-95.
- Sinnott, R.K., Towler, G., 2009. *Chemical engineering design*, 4th ed. Elsevier, Oxford, UK, pp. 243-268.
- Sklyar, V., Epov, A., Gladchenko, M., Danilovich, D., Kalyuzhnyi, S., 2003. Combined biologic (anaerobic-aerobic) and chemical treatment of starch industry wastewater. *Applied Biochemistry and Biotechnology* 109, 253-262.
- Sompech, K., Chisti, Y., Srinophakun, T., 2012. Design of raceway ponds for producing microalgae. *Biofuels* 3, 387-397.
- Sonune, A., Ghate, R., 2004. Developments in wastewater treatment methods. *Desalination* 167, 55-63.
- Sorokin, C., Krauss, R.W., 1958. The effects of light intensity on the growth rates of green algae. *Plant Physiology* 33, 109-113.
- Spolaore, P., Joannis-Cassan, C., Duran, E., Isambert, A., 2006. Commercial applications of microalgae. *Journal of Bioscience and Bioengineering* 101, 87-96.
- Steele, J.H., 1977. Microbial kinetics and dynamics, in: Lapidus, L., Amunson, N.R. (Eds.), *Chemical reactor theory*. Prentice-Hall, Englewood Cliffs, New Jersey, pp. 405-483.
- Stumm, W., Morgan, J.J., 2012. *Aquatic chemistry: chemical equilibria and rates in natural waters*. John Wiley & Sons, New Jersey.

- Su, Y., Mennerich, A., Urban, B., 2011. Municipal wastewater treatment and biomass accumulation with a wastewater-born and settleable algal-bacterial culture. *Water Research* 45, 3351-3358.
- Su, Y., Mennerich, A., Urban, B., 2012a. Coupled nutrient removal and biomass production with mixed algal culture: impact of biotic and abiotic factors. *Bioresource Technology* 118, 469-476.
- Su, Y., Mennerich, A., Urban, B., 2012b. Synergistic cooperation between wastewater-born algae and activated sludge for wastewater treatment: influence of algae and sludge inoculation ratios. *Bioresource Technology* 105, 67-73.
- Subashchandrabose, S.R., Ramakrishnan, B., Megharaj, M., Venkateswarlu, K., Naidu, R., 2011. Consortia of cyanobacteria/microalgae and bacteria: biotechnological potential. *Biotechnology Advances* 29, 896-907.
- Suh, I.S., Lee, C.-G., 2003. Photobioreactor engineering: design and performance. *Biotechnology and Bioprocess Engineering* 8, 313-321.
- Sydney, E.B., Novak, A.C., Carvalho, J.C., Soccol, C.R., 2014. Respirometric balance and carbon fixation of industrially important algae, in: Pandey, A., Lee, D.-J., Chisti, Y., Soccol, C.R. (Eds.), *Biofuels from algae*. Elsevier, Burlington, Massachusetts, USA, pp. 67-84.
- Sydney, E.B., Sturm, W., de Carvalho, J.C., Thomaz-Soccol, V., Larroche, C., Pandey, A., Soccol, C.R., 2010. Potential carbon dioxide fixation by industrially important microalgae. *Bioresource Technology* 101, 5892-5896.
- Taher, H., Al-Zuhair, S., Al-Marzouqui, A.H., Haik, Y., Farid, M.M., 2011. A review of enzymatic transesterification of microalgal oil-based biodiesel using supercritical technology. *Enzyme Research* 2011, 1-25.
- Talbot, P., De la Noüe, J., 1993. Tertiary treatment of wastewater with *Phormidium bohneri* (Schmidle) under various light and temperature conditions. *Water Research* 27, 153-159.
- Tampion, J., Tampion, M.D., 1987. *Immobilized cells: principles and applications*. Cambridge University Press, Cambridge, UK, pp. 257.

- Tang, D., Han, W., Li, P., Miao, X., Zhong, J., 2011. CO₂ biofixation and fatty acid composition of *Scenedesmus obliquus* and *Chlorella pyrenoidosa* in response to different CO₂ levels. *Bioresource Technology* 102, 3071-3076.
- Tans, P., Keeling, R., 2015. Trends in atmospheric carbon dioxide, www.esrl.noaa.gov/gmd/ccgg/trends/, (accessed 08-10-2015).
- Tarlan, E., Dilek, F.B., Yetis, U., 2002. Effectiveness of algae in the treatment of a wood-based pulp and paper industry wastewater. *Bioresource Technology* 84, 1-5.
- Teoh, M.-L., Chu, W.-L., Marchant, H., Phang, S.-M., 2004. Influence of culture temperature on the growth, biochemical composition and fatty acid profiles of six Antarctic microalgae. *Journal of Applied Phycology* 16, 421-430.
- Teoh, M.-L., Phang, S.-M., Chu, W.-L., 2013. Response of Antarctic, temperate, and tropical microalgae to temperature stress. *Journal of Applied Phycology* 25, 285-297.
- The Engineering Toolbox, 2015. Fans - efficiency and power consumption, http://www.engineeringtoolbox.com/fans-efficiency-power-consumption-d_197.html, (accessed 22-12-2015).
- Thomas, W.H., Gibson, C.H., 1990. Effects of small-scale turbulence on microalgae. *Journal of Applied Phycology* 2, 71-77.
- Tomaseli, L., 2004. The microalgal cell, in: Richmond, A. (Ed.) *Handbook of microalgal culture: biotechnology and applied phycology*. Blackwell Science Ltd, Oxford, UK, pp. 3-19.
- Tran, K.C., Mendoza Martin, J.L., Heaven, S., Banks, C.J., Acien Fernandez, F.G., Molina Grima, E., 2014. Cultivation and anaerobic digestion of *Scenedesmus* spp. grown in a pilot-scale open raceway. *Algal Research* 5, 95-102.
- Tredici, M.R., Bassi, N., Prussi, M., Biondi, N., Rodolfi, L., Chini Zittelli, G., Sampietro, G., 2015. Energy balance of algal biomass production in a 1-ha “Green Wall Panel” plant: how to produce algal biomass in a closed reactor achieving a high Net Energy Ratio. *Applied Energy* 154, 1103-1111.
- Tredici, M.R., Materassi, R., 1992. From open ponds to vertical alveolar panels: the Italian experience in the development of reactors for the mass cultivation of phototrophic microorganisms. *Journal of Applied Phycology* 4, 221-231.

- Tripathi, B.D., Shukla, S.C., 1991. Biological treatment of wastewater by selected aquatic plants. *Environmental Pollution* 69, 69-78.
- Ugwu, C., Aoyagi, H., Uchiyama, H., 2007. Influence of irradiance, dissolved oxygen concentration, and temperature on the growth of *Chlorella sorokiniana*. *Photosynthetica* 45, 309-311.
- Ugwu, C., Aoyagi, H., Uchiyama, H., 2008. Photobioreactors for mass cultivation of algae. *Bioresource Technology* 99, 4021-4028.
- Unnithan, V.V., Unc, A., Smith, G.B., 2014. Mini-review: a priori considerations for bacteria-algae interactions in algal biofuel systems receiving municipal wastewaters. *Algal Research* 4, 35-40.
- Uusitalo, J., 1996. Algal carbon uptake and the difference between alkalinity and high pH ("alkalinization"), exemplified with a pH-drift experiment. *Scientia Marina* 60, 129-134.
- Valderrama, L.T., Del Campo, C.M., Rodriguez, C.M., de-Bashan, L.E., Bashan, Y., 2002. Treatment of recalcitrant wastewater from ethanol and citric acid production using the microalga *Chlorella vulgaris* and the macrophyte *Lemna minuscule*. *Water Research* 36, 4185-4192.
- Valladares, F., 2004. Photosynthetic acclimation to simultaneous and interacting environmental stresses along natural light gradients: optimality and constraints. *Plant Biology* 6, 254-268.
- Van Den Hende, S., Vervaeren, H., Desmet, S., Boon, N., 2011. Bioflocculation of microalgae and bacteria combined with flue gas to improve sewage treatment. *New Biotechnology* 29, 23-31.
- Vandamme, D., 2013. Flocculation based harvesting processes for microalgae biomass production. Dissertation for obtention of the degree of Doctor of Philosophy, Faculty of Bioscience Engineering, KU Leuven, Belgium.
- Vandamme, D., Foubert, I., Fraeye, I., Meesschaert, B., Muylaert, K., 2012. Flocculation of *Chlorella vulgaris* induced by high pH: role of magnesium and calcium and practical implications. *Bioresource Technology* 105, 114-119.

- Wahlen, B.D., Willis, R.M., Seefeldt, L.C., 2011. Biodiesel production by simultaneous extraction and conversion of total lipids from microalgae, cyanobacteria, and wild mixed-cultures. *Bioresource Technology* 102, 2724-2730.
- Wang, B., Lan, C.Q., 2010. Biofixation of carbon dioxide (CO₂) by microorganisms, in: Maroto-Valer, M.M. (Ed.) *Developments and innovation in carbon dioxide (CO₂) capture and storage technology*. Woodhead Publishing Limited, Cambridge, UK.
- Wang, L., Min, M., Li, Y., Chen, P., Chen, Y., Liu, Y., Wang, Y., Ruan, R., 2010. Cultivation of green algae *Chlorella* sp. in different wastewaters from municipal wastewater treatment plant. *Applied Biochemistry and Biotechnology* 162, 1174-1186.
- Wang, M., Kuo-Dahab, W.C., Dolan, S., Park, C., 2014. Kinetics of nutrient removal and expression of extracellular polymeric substances of the microalgae *Chlorella* sp. and *Micractinium* sp. in wastewater treatment. *Bioresource Technology* 154, 131-137.
- Wang, X.-J., Xia, S.-Q., Chen, L., Zhao, J.-F., Renault, N., Chovelon, J.-M., 2006. Nutrients removal from municipal wastewater by chemical precipitation in a moving bed biofilm reactor. *Process Biochemistry* 41, 824-828.
- Wilkie, A.C., Mulbry, W.W., 2002. Recovery of dairy manure nutrients by benthic freshwater algae. *Bioresource Technology* 84, 81-91.
- Williams, P.J.I.B., Laurens, L.M., 2010. Microalgae as biodiesel & biomass feedstocks: review & analysis of the biochemistry, energetics & economics. *Energy & Environmental Science* 3, 554-590.
- Woertz, I., Feffer, A., Lundquist, T., Nelson, Y., 2009. Algae grown on dairy and municipal wastewater for simultaneous nutrient removal and lipid production for biofuel feedstock. *Journal of Environmental Engineering* 135, 1115-1122.
- Xia, J.-R., Gao, K.-S., 2005. Impacts of elevated CO₂ concentration on biochemical composition, carbonic anhydrase, and nitrate reductase activity of freshwater green algae. *Journal of Integrative Plant Biology* 47, 668-675.
- Xin, L., Hong-ying, H., Ke, G., Jia, Y., 2010. Growth and nutrient removal properties of a freshwater microalga *Scenedesmus* sp. LX1 under different kinds of nitrogen sources. *Ecological Engineering* 36, 379-381.

- Xin, L., Hong-ying, H., Yu-ping, Z., 2011. Growth and lipid accumulation properties of a freshwater microalga *Scenedesmus* sp. under different cultivation temperature. *Bioresource Technology* 102, 3098-3102.
- Xu, L., Weathers, P.J., Xiong, X.-R., Liu, C.-Z., 2009. Microalgal bioreactors: challenges and opportunities. *Engineering in Life Sciences* 9, 178-189.
- Xu, N., Zhang, X., Fan, X., Han, L., Zeng, C., 2001. Effects of nitrogen source and concentration on growth rate and fatty acid composition of *Ellipsoidion* sp.(Eustigmatophyta). *Journal of Applied Phycology* 13, 463-469.
- Yang, G.-J., Luan, Z.-Q., Zhou, X.-H., Mei, Y., 2010. The researching of the effect of temperature on *Chlorella* growth and content of dissolved oxygen and content of chlorophyll. *Mathematical and Physical Fisheries Science* 8, 68-74.
- Yang, J., Li, X., Hu, H., Zhang, X., Yu, Y., Chen, Y., 2011. Growth and lipid accumulation properties of a freshwater microalga, *Chlorella ellipsoidea* YJ1, in domestic secondary effluents. *Applied Energy* 88, 3295-3299.
- Yang, Y., Gao, K., 2003. Effects of CO₂ concentrations on the freshwater microalgae, *Chlamydomonas reinhardtii*, *Chlorella pyrenoidosa* and *Scenedesmus obliquus* (Chlorophyta). *Journal of Applied Phycology* 15, 379-389.
- Yeesang, C., Cheirsilp, B., 2011. Effect of nitrogen, salt, and iron content in the growth medium and light intensity on lipid production by microalgae isolated from freshwater sources in Thailand. *Bioresource Technology* 102, 3034-3040.
- Yeh, K.-L., Chang, J.-S., chen, W.-m., 2010. Effect of light supply and carbon source on cell growth and cellular composition of a newly isolated microalga *Chlorella vulgaris* ESP-31. *Engineering in Life Sciences* 10, 201-208.
- Yeh, K.L., Chang, J.S., 2011. Nitrogen starvation strategies and photobioreactor design for enhancing lipid content and lipid production of a newly isolated microalga *Chlorella vulgaris* ESP-31: implications for biofuels. *Biotechnology Journal* 6, 1358-1366.
- Yen, H.-W.Y., Hu, I.-C., Chen, C.-Y., Chang, J.-S., 2013. Design of photobioreactors for algal cultivation, in: Pandey, A., Lee, D.-J., Chisti, Y., Soccol, C.R. (Eds.), *Biofuels from Algae*. Elsevier, USA, pp. 23-46.

- Yoon, H.S., Hackett, J.D., Ciniglia, C., Pinto, G., Bhattacharya, D., 2004. A molecular timeline for the origin of photosynthetic eukaryotes. *Molecular Biology and Evolution* 21, 809-818.
- Yun, Y.-S., Lee, S.B., Park, J.M., Lee, C.-I., Yang, J.-W., 1997. Carbon dioxide fixation by algal cultivation using wastewater nutrients. *Journal of Chemical Technology and Biotechnology* 69, 451-455.
- Zafiriadis, I., Ntougias, S., Mirelis, P., Kapagiannidis, A.G., Aivasidis, A., 2012. Molecular characterization of denitrifying bacteria isolated from the anoxic reactor of a modified DEPHANOX plant performing enhanced biological phosphorus removal. *Water Environment Research* 84, 475-484.
- Zamalloa, C., Vulsteke, E., Albrecht, J., Verstraete, W., 2011. The techno-economic potential of renewable energy through the anaerobic digestion of microalgae. *Bioresource Technology* 102, 1149-1158.
- Zang, Y., Li, Y., Wang, C., Zhang, W., Xiong, W., 2015. Towards more accurate life cycle assessment of biological wastewater treatment plants: a review. *Journal of Cleaner Production* 107, 676-692.
- Zhou, Q., Takenaka, S., Murakami, S., Seesuriyachan, P., Kuntiya, A., Aoki, K., 2007. Screening and characterization of bacteria that can utilize ammonium and nitrate ions simultaneously under controlled cultural conditions. *Journal of Bioscience and Bioengineering* 103, 185-191.
- Zhu, L., Wang, Z., Shu, Q., Takala, J., Hiltunen, E., Feng, P., Yuan, Z., 2013. Nutrient removal and biodiesel production by integration of freshwater algae cultivation with piggery wastewater treatment. *Water Research* 47, 4294-4302.
- Zita, A., Hermansson, M., 1994. Effects of ionic strength on bacterial adhesion and stability of flocs in a wastewater activated sludge system. *Applied and Environmental Microbiology* 60, 3041-3048.
- Zwietering, M., Jongenburger, I., Rombouts, F., Van't Riet, K., 1990. Modeling of the bacterial growth curve. *Applied and Environmental Microbiology* 56, 1875-1881.

Annexes

Annex I

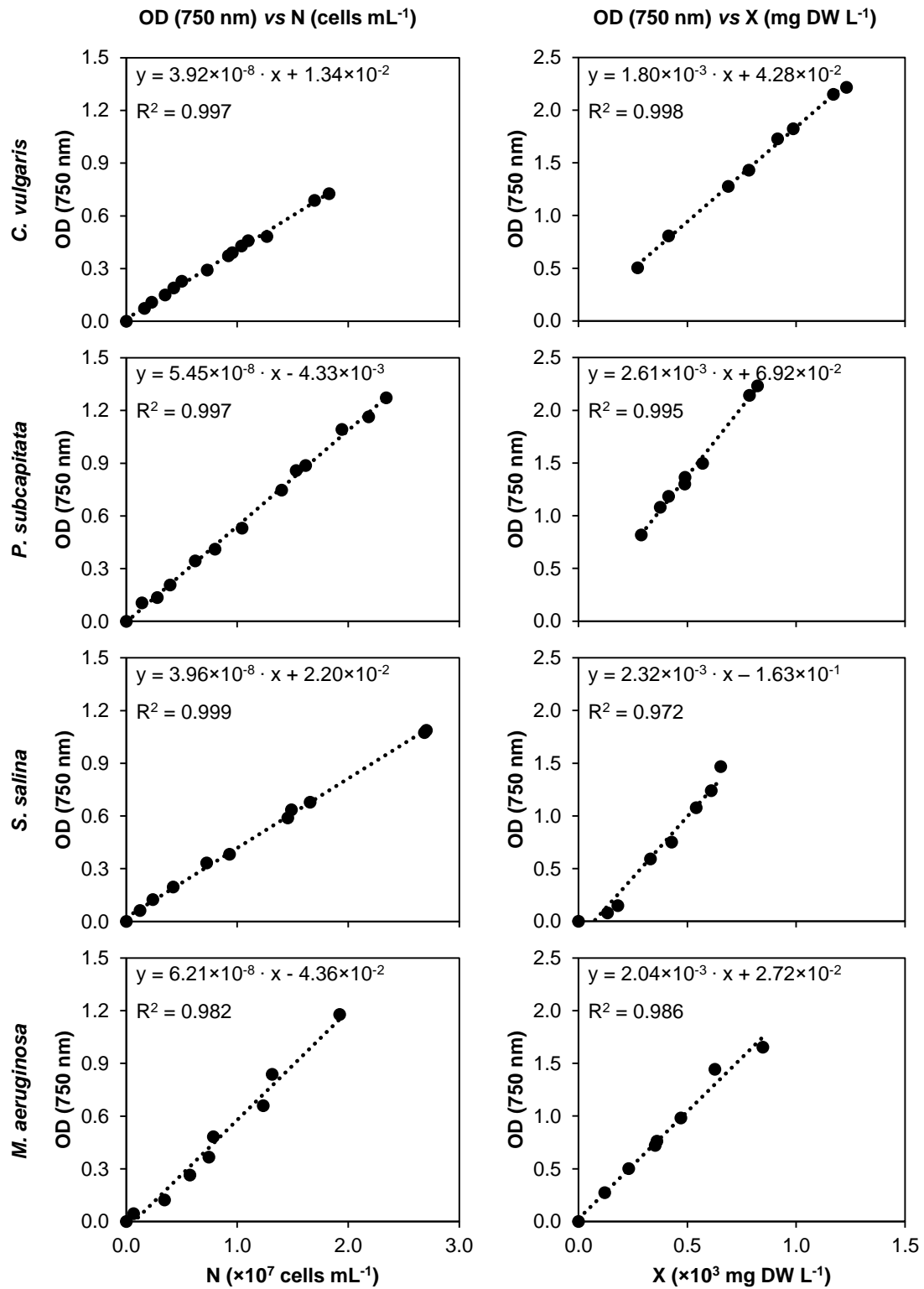


Figure I.1. Linear regression between OD (750nm) and cell concentrations (N , in cells mL^{-1}) and between OD (750 nm) and biomass concentrations (X , in mg DW L^{-1}) for each of the studied microorganisms.

Table I.1. Values of temperature, average daily light irradiance and specific growth rate used to determine optimal light and temperature conditions for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* through mathematical modelling

T (°C)	I ($\mu\text{E m}^{-2} \text{s}^{-1}$)	μ (d^{-1})				Reference
		<i>C. vulgaris</i>	<i>P. subcapitata</i>	<i>S. salina</i>	<i>M. aeruginosa</i>	
15	15	0.142	0.099	0.126	0.203	This study
	36	0.328	0.256	0.119	0.160	This study
	75	0.170	0.246	0.133	0.294	This study
	180	0.357	0.601	0.438	0.486	This study
25	15	0.267	0.230	0.214	0.296	This study
	15	-	0.201	-	-	(Pires et al., 2014)
	21	0.544	-	-	-	(Pires et al., 2014)
	21	0.456	0.370	0.327	0.401	This study
	25	0.400	0.441	0.430	0.415	This study
	30	0.387	0.324	-	-	(Pires et al., 2014)
	35	0.875	0.775	0.700	0.702	This study
	36	0.425	-	-	-	(Pires et al., 2014)
	36	0.704	0.321	-	-	This study
	40	0.367	0.324	-	-	(Pires et al., 2014)
	42	-	0.465	-	-	(Pires et al., 2014)
	50	0.479	-	-	-	(Li et al., 2011b)
	50	0.495	0.487	0.344	0.435	This study
	53	0.469	0.354	-	-	(Pires et al., 2014)
	56	0.659	0.635	-	-	(Pires et al., 2014)
	60	0.868	0.976	0.932	0.810	This study
	70	0.751	0.672	0.729	0.653	This study
	72	-	0.417	-	-	(Pires et al., 2014)
	74	0.485	0.543	-	-	(Pires et al., 2014)
	75	0.528	-	0.517	0.521	This study
	96	0.738	-	-	-	(Pires et al., 2014)
	105	1.11	0.990	-	-	This study
	120	-	-	1.13	1.03	This study

T - temperature (in °C); I - average daily light irradiance (in $\mu\text{E m}^{-2} \text{s}^{-1}$); μ - specific growth rate (in d^{-1}).

Table I.1. (Continued)

T (°C)	I ($\mu\text{E m}^{-2} \text{s}^{-1}$)	μ (d^{-1})				Reference
		<i>C. vulgaris</i>	<i>P. subcapitata</i>	<i>S. salina</i>	<i>M. aeruginosa</i>	
25	126	-	0.421	-	-	(Pires et al., 2014)
	138	1.30	-	-	-	(Yeh et al., 2010)
	180	-	1.14	1.14	1.01	This study
	193	1.10	-	-	-	(Yeh et al., 2010)
35	15	0.0666	0.0188	0.140	0.185	This study
	36	0.193	0.0616	0.308	0.332	This study
	75	0.424	0.577	0.392	0.307	This study
	180	0.745	0.572	0.596	0.528	This study

T - temperature (in °C); I - average daily light irradiance (in $\mu\text{E m}^{-2} \text{s}^{-1}$); μ - specific growth rate (in d^{-1}).

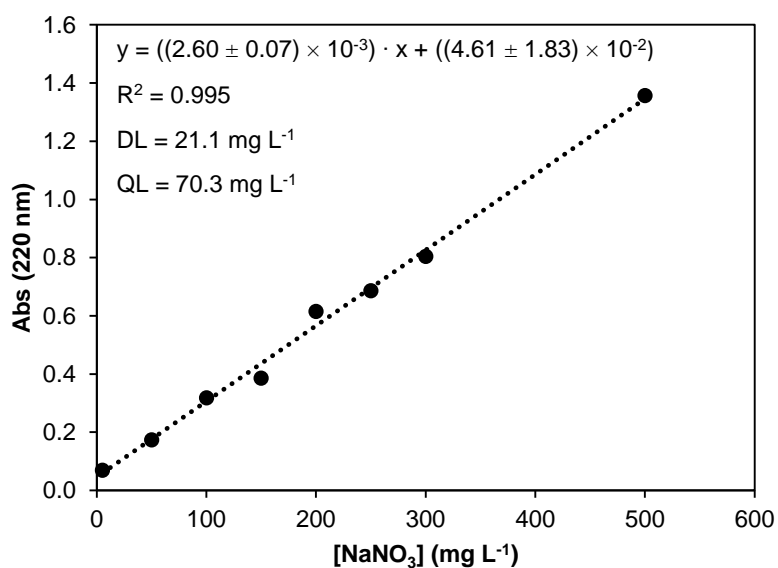


Figure I.2. Calibration curve of absorbance measured at 220 nm as a function of NaNO₃ concentration (in mg L⁻¹).

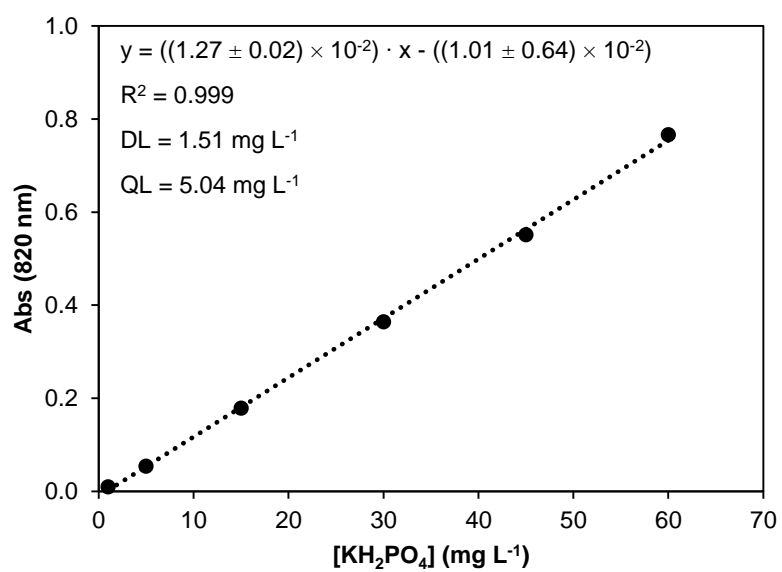


Figure I.3. Calibration curve of absorbance measured at 820 nm as a function of KH₂PO₄ concentration (in mg L⁻¹).

Annex II

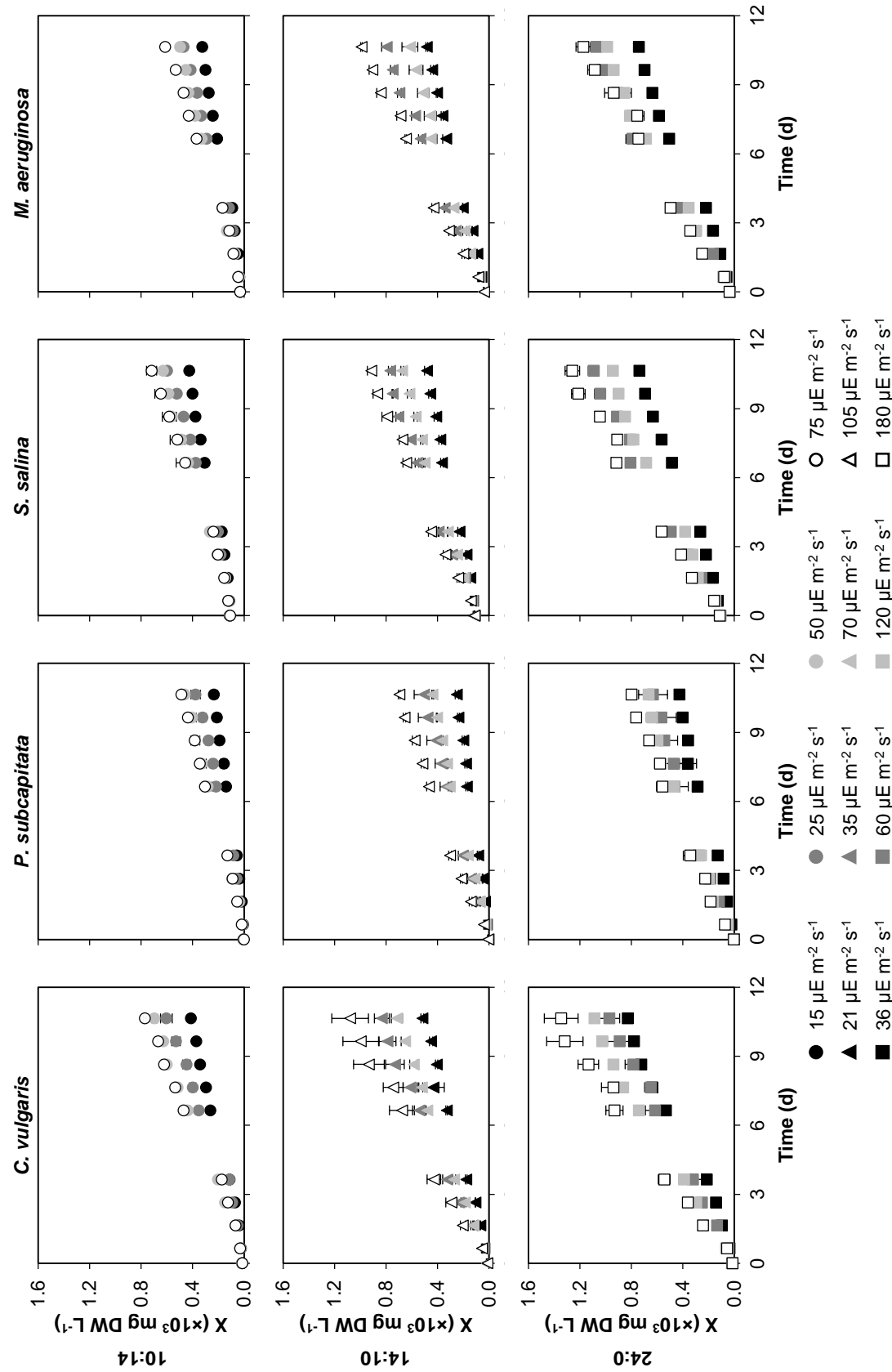


Figure II.1. Growth curves obtained for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* grown under different light conditions. Error bars correspond to the standard deviation of the mean determined for two independent experiments.

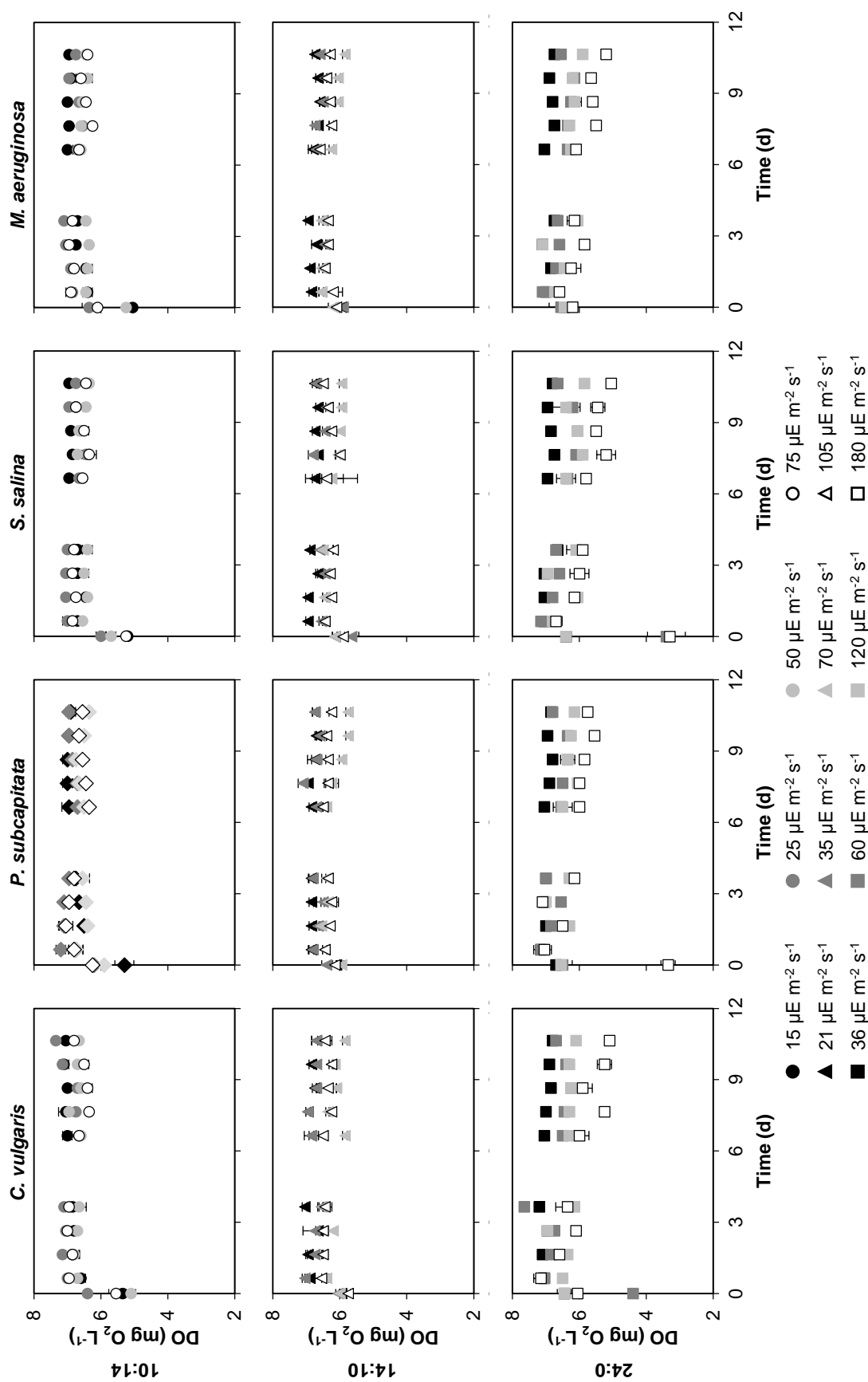


Figure II.2. Time-course evolution of dissolved oxygen concentration (DO , in $\text{mg O}_2 \text{L}^{-1}$) in the culture medium for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* cultures grown under different light conditions. Error bars correspond to the standard deviation of the mean determined for two independent experiments.

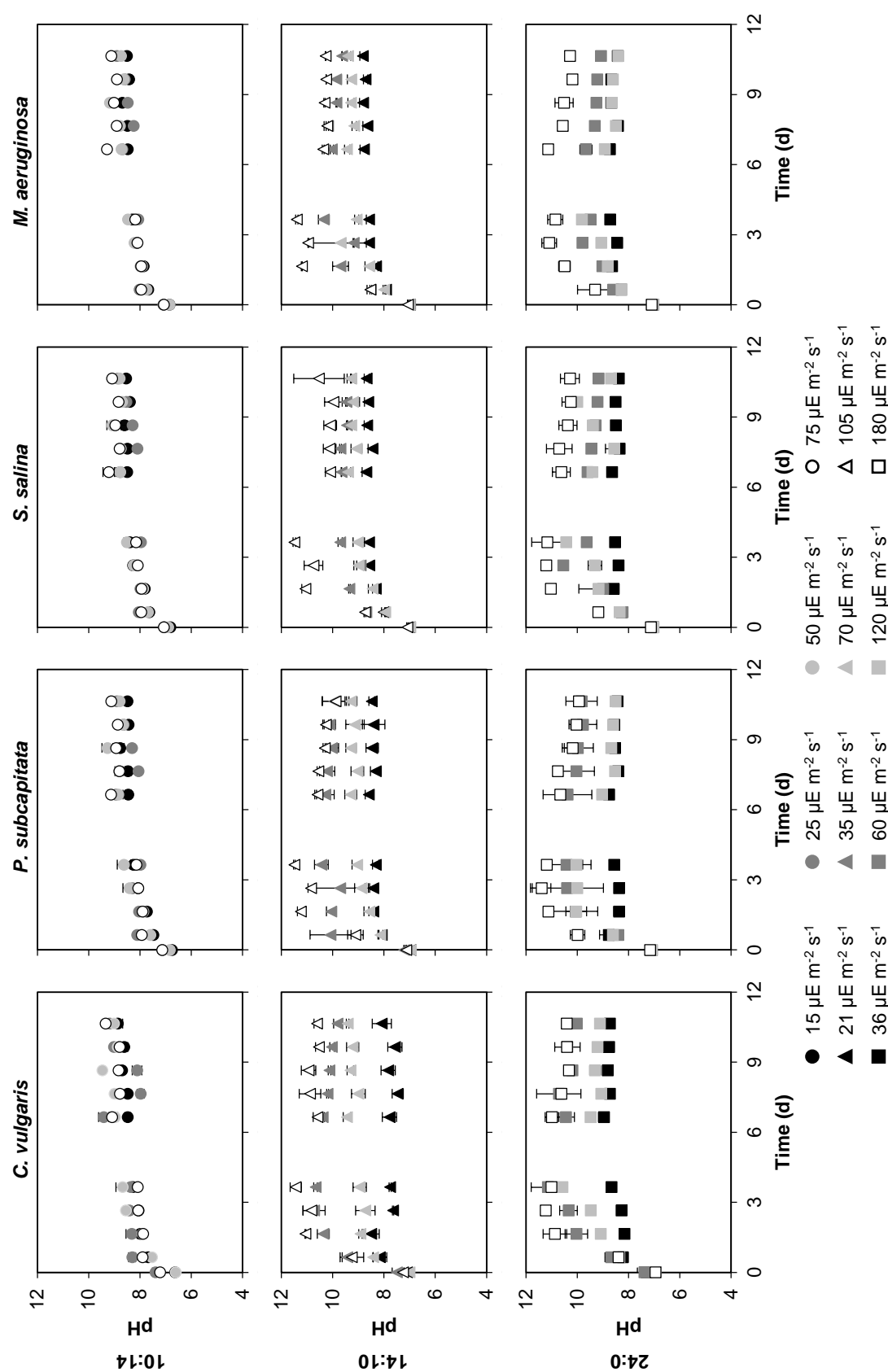


Figure II.3. Time-course evolution of pH in the culture medium for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* cultures grown under different light conditions. Error bars correspond to the standard deviation of the mean determined for two independent experiments.

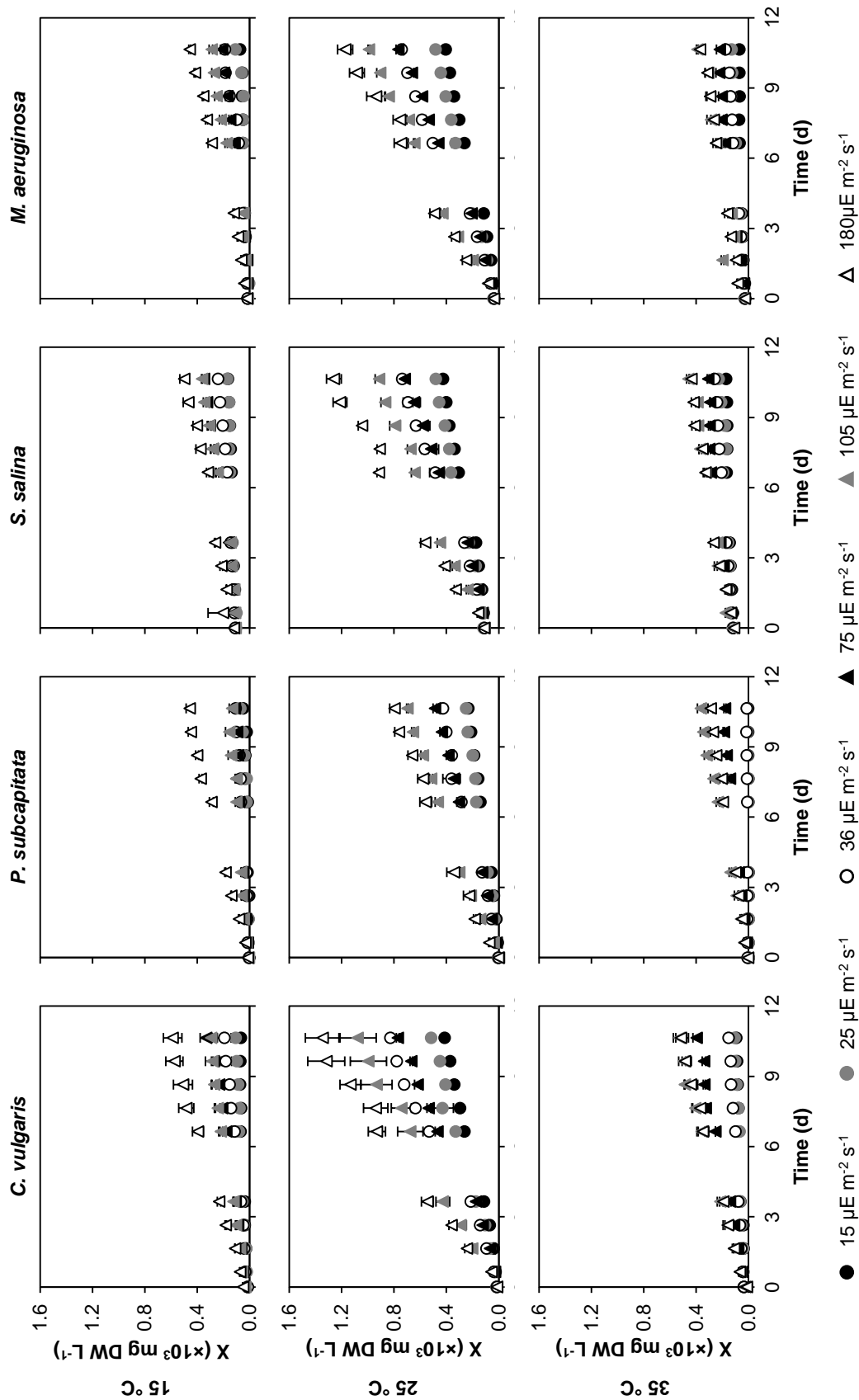


Figure III.1.1. Growth curves obtained for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* grown under different light and temperature conditions. Error bars correspond to the standard deviation of the mean determined for two independent experiments.

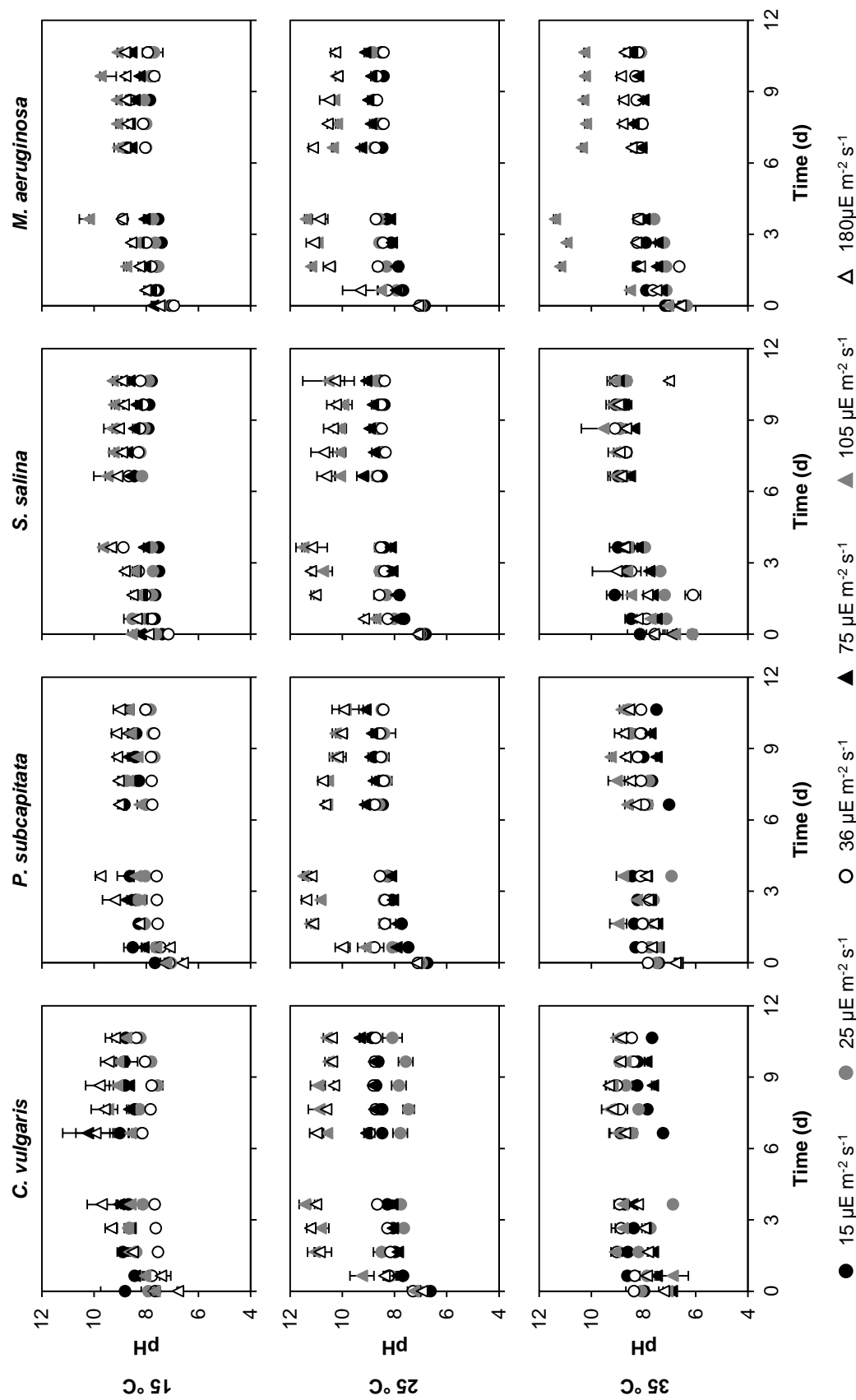


Figure III.2. Time-course evolution of pH in the culture medium for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* cultures grown under different light and temperature conditions. Error bars correspond to the standard deviation of the mean determined for two independent experiments.

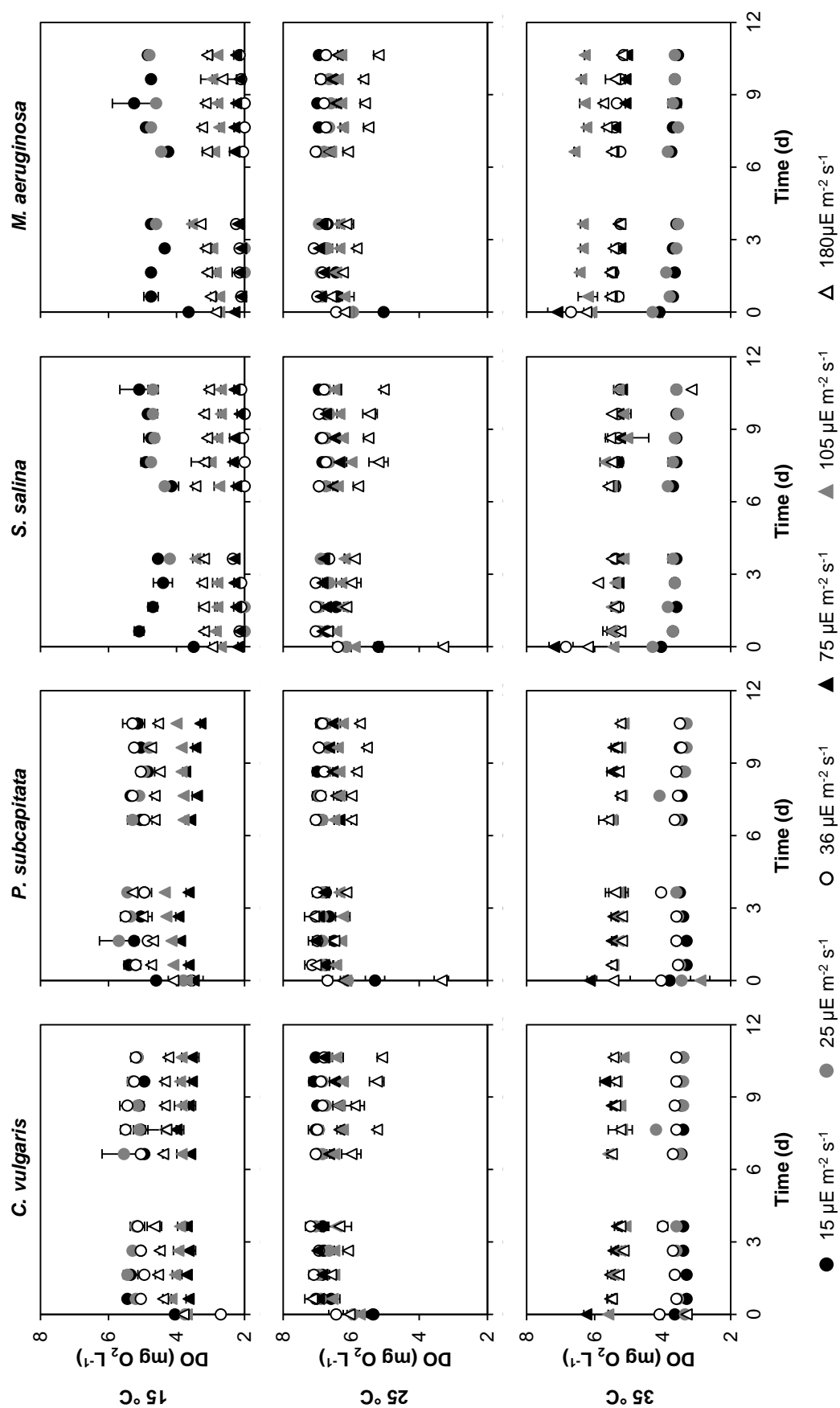


Figure III.3. Time-course evolution of dissolved oxygen concentration (DO , in $\text{mg O}_2 \text{L}^{-1}$) in the culture medium for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* cultures grown under different light and temperature conditions. Error bars correspond to the standard deviation of the mean determined for two independent experiments.

Table III.1. Regression coefficients (β) considered statistically significant determined through multiple linear regression to evaluate the response of nitrogen and phosphorus mass fractions to different light and temperature conditions

Parameter	Microorganisms	β_0	β_1	β_2	β_3	β_4	β_5
α_N	<i>C. vulgaris</i>	0.0425	-0.0155	n.a.	n.a.	n.a.	n.a.
	<i>P. subcapitata</i>	0.0459	n.a.	n.a.	n.a.	n.a.	n.a.
	<i>S. salina</i>	0.0476	-0.0234	n.a.	n.a.	n.a.	n.a.
	<i>M. aeruginosa</i>	0.0490	-0.0378	-0.0405	n.a.	n.a.	0.0482
α_P	<i>C. vulgaris</i>	0.00991	-0.0361	n.a.	0.0371	n.a.	-0.00328
	<i>P. subcapitata</i>	0.00853	-0.00639	-0.00973	n.a.	n.a.	0.0111
	<i>S. salina</i>	0.00982	-0.0295	n.a.	0.0306	n.a.	n.a.
	<i>M. aeruginosa</i>	0.00978	-0.0365	n.a.	0.0371	n.a.	n.a.

α_N – nitrogen mass fraction (in % w/w); α_P – phosphorus mass fraction (in % w/w); n.a. - not applicable ($p>0.05$).

Table III.2. Values of temperature, average daily light irradiance and specific growth rate used to validate the models determined for *C. vulgaris*, *P. subcapitata*, *S. salina* and *M. aeruginosa* (validation data set)

T (°C)	I ($\mu\text{E m}^{-2} \text{s}^{-1}$)	μ (d^{-1})				Reference
		<i>C. vulgaris</i>	<i>P. subcapitata</i>	<i>S. salina</i>	<i>M. aeruginosa</i>	
15	21	0.131	0.245	0.311	0.375	This study
	105	0.313	0.453	0.165	0.275	This study
25	15	0.267	-	-	-	(Pires et al., 2014)
	21	-	0.516	-	-	(Pires et al., 2014)
	36	-	0.662	-	-	(Pires et al., 2014)
	36	-	-	0.620	0.560	This study
	42	0.428	-	-	-	(Pires et al., 2014)
	72	0.523	-	-	-	(Pires et al., 2014)
	75	-	0.668	-	-	(Pires et al., 2014)his study
	96	-	0.496	-	-	(Pires et al., 2014)
	105	-	-	0.918	0.857	This study
	120	1.17	1.13	-	-	This study
35	180	1.19	-	-	-	This study
	21	0.0799	0.0475	0.220	0.226	This study
	105	0.769	0.558	0.392	0.857	This study

T - temperature (in °C); I - average daily light irradiance (in $\mu\text{E m}^{-2} \text{s}^{-1}$); μ - specific growth rate (in d^{-1}).

Annex IV

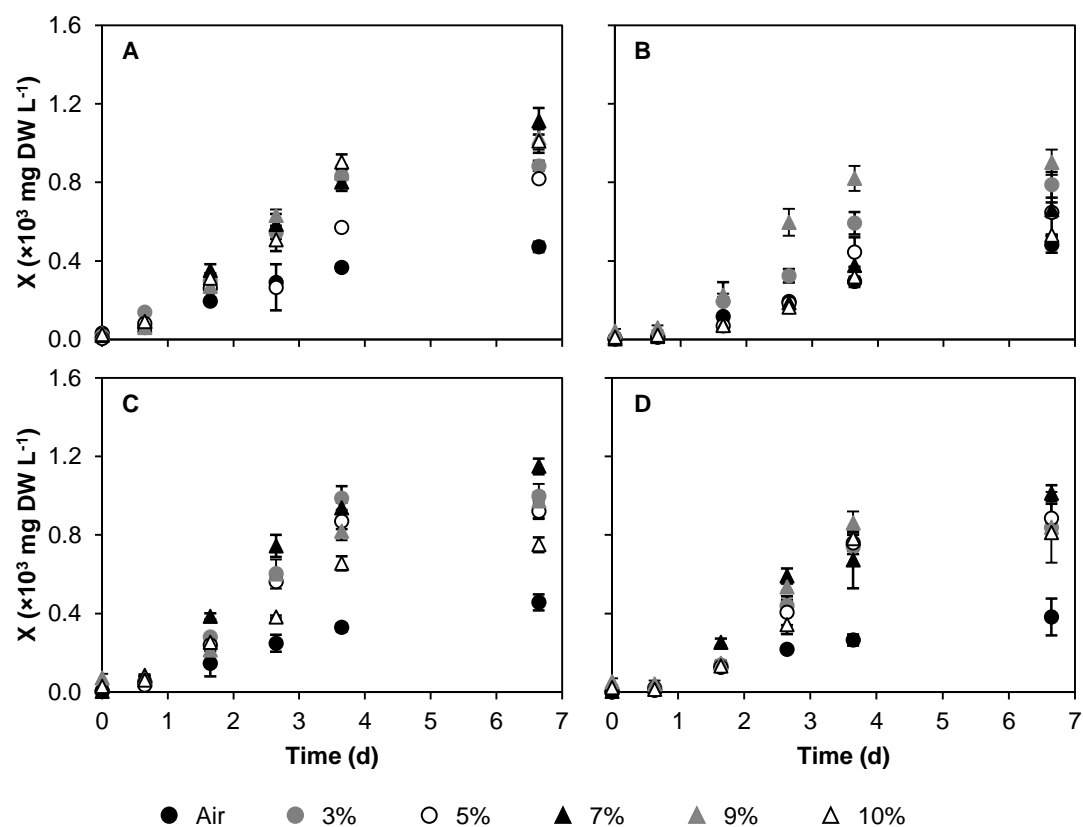


Figure IV.1. Growth curves obtained for *C. vulgaris* (A), *P. subcapitata* (B), *S. salina* (C) and *M. aeruginosa* (D) grown with different CO₂ concentrations in the air stream. Error bars correspond to the standard deviation of the mean determined for two independent experiments.

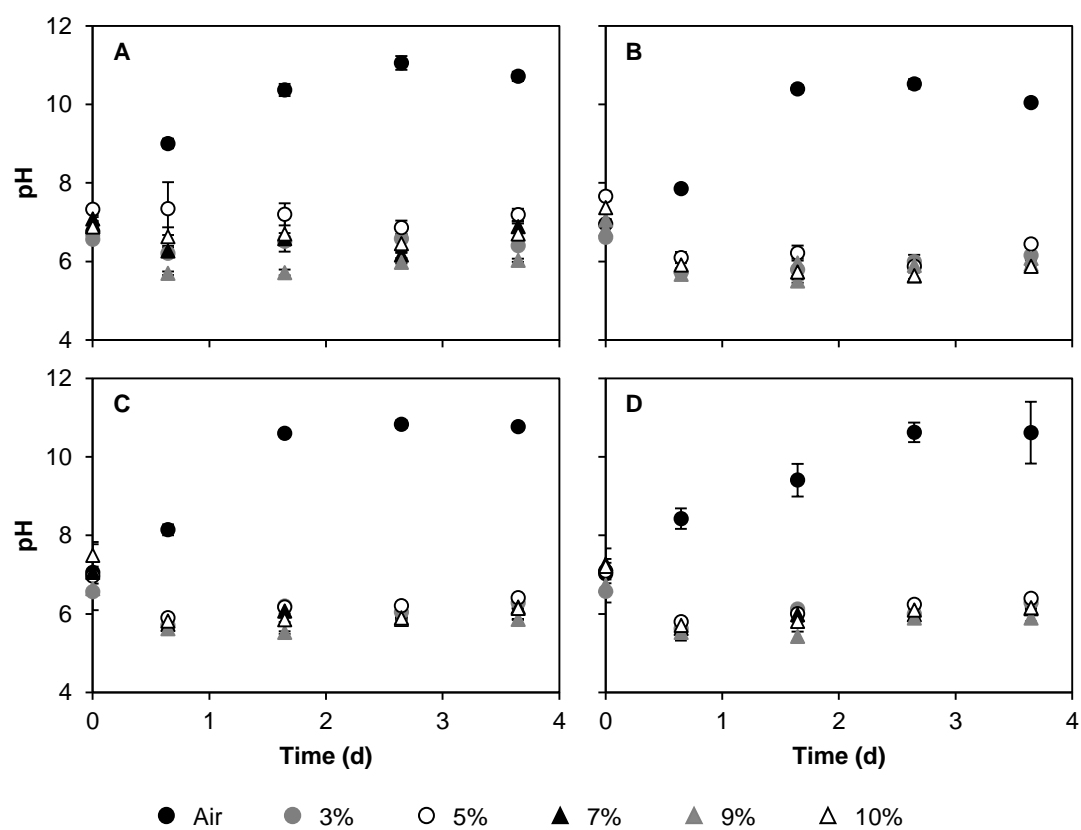


Figure IV.2. Time-course evolution of pH in the culture medium for *C. vulgaris* (A), *P. subcapitata* (B), *S. salina* (C) and *M. aeruginosa* (D) cultures grown with different CO₂ concentrations in the air stream. Error bars correspond to the standard deviation of the mean determined for two independent experiments.

Annex V

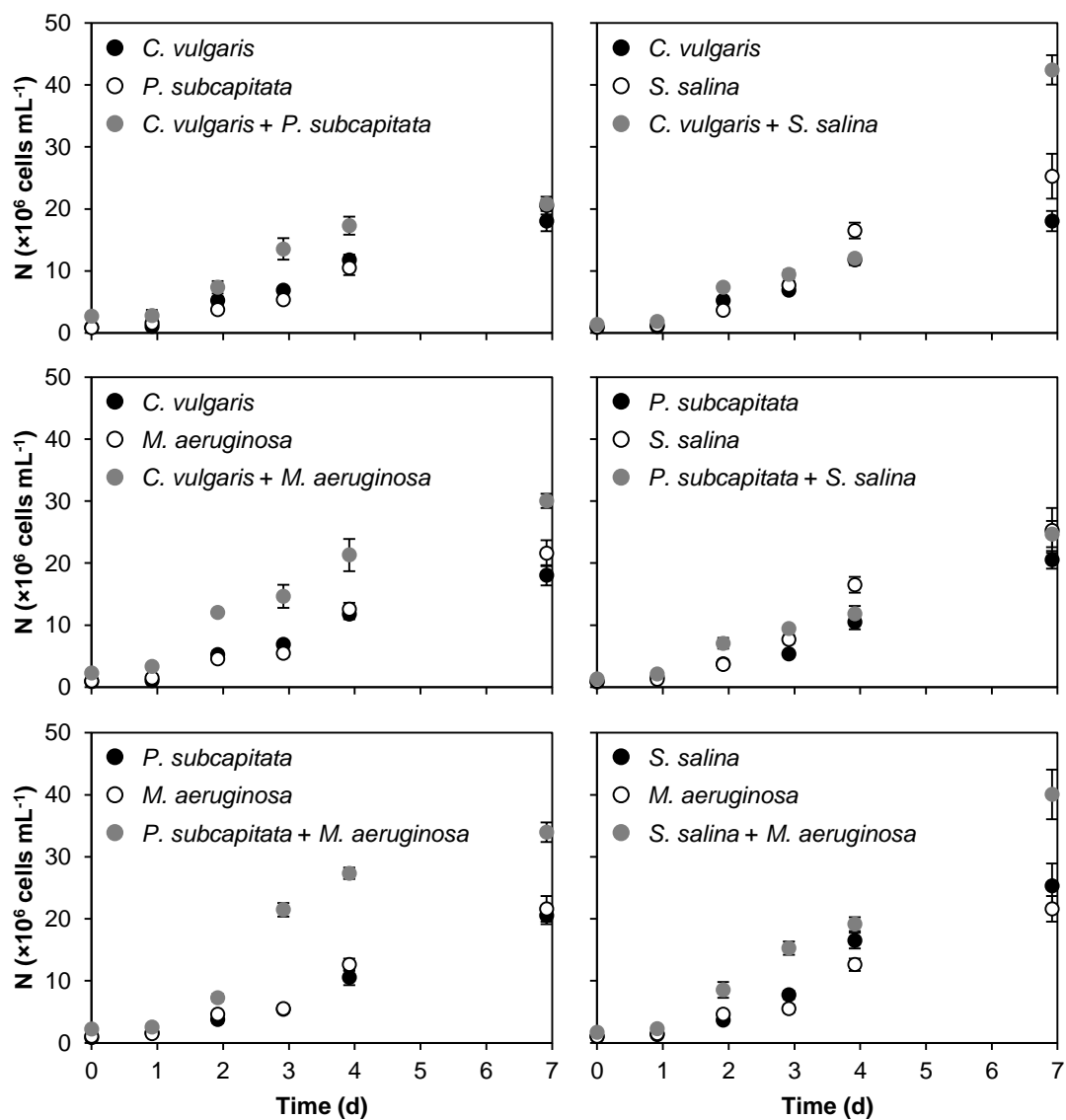


Figure V.1. Growth curves obtained for single and dual-species cultures of microalgae. Error bars correspond to the standard deviation of the mean determined for four independent experiments.

Table V.1. Kinetic growth parameters (μ , in d^{-1} , N_{\max} , in cell mL^{-1} , and P , in $\text{mg DW L}^{-1} \text{d}^{-1}$), carbon contents (α_c , in % w/w) and CO_2 uptake rates (R_c , in $\text{mg CO}_2 \text{L}^{-1} \text{d}^{-1}$) determined for single and dual-species cultures of microalgae

Microorganisms	μ (d^{-1})	N_{\max} (cells mL^{-1})	P (mg DW $\text{L}^{-1} \text{d}^{-1}$)	α_c (% w/w)	R_c (mg $\text{CO}_2 \text{L}^{-1} \text{d}^{-1}$)
<i>C. vulgaris</i>	0.950 ± 0.107	18.0 ± 1.6	47.0 ± 3.1	44.31	76.3 ± 5.0
<i>P. subcapitata</i>	0.665 ± 0.060	20.5 ± 1.4	41.2 ± 9.2	47.32	71.4 ± 16.0
<i>S. salina</i>	0.690 ± 0.028	25.3 ± 3.6	60.2 ± 9.4	42.51	93.8 ± 14.7
<i>M. aeruginosa</i>	0.702 ± 0.047	21.6 ± 2.1	49.9 ± 3.1	42.90	78.4 ± 4.8
<i>C. vulgaris</i> + <i>P. subcapitata</i>	0.346 ± 0.043	20.8 ± 0.7	97.6 ± 1.0	45.69	163 ± 2
<i>C. vulgaris</i> + <i>S. salina</i>	0.811 ± 0.045	42.4 ± 2.4	96.9 ± 8.2	42.38	150 ± 13
<i>C. vulgaris</i> + <i>M. aeruginosa</i>	0.249 ± 0.021	30.0 ± 1.1	83.1 ± 1.0	42.88	131 ± 2
<i>P. subcapitata</i> + <i>S. salina</i>	0.643 ± 0.044	24.7 ± 2.1	63.6 ± 4.1	47.68	111 ± 7
<i>P. subcapitata</i> + <i>M. aeruginosa</i>	0.467 ± 0.045	34.0 ± 1.6	70.1 ± 5.1	47.30	122 ± 9
<i>S. salina</i> + <i>M. aeruginosa</i>	0.696 ± 0.018	40.1 ± 4.0	83.9 ± 10.2	42.64	131 ± 16

Values are presented as the mean \pm standard deviation of two independent experiments.

Table V.2. Average nitrogen and phosphorus removal rates (R_S , in $\text{mg S L}^{-1} \text{d}^{-1}$) and removal efficiencies (R , in %) determined for single and dual-species cultures of microalgae

Microorganisms	Nitrogen		Phosphorus	
	R_N ($\text{mg N L}^{-1} \text{d}^{-1}$)	R (%)	R_P ($\text{mg P L}^{-1} \text{d}^{-1}$)	R (%)
<i>C. vulgaris</i>	0.102±0.006	70.6±4.1	0.116±0.004	80.2±2.8
<i>P. subcapitata</i>	0.0912±0.0135	62.9±9.3	0.0730±0.0036	50.4±2.5
<i>S. salina</i>	0.0758±0.0032	52.3±2.2	0.112±0.005	77.0±3.6
<i>M. aeruginosa</i>	0.0722±0.0054	49.8±3.7	0.118±0.004	81.3±3.0
<i>C. vulgaris</i> + <i>P. subcapitata</i>	0.0990±0.0019	68.3±1.3	0.104±0.002	71.8±1.6
<i>C. vulgaris</i> + <i>S. salina</i>	0.122±0.003	84.5±2.3	0.124±0.004	85.9±2.7
<i>C. vulgaris</i> + <i>M. aeruginosa</i>	0.117±0.016	80.8±11.2	0.0902±0.0011	62.3±0.7
<i>P. subcapitata</i> + <i>S. salina</i>	0.104±0.008	72.0±5.3	0.133±0.002	91.8±1.3
<i>P. subcapitata</i> + <i>M. aeruginosa</i>	0.112±0.002	77.6±1.1	0.0983±0.0018	67.8±1.3
<i>S. salina</i> + <i>M. aeruginosa</i>	0.113±0.001	77.7±0.9	0.141±0.003	97.2±1.9

Values are presented as the mean ± standard deviation of two independent experiments.

Annex VI

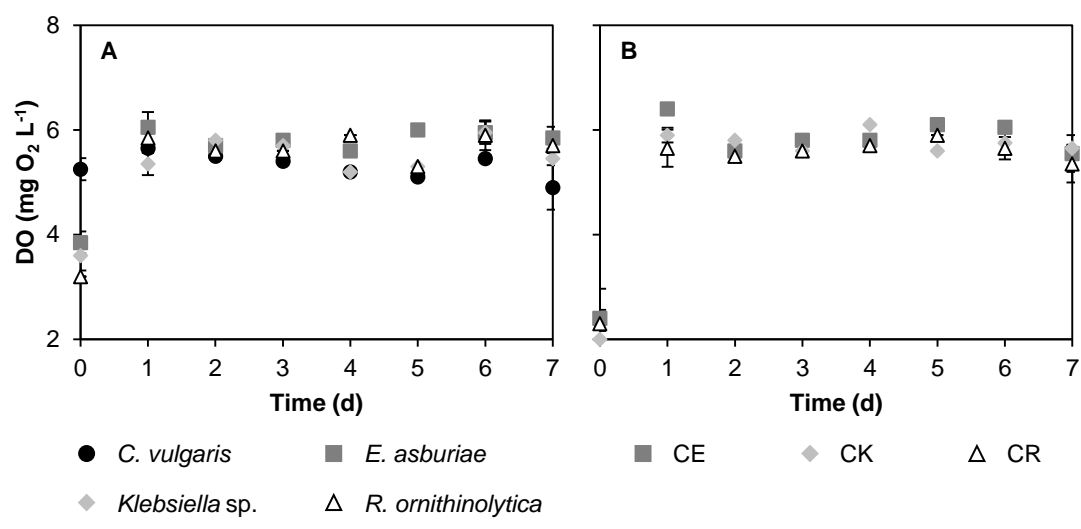


Figure VI.1. Time-course evolution of dissolved oxygen concentration (DO , in $\text{mg O}_2 \text{ L}^{-1}$) in the culture medium obtained for the studied microorganisms when grown in single (A) and dual-species (B) cultures containing *C. vulgaris*. Error bars correspond to the standard deviation of the mean determined for four independent experiments.

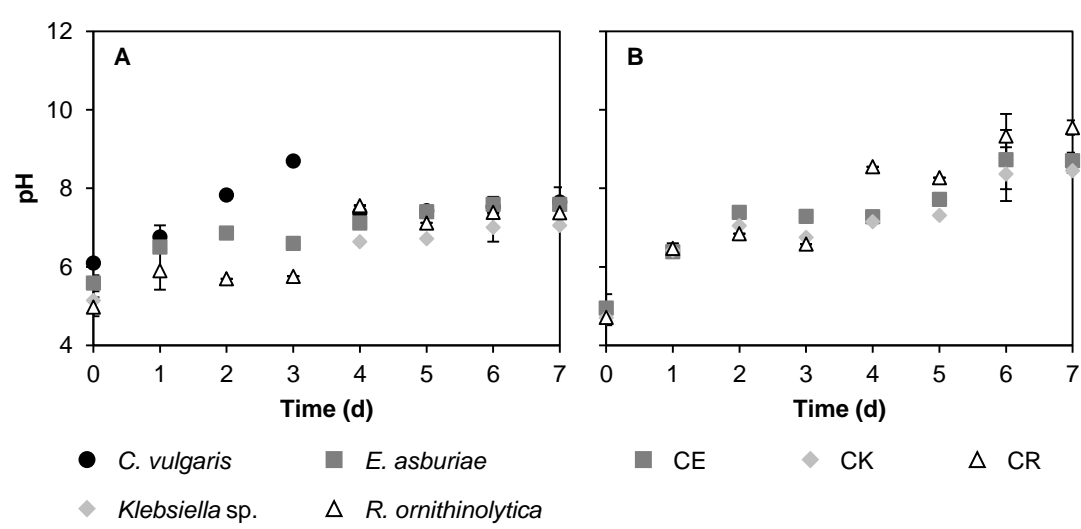


Figure VI.2. Time-course evolution of pH in the culture medium obtained for the studied microorganisms when grown in single (A) and dual-species (B) cultures containing *C. vulgaris*. Error bars correspond to the standard deviation of the mean determined for four independent experiments.

Annex VII

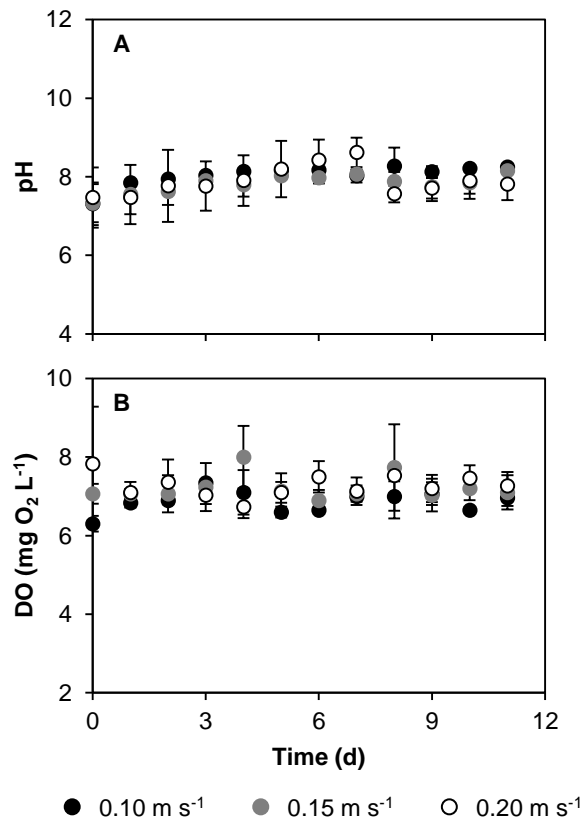


Figure VII.1. Time-course evolution of pH (A) and dissolved oxygen concentration (DO, in mg O₂ L⁻¹) (B) in the culture medium for *C. vulgaris* grown in the raceway pond under different fluid velocities. Error bars correspond to the standard deviation of the mean determined for two independent experiments.