

MICROALGAE-BASED BIOREMEDIATION

ASSESSMENT OF WASTEWATER TREATMENT IN THE PORTO WASTE MANAGEMENT SERVICE

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Resumo

Os resíduos verdes, juntamente com os resíduos alimentares, constituem a maior fração dos resíduos urbanos. Geralmente estes são mantidos em pilhas pelos serviços de gestão de resíduos antes da trituração, o que garante um melhor desempenho na sua compostagem ou na produção de energia. É frequente a formação de um lixiviado rico em nutrientes nessas pilhas, pelo que é necessário um tratamento do efluente para evitar um escoamento descontrolado e a poluição dos cursos de água. Atualmente, esses efluentes são em grande maioria redirecionados para estações de tratamento de águas municipais, o que acarreta altos custos de transporte e tratamento. Consequentemente, existe a necessidade de um processo de remediação de lixiviados barato e aplicável localmente que possa permitir, por exemplo, a posterior descarga do efluente tratado.

Neste trabalho é proposto um processo de biorremediação à base de microalgas aplicável no local para lidar com um lixiviado de resíduos verdes da LIPOR - Serviço Intermunicipalizado de Gestão de Resíduos do Grande Porto, com base na literatura disponível e recente. Para o crescimento de microalgas, sugere-se um sistema de *high rate algal pond*. Para a etapa de colheita, consideram-se as técnicas de autofloculação através da precipitação com hidróxido de magnésio e centrifugação. Estima-se que o sistema seja capaz de produzir 208 kg de biomassa seca de microalgas por ano. Para o efluente tratado é proposto um sistema de desinfecção solar para garantir a remoção de agentes patogénicos exigida pela legislação. Além disso, são exploradas as aplicações para os produtos resultantes do processo. O desenvolvimento de um corretivo orgânico de solos com propriedades bioestimulantes a partir da biomassa e o uso do efluente tratado para a promoção da biodiversidade são apontados como as opções mais indicadas. Para os lixiviados produzidos na LIPOR, o uso para irrigação é permitido pela legislação antes e depois da remediação. Um diagrama resumindo todo o processo é apresentado. É também feita uma avaliação económica que permite concluir que após dois anos de operação, a redução de custos advinda da alteração da gestão atual do lixiviado cobre os custos de investimento. Após isso, o processo é sustentável, porém economicamente frágil, pois apresenta uma margem de lucro muito baixa.

Uma prova de conceito foi produzida usando *Chlorella vulgaris* e os lixiviados produzidos na LIPOR. O trabalho experimental permitiu verificar que a microalga *Chlorella vulgaris* pode crescer em meios de cultura à base de lixiviados, porém, para a remoção de nutrientes (nitratos, fosfatos, CQO e CBO₅) há evidências de que a

metodologia definida não foi adequada o suficiente para avaliá-la corretamente. Foram propostas sugestões para o ajuste da metodologia para trabalhos futuros. Não obstante, em alguns ensaios houve remoção parcial de nitratos (6%) e fosfatos (11-21%), mostrando assim o potencial do processo para remediação de efluentes, bem suportado pela maior parte da bibliografia. Este estudo foi o primeiro contributo para a simultânea remediação e valorização de lixiviados de resíduos verdes na LIPOR.

Palavras-chave: Lixiviado de resíduos verdes; Microalgas; Biorremediação; Avaliação tecno-econômica; Tratamento de águas.

Abstract

Green waste along with food waste constitutes the highest fraction of urban waste. This is usually maintained in a large pile by waste management services prior to size reduction to ensure better composting or energy production performance. As a leachate rich in nutrients is often formed in these piles, an effluent treatment is required to avoid uncontrolled runoff and pollution of watercourses. Nowadays, these wastewaters are mostly redirected to municipal wastewater treatment plants, which entail high transportation and treatment costs. Therefore, there is a need for a low-cost and on-site leachate remediation process that may allow, for example, the discharge of treated effluent into a watercourse.

In this work, an on-site microalgae-based bioremediation process is proposed to deal with a green waste leachate from LIPOR - Serviço Intermunicipalizado de Gestão de Resíduos do Grande Porto, based on the available and recent literature. For the microalgae growth, a high rate algal pond system is suggested. For the harvesting and dewatering steps, microalgae autoflocculation via magnesium hydroxide precipitation and centrifugation are considered. The whole system is estimated to produce 208 kg of dry microalgae biomass per year. For the treated effluent, a solar disinfection system is proposed to ensure the removal of pathogens demanded by regulation. Moreover, applications for the resulting products are explored. The development of an organic soil improver with biostimulant properties based on biomass and the use of the treated effluent for biodiversity promotion are pointed out as the most suitable options. For the leachates produced at LIPOR, the use for irrigation is allowable by the legislation before and after the remediation. A diagram summarizing the entire process is presented. An economic assessment is also made, which allows to conclude that after two years of operation, the savings from avoiding the current treatment and transportation costs cover the investment costs. Afterwards, the process is sustainable, however economically fragile since it presents a very low profit margin.

A proof of concept was produced using *Chlorella vulgaris* and the leachates produced at LIPOR. The experimental work allowed to verify that the microalga *Chlorella vulgaris* can grow in leachate-based growth media, however, for the removal of nutrients (nitrates, phosphates, COD and BOD₅) there is evidence that the defined methodology was not adequate enough to properly evaluate it. Suggestions for the adjustment of the methodology for future works were proposed. Nevertheless, in some

experiments there was partial removal of nitrates (6%) and phosphates (11-21%), showing the potential of the process for effluent remediation, well supported in most of the literature. This study was the first contribution to the simultaneous remediation and valorisation of green waste leachates at LIPOR.

Keywords: Green waste leachate; Microalgae; Bioremediation; Techno-economic assessment; Wastewater treatment.

Declaração

Declaro, sob compromisso de honra, que este trabalho é original e que todas as contribuições não originais foram devidamente referenciadas com identificação da fonte.

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Nomenclature

Acronyms

AS	Activated sludge
BOD	Biochemical oxygen demand
C:N	Carbon to nitrogen ratio
CAPEX	Capital expenditures
COD	Chemical oxygen demand
CPC	Compound parabolic collector
GW	Green waste
GWL	Green waste leachate
HRAP	High rate algal pond
HRT	Hydraulic residence times
LED	Light emitting diode
MPN	Most probable number
OECD	Organization for Economic Co-operation and Development
OPEX	Operational expenditures
PBR	Photobioreactor
PVC	Polyvinyl chloride
SLL	Sanitary landfill leachate
SODIS	Solar disinfection
TN	Total nitrogen
TOC	Total organic carbon
TP	Total phosphorous
TSS	Total soluble solids
WWTP	Wastewater treatment plant

Notation

A	Surface area (m^2)
Abs _{220nm}	Absorbance at 220 nm
Abs _{820nm}	Absorbance at 820 nm
C _{KH₂PO₄}	Concentration of KH ₂ PO ₄ ($\text{mg}\cdot\text{L}^{-1}$)
C _{NaNO₃}	Concentration of NaNO ₃ ($\text{mg}\cdot\text{L}^{-1}$)
d _h	Hydraulic diameter (m)
e	Motor efficiency (%)
f _M	Manning friction factor ($\text{s}\cdot\text{m}^{-1/3}$)
g	Gravitational acceleration ($\text{m}\cdot\text{s}^{-2}$)
OD _{750nm}	Optical density at 750 nm
P	Average biomass productivity ($\text{mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$)
p	Straight channel length (m)
P _p	Paddlewheel power requirement (W)
q	Reactor total width (m)
R	Nutrients removal efficiency (%)
R _s	Average nutrients removal rates ($\text{mg}\cdot\text{L}^{-1}$)
S _f	Nutrients concentration at the end of the cultivation time ($\text{mg}\cdot\text{L}^{-1}$)
S _i	Nutrients concentration in the beginning of the cultivation time ($\text{mg}\cdot\text{L}^{-1}$)
t _f	End of the cultivation time (d)
t _i	Beginning of the cultivation time (d)
u	Flow velocity ($\text{m}\cdot\text{s}^{-1}$)
V	Reactor volume (m^3)
X	Biomass concentration in dry weight ($\text{mg}\cdot\text{L}^{-1}$)
X _f	Biomass concentration at the end of the cultivation time ($\text{mg}\cdot\text{L}^{-1}$)
X _i	Biomass concentration in the beginning of the cultivation time ($\text{mg}\cdot\text{L}^{-1}$)
ρ	Culture broth density ($\text{kg}\cdot\text{m}^{-3}$)

1. Introduction

1.1. Framework

Green waste (GW) along with food waste constitutes the highest fraction of urban waste – 44% of world's municipal solid waste (Kumar et al., 2010; Sharma & Jain, 2020). The conversion of these organic wastes into useful products is essential to improve the sustainability of agricultural production and the conservation of natural resources (Muscolo et al., 2021). There are two main technologies to manage and treat these residues: composting by aerobic digestion and bioenergy production by anaerobic digestion (Viretto et al., 2021). However, composting, i.e., a controlled biological process used to stabilize and transform organic wastes into a valuable soil amendment (Barrington et al., 2003), is by far the most-used method (Bustamante et al., 2016; López et al., 2010).

GW, i.e., pruning yard wastes, fallen leaves, grass clippings among other vegetal materials and soil, is expensive to transfer and unattractive, due to its low bulk density and its low value, respectively. It is usually maintained in a large pile by waste management services prior to size reduction (e.g. by shredding), which ensures better composting performance (Reyes-Torres et al., 2018). Although it depends on the presence of high moisture content wastes (e.g. fruit and vegetables) and weather, a leachate is often formed in these piles due to the beginning of aerobic and anaerobic microbial decomposition and water percolation (Gupta et al., 2014). If the deposit is over an impermeable surface, as it is in Greater Porto waste management service, this leachate must be collected and stored to avoid uncontrolled runoff and pollution of watercourses (Tyrrel et al., 2008). Nowadays, this wastewater is mostly redirected to municipal wastewater treatment plants - a high cost option mainly due to transportation and operating costs (Brennan et al., 2017; Savage & Tyrrel, 2005). On-site treatment is an alternative to paying high tank transport costs, however, to date, few studies have been published on the subject (Savage & Tyrrel, 2005; Tyrrel et al., 2008).

In the two last decades, the generation of urban green waste has raised worldwide (Boldrin et al., 2011; Eades et al., 2020; Viretto et al., 2021). Consequently, the still very centralized waste management services have to deal with increasing quantities of GW and GW leachate (Angouria-Tsorochidou et al., 2021). Therefore, there is a need for a low-cost and on-site leachate remediation process that may permit, for example, the

discharge of treated effluent into a watercourse. As these wastewaters commonly present high concentration of nutrients, i.e., high values of BOD₅ (biochemical oxygen demand) and ammoniacal nitrogen (Frederickson, 1997), biological treatments (bioremediation) are more suitable (Burton et al., 2013). Although the microalgae high-potential for leachate remediation (phycoremediation) has been demonstrated in several works (Khanzada & Övez, 2017; Koul et al., 2022; Paiva et al., 2021), its growth is highly-dependent on leachates' composition profile (Paskuliakova et al., 2018). Thus, a proof-of-concept should be always obtained for a given combination of leachate and microalgae strain.

1.2. LIPOR - Waste Management Service of Porto

LIPOR - Serviço Intermunicipalizado de Gestão de Resíduos do Grande Porto (Intermunicipalized Waste Management Service of Porto) - is the entity responsible for the management, recovery and treatment of urban waste produced by the eight municipalities that comprise it: Espinho, Gondomar, Maia, Matosinhos, Porto, Póvoa de Varzim, Valongo and Vila do Conde (Figure 1). It was established in 1982 as an association of municipalities and, since then, it has been implementing an Integrated Solid Waste Management – *“a strategic approach to sustainable management of solid wastes covering all sources and aspects, such as generation, segregation, transfer, sorting, treatment, recovery and disposal in an integrated manner, with an emphasis on maximizing resource use efficiency”* (International Environmental Technology Centre - United Nations Environment Programme) – through the recovery, expansion and construction of new infrastructures, complemented with public awareness campaigns. Currently, this strategy is based on four main components: Multimaterial, Organic and Energy Recovery, complemented by a Sanitary Landfill to receive process rejects and previously prepared waste. The first two are located at LIPOR I (Gondomar) and the last two at LIPOR II (Maia). Every year, 500,000 tons of urban waste produced by about 1 million inhabitants are managed and treated at LIPOR's facilities. In addition to waste management, LIPOR also seeks to fulfill a sustainable development agenda based on three pillars - environmental, economic and social - through *“valuing People, being in harmony with the Planet, projecting Prosperity and creating value through Partnerships”* (www.lipor.pt). To implement an innovation strategy, the Research, Development and Innovation Unit was created, where the present work was developed.

1.3. Main objectives

In this work, an on-site microalgae-based bioremediation process is proposed, designed and dimensioned to deal with a GW leachate from LIPOR, based on the available and recent literature. In addition, applications are explored for the resulting biomass and treated effluent, in order to present the best option to the company, considering all of the LIPOR guidelines and principles. An economic assessment is carried out to evaluate the viability of the project. Moreover, a proof-of-concept was produced using a chosen microalgae strain and the GW leachate. Two landfill leachates were also assessed to verify whether the proposed system can be scaled up using other effluents produced at LIPOR's facilities.

The remainder of this thesis is outlined as follows: in Section 2 a review of the literature is presented; in Section 3 the proposal of the phycoremediation process is made and supported; Section 4 presents the methods used during the production of the proof-of-concept; in Section 5 the results obtained using the methodology presented in section 4 and respective discussion are presented; in Sections 6 and 7 the main conclusions and suggestions for future work are presented, respectively.



Figure 1 - Area of LIPOR action (light green) and facilities.

2. Literature review

In this chapter, a brief and directed literature review is made in order to present the most suitable alternative for the construction and implementation of a leachate phycoremediation system at LIPOR's facilities (see section 3). Moreover, harvesting and dewatering techniques and applications for the resulting microalgae biomass are also explored.

2.1. Microalgae

Microalgae are microscopic algae (1–10 μm) found as part of phytoplankton and in nearly all aquatic, terrestrial and sub-aerial surfaces, including all types of soil (Larkum et al., 2012). They represent a large group of either eukaryotic (e.g. green algae, diatoms, euglenoids) or prokaryotic (cyanobacteria), typically photoautotrophic microorganisms, which occupy very different phylogenetic positions (Alvarez et al., 2021; Metting, 1996). Morphology, physiology and biochemical characteristics of these organisms are highly diverse (Reimann et al., 2020). They are found under several different combinations of environmental conditions of temperature, salinity, sunlight exposure, acidity and alkalinity (Gouveia, 2011; Larkum et al., 2012; Soru et al., 2019; Subashchandrabose et al., 2013). It is estimated that there are more than 350,000 algal species, most of which are unicellular (Brodie, 2007).

Microalgae grow mainly autotrophically through photosynthesis - using solar light as source of energy and atmospheric carbon dioxide (CO_2) as source of carbon, releasing oxygen (O_2) in the process - however, some species can grow heterotrophically, using available organic carbon as carbon source, and mixotrophically, using simultaneously CO_2 and organic carbon as carbon sources under sunlight (John et al., 2011; Subashchandrabose et al., 2013).

Nowadays, microalgae are extensively studied due to their great diversity and potential for biotechnological applications. Current research can be divided into four main fields (Levasseur et al., 2020): i) Biofuel production: microalgae have been studied as a third-generation feedstock, which possess several advantages such as high lipid content and sustainable lipid productivity, no competition and interference with food production, high biomass productivity and capacity of growing in polluted municipal wastewaters (Mathimani et al., 2019). In comparison with other available feedstocks, microalgae

photosynthetic efficiency is higher (Blankenship Robert et al., 2011) and its production is simpler (Mata et al., 2010). ii) CO₂ sequestration: by far the topic with less research effort (Levasseur et al., 2020). However, there are some studies that point phycocapture of CO₂ as a reliable option to reduce greenhouse gases in metropolitan areas (López-Pacheco et al., 2021). iii) High-added value molecules production: these organisms are important sources of a wide variety of biotechnological products, including pigments, polyunsaturated fatty acids, lipids, polysaccharides, polyphenols, vitamins, and peptides (Castillo et al., 2021; Galasso et al., 2019). They have been attracting attention as a sustainable and renewable feedstock of these chemicals for the food, pharmaceutical and cosmetic industries (Barsanti & Gualtieri, 2018). iv) Wastewater treatment: the use of microalgae for bioremediation of nutrients, such as nitrogen, phosphorus and carbon, from various wastewater streams has been successfully demonstrated with various species at different scales and systems (Geremia et al., 2021; Larsdotter, 2006). Furthermore, phycoremediation has shown also great potential in the removal of the so-called emerging contaminants (e.g. pharmaceuticals, personal care products and pesticides) (Sutherland & Ralph, 2019).

Due to the still high costs of production and biomass separation - low biomass concentration in autotrophic cultures has a strong impact on downstream processing -, research and publication trends are now towards microalgae-based production of high-added value molecules and phycoremediation of wastewaters (Levasseur et al., 2020). Both topics show great potential to make microalgae cultivation economically sustainable and profitable: the former increasing the value of end-products, the latter reducing the cultivation costs (Rizwan et al., 2018). Despite the different approach, these two trending topics still allow CO₂ sequestration and biomass energetic valorization. For example, through anaerobic digestion of residual biomass after extracting target compounds (Molazadeh et al., 2019; Sialve et al., 2009).

Although some species have already been deeply studied, the workhorse for process development for microalgae have been *Chlorella vulgaris* (Larkum et al., 2012). The same is verifiable in the wastewater phycoremediation research topic, the subject of this work (Al-Jabri et al., 2021; Santos & Pires, 2018; Sutherland & Ralph, 2019). Here, this is the species considered, both in the phycoremediation process design and in the proof-of-concept. Working with a well-known species guarantees a greater probability of success in the implementation of similar processes at LIPOR's facilities.

2.1.1. *Chlorella vulgaris*

Chlorella vulgaris is the widely cultivated and studied microalgae species (Masojídek & Torzillo, 2014). It is characterized as a green eukaryotic microalga, with the following taxonomic classification: Domain: Eukaryota, Kingdom: Protista, Division: Chlorophyta, Class: Trebouxiophyceae, Order: Chlorellales, Family: Chlorellaceae, Genus: *Chlorella*, Specie: *Chlorella vulgaris* (Safi et al., 2014). Morphologically, it is a unicellular, spherical microscopic cell with 2–10 µm of diameter and with many cell structures similar to higher plants (Figure 2), such as chloroplasts, cell wall and vacuoles, that grows in fresh water (Illman et al., 2000).

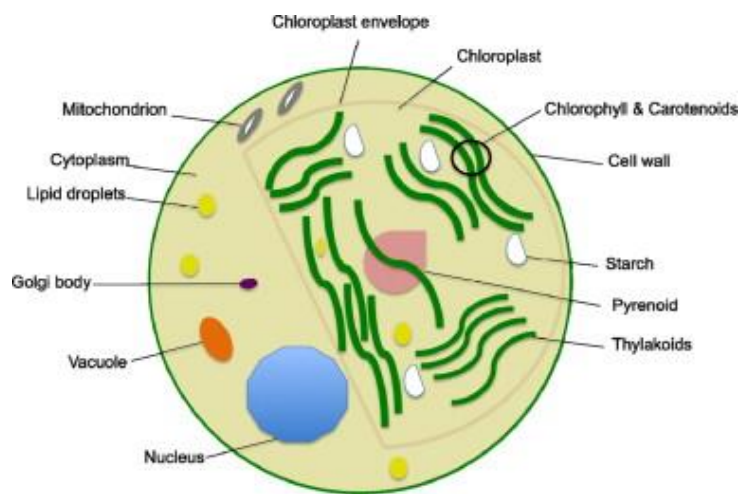


Figure 2 - Schematic cell structure of *C. vulgaris* (adapted from Safi et al., 2014).

C. vulgaris is a non-motile reproductive cell that reproduces asexually and rapidly by autosporulation, the most common asexual reproduction in microalgae, forming autospores - daughter cells with their own cell walls, in contrast to zoospores and aplanospores which are flagellate cells and spores without cell wall, respectively (Yamamoto et al., 2004).

Nowadays, a total annual production of dry biomass of *C. vulgaris* of about 5000 tons is estimated (Masojídek & Torzillo, 2014). Because of its content of nutrients and positive health effects, it is now produced and marketed as a health food supplement in many countries, like China, Japan, Europe, and the United States, even though *Chlorella* sp, as well as other microalgae species, are historically used as food ingredients (Milledge, 2011). Biochemical composition of the biomass (% dry weight) consists approximately of 56.8% of protein, 16.9% of lipids, 5.9% of carbohydrates and 1-2% of pigments such as carotenoids and chlorophylls (Niccolai et al., 2019). *C. vulgaris* also synthesizes all essential amino acids for humans (Safi et al., 2013).

This species presents several advantages such as strong growth rate, adaptability, metabolic diversity and high resistance to adverse conditions (e.g. high levels of heavy metals) and invaders (Romero et al., 2020; Silva et al., 2022). Its versatility allows for the targeted production of nutrients and other high-value molecules. While lipid and starch production can be increased during unfavorable growth conditions (e.g. nitrogen and phosphorous limitation or high CO₂ concentration and temperature), protein production increases under optimized conditions, such as nitrogen supplementation (Safi et al., 2014). On the other hand, mainly due to its high resistance, *C. vulgaris* is one of the most commonly used species in the phytoremediation of wastewater. Several studies show removal efficiencies of 80-90% for nitrogen and phosphorous, >50% for chemical oxygen demand (COD) and also high capability of adsorbing heavy metals, resisting pollutants and removing crude oil hydrocarbons (Silva et al., 2022). Other applications of *C. vulgaris* biomass include biofuel production (Brennan & Owende, 2010), animal feed (Becker, 2007) and crop biofertilization and biostimulation (Faheed & Fattah, 2008; Viegas et al., 2021).

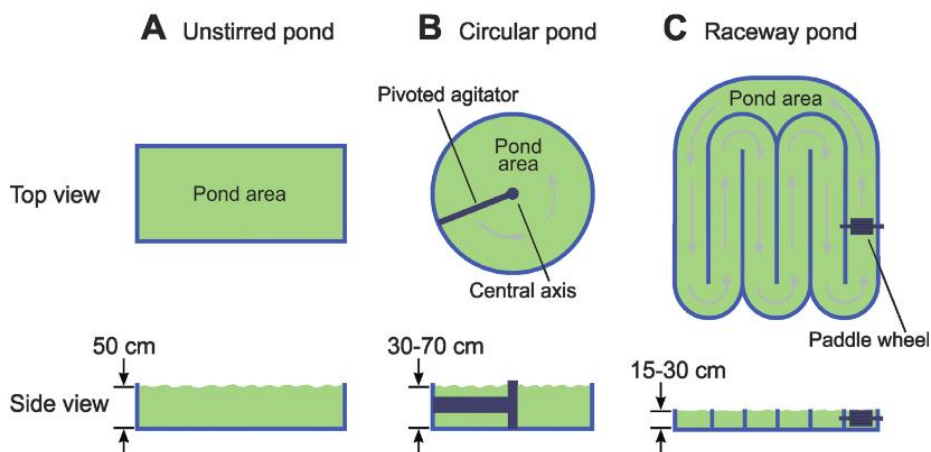
2.2. Microalgae cultivation

Current approaches to microalgae cultivation are highly diverse. Several systems with significant different strategies were already explored, however, two main variables stand out, of which the others are conditioned: suspended versus non-suspended cultivation and open versus closed system. Regarding the first one, Table 1 presents the main advantages and limitations of both suspended- and immobilized-cells cultivation systems (Gonçalves, 2017). Using the non-suspended cell mode, two subtypes arise: biomass can grow enclosed in a matrix, using well-established techniques for enzymes and organelles entrapment, or form a biofilm on a surface (non-enclosure methods). While entrapment methods require expensive polymeric matrixes, non-immobilized methods require high surface areas (de-Bashan & Bashan, 2010). With non-suspended cultivation, it is much easier to separate microalgae biomass from the medium, which is the main motivation for its development, however, this system has not yet been extensively studied and optimized (Katarzyna et al., 2015; Sousa et al., 2022). In this work, a suspended-cell approach was considered. Despite the difficulty of harvesting the biomass (discussed in section 2.3.), these systems are generally easier to design, scale-up and optimize (Gonçalves, 2017; Katarzyna et al., 2015).

Table 1 - Advantages and limitations of suspended- and immobilized-cells cultivation systems (adapted from Gonçalves, 2017).

Cultivation systems	Advantages	Limitations
Suspended-cells cultivation systems	• Widely studied and optimized • Increased biomass productivities • Applicable in large-scale operations	• Microalgal harvesting can be more expensive and/or time-consuming
Immobilized-cells cultivation systems	• 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	• 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

Regarding the second variable, suspended microalgae cultures, the most studied approach also in wastewater treatment (Pires et al., 2013), can operate both in open or closed reactors. Open reactors are stirred or unstirred tanks, shallow big ponds, circular ponds and raceway ponds (Figure 3). While open unstirred ponds and tanks are the simplest, most economical, but also most ineffective methods, stirred reactors are much more efficient (Rehman et al., 2022). Raceway ponds are well-established and already considered a conventional method for microalgae cultivation (Aliyu et al., 2021). These are shallow, generally hairpin-shaped, closed-loop flow channels, usually 0.15–0.30 m deep, internally lined with cement, polyvinyl chloride or clay, with mixing and aeration provided through circulation by a rotating paddlewheel (Cunha et al., 2020).

**Figure 3** - Examples of different types of open pond systems. A) Unstirred pond. B) Circular pond. C) Raceway pond (adapted from Hallmann, 2015).

Closed-culture systems, mostly referred as photobioreactors (PBR), also present several geometries, such as plate, column, annular and tubular reactors. Each geometry

presents its own advantages and limitations, however, compared to open ponds, PBRs allow higher biomass productivities with shorter retention times, mainly because growth conditions and contaminations can be better controlled and avoided, respectively (Okoro et al., 2019). Table 2 compares the estimated biofuel production cost from microalgae with an ideal open pond and PBR system. This comparison was performed based on a batch mode cultivation approach for 300 days.

Table 2 - Comparisons of algae cultivation cost using open pond and PBR system (Slade & Bauen, 2013).

Cultivation approach	Open pond	PBR
Power	1.55–1.58	6.60–8.40
Labour	1.30–1.50	6.40–6.60
Carbon dioxide	0.40–1.30	6.20–6.40
Purchase Tax	1.58–1.60	8.40–9.80
Other cost (Installation)	1.60–1.80	9.80–10.20
Depreciation	0.25	6.00

Note: Unit cost = algae biomass production cost (USD/kg).

Besides the significant higher implementation and operation costs, the implementation of a PBR system is still considered the best approach for microalgae production, as the biomass productivity is higher compared to open pond cultivation (Benvenuti et al., 2016). However, if biomass production is not a top priority, cost savings in terms of energy and materials through open pond cultivation are a great advantage, even though that there is a high risk of contamination and insufficient control of growth conditions (Peter et al., 2022). The intended application for the produced biomass also conditions the reactor choice. If the target sectors are the food, feed and pharmaceutical industries, a closed reactor approach is almost mandatory. In wastewater treatments plants, even though the production of by-products can positively contribute to the economic sustainability of the process, obtaining a treated effluent is the ultimate goal (Park et al., 2011). Remediation process designs aim to reduce construction and operating costs while meeting this objective, not increase them to obtain a valuable product that may require expensive post-processing and high capital investment (Bravo-Fritz et al., 2016). Open pond technology has already proven its high potential for wastewater treatment. The recognition of the benefit of algal biomass for energy recovery from these systems is an economic asset (Ho & Goethals, 2020).

Raceway designs are currently the preferred option for microalgae-based wastewater treatment because of the potential to process larger volumes of water and their low cost, ease of operation and maintenance. Thin-layer cascade bioreactors (raceway-

type reactor) are cheap and simple to operate, allow for higher biomass yields than typical designs, but, because of the low culture depth, present a lower working volume per surface area (Morillas-España et al., 2020; Morillas-España et al., 2021). On the other hand, high rate algal ponds, also raceway-type ponds but with greater depths, process higher effluent flows and are the most commonly used strategy (Larsdotter, 2006; Li et al., 2019).

2.2.1. High rate algal ponds

High rate algal ponds (HRAPs) are systems that combine wastewater treatment and microalgae production for feedstock or bioenergy production. Nowadays, it is widely accepted that this combination is the only economically viable way to produce microalgae derived biofuels (Lundquist et al., 2010; Park et al., 2011). These are characterized as raceway-type ponds with shallow depths of 0.2–1 m. To ensure the microalgae proliferation, mixing is provided by a paddlewheel to give a mean horizontal water velocity of approximately $0.15\text{--}0.3\text{ m}\cdot\text{s}^{-1}$, preventing microalgae sedimentation and maximizing its solar exposure. The configuration may have one or multiple loops around central dividing walls (Park et al., 2011).

These systems employ the symbiotic relationship between algae (in photoautotrophic metabolic mode) and bacteria to remove organic matter and nutrients from wastewater via assimilation and degradation. Oxygen derived from microalgal photosynthesis drives aerobic bacterial degradation of organic compounds, which, in turn, provides the CO_2 required by the microalgae for growth and subsequent nutrient uptake (Oswald, 1991). In this way, HRAPs result in combined secondary and tertiary wastewater treatments, removing organic matter and nutrients such as nitrogen and phosphorus, and ensuring improved natural disinfection rates compared to conventional ponds (Sutherland et al., 2021).

Compared to activated sludge (AS), the conventional method for secondary wastewater treatment, HRAPs consume almost two-thirds less energy - microalgae-based systems do not require external aeration due to photosynthesis (Ortiz et al., 2022) - while producing almost four times more biomass as reported by Taylor et al., 2021. Moreover, HRAPs convert a greater portion of nitrogen from effluents into biomass, making it more suitable for nitrogen recovery and biomass soil applications, assimilate CO_2 and require unskilled operators (Craggs et al., 2014). Main disadvantages include the need for high

surface area, which reflects into greater use of land area, and vertical limitation (i.e., there is a maximum operational depth). While AS is ideal for applications where free area and options for biomass disposal are not available, HRAPs are suitable for effluent treatment where space is not a constraint and there are viable options to reuse the residual biomass, such as the generation of energy or nutrient recovery through soil fertilization (Taylor et al., 2021). Due to microalgal-bacterial consortia present in HRAPs, these systems are also better suited to handle effluents with higher carbon to nitrogen ratios (when compared to other microalgae-based approaches) and, consequently, have less need for an external carbon source (CO_2) to enhance productivity and nutrient removal (Arbib et al., 2017). According to Oswald (1988), properly designed and operated HRAPs are capable of removing more than 90% of the biochemical oxygen demand (BOD) and up to 80% of the nitrogen and phosphorus.

However, due to their lower efficiency compared to conventional processes (i.e., with higher hydraulic residence times, HRT) and dependence on climatic conditions, HRAPs have not been widely implemented and expanded to an industrial scale. According to Ortiz et al. (2022), in order to outcompete with conventional treatment systems, the HRT and power consumption of HRAPs has to be reduced at least below 7 days and $0.5 \text{ kWh}\cdot\text{m}^{-3}$, respectively, which are the average values of conventional wastewater treatment by AS (Acién et al., 2016). The same study concluded that a circuit with two baffles and tear-shapes with a diameter equal to $\frac{1}{4}$ of the channel width is the most efficient configuration evaluated (Figure 4). In addition, the difficulty of harvesting microalgae is a major obstacle to the normalization of these systems (Rawat et al., 2011; Young et al., 2021).

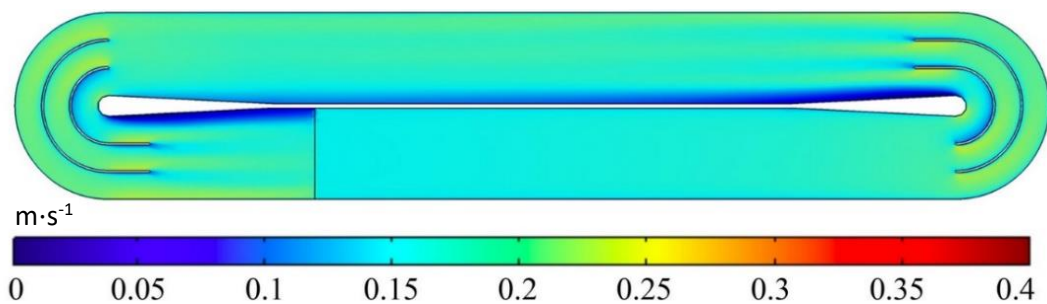


Figure 4 - Velocity field of an optimal HRAP design (adapted from Ortiz et al., 2022).

The optimal depth for a HRAP acting as a secondary wastewater treatment system is pointed as 0.4 m. Maximum reported BOD_5 , total nitrogen, ammonium, total phosphorous and orthophosphate removal rates are 93.4, 75.7, 94, 97.2 and 71%, respectively. Maximum reported $\log_{10}$ reduction value for *Escherichia coli* is 3.01 $\log_{10}$

E. coli MPN (Most Probable Number) 100 ml⁻¹. Typical HRT range between 4 and 10 days (Young et al., 2017). Algal productivities measured in both commercial production and wastewater treatment HRAPs range widely from 12–40 g·m⁻²·d⁻¹, depending on algal species, climate and operation. Wastewater treatment HRAPs present an average productivity of 20 g·m⁻²·d⁻¹ (Park et al., 2011).

2.2.2. Main factors influencing microalgal growth

Microalgae growth is influenced by several factors. These can be classified as abiotic, biotic and processual factors. Abiotic factors include temperature, pH, light, concentration of nutrients, CO₂ and O₂, cultivation medium and salinity. Biotic factors include the presence of viruses, fungi and bacteria (pathogenic or innocuous), microalgae strain and competition with other strains. Operational factors, such as HRT, mixing rate, harvesting frequency and biomass concentration, also affect significantly microalgal growth since these control light exposure, CO₂ availability and O₂ concentration (Chisti, 2008; Okoro et al., 2019).

Light

Photoautotrophic growth is driven by light. In the absence of nutrient limitation, the increasing light irradiation promote higher photosynthetic activity and, consequently, higher algal growth rate, until a light saturation point. Beyond this point, light can damage the light receptors of microalgae by photo-oxidation, therefore decreasing the photosynthetic rate and productivity (Richmond et al., 2003). Mainly in deep ponds, the increase in biomass concentration originates a shading effect over the cells in deeper zones (Cao et al., 2020). In HRAPs, paddlewheel mixing provides a significant degree of vertical mixing through the pond depth thus ensuring that the algal biomass is intermittently exposed to light, maximizing solar radiation exposure (Park et al., 2011).

Temperature

On temperature parameter, microalgal productivity also increases until a maximum temperature value above which the cell activity is inhibited (Pulz, 2001). The optimal temperature range varies between microalgae species and according to the biogeographical origin of the strains (Teoh et al., 2013). Moreover, the optimal range can vary when there is a nutrient or light limitation, and growth often declines when algae are

subjected to a sudden temperature change (Soeder et al., 1985). While the upper temperature limit for eukaryotic algae is 62 °C (thermophiles), some snow and ice algae grow at 1 °C (psychrophiles) (Varshney et al., 2015). The microalgae huge diversity helps to promote these extreme temperature tolerance regimes. The upper temperature limit for the growth of *Chlorella* strains is pointed in the range of 26 to 30 °C and the optimal growth temperature for *C. vulgaris* is often stated between 25-30 °C (Serra-Maia et al., 2016). Temperature also affects other abiotic factors such as pH and gas solubility (O₂ and CO₂). In HRAPs, paddlewheel mixing provides a significant degree of vertical mixing, thus preventing thermal stratification of the medium.

pH and CO₂ concentration

The pH of the growing medium affects CO₂ solubility and many of the biochemical processes associated with microalgae growth and metabolism, including the availability and uptake of nutrient ions (Park et al., 2011). HRAP pH is a function of the ionic composition of the medium and microalgal/bacterial respiration, autotrophic and heterotrophic activities (Garcia et al., 2000). It is also a function of microalgal productivity: microalgae photosynthesis raises pH by consumption of CO₂ and HCO₃⁻. In HRAPs, pH often exceeds 11, which has a disinfecting effect on the wastewater (Heubeck et al., 2007; Larsdotter, 2006). An elevated pH can enhance ammonium nitrogen and phosphorus removal from the pond liquid via ammonia volatilization and phosphate precipitation with unchelated ferric iron, calcium and magnesium, respectively (Craggs, 2005). However, heterotrophic bacteria present in HRAPs have an optimum pH of 8.3, above which bacterial activity is increasingly inhibited, and freshwater microalgae have an optimal growth at a pH of about 8 (*C. vulgaris* included) (Rachlin & Grosso, 1991; Kong et al., 2009). An increase in the pH can also induce microalgal flocculation, which can be very useful in the harvesting step, but not during microalgae growth (Vandamme et al., 2012; Wu et al., 2012). The CO₂ supply is usually used not only to enhance microalgae growth and productivity, but also to keep pH in an optimal range – the formation of carbonic acid decreases the pH of the medium. In biomass production systems, the feed rate is strictly controlled to avoid abrupt decreases (high CO₂ feed rate) and increases (low CO₂ feed rate in comparison to microalgae assimilation) in pH values (Posadas et al., 2015). However, in wastewater treatment HRAPs, CO₂ external supply is not often used due to the associated costs (Park et al., 2011). In these systems, CO₂ availability strongly depends on the heterotrophic oxidation of organic compounds by

bacteria. As an alternative, waste gaseous emissions such as exhaust gas ($\sim 12\%$ CO_2) can be used to increase productivity (Sharma & Maréchal, 2019).

Nutrients

The carbon to nitrogen ratio (C:N) of wastewater should be evaluated to verify if it is appropriate to fully support optimal microalgae production, considering whether or not should be additional supplementation of CO_2 (Zheng et al., 2019). Microalgae biomass typically presents a C:N ratio in the range of 6-15 (Benemann, 2003). The adjustment of the wastewater C:N ratio for microalgae cultivation is possible through dilution in freshwater or through the addition of an external carbon source (Lu et al., 2018; Zheng et al., 2019). In these systems, the use of fertilizers is not necessary, as the effluents are often rich enough in nitrogen and phosphorous – both essential for microalgae growth. The same is valid for micronutrients (e.g. Mg, Ca, Zn and other metals).

O₂ concentration

High microalgae activity, i.e., photosynthetic activity, also promotes high levels of O_2 in the medium, which may reach $>200\%$ saturation (Park & Craggs, 2010). This effect inhibits microalgae growth and its extent is species-specific (Sforza et al., 2020). Excessive concentrations of dissolved oxygen can be effectively controlled through aeration, similarly to an air stripping process. However, CO_2 concentration can be also significantly reduced (Kazbar et al., 2019).

Salinity

Salinity is not a widely discussed parameter on microalgae-based wastewater treatments, since most wastewaters do not have significant levels of salinity, presenting only concentrations that allow the normal growth of microalgae. However, there are extensive reviews about salt-tolerant microalgae species on dealing with specific effluents with high salt concentrations (e.g. 3-15%) (Haris et al., 2022; Mohseni et al., 2021; Vo et al., 2020). Overall, high salinities usually inhibit microalgae growth. For example, *C. vulgaris* growth is inhibited at 0.4 M NaCl (Pandit et al., 2017). To control salt levels, especially in dry and hot geographical regions, freshwater is added to open culture systems, also to compensate for water losses through evaporation (García-González et al., 2003).

Biotic factors

The presence of microalgal-bacterial consortia of multiple species in HRAPs, in addition to a metabolic symbiosis and cooperative effects, also promotes inhibition and competition effects. Among the competitive effects, there are the excretion of algicidal and bactericidal compounds by bacteria and microalgae, respectively, the increase of pH value by microalgae, and the natural competition for nutrients by different species (Gonçalves, 2017; Larsdotter, 2006). Positive or cooperative effects include the development of a robust system, able to resist to environmental fluctuations and invasion by other species, and nutrient exchange: microalgae produce O₂ for bacterial respiration and bacteria produces CO₂ for microalgae anabolism. Moreover, microalgae create habitat for bacteria, which produce growth factors to promote microalgae proliferation (Chan et al., 2022).

HRAPs are also susceptible to grazing by herbivorous protozoa and zooplankton (e.g. rotifers and cladocerans), and to fungal and viral infections, which can significantly reduce microalgae production (Di Caprio, 2020; Molina-Grima et al., 2022). These problems can be controlled through physical treatments, such as filtration, and chemical treatments, which include the application of biocides, bleach (hypochlorite) and increase of pH, which microalgae naturally promote (Benemann et al., 1978). Chemical treatments are the most effective and broadly implemented methods. However, it is important to ensure that the final product and its application is not compromised and the treatment is environmentally acceptable (Molina-Grima et al., 2022). pH adjustment to high values (~11) is a suitable and easy to implement option to control most zooplankton, one of the main biotic sources of biomass productivity loss in HRAPs (Montemezzani et al., 2015, 2017; Park et al., 2011).

For fast-growing species, such as *Chlorella vulgaris*, the risk of significant competition is low, since their proliferation easily outcompete potential contaminants, such as other microalgae species and strains (Chisti, 2006).

2.3. Microalgae harvesting

After microalgae cultivation, biomass must be harvested to separate it from the treated effluent. This step is essential to deal with the biomass and treated effluent as separated products, however, it is not easily achieved, and is consequently a cost

expensive part of the whole process, representing a cost of up to 50% of the algal biomass (Larsdotter, 2006; Young et al., 2017). Microalgae harvesting is therefore considered one of the biggest limitations to cost-effective production of microalgae via HRAPs treating wastewaters (Young et al., 2021). The difficulty comes from their small size ($< 20 \mu\text{m}$), low density similar to water ($1.08\text{--}1.13 \text{ g}\cdot\text{mL}^{-1}$), strong negative surface charge and relatively low concentrations in the medium ($0.5\text{--}5 \text{ g}\cdot\text{L}^{-1}$) (Muylaert et al., 2017).

Several options have been proposed, such as filtration, centrifugation, sedimentation and floatation, however, these greatly increase operational costs for algal production and are only economically feasible for production of high value products (Park et al., 2011). On the other hand, flocculation has been suggested as a most suitable method, due to its low-cost and high efficiency (Lam & Lee, 2012; Vandamme et al., 2018). It is defined as the aggregation of single cells into larger, $1.0 - 5.0 \text{ mm}$, and heavier particles called flocs that can be easily separated from the medium via gravity sedimentation (Vandamme et al., 2016). Nevertheless, harvesting microalgae with flocculants can contaminate the slurry concentrate, which still need additional processing – dewatering and drying.

Flocculation techniques for harvesting microalgae can be classified into physicochemical, biological and autoflocculation methods. Physicochemical methods include the addition of metal ions and polymers, which neutralize the negative charge on the microalgal surface and bridge individual cells to induce microalgal flocs, and electroflocculation. On the other hand, bioflocculation can be achieved by co-culturing different species with different rates of flocculation, co-culturing microalgae with fungi or bacteria that produce extracellular polymers, or by adding a biopolymer. The use of bioflocculation is associated with less chemical contamination of the biomass (except when a biopolymer is added, even if it is biodegradable) and it is generally cheaper than physicochemical methods (Min et al., 2022; Okoro et al., 2019). Chitosan, for example, the second-most abundant natural biopolymer, mainly derived from the shells of crustaceans (Kritchenkov et al., 2020), is widely used for microalgae flocculation due to its positive surface charge (Pan et al., 2006; Zhou et al., 2016). Nevertheless, autoflocculation, a simple, low-cost and eco-friendly strategy, is pointed as the method with the greatest potential for harvesting microalgae (Matter et al., 2019; Park et al., 2011; Young et al., 2021). It consists of the spontaneous aggregation of microalgae cells under a pH change ($\text{pH} < \approx 4$ or $\text{pH} > \approx 11$), which induces gravitational sedimentation (Matter et al., 2019). Although it can ensure $\sim 90 \%$ harvesting efficiency (Ummalyma et al.,

2016), this method has the disadvantages of high species-specificity and slowness when compared to mechanical methods (Matter et al., 2018). Figure 5 shows the potential of autoflocculation for microalgae harvesting.

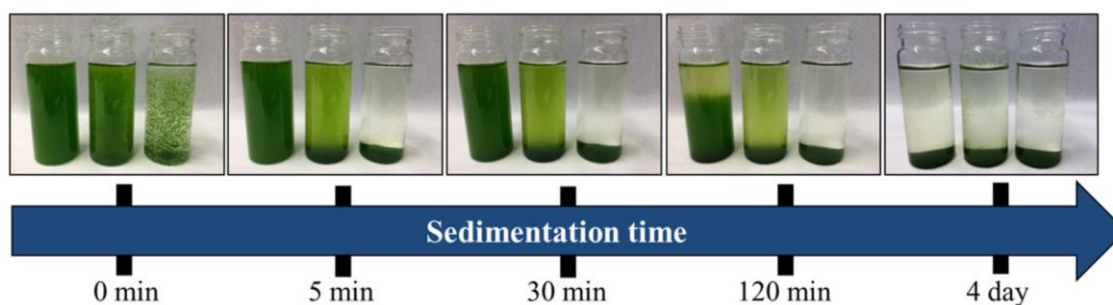


Figure 5 - Comparison of settling times between three different degrees of autoflocculation (adapted from Nguyen et al., 2017).

Autoflocculation via magnesium hydroxide precipitation currently seems to be the autoflocculation method with the greatest potential (Young et al., 2021). It consists of the flocculation of microalgae cells caused by the precipitation of magnesium hydroxide at $\text{pH} > 9$. Even though it requires the use of additives - magnesium chloride to increase magnesium concentration and calcium hydroxide to increase the pH value - (in contrast to free and spontaneous autoflocculation), this method is still economically advantageous, as these present a low cost. Moreover, these also have the advantages of being low in toxicity, environmentally friendly, recoverable and reusable. After flocculation induced by precipitation of magnesium hydroxide at high pH (10-10.5) and pre-concentration of the microalgal biomass by gravity sedimentation, the precipitated magnesium hydroxide can be removed from the biomass by mild acidification (pH 7-8) (Vandamme et al., 2015). A harvesting efficiency of about 90% has already been achieved with different microalgae species, including *Chlorella vulgaris* (Aléman-Nava et al., 2017; Vandamme et al., 2015). Furthermore, this method has been successfully tested in wastewater HRAPs at different scales (Young et al., 2021); inclusively with *Chlorella* sp. (Drira et al., 2016).

Despite its efficiency, flocculation is mostly considered as a pre-harvesting step in order to facilitate or complement other harvesting methods like centrifugation or filtration (Junior et al., 2020; Safi et al., 2014). For example, the high energy requirement of centrifugation makes it only economically viable for secondary thickening of harvested algae (Park et al., 2011). A techno-economic evaluation of microalgae harvesting and dewatering systems conclude that the combination of flocculation with other operations is the most economically competitive alternative, in contrast to single-step systems (harvest and dewater step) and to other process combinations (Fasaei et al., 2018).

Moreover, it concluded that flocculation for harvesting followed in combination with a second unit operation for dewatering require less than $0.1 \text{ kWh} \cdot \text{kg}^{-1}$ algae. In terms of energy demand and total costs, pressure filtration is the best option to link with flocculation for the dewatering step (Fasaei et al., 2018; Min et al., 2022). It is a process with low energy demand and high recovery, however, it operates discontinuously and, similarly to other filtration types, there are problems of deposition of microalgae on the membrane, which decrease the filtration rate and demand membrane cleaning or replacement (Min et al., 2022; Molina Grima et al., 2003). On the other hand, centrifugation, which has higher but close energy requirement and total costs when associated with flocculation as a dewatering step for harvest microalgae biomass (Fasaei et al., 2018), is also a good option. Although it requires a higher capital investment, it is still a very robust and widely implemented method that efficiently ensures high recoveries (Garofalo, 2009; Pahl et al., 2013).

After harvesting and dewatering, a drying step may be needed, depending on the final application of the microalgae biomass, to increase product stability and allow further processing (Aliyu et al., 2021). When valuable products are intended to be extracted from the biomass, a gentle and slow drying method is mandatory to avoid the degradation of the target substances. Similarly, if the biomass is the final product, a drying process that minimizes biomass losses per thermal degradation should be chosen (de Carvalho et al., 2020). Nevertheless, this step may represent between 60 and 80 % of whole harvesting process cost (flocculation, dewatering and drying) (Aliyu et al., 2021; Min et al., 2022). Of the main methods proposed in the literature for drying microalgae - solar drying, microwave drying, convective drying, freeze drying, conduct/contact drying and spray drying - the one which entails fewer costs is the solar drying, however, it implies a high risk of contamination, is highly dependent on weather conditions and is not considered suitable for most projects also due to land area requirements (Fasaei et al., 2018; Show et al., 2015). Table 3 presents an overview of microalgae drying technologies with their main technical and economic characteristics.

Excluding freeze and microwave drying due to their high CAPEXs, the other four methods present in Table 3 are suitable for processes based on wastewater treatment. Process-specific variables, such as product application, biomass concentration before and after drying and production scale should be considered to choose the most appropriate technology (Show et al. 2015; Carvalho et al. 2020).

Table 3 - Overview of microalgae drying technologies with their main technical and economic characteristics (de Carvalho et al., 2020).

Drying method	Principle	Effect on biomass	OPEX (energy, air, vacuum)	Pros	Cons
Solar	Solar heating of the biomass	Compaction, some degradation during slow drying	Low	Low energy cost	Slow, requires large areas, depends on the weather
Microwave	Microwave heating	Variable. Compaction, expansion or degradation can occur	Moderate	Fast, highly controllable	High CAPEX, higher safety concerns
Convective (oven, tray, tunnel)	Warm air heats the biomass and carries the water	Compaction, degradation at higher temperatures	Moderate	Practical	Can degrade biomass
Spray	Warm liquid and air carry the water	Enhanced permeation and some degradation can occur	High	Fast, generates powder.	Higher energy intensiveness, requires large air volumes
Conduction (drum, tray)	Warm surfaces heat the biomass	Compaction, degradation likely	Moderate	Fast	Degrades biomass
Freeze	Warm surfaces heat frozen biomass. Water sublimates.	Low, can maintain cell viability. Biomass cake very porous.	High	High-quality biomass	Slow, high CAPEX,

2.4. Microalgae biomass applications

Microalgae biomass has several applications. The broadest is the direct use in feed and food industry, mainly in aquaculture (Chen et al., 2021), or as a food supplement, due to its high nutritional value and health benefits. It is commercialized in dry and wet forms and even incorporated into common products, such as yogurts and cookies (Junior et al., 2020). However, because of contamination and regulatory issues, microalgae grown in wastewater are not recommended for these industries (Young et al., 2017).

A more suitable application is the energy recovery of biomass, which is widely studied. For that purpose, three kinds of biofuels can be produced: solid, liquid and gaseous (Khan et al., 2018). One of the solid biofuels produced from microalgae biomass is called biochar - a carbon-rich charcoal of any kind of biomass produced by thermal decomposition (e.g. pyrolysis) of an organic feedstock under limited oxygen supply (Yu et al., 2017). Rather than using this material as process fuel in energy conversion, it can also be used to improve soil fertility. It has been demonstrated that biochar amendment can improve water holding capacity and nutrient status of soils. Due to its physiochemical

properties, i.e., mechanical and thermal stability, high reactive surface area and electrical conductivity, this material has been also studied as an electrode, electrochemical sensor and catalyst (Binda et al., 2020). It also shows great performance as an adsorbent in the immobilization of pollutants (Uchimiya et al., 2010). During thermal decomposition, a thermochemical process, bio-oil and biogas are also produced (Grierson et al., 2013). Other solid biofuels production is based on physical treatments such as crushing, grinding, drying and pelletizing (Ong et al. 2019). On the other hand, biochemical treatments allow the production of liquid biofuels like bioethanol through fermentation and gaseous biofuels (e.g. biogas, biomethane) through anaerobic digestion (Silva & Bertucco, 2016). The material that remains after digestion - residual biomass - can be further valued, for example, again by thermal decomposition. Chemical processes are used to convert microalgae lipids into biodiesel by transesterification, which is usually catalysed by enzymes (Mota et al., 2022). Along with biogas, the production of this type of biofuel has been the focus of most research on the subject, due to the high lipid content in microalgae (Silva & Bertucco, 2016).

One concept of current particular relevance is the concept of biorefineries - chemical factories in which all biomass fractions (proteins, glycosides and lipids) are utilized to generate different types of products and energy. The production of compounds with higher commercial value is pointed out as one solution to make the production of microalgae on an industrial-scale economically viable (Bravo-Fritz et al., 2016). These are promising sources of bioactive compounds for pharmaceutical, cosmeceutical and nutraceutical applications, such as pigments, polyunsaturated fatty acids, vitamins and peptides (Junior et al., 2020; Khan et al., 2018; Russell et al., 2022). However, considering the common applications of the produced high-value compounds, wastewater treatments plants are often not recommended as a source of biomass.

In addition to energy recovery, microalgae biomass produced in wastewater treatments plants is also suitable for soil application in the form of fertilizing products, such as organic fertilisers, soil improvers, biostimulants, and biofertilisers (Paiva et al., 2021). There is a terminology slip present in most literature where microalgae biomass may be used as a biofertiliser based on its nutrient concentration (Castro et al., 2020; Khan et al., 2019), which may be higher than commercial chemical/inorganic fertilizers (Choudhary et al., 2017). However, according to the available definitions (Table 4), the correct term for this product is organic fertilizer (Ronga et al., 2019). Nevertheless, microalgae can be used as real biofertilizers/microbial biostimulants (Chatterjee et al.,

2017). For example, one study reports that rice cultivation fields inoculated with the microalgae *Chlorella vulgaris* or *Arthrospira platensis* led to 7-21% higher rice yields (Dineshkumar et al., 2018). The key concepts were considered here defined as shown in Table 4.

According to the primary nutrient content of the biomass (nitrogen, phosphorus and potassium), this can be considered an organic fertiliser - high nutrient concentration (>1% by mass of each one or >2/2.5% of a single (EU Regulation 2019/1009)) – or an organic soil improver – low nutrient concentration – provided that it also shows beneficial effects on the soil. Independently of its nutrients content, if the biomass improves plants' nutrient use efficiency, tolerance to abiotic stresses, quality traits or availability of confined nutrients in the soil or rhizosphere, then it can be considered a biostimulant. However, biostimulant is a broad concept, thus, for example, an organic soil improver or a growth medium enriched with an organic soil improver can easily exhibit biostimulant properties. These properties are ensured by the presence, even at low quantities, of beneficial substances, such as humic and fulvic acids, amino-acids and peptides mixtures, inorganic salts, carbohydrates, hormones and betaines, and beneficial microorganisms: bacteria, mainly plant growth-promoting rhizobacteria, and fungi (Jardin, 2015; Khan et al., 2009). Microalgae-based biostimulants contain macro and micronutrients, phytohormones, carotenoids, amino acids, antifungal substances, growth regulators, polyamines, natural enzymes, carbohydrates, proteins and vitamins which enhance plant growth, being able to fix nitrogen, solubilize phosphate and promote the growth of microorganisms demanded for the plants optimal development (Ronga et al., 2019). The application of biostimulants is pointed as a good approach to reduce the widely use of chemical fertilizers, being able to be applied as an alternative technique, or used in conjunction with fertilisers (inorganic and organic), reducing the quantities needed and, thus, promoting a more ecological and resource-efficient use of them (Sutton et al., 2013). The direct use of *Chlorella vulgaris* biomass as a soil additive has already demonstrated a significant biostimulant activity on maize and wheat plants (Shaaban, 2001; Viegas et al., 2021).

Besides the economic and environmental advantages arising from wastewaters phycoremediation and the consequent production of biomass rich in macro and micro nutrients essential for an optimal crop growth, accurate chemical analysis should be performed to certify these soil application products, since microalgae also have the ability to accumulate heavy metals and other contaminants (Ronga et al., 2019).

Table 4 - Key concepts and respective definitions about fertilising products.

Concept	Definitions
Fertilising product	“A substance, mixture, micro- organism or any other material, applied or intended to be applied on plants or their rhizosphere or on mushrooms or their mycosphere, or intended to constitute the rhizosphere or mycosphere, either on its own or mixed with another material, for the purpose of providing the plants or mushrooms with nutrient or improving their nutrition efficiency” (EU Regulation 2019/1009).
Fertiliser	“A fertiliser shall be a fertilising product the function of which is to provide nutrients to plants or mushrooms” (EU Regulation 2019/1009).
Organic fertiliser	“An organic fertiliser shall contain organic carbon and nutrients of solely biological origin” (EU Regulation 2019/1009). “An organic fertilizer is a fertilizer that is derived from organic sources, including organic compost, cattle manures, poultry droppings and domestic sewage, in contrast to chemical fertilizers” (Green, 2015; Jardin, 2015).
Soil improver	“A soil improver shall be a fertilising product the function of which is to maintain, improve or protect the physical or chemical properties, the structure or the biological activity of the soil to which it is added” (EU Regulation 2019/1009).
Organic soil improver	“An organic soil improver shall consist of material 95 % of which is of solely biological origin” (EU Regulation 2019/1009).
Growing medium	“A growing medium shall be a fertilising product other than soil in situ, the function of which is for plants or mushrooms to grow in” (EU Regulation 2019/1009).
Plant biostimulant	“A plant biostimulant shall be a fertilising product the function of which is to stimulate plant nutrition processes independently of the product’s nutrient content with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere: nutrient use efficiency; tolerance to abiotic stress; quality traits; availability of confined nutrients in the soil or rhizosphere” (EU Regulation 2019/1009). “A plant biostimulant is any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content. By extension, plant biostimulants also designate commercial products containing mixtures of such substances and/or microorganisms” (Jardin, 2015). Note: Plant biostimulants are divided in two categories: microbial and non-microbial (EU Regulation 2019/1009).
Biofertiliser	“Product whose active principle are live microorganisms, non-pathogenic to humans, animals or plants, nor opportunistic human pathogens, which favour the nutrition and/or development of plants, without affecting the biological diversity of the soil and the environment, to the exclusion of the so-called biological control agents, biofungicides, bionematicides and bioinsecticides” (Decreto-Lei n. ° 103/2015). “A biofertiliser is any bacterial or fungal inoculant applied to plants with the aim to increase the availability of nutrients and their utilization by plants, regardless of the nutrient content of the inoculant itself” (Jardin, 2015). Note: In the European Union, there are currently no legal definitions for the term biofertiliser (Malusá & Vassilev, 2014), even in the latest regulation (EU Regulation 2019/1009). According to the Portuguese legislation and the last European regulation on the subject, the concept of biofertilizer is under the concept of microbial plant biostimulant (Decreto-Lei n. ° 103/2015; EU Regulation 2019/1009).

3. Phycoremediation process proposal

The selection of a suitable cultivation system for microalgae production is mainly influenced by the microalgal strain, source of nutrients, desired product and investment costs (Klinthong et al., 2015). In this case-study, since *Chlorella vulgaris* is a very versatile species (Silva et al., 2022), a wastewater (GW leachate) is the source of nutrients, the desired product is a treated effluent and the available investment capital is low, a raceway-type reactor, more specifically an HRAP, was considered. Taking into account that the effluent has a considerable amount of organic matter, the HRAP approach again proves to be a suitable system, as it also promotes bacterial growth and consequently a greater metabolic diversity that allows the degradation of most organic compounds, mainly in the deeper zone (Oswald, 1991). After establishing the reactor type, the project priority becomes to maximize the microalgae production, in order to obtain a more treated effluent (i.e. with lower nutrient and pollutant concentrations), facilitate the harvesting process, and increase the benefits from biomass valorization through energy recovery or new product development for soil application - the low quality of the biomass, due to the potential contamination by pathogens in the wastewater and the difficulty in maintaining monocultures in an open system make it more suitable for these types of valorization. For that, optimal and already tested designs present in the literature were considered in this project. In this chapter, more than the technical feasibility, the design and economic viability is also assessed.

3.1. Problem description

At LIPOR I (Figure 6), in Gondomar municipality, two leachates are produced resulting from the company current and previous activities: a sanitary landfill leachate (SLL) and a GW leachate (GWL). The first is formed mainly due to anaerobic microbial decomposition and water percolation through urban waste (constituted by organic and inorganic matter such as plastic, glass, paper, metal, etc.) deposited in the now sealed and closed sanitary landfill of Ermesinde/Baguim do Monte, which received since the 1970s a large part of the waste produced in the LIPOR's associated municipalities. Nowadays, after a landscape recovery, the landfill is known as Parque Aventura, a green and ludic place for the population. The second, GWL, is mainly formed by the same phenomena, even though aerobic microbial decomposition is also significant, but in GW piles. Before

being shredded for use in composting, GW is kept on an impermeable surface (Parque de Verdes), where the leachate is formed and where it is collected into a 50 m³ reservoir tank (Figure 7). In turn, the SLL is collected into two reservoir tanks of 150 m³ each. Since both tanks are fed from different sections of the landfill, the leachate collected in each one present some difference in composition. Tank I is fed from the upper section of the landfill, where the waste is more recent and a controlled discharge has been made, while tank II is fed from the lower section, which is more stabilized despite having had a less selective discharge.



Figure 6 - LIPOR I (yellow polygon: LIPOR; red polygon: Parque Aventura; green polygon: Parque de Verdes).

Nowadays, these leachates are periodically picked up from the storage tanks to be treated in wastewater treatment plants (WWTPs). According to their composition (Table 5) and the concentration limits imposed by Portuguese legislation (Decreto-Lei n.º 236/98) for discharge of wastewaters (Table I.1, Annex I), there is no option than reduce the concentration of nutrients in the leachates to discharge them. However, for the parameters assessed, these fully comply with the legislation to be used as irrigation waters (Table I.2, Annex I). The leachates' characterization was carried out by a previous master's student during an internship at LIPOR (Bragança, 2018). The concentrations of metals were also previously assessed, but they are not relevant to this work. All, except iron concentration for wastewater discharge, are below the limits present in the legislation.

Table 5 - Characterization of the LIPOR I leachates (Bragança, 2018).

Leachate	pH	BOD ₅ (mg O ₂ /L)	TOC (mg/L)	TSS (mg/L)	COD (mg O ₂ /L)	TP (mg/L)	PO ₄ ³⁻ (mg-P/L)	TN (mg/L)
SLLI	7,2±0,1	<4	147±1	256±8	420±8	6,3±0,1	0,5±0,1	462±10
SLLII	7,1±0,1	<10	139±1	122±20	388±20	0,6±0,1	0,2±0,1	547±11
GWL	7,3±0,1	114±2	306±20	142±40	1294±90	6,3±0,1	5,8±0,3	>500

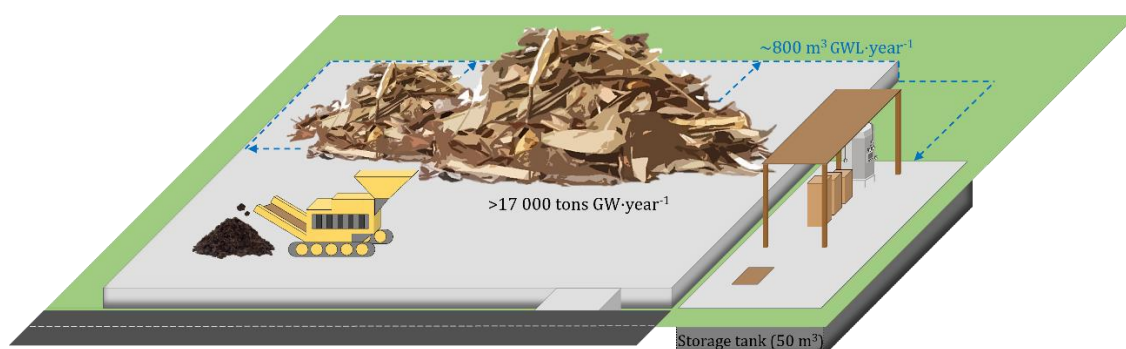
TOC - Total organic carbon; TSS - total soluble solids; COD - Chemical oxygen demand; TP – Total phosphorous; TN – Total nitrogen.

In 2021, 10775 m³ of leachate left the LIPOR I facilities. Approximately 7 % were GWL. Table 6 presents all the relevant data about leachate production and costs.

Table 6 - Leachates' production and leachates' processing costs at LIPOR I.

Parameters (from 2021)	Value
Tank volume for SLL storage (m ³)	300
Tank volume for GWL storage (m ³)	50
2021 production of leachate (m ³)	10775
Annual production of SLL (m ³)	~ 10000
Annual production of GWL (m ³)	~ 800
Transportation cost (€/m ³)	4.57
Treatment cost (€/m ³)	0.68 - 1.7

It is important to note that the leachates' composition is not constant throughout the year. In winter, with increased precipitation, more leachate is produced, although less concentrated. The opposite is valid for the summer season, where a more concentrated wastewater is produced in lower quantities. This is one of the reasons for the variation in the treatment cost (see Table 6). The other is the differentiated price table for the different WWTPs. The aforementioned leachates' characterization was carried out with samples collected in March, while here they were collected in the first week of April.

**Figure 7** - Scheme of the Parque de Verdes (GWL in blue).

In total, approximately €62200 is spent annually by LIPOR on dealing with these

effluents, of which €4600 related to GWL. This work aims to suggest technical options to reduce this expense. In addition, the possibility of income from the valorization of by-products and final products is also assessed. The carbon cost reduction in transportation is considered.

3.2. Solution

A HRAP reactor inoculated with *Chlorella vulgaris* was considered for the microalgae-based bioremediation of the GW leachate produced at LIPOR's facilities. This choice is well justified throughout the directed literature review present in this work (see section 2.2.) as the choice of the harvesting method - autoflocculation via magnesium hydroxide precipitation plus filtration or centrifugation (see section 2.3.).

3.2.1. Reactor design

For this microalgae-based system, a variable HRT between the typical range of 4-10 days, where in summer season is equal to 5.5; in spring-like season to 7 and in winter to 9 days, as in (Sutherland et al., 2014), should be appropriate. However, as this will operate in semi-continuous mode, since operating it in continuous mode may be difficult to maintain due to the low wastewater production (mainly in summer), and the channel depth is fixed (Table 7), a constant operation time of 7 days is considered to allow fixing the final dimensions. The option of operating it in full batch mode was not taken into account because it demands a new inoculation for each batch, which is not practical. In semi-continuous regime, a percentage of the broth is left as inoculum for the next cycle. Here, a 10% of culture broth was considered to be left in the reactor as inoculum (Morillas-España et al., 2021). Periodically, whether necessary, a completely new inoculum is produced and the entire volume of the reactor is harvested.

Along a whole year, 52 cycles of 7 days are possible. Considering the annual production of GWL ($\sim 800 \text{ m}^3$), a minimum reactor volume (V) of 15.4 m^3 is required ($800 \text{ m}^3 / 52$), which means a total surface area (A) of 38.5 m^2 ($15.4 \text{ m}^3 / 0.4 \text{ m}$). No further allowances on reactor's volume and surface area were assumed because the leachate production was already slightly overestimated. Here, a possible dilution of the leachate was not considered. Future works may conclude that it is necessary and that the HRAP size must be adjusted. However, that is unlikely regarding that several studies have shown

successful treatments on undiluted effluents (Young et al., 2017). The evaporation rate can be compensated through the addition of tap or rain water (Morillas-España et al., 2021). Such a system can be built to be automatic according to the medium level.

HRAPs with semi-circular ends are the most common configuration for cultivating microalgae (Figure 8). These generally have vertical walls and a flat bottom. In such ponds, curved baffles or flow deflectors are generally installed at both ends. The baffles ensure that the flow remains uniform throughout the curved bends and minimize the development of dead zones (Chisti, 2013). An advanced biokinetic and hydrodynamic modelling to optimize the design of full-scale HRAPs concluded that the presence of two baffles at both ends and tear-shapes with a diameter equal to $\frac{1}{4}$ of the channel width is the most efficient configuration (Figure 4), i.e., that minimize the presence of low velocity regions (Ortiz et al., 2022). The two baffles are equally spaced from the walls and from each other. A standard configuration, which has a full channel width paddlewheel, was considered. This proved to be more efficient in microalgae cultivation compared to systems where there is a narrowing in the paddlewheel zone (Cunha et al., 2020). The constructive material in contact with the broth was considered PVC (Polyvinyl chloride; with manning friction factor, f_M , of $0.013 \text{ s} \cdot \text{m}^{-1/3}$) (Ortiz et al., 2022).

According to Chisti (2013), the ratio of the straight channel length to the width of the whole reactor (p/q ratio, see Figure 8) should be 10 or greater to avoid flow disturbances. Considering it 10, it is possible to determine p and q through Equations 1 and 2 (assuming a negligible thickness for the central dividing wall).

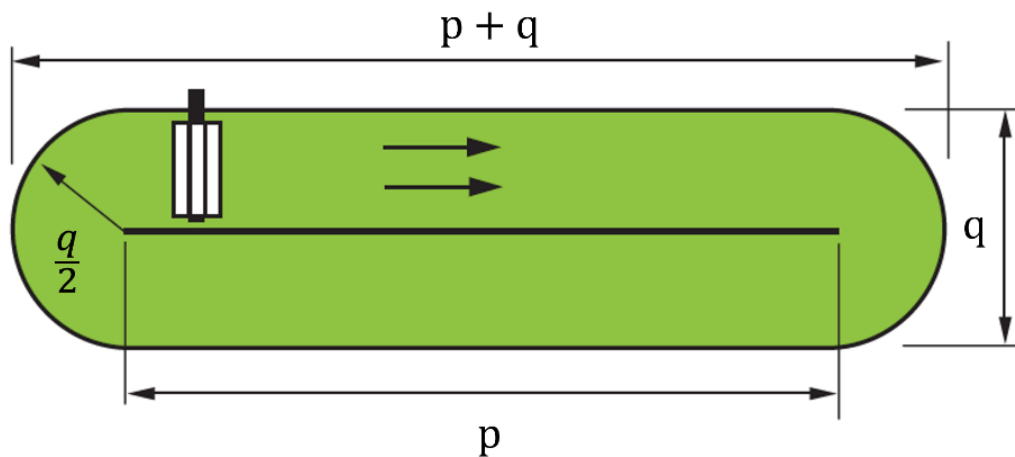


Figure 8 - Areal view of a raceway pond with semicircular ends (adapted from Chisti, 2013).

$$A = \frac{\pi q^2}{4} + pq \quad (1)$$

$$\frac{p}{q} = 10 \quad (2)$$

In this project, a sump was not considered since this system will not have a CO₂ external supply. A sump is defined as a channel depression, baffled or not, where the CO₂ supply is enhanced due to the longer contact time between the bubbles and the taller broth column. This may be necessary to increase biomass productivity (Mendoza et al., 2013), but for HRAPs intended to treat wastewaters with high carbon loads (Table 5) constitutes an unnecessary expense.

Typical systems from microalgae production operate with a flow velocity (u) of 0.3 m/s (Cunha et al., 2020), however, a flow velocity of 0.2 m·s⁻¹ is enough to prevent thermal stratification and biomass sedimentation (Chisti, 2013). This velocity is also widely used in wastewater treatment HRAPs (Craggs et al., 2012). With these conditions, turbulent flow is guaranteed in the reactor: $Re > 17000$; far above 8000, the limit to achieve turbulent regime in open channels (Ortiz et al., 2022).

Paddlewheels have become established as the most effective way of generating flow in raceways (Sutherland et al., 2015). The blades are generally flat, may be made of steel or fiberglass-reinforced plastic and should be supported on the shaft in such a way that they do not bend because of the water pressure during rotation (Chisti, 2013). Typically, only a single eight-bladed paddlewheel is used per raceway pond to avoid interference between paddlewheels (Craggs et al., 2012). The position in the reactor is shown in Figure 8. More efficient paddlewheel designs prevent the water escaping backwards through the blade clearance at the bottom of pond. Such high-efficiency paddlewheels require the bottom of the pond to have a curvature of a slightly greater radius than the radius of the paddlewheel (in contrast to flat bottom typical designs) and a close clearance between the bottom of the pond and the paddlewheel (2 cm) (Chisti, 2013; Craggs et al., 2012). This is the design here considered (Figure 9). A clearance of 5 cm was considered for the sides. Regarding the dimensions, the height of the paddle is calculated as the sum of the static water depth (0.4 m), the maximum head generated by rotation and an additional allowance (e.g. 0.1 m). Considering a low head generation, a height of 0.7 m was considered for the paddles and a total diameter of 1.4 for the paddlewheel. The power requirement P_p (W) for a paddlewheel can be given by Equation 3, where ρ , g , e and d_h represent the culture broth density (1000 kg·m⁻³), gravitational acceleration (9.81 m·s⁻²), efficiency of the motor (0.17) and channel's hydraulic diameter (0.82 m), respectively (Chisti, 2013). The blades of the paddlewheel present a thickness of 0.2 cm (Singha et al., 2017).

$$P_p = \frac{1.59A_p g u^3 f_M^2}{e d_h^{0.33}} \quad (3)$$

According to Equation 3, the paddlewheel motor for this HRAP will need to have a minimum power output of 5.1 W. However, this value is obviously underestimated. Assuming instead an average shaft power per unit area of $4 \text{ W}\cdot\text{m}^{-2}$ (relative to flat-bladed paddlewheels) and an efficiency of 30% (relative to flat-bladed paddlewheels generating a flow at a velocity of $0.2 \text{ m}\cdot\text{s}^{-1}$), the paddlewheel motor will need to have a minimum power output of 205 W (Li et al., 2014). This value is on the right scale for the mixing energy requirements for HRAPs reported in the literature (Milledge, 2013), however, field tests must be carried out to verify if that much power is required or enough. The motor and drive should allow a variable speed operation, except when the operational performance of the raceway has already been validated at a given flow velocity at the operational scale over an extended period, which is not the case here. Ideally, the motor should present the minimum power capacity and work at the minimum power required to achieve the target flow velocity ($0.2 \text{ m}\cdot\text{s}^{-1}$) in order to avoid unnecessary energy costs.

The considered HRAP design parameters are presented in Table 7 along with the respective bibliographic references and notes.

Table 7 - Main HRAP design parameters

Parameter	Value	Notes	Reference
Cycle time	7 days	Average of suitable HRT values for different seasons between 5.5-9.	(Sutherland et al., 2014)
Flow velocity	$0.2 \text{ m}\cdot\text{s}^{-1}$	Enough to prevent thermal stratification and biomass sedimentation	(Chisti, 2013)
Working volume	15.4 m^3	-	-
Surface area	38.5 m^2	-	-
Depth	0.4 m	Optimal depth for a HRAP acting as a secondary wastewater treatment system.	(Young et al., 2017)
Freeboard	0.2 m	-	(Young et al., 2017)
Straight channel length	18.9 m	Equations 1 and 2	-
Total width	1.9 m	Equations 1 and 2	-
Paddle's height	0.7 m	Sum of the static water depth, the maximum head generated and an additional allowance	(Chisti, 2013)
Paddlewheel's width	1.8 m	-	-

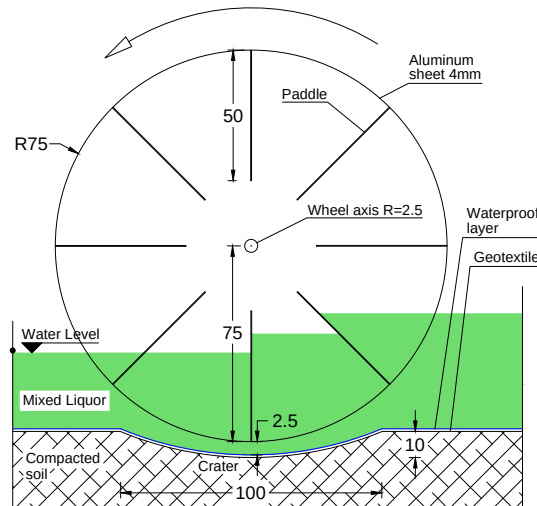


Figure 9 - Cross section of a highly efficient paddlewheel. Dimensions are in cm but do not refer to this work (adapted from Ortiz et al., 2022).

3.2.2. Harvest system design

For the harvesting step, considered here as a combination of two operations, harvesting and dewatering, the methods of microalgae autoflocculation via magnesium hydroxide precipitation and centrifugation were considered, respectively. Centrifugation was chosen over filtration because of its simplicity and robustness.

After the defined cycle time for the phycoremediation, the broth is enriched in magnesium and 90% of it is transferred by a centrifugal pump to a sedimentation tank or a series of sedimentation tanks (equipment already available at LIPOR I), where the pH value will be increased to promote flocculation. According to (Vandamme et al., 2015), the concentration of Mg^{2+} has to be increased to $\sim 1 \text{ mM}$ ($24.3 \text{ mg}\cdot\text{L}^{-1}$) and pH to 10.5-11. In (Young et al., 2021), $80 \text{ mg}\cdot\text{L}^{-1}$ were used but the authors recognized that it was excessive and that the same results could be achieved with half the magnesium concentration. Here, $30 \text{ mg}\cdot\text{L}^{-1}$ of Mg^{2+} is considered the target concentration. Magnesium chloride (MgCl_2) is the reagent suggested in the literature to increase magnesium concentration and calcium hydroxide (slaked lime, a cost-efficient base which poses a low health risk) to increase the pH value (Young et al., 2021). MgCl_2 is added to the broth still in the raceway pond to take advantage from the mixing system. The concentration of magnesium in the leachate must be monitored to determine how much needs to be added. It is possible that GWL already has the amount of Mg^{2+} necessary to ensure an efficient flocculation. According to (García-Pérez et al., 2014), lower concentrations of Mg^{2+} ($< 5 \text{ mM}$) and not very extreme pH values (~ 10.5) promote

smaller volumes of sludge, i.e., higher concentration factors. Here, it is assumed a concentration factor of 35, considering that this is a process that still has room to be optimized. After flocculation, it takes three hours for the biomass to settle (Young et al., 2021).

According to López et al. (2019), $20 \text{ g}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ is the threshold for the average annual productivity of a raceway pond. Park et al. (2011) reports similar areal productivities for microalgae-based WWTPs. Assuming that productivity, after seven days a microalgae concentration of $0.35 \text{ g}\cdot\text{L}^{-1}$ is reached, however, a lower final concentration was considered, $0.25 \text{ g}\cdot\text{L}^{-1}$, as in (García-Pérez et al., 2014), which corresponds to the typical final value of dry matter for open pond systems of 0.05% (Fasaei et al., 2018). The assumed final concentration of biomass is in compliance with the results of a year-long evaluation of microalgae production in wastewater using pilot-scale raceways (Morillas-España et al., 2021). A modest production is considered ($0.5 \text{ g}\cdot\text{L}^{-1}$ has already been reached in other works (Fernández et al., 2013), generally with higher cycle times) because too many variables may affect this process and have not yet been assessed on a pilot-scale.

After separating the treated effluent by decanting, 0.44 m^3 of sludge ($\sim 2.5\%$ dry matter) passes to the next step, centrifugation (Fasaei et al., 2018). An intermediate magnesium recovery step may be considered depending on whether there is a problem with the biomass presenting a median Mg^{2+} concentration ($< 5\%$ wt.) or if it is economically advantageous to recover magnesium chloride instead of buying more. Magnesium recovery is possible through a mild acidification (pH 7 to 8), which can be done in a second settling tank, where the new supernatant retains most of the magnesium previously precipitated in the biomass (Vandamme et al., 2015). Ultimately, the presence of this step depends on the final application of the biomass. In any case, it is assumed that a concentration factor of 6 is reached on centrifugation (Fasaei et al., 2018). Per cycle, approximately 0.075 m^3 (75 L) of this final product are produced, which corresponds to 4 kg of microalgae biomass. Annually, which corresponds to 52 cycles, 208 kg of dry microalgae biomass are produced.

Regarding the treated effluent, produced at a rate of $\sim 15 \text{ m}^3$ per cycle, it also needs further processing. Although wastewater treatments based on microalgae cultures have the potential to reduce bacterial concentration (García et al., 2008), in most cases, that plus the subsequent harvesting processes cannot achieve the level of pathogen removal demanded by regulation. Therefore, a specific disinfection treatment is required in order

to reuse the water (Gutiérrez-Alfaro et al., 2018).

UV technology is a well-established method for water disinfection (Tsenter et al., 2022), however, as drawback, lamps require significant amounts of energy throughout their lifetime, including their fabrication and operation, maintenance, and disposal. Solar disinfection (SODIS) is pointed as one of the best solutions: it is exclusively based on solar UV radiation energy utilisation, and its effectiveness for the elimination of pathogens from water has been widely demonstrated (Juvakoski et al., 2022; Pichel et al., 2019). The devices most used for disinfecting wastewater using solar radiation are the compound parabolic collectors (CPCs): inexpensive and low maintenance static reactors which efficiently concentrate both direct and diffuse solar radiation (Malato et al., 2009) (Figure 10). Incorporating a SODIS unit substantially increases the possibilities for reuse of the treated water after receiving a cumulative UV radiation dose of $25 \text{ Wh}\cdot\text{m}^{-2}$ (50-90 min of solar illumination) (Gutiérrez-Alfaro et al., 2018; Juvakoski et al., 2022).

In this work, where microalgae biomass is produced based on a wastewater treatment plant, the recommended applications for it consist of energy recovery and soil application. Therefore, the drying process choice is of secondary importance since the biomass will not have a noble application where high quality traits are mandatory (e.g. food, feed and pharmaceutical industries); see section 3.4. Moreover, some soil applications do not need much further concentration of the biomass since it can be sold as a biostimulant in form of a liquid product. Some examples include allfertis by Allmicroalgae® (Portugal) and AgriAlgae® Original Soil (Spain). Here, the design of the drying process was skipped. In future works, according to the intended future application established for the biomass, this step should be designed, if needed, in order to meet LIPOR's objectives.



Figure 10 - Compound parabolic collector used in solar disinfection (adapted from Gutiérrez-Alfaro et al., 2018).

3.2.3. End products

After bioremediation and biomass separation, two end-products are formed: biomass itself and a treated effluent.

Despite all the stated applications for the microalgae biomass (see section 2.4), most are not suitable for this study. Green waste leachate phycoremediation at LIPOR does not produce enough biomass (annual production of 208 kg) to ensure a viable process of energy recovery (Rafa et al., 2021). Besides the high infrastructure construction costs, annual production is so low that even operating costs are unlikely to be covered. Low production rate also makes it difficult to sell, mainly in food and feed sector. Moreover, and more importantly, the low quality of the biomass, due to the potential contamination by pathogens in the wastewater and the difficulty in maintaining monocultures in an open system make it not suitable for these types of valorization, including the production of high-value compounds for pharmaceutical and cosmetic industries.

The development of a new product with biostimulant activity for soil application is, however, a promising option, since these products only require small portions of biomass. The biostimulant activity of microalgae biomass in plants has already been verified at concentrations of 0.5 to 2 g·L⁻¹ (Ferreira et al., 2021; Mazepa et al., 2021). The LIPOR's infrastructure and know-how about composting and organic growing media formulation is a great advantage on this subject.

LIPOR is nowadays producer of two main kinds of products: organic soil improvers and organic growing media. The first are complex products which are applied in low quantities to the soil. They are widely used in agricultural soils to improve their properties for food production but are also used in gardens and vegetable gardens to enhance plant growth. The second are simpler (mainly constituted by organic fibers such as coconut fiber and peat) and are applied as a substitute of the soil. The main applications include its use in sowing and potting seedlings for crop production and in urban gardening. Considering this plus the relatively low production of microalgae biomass predicted in this project, the base-product for the development of a new one should be organic growing media. These are not extensively used as organic improvers and the biostimulant activity of the biomass is best used in a product aimed at seed germination and seedling growth (Ferreira et al., 2021). Further work is mandatory to assess not only the biostimulant activity of the produced biomass but also to corroborate the possibility

of developing a new product including LIPOR's current products – organic soil improvers and organic growing media Nutrimais®.

In other hand, the treated effluent, which should be monitored to verify whether its parameters are below the emission limit values imposed by the legislation in force for a given application (Table I.1 and I.2, Annex I), can be redirected in many ways. A simple usage implies the forwarding of the effluent to the irrigation system as irrigation water. According to the values transposed in Table 8 and as already discussed in section 3.1., the leachates studied comply with the legislation for this application in the parameters previously evaluated (the same is not valid for its discharge). However, the system here proposed is not suitable due to the also relatively low production of treated effluent ($\sim 780 \text{ m}^3/\text{year}$), which is significantly lower than LIPOR irrigation requirements ($\sim 7200 \text{ m}^3/\text{year}$). On the other hand, the SLLI and SLLII leachates (produced at a rate of $\sim 10000 \text{ m}^3/\text{year}$) can be redirected directly to this application without any further treatment, as far as is known. In any case, the possibility of produce value from these leachates in the form of microalgae biomass before its use as irrigation water should be assessed, as proposed in this work for the GWL. Their application as irrigation effluent is always guaranteed, before or after biomass production. However, treating different wastewaters through bioremediation always requires new research and testing work.

Table 8 - Effluent emission limit values for BOD_5 , COD, PO_4^{3-} and NO_3^- (Decreto-Lei n.º 236/98).

Water use	BOD_5 (mg O_2/L)	COD (mg O_2/L)	PO_4^{3-} (mg/L)	NO_3^- (mg/L)
Irrigation	-	-	-	-
Discharge	40	150	-*	50

*Total phosphorus has a concentration limit: 10; 3 in waters that feed ponds and dams; 0.5 in ponds and dams

Another possible use, and also easy to implement, take in account the need to promote biodiversity, even in urban green spaces, in order to increase physical and psychological urban health, environmental awareness and city's capacity to adapt to changing environmental conditions (Castelli et al., 2021; Nilon et al., 2017). LIPOR's facilities in Baguim do Monte include a 19 hectare green space called Parque Aventura (Figure 11). This is the result of closure, sealing and landscaping recovery of Ermesinde/Baguim do Monte sanitary landfill, that received since the 1970s' a large part of the waste produced in the associated municipalities. It is intended to be used freely by the local population as a ludic space, where physical activities, healthy eating, respect for

the environment and socialization are promoted. Associated with this park, there is a small trail called Trilho Ecológico (Ecologic Trail) that allows the population to get to know LIPOR and what is done in its facilities and to learn about biodiversity and its preservation. Although this whole infrastructure already presents a section of a small river passing through it (Rio Tinto), which ensures a good support for riparian flora and fauna, the creation of a network of small ponds with the treated effluent has the potential to further promote biodiversity while maintaining other species specific to this habitat (e.g. some groups of dragonflies and aquatic insects and amphibians). These ponds can be purposely colonized with native aquatic plants, also specific to this habitat, to enhance this effect. Not many native species can proliferate in both rivers and ponds, thus, the diversity of freshwater habitats leads to higher richness of species and therefore greater biodiversity, which is in decline globally (Dudgeon et al., 2006; Hermoso et al., 2022). Actions like this contribute to fulfilling the goals present in the commitment that LIPOR signed in 2020 with act4nature Portugal - an initiative promoted by BCSD Portugal (Business Council for Sustainable Development) within the scope of act4nature international, launched in France in 2018, with the aim of mobilizing companies to protect, promote and restore biodiversity. The objectives include promoting the integrated protection of biodiversity in the company's global strategy and carrying out activities to raise awareness and conserve biodiversity, which can be done using the green and blue infrastructures created at LIPOR.



Figure 11 - LIPOR I with Parque Aventura and Trilho Ecológico highlighted.

3.3. Project diagram

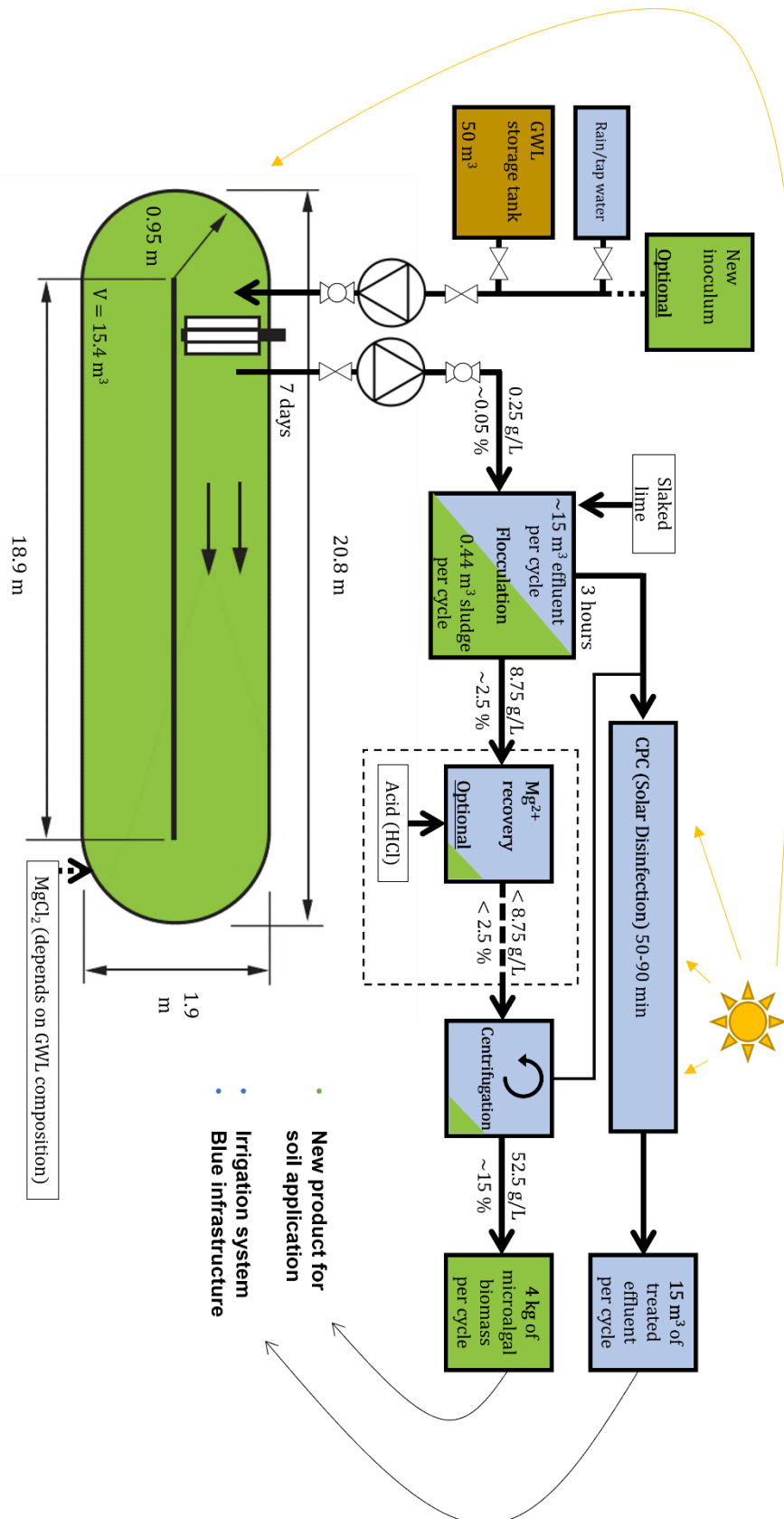


Figure 12 - Diagram of the proposed microalgae-based bioremediation process. $\text{g}\cdot\text{L}^{-1}$ indicates g of biomass per litre and % indicates % of dry weight. Although not explicit here, the HRAP configuration considered is the one presented in Figure 4.

3.4. Economic assessment

Similarly to other industrial processes, costs of wastewater treatment using a HRAP can be divided in capital costs (investment costs) and operational costs, which include energy, labour and maintenance costs.

Capital costs for the installation of a HRAP are often stated in the literature as a function of the total surface area of the reactor. (Park et al., 2011) refers the range 0.1(unlined)-0.25(lined) \$US million/ha. This project consider a lined reactor, i.e., impermeable and sealed to prevent fluid leakage and contaminations of the soils. In other hand, (Morillas-España et al., 2022), a more recent work, refers the range 0.13-0.37 million€/ha. (Ortiz et al., 2022) projected a HRAP with 667 m² with a total capital cost of 0.85 million€/ha, however, this cost seems overestimated since 54 % of it is relative to the waterproof layer (34.5%) and the paddlewheel (19.5%, without installation). Plastic pond liners have been estimated to represent a fraction of around 25% of the capital cost (Efroymson et al., 2020). In this work, a cost of €0.5 million/ha was assumed, which corresponds to a capital cost of 50 €/m² and an investment of 1925 € in this particular project. According to Ortiz et al. (2022), 54% of that cost refers to HRAP construction, 5% to hydraulic facilities, 14% to electrical facilities and 27% to mixing system. The same work estimated an operating cost for the HRAP of 0.163 €/m³ of effluent, considering the energy and labour requirements. Land cost was not considered because it is assumed that LIPOR I facilities has enough free area to proceed with this project.

Regarding operational costs, for autoflocculation, a cost of 1.40 €/kg of biomass is estimated based on the amounts needed of slaked lime and magnesium chloride and respective costs (Young et al., 2021). This is significantly higher than the costs predicted in (Fasaei et al., 2018) for other flocculants: 0.66 €/kg of biomass for cationic flocculants (average dose of 165 mg/g of biomass and average price of 4 €/kg) and 0.37 €/kg of biomass for chitosan (average dose of 21 mg/g of biomass and average price of 17.5 €/kg). Therefore, it is likely overestimated. A concentration of 30 mg/L of MgCl₂, the defined target concentration, represents a need to add less than 500 g per cycle (assuming an initial concentration of zero). Knowing that food grade MgCl₂ is sold at a price in the range of US\$ 0.2-1/kg (Focus Technology Co., Ltd., 2022), less than 0.5 € are required in magnesium chloride per cycle, i.e., per 4 kg of biomass (0.125 €/kg of biomass). To include transportation costs, slaked lime cost, which is almost negligible due the low quantities required per cycle, and the energy and labour costs, 0.5 €/kg of biomass was

considered, the average cost predicted in Fasaei et al. (2018) for other flocculants and for the whole step of flocculation. The same work estimates a total cost of 0.8 €/kg of biomass for the entire process of harvesting and dewatering using the same methods here considered, i.e., flocculation plus centrifugation. This global cost includes the types of cost mentioned above for the flocculation step.

Energy and other costs of a possible drying unit were not estimated because the need for such a unit is not confirmed. It depends on the final application for the biomass which is not yet defined. In any case, the most likely scenario, which is the use of the biomass for the development of a soil application product, does not require further drying after centrifugation.

Table 9 sums up the assumptions already stated for capital and operational costs and includes the quantification of the installation and indirect capital costs based on cost factors reported in the literature (Acién et al., 2012). Capital costs of harvesting and dewatering equipment, including acquisition and installation costs, were determined assuming that half of the total capital cost is due to the biomass and effluent processing units, as predicted in Gonçalves (2017). Annual production/operational costs are also presented in Table 9. These include energy and labour costs and were determined considering that the biomass production is constant throughout the year. Maintenance costs were considered 5% of the total capital costs (Gonçalves, 2017). An allowance of 100% was considered in the previously determined annual production costs to include miscellaneous materials required, insurance, utilities (e.g. water for washing) and additional labour and supervision costs.

Assuming that a treated effluent is obtained, the major aim of this project is the production of biomass to ensure project's economic viability. As mentioned in section 3.2.3, the more suitable application for the produced biomass is the development of a new product with biostimulant activity based on organic growing media. All three organic growing media produced at LIPOR (Figure 13) are sold at a price of €3.5 per bag of 40 L. Considering the addition of 80 g of microalgae biomass per bag of these products, in order to reach a concentration of $2 \text{ g} \cdot \text{L}^{-1}$, enough to guarantee biostimulant activity in seed germination and seedling growth (Mazepa et al., 2021; Ranglová et al., 2021), and a final selling price of 4.0 € per bag, 2600 bags ($208/0.080$) of new product can be produce per year with a total additional revenue from microalgae biomass production of €1300 ($0.05\text{€} \times 2600$). The sale of these new products is the only revenue source considered in this assessment. However, LIPOR is currently spending €4600 annually on dealing with

GWL. This expense is totally eliminated with the implementation of the process proposed here, which guarantees a payback time of only 2 years, considering a total investment of €6972 and that revenue minus costs is positive and almost negligible (€12). After payback time, the process is sustainable but not significantly profitable. The objective of reducing/eliminating LIPOR's expenses on dealing with GWL (transportation plus treatments costs) is, however, achieved.

Table 9 - Capital and annual production costs.

Capital costs	Factor ^a	Value (€)	Annual production costs	Value (€)
HRAP			HRAP	
Direct costs			Energy and labour	130
HRAP construction (A)	-	1040	Maintenance	174
Hydraulic facilities (B)	-	96		
Electrical facilities (C)	-	270		
Mixing system (D)	-	520		
Equipment installation	0.20	385		
Instrumentation and control	0.15	289		
Indirect costs			Harvesting and dewatering	
Engineering and supervision	0.30	578	Energy and labour	166
Construction expenses	0.05	96	Maintenance	174
Contractor's fee	0.03	58		
Contingency	0.08	154		
Subtotal		3486	Subtotal	644
non-HRAP ^b		3486	Allowance	644
Total		6972	Total	1288

^a Fraction of the total purchased costs (A+B+C+D) (Acién et al., 2012).

^b These include the costs of flocculation, centrifugation and effluent disinfection units, and the costs inherent to the electrical and hydraulic equipment required.

Moreover, the implementation of this process allows the reduction of carbon emissions associated with the processing of the leachate. Roughly assuming the same energetic consumption per m³ of leachate in a municipal WWTP and in the process proposed here (which is usually higher in conventional WWTPs - Ortiz et al., 2022), the differences in carbon emissions stem from differences in transport (this process does not require the transport of GWL outside LIPOR's facilities) and in the biological process: microalgae growth captures atmospheric CO₂, in contrast to AS which releases CO₂ due to the predominant bacterial activity. Currently, tanker trucks are used to take the GWL from LIPOR's facilities to WWTPs.

The average reference emissions for truck transport amount to 52.7 gCO₂/tkm (tonne-kilometre, abbreviated as tkm, is a unit of measure of freight transport which

represents the transport of one tonne of goods by a given transport mode a distance of one kilometre) (Suzan, 2021). The annual transport emissions can be determined by multiplying this reference value by the total kilometres travelled in a year between LIPOR I and the WWTP (ETAR de Parada) and the total mass of effluent transported. Knowing that LIPOR I is 7 km away from the WWTP used, and that ~800 tons of GWL are transported per year (a density of $1000 \text{ kg}\cdot\text{m}^{-3}$ is considered), 0.3 tons of CO_2 are annually emitted for the atmosphere as a result of the current management of the target effluent. An equivalent calculation can be made for SSLI and SLLII: 3.7 tons of CO_2 emissions annually. Considering all this, the balance is clearly positive, at least in terms of eliminating CO_2 emissions associated with transport of GWL.

The use of the treated effluent for irrigation is not here considered as an income since all the water currently used for that purpose at LIPOR's facilities come from wells. The fixed costs inherent to having wells are heavily diluted, as only a very small fraction of the collected water is used for irrigation; most of it is used for cleaning trucks and equipment and other processes. The same if the treated effluent is used to promote biodiversity – it is not possible to determine a direct economic income for LIPOR.



Figure 13 - Organic growing media produced at LIPOR.

4. Materials and methods

4.1. Leachate collection

Three leachates produced at LIPOR I facilities were assessed in this work, two from the sanitary landfill now called Parque Aventura (SLL I and II) and one from the Parque de Verdes (GWL) (Table 5). A sample of 15 L of each SLL was collected on April 1st, 2022, and a sample of GWL on April 4th, using plastic containers. This task was carried out under the supervision of elements belonging to LIPOR's staff. The location of the storage tanks for each leachate is shown in Figure 6. The samples were stored at room temperature in the dark. No significant microbial activity was detected in the containers during this work.

4.2. Culture media

The experiments of microalgae growth were performed in different culture media. These were formulated based on different combinations and dilutions of the three leachates evaluated. Dilutions were made to ensure that the culture media were not too resistant to light penetration. Tap water was used for the dilutions. Table 10 shows the proportion of each leachate in every formulation produced and tested. Table 5 presents some leachates characterization parameters obtained by Bragança (2018).

Table 10 - Tested culture media formulations.

Code	Proportion (%)			
	GWL leachate	SLLI leachate	SLLII leachate	Tap water
PV-10	10	-	-	90
PV-25	25	-	-	75
PV-50	50	-	-	50
A1-10	-	10	-	90
A2-10	-	-	10	90
A1-50	-	50	-	50
A2-50	-	-	50	50
PV-10A1	10	90	-	-
PV-10A2	10	-	90	-

For the control tests, microalgae growth was performed in an adapted version of Organization for Economic Co-operation and Development (OECD) test medium with

the following composition (per litre): 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 $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 50 mg NaHCO_3 , 250 mg NaNO_3 and 45 mg KH_2PO_4 . Only control medium was sterilized. Leachates were not sterilized. No work of isolation and identification of additional organisms present in unsterilized media was performed.

4.3. Microalgae

The microalgae strain used in this study was *Chlorella vulgaris* AGF002. This was obtained at ALiCE - Associate Laboratory in Chemical Engineering, hosted at the Faculty of Engineering of University of Porto, already in the right concentration for inoculation under the conditions evaluated in this work.

4.4. Experimental setup and cultivation conditions

To produce a proof-of-concept that microalgae *Chlorella vulgaris* can grow in LIPOR's leachates, batch experiments were performed in 500 mL flasks (Boeco, Germany) with a working volume of 200 mL. Microalgae were cultivated in different culture media (Table 10) for 10 days in the following conditions: initial microalgae cell concentration of $0.05 \text{ g} \cdot \text{L}^{-1}$ (dry weight); room temperature ($\sim 20^\circ\text{C}$); light irradiance of $30 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$; and continuous mixing and aeration with the injection of atmospheric air at the bottom of the flasks. The same conditions were guaranteed in the control tests. The light supply was given by four LED strips. The light irradiance was measured using an ISO-TECH 1335 Digital Light Meter (RS Components, United Kingdom). The photoperiod was kept constant and equal to 24 h for all tests. The air compression was ensured by two different air pumps with similar outputs of $4800 \text{ cm}^3 \cdot \text{min}^{-1}$ (Maxima R A-806, Hagen Inc., United Kingdom and air 275 R plus, Sera, Germany). Triplicates for each condition were performed. Figure 14 shows the apparatus developed for the experiments. A box lined with a reflective material (aluminium foil) was covered with a plate where the LED strips were fixed. The flasks were left inside the apparatus during the experiments.



Figure 14 - Experimental setup used for the evaluation of different cultivation conditions in microalgae growth. A - Open; B - Closed.

4.5. Microalgae growth monitoring and parameters

In all experiments, samples were collected in the last day of culturing. To quantify microalgae growth, the optical density at 750 nm (OD_{750nm}) was measured in a V-1200 spectrophotometer (VWR, Portugal), and then converted to dry weight of biomass (X , $mg \cdot L^{-1}$) through the Equation 4 ($R^2 = 0.998$) (Gonçalves, 2017).

$$OD_{750nm} = (1.80 \times 10^{-3})X + (4.28 \times 10^{-2}) \quad (4)$$

Biomass concentration values were used to determine average biomass productivities (P , in $\text{mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$). These were calculated from the variation in biomass concentration within the cultivation time, as shown in Equation 5:

$$P = \frac{X_f - X_i}{t_f - t_i} \quad (5)$$

where X_f and X_i correspond to biomass concentrations (in $\text{mg}\cdot\text{L}^{-1}$) at times t_f and t_i (in d), the end and beginning of cultivation time, respectively. The initial biomass concentration for all experiments is known and similar: $50 \text{ mg}\cdot\text{L}^{-1}$. A control for microalgae growth was carried out in control medium for every set of experiments.

4.6. Nutrient concentration and removal kinetics

Nutrients removal was evaluated by quantification of NO_3^- and PO_4^{3-} concentrations, and soluble BOD_5 and soluble COD in the culture medium. In all experiments, samples were collected in the last day of culturing. Initial values for these parameters were determined based on the leachate characterization through the same methods, according to the proportions present in Table 10.

For nitrate quantification, supernatants were recovered after samples centrifugation at $4000 \times g$ for 5 min. Supernatant absorbance was measured at 220 nm ($\text{Abs}_{220\text{nm}}$) in quartz cuvettes in a T80 UV/VIS spectrophotometer (PG Instruments, United Kingdom). A calibration curve from Sousa et al. (2021) was used (Equation 6). This was prepared with NaNO_3 solutions with concentrations ranging from 0.5 to 500 mg/L ($R^2 = 0.995$). The nitrate concentration was obtained by multiplying C_{NaNO_3} by the factor 0.73.

$$\text{Abs}_{220\text{nm}} = (2.60 \times 10^{-3})C_{\text{NaNO}_3}(\text{mg} \cdot \text{L}^{-1}) + (4.61 \times 10^{-2}) \quad (6)$$

To quantify phosphorus concentration, the collected samples were centrifuged, and the supernatants were stored at -20°C until being analyzed. Then, a mixture was prepared by adding ascorbic acid (10% w/v) to $(\text{NH}_4)_2\text{MoO}_4 \cdot 4\text{H}_2\text{O}$ (0.42% w/v) with H_2SO_4 (1 M), in a 1:6 ratio. For this purpose, 700 μL of the mixture was added to 300 μL of supernatant (diluted firstly if necessary) and further incubated for 1 h. Then, the absorbance was read at 820 nm. A calibration curve from Sousa et al. (2021) was used

(Equation 7). This was prepared with KH_2PO_4 solutions with concentrations ranging from 0.5 to 60 $\text{mg}\cdot\text{L}^{-1}$ ($R^2 = 0.997$). The phosphate concentration was obtained by multiplying $C_{\text{KH}_2\text{PO}_4}$ by the factor 0.70.

$$\text{Abs}_{820\text{nm}} = (1.27 \times 10^{-2})C_{\text{KH}_2\text{PO}_4}(\text{mg} \cdot \text{L}^{-1}) - (1.01 \times 10^{-2}) \quad (7)$$

The quantifications of soluble COD and BOD_5 were performed in an external laboratory belonging to Eurofins Scientific following the reference methods ISO 15705 and NF EN ISO 5815-1, respectively. In contrast to nitrate and phosphate quantifications, COD and BOD_5 measurements were made for only one of the replicates. For that, the replica with higher final biomass concentration was selected in each experiment.

Regarding nutrient removal kinetics, average removal rates (R_s , in $\text{mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$) and nutrients removal efficiencies (R , in %) were determined. Average removal rates were calculated according to Equation 8:

$$R_s = \frac{S_i - S_f}{t_f - t_i} \quad (8)$$

where S_i and S_f correspond to nutrients concentrations (in $\text{mg}\cdot\text{L}^{-1}$) at times t_i and t_f (in days), respectively. Nutrients removal efficiencies were determined according to Equation 9:

$$R = \frac{S_i - S_f}{S_i} \times 100 \quad (9)$$

5. Results and Discussion

5.1. Media characterization

The different media formulations produced were characterized based on the characterization of the leachates tested according to Table 10. This consisted of the quantification of dissolved nitrate and phosphate concentrations and chemical and biochemical oxygen demands. The values obtained for these four parameters for leachates and tested formulations are presented in Table 11.

Table 11 - Characterization of leachates and tested media.

Sample	Parameters			
	NO ₃ ⁻ (mg/L)	PO ₄ ³⁻ (mg/L)	COD (mgO ₂ /L)	BOD ₅ (mgO ₂ /L)
GWL	1.41×10 ³	5.51×10 ¹	5.84×10 ²	2.20×10 ¹
SLLI	2.01×10 ³	1.10×10 ¹	4.39×10 ²	3.90×10 ¹
SLLII	2.58×10 ³	1.27×10 ¹	5.80×10 ²	1.30×10 ¹
Tap water*	0	0	0	0
A1-10	2.01×10 ²	1.10×10 ⁰	4.39×10 ¹	3.90×10 ⁰
A2-10	2.58×10 ²	1.27×10 ⁰	5.80×10 ¹	1.30×10 ⁰
A1-50	1.00×10 ³	5.51×10 ⁰	2.20×10 ²	1.95×10 ¹
A2-50	1.29×10 ³	6.34×10 ⁰	2.90×10 ²	6.50×10 ⁰
PV-10A1	1.95×10 ³	1.54×10 ¹	4.54×10 ²	3.73×10 ¹
PV-10A2	2.46×10 ³	1.69×10 ¹	5.80×10 ²	1.39×10 ¹
PV-10	1.41×10 ²	5.51×10 ⁰	5.84×10 ¹	2.20×10 ⁰
PV-25	3.53×10 ²	1.38×10 ¹	1.46×10 ²	5.50×10 ⁰
PV-50	7.07×10 ²	2.76×10 ¹	2.92×10 ²	1.10×10 ¹

*No measurements were made for tap water (0 mg·L⁻¹ was assumed for the given parameters).

In general, when compared to the characterization reported in Bragança (2018) for the same leachates, all of them now show a slight increase in the concentrations of the parameters assessed in both studies, i.e., COD, BOD₅ and PO₄³⁻ (see Table 5). The cause of this fact can be attributed to the lack of precipitation in the first quarter of 2022. As reported by IPMA (Instituto Português do Mar e da Atmosfera), in comparison to the first quarter of 2018, when leachate samples were collected for Bragança (2018) work, the first three month of 2022 were dry (Figure 15). As leachates are largely formed by water percolation, the decrease in precipitation leads to more concentrated effluents (Gupta et al., 2014). The exception is the decrease of COD and BOD₅ in GWL in the same period. This result derives from other two variables that affect leachate's composition: the waste constitution and its residence time in the pile (Savage & Tyrrel, 2005). Both variables are not applicable for SLLI and SLLII since the sanitary landfill is closed and sealed and so

the waste from which the leachate is generated is the same over the years. However, for the GWL these are particularly important because the GW pile composition and GW residence time in the pile change constantly according to the waste generated in the area managed by LIPOR and the shredding rate for its use in composting process, respectively. Consequently, the GWL parameters are also variable, as proven here, even if precipitation rates point to the opposite effect, i.e., leachate concentration due to lack of precipitation in this particular case.

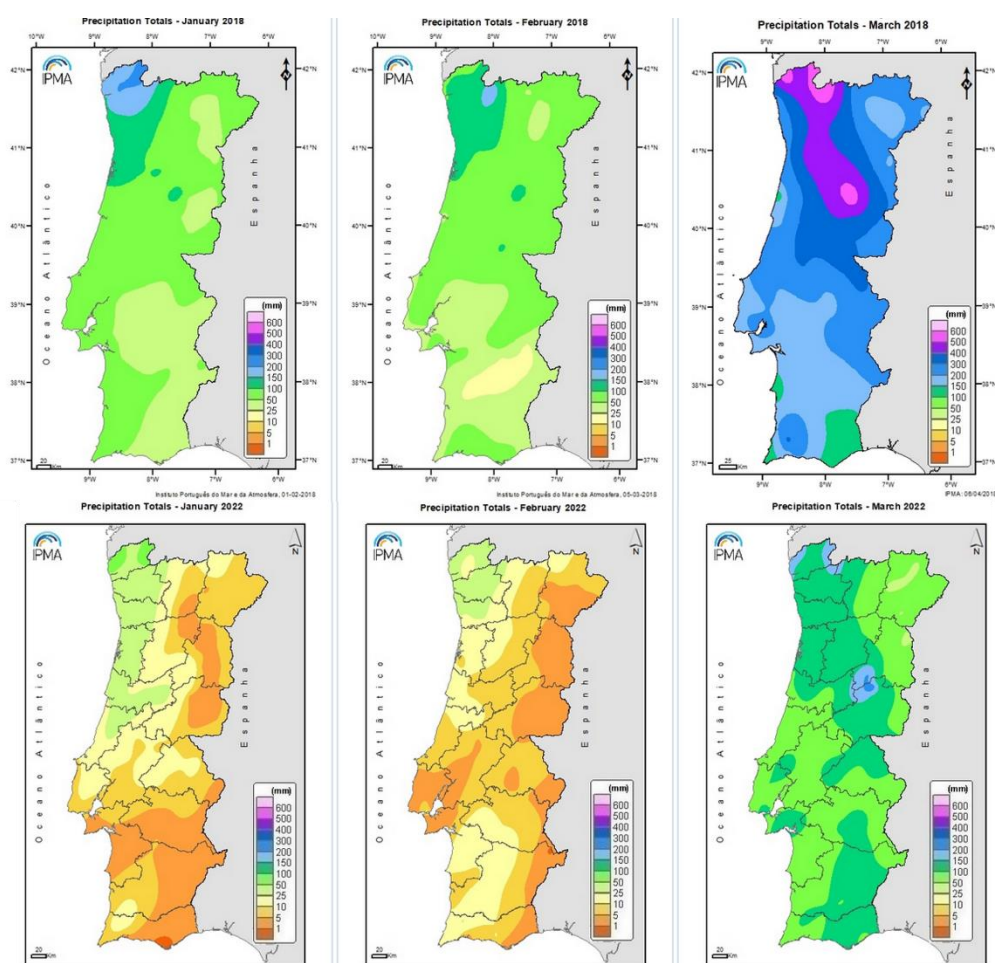


Figure 15 - Total monthly precipitation in Portugal in the first quarter of 2018 (up) and 2022 (down) (source: www.ipma.pt).

Regarding the formulations produced, it is clear that PV-10A1 and PV-10A2 are the most concentrated media for all parameters evaluated, and that there are no significant differences in COD and BOD₅ between all formulations. The main difference stands on the ratio between nitrate and phosphate concentrations. This is significantly higher in the PV formulations than in the A1 and A2 formulations, as the former are made up of GWL at different dilutions, which is five times richer than SLLI and SLLII in phosphates and 1.5 times poorer in nitrates.

5.2. Microalgal growth

Two points were collected to measure microalgae growth during the experiments, the start point, where the concentration was the same for all the experiments and equal to $50 \text{ mg}\cdot\text{L}^{-1}$, and the final point (X_F , in $\text{mg}\cdot\text{L}^{-1}$), after 10 days. With those, the average productivity in biomass (P , in $\text{mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$) of each experiment was determined. The results are presented in Table 12. These should be analyzed taking into account the characterization of the tested media shown in Table 12.

Table 12 - Maximum biomass concentrations (X_F , in $\text{mg}\cdot\text{L}^{-1}$) and average biomass productivities (P , in $\text{mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$) determined for *C. vulgaris* under different medium conditions.

Medium	X_F ($\text{mg}\cdot\text{L}^{-1}$)	P ($\text{mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$)
A1-10	$(2.00\pm 1.68) \times 10^2$	$(1.50\pm 1.68) \times 10^1$
A2-10	$(3.72\pm 2.25) \times 10^2$	$(3.22\pm 2.25) \times 10^1$
A1-50	$(2.40\pm 1.13) \times 10^2$	$(1.90\pm 1.13) \times 10^1$
A2-50	$(6.60\pm 2.61) \times 10^1$	$(2.79\pm 2.28) \times 10^0$
PV-10A1	0	0
PV-10A2	0	0
PV-10	$(3.53\pm 2.25) \times 10^2$	$(3.03\pm 2.25) \times 10^1$
PV-25	$(2.91\pm 1.21) \times 10^2$	$(2.41\pm 1.21) \times 10^1$
PV-50	$(3.99\pm 0.41) \times 10^2$	$(3.49\pm 0.47) \times 10^1$
Control (n = 7)	$(4.66\pm 0.93) \times 10^2$	$(4.16\pm 0.93) \times 10^1$

Values, except for control medium, are presented as the mean $\pm$ standard deviation of three independent experiments

Concerning PV-10A1 and PV-10A2 media, no microalga growth was detected. This is most probably associated with the too high nutrient concentrations present in these formulations. It is known that high concentration of nitrates and phosphates can inhibit microalgae proliferation, in particular *Chlorella vulgaris* (Li et al., 2018; Sousa et al., 2021). The highest COD, N, and P concentrations microalgae could tolerate are 3750, 251, and 215 mg L^{-1} , respectively, according to Su (2021). Moreover, some parameters that were not assessed in this study may also have influenced these results. An example is the concentration of ammonia (NH_4^+), which can have a strong inhibition effect on microalgae (Praveen et al., 2018; Su, 2021). This is particularly relevant considering that these formulations were the only ones tested composed only of leachates, i.e., undiluted effluents. The same arguments are valid to justify the low biomass productivity in the A2-50 medium, the lowest among the experiments where biomass growth was detected, although this is composed of a 1:1 dilution of the leachate SLLII with tap water.

Among all formulations, the PV group (PV-10, PV-25 and PV-50) stood out for the higher biomass productivities achieved in comparison to the tested media consisting

of diluted SLL (A1-10, A1-50, A2-10 and A2-50). In addition, a trend towards higher yields associated with higher concentrations of GWL in PV formulations is notorious. These results are most likely associated with a better N:P ratio in PV formulations than in A1 and A2 formulations. Choi & Lee (2015) work reports an optimum N:P ratio of 10 to maximize biomass production in wastewater treatments using microalgae. However, in the same work, it is stated that the optimum ratio for biomass productivity and nutrient removal varies from 5 to 30, depending on the ecological conditions in the wastewater. According to the N:P ratio in algal biomass, which can range from about 4:1 to almost 40:1, any value between these two may be suitable and the optimum to maximize microalgae growth, although considering the typical composition ($C_{106}H_{181}O_{45}N_{16}P$), a ratio of 16 would be the most likely optimum (Craggs, 2005; Park et al., 2011). On average, the A1 and A2 formulations present an N:P ratio of 193, in contrast to PV formulations that present a ratio of 26, which is far closer to the optimum stated in the literature and within the optimal range considered. Regarding the oxygens demands, these probably had little effect on the results between the tested formulations, as they are small when compared to the values present in the literature for microalgae-based wastewater treatments ($> 1000 \text{ mg}\cdot\text{L}^{-1}$), and because C:N ratios are similar in all formulations where microalgae growth was detected (Gupta et al., 2017; Mendonça et al., 2022). However, these certainly affected microalga growth in this work as a whole because C:N ratio is too low ($0.3 < 10$) to ensure a good CO_2 availability as a result of heterotrophic oxidation by bacteria (Park et al., 2011). This was supplied mainly by aeration.

According to the results obtained, GWL is suitable for the growth of microalgae, which meets the aim of the experimental segment of this work that is to verify the possibility of taking value from this effluent in the form of biomass. Considering the average productivity of PV-50 ($34.9 \text{ mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$), it is verifiable that a final concentration of $0.25 \text{ g}\cdot\text{L}^{-1}$ is achievable in 7 days of culturing, as assumed in the process proposed in section 3. This is part of the proof of concept. On the other hand, SLLI and SLLII are not so suitable, however, these can still be used directly as irrigation waters, as far as is known. In any case, new formulations based on these three leachates should be tested in future works in order to try to value SLL leachates before their final use.

No formulation allowed to reach a productivity similar to the control medium. This result was expected since the control medium is optimized for the microalgae proliferation while the tested formulations are made from wastewaters without completely known composition.

5.3. Nutrients removal

Two points were collected to measure nutrients removal during the experiments: the start point - the initial concentration for each parameter is presented in Table 11 for all the experiments - and the final point, after 10 days. With those, average removal rates (R_s , in $\text{mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$) and nutrients removal efficiencies (R , in %) for each experiment and for each nutrient (NO_3^- , PO_4^{3-} , COD, BOD_5) were determined. The results for NO_3^- and PO_4^{3-} are presented in Table 13, and for COD and BOD_5 are presented in Table 14.

Table 13 - Average NO_3^- and PO_4^{3-} removal rates (R_s , in $\text{mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$) and removal efficiencies (R , in %) determined for *C. vulgaris* under different medium conditions.

Medium	NO_3^-		PO_4^{3-}	
	R_s ($\text{mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$)	R (%)	R_s ($\text{mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$)	R (%)
A1-10	$(-2.58\pm1.34)\times10^1$	-128 ± 67	$(-6.44\pm3.59)\times10^1$	-580 ± 330
A2-10	$(-2.77\pm0.16)\times10^2$	-1070 ± 61	$(-9.94\pm1.27)\times10^1$	-780 ± 100
A1-50	$(-1.70\pm1.16)\times10^2$	-169 ± 115	$(-5.51\pm5.26)\times10^1$	-100 ± 95
A2-50	$(-2.34\pm3.47)\times10^3$	-1810 ± 2690	$(-9.46\pm4.69)\times10^1$	-150 ± 74
PV-10A1	$(-2.00\pm2.15)\times10^1$	-10 ± 11	$(-0.37\pm2.49)\times10^1$	-2 ± 16
PV-10A2	$(-6.91\pm2.88)\times10^1$	-28 ± 12	$(-1.12\pm0.78)\times10^0$	-66 ± 46
PV-10	$(-1.77\pm0.39)\times10^1$	-125 ± 28	$(-0.92\pm5.52)\times10^1$	-17 ± 100
PV-25	$(-6.80\pm3.94)\times10^0$	-19 ± 11	$(2.75\pm3.61)\times10^1$	20 ± 26
PV-50	$(4.02\pm5.53)\times10^0$	6 ± 8	$(2.94\pm6.39)\times10^1$	11 ± 23

Values are presented as the mean $\pm$ standard deviation of three independent experiments ($n = 3$).

Overall, a major result is noted for almost all media and parameters tested: the average removal rates and nutrient removal efficiencies are negative instead of positive, i.e. the concentration of the assessed parameters increased during the experiments instead of decreasing. This is most likely related to the fact that all experiments were not performed under sterile conditions and, therefore, the microalgae cultures were not axenic. This is corroborated by the fact that leachates, effluents originating from decomposing material, are rich in microorganisms and were not previously autoclaved. The presence of bacteria under preferential culture conditions for microalgae may promote their death and the consequent release of intracellular material into the medium. This intracellular content is not accounted for the initial concentration of nutrients stated in Table 11 because it is released afterwards during the experiments (samples are centrifuged to remove the biomass pellet before measurements, see section 4). According

to the results, even that microalgae uses part of these nutrients for its growth, 10 days under the conditions tested are not enough to remove them completely and reach the measured initial concentrations of the assessed parameters in most experiments. Additionally, the few suspended material present in the media, which come from the studied leachates. is decomposed during the experiments, thus contributing to increase the concentrations of dissolved nutrients.

Table 14 - Average COD and BOD₅ removal rates (R_s , in $\text{mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$) and removal efficiencies (R , in %) determined for *C. vulgaris* under different medium conditions.

Medium	COD		BOD ₅	
	R_s ($\text{mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$)	R (%)	R_s ($\text{mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$)	R (%)
A1-10	-3.88×10^1	-884	-1.16×10^1	-2977
A2-10	-1.70×10^1	-293	-9.70×10^{-1}	-746
A1-50	-2.75×10^1	-125	-2.05×10^0	-105
A2-50	-7.30×10^1	-252	-3.55×10^0	-546
PV-10A1	-2.45×10^0	-5	2.73×10^0	73
PV-10A2	1.17×10^1	20	-3.10×10^{-1}	-22
PV-10	-3.79×10^1	-648	-1.08×10^1	-4900
PV-25	-1.03×10^1	-71	-1.05×10^1	-1900
PV-50	-4.12×10^1	-141	-1.19×10^1	-1082

Concerning nitrates and phosphates, PV group stood out again, but now for the higher removal rates and removal efficiencies achieved in comparison to the tested media consisting of diluted SLL. In addition, a trend towards higher rates associated with higher concentrations of GWL in PV formulations is also notorious. The higher microalgae growth obtained in PV formulations allowed to remove almost all the additional nutrients released during the experiments (PV-10 and PV-25), and further reduce their concentrations to lower values than the initial ones (PV-50). Another possible complementary explanation to these differences between the results from PV group and A1 and A2 formulations is the discoordination of growth phases after 10 days of culturing. Microalgae, as any microorganism cultivated in batch mode, have four main phases of growth: lag-phase, exponential phase, stationary phase and death phase (Vonshak, 1985). Since the microalgae concentration was not monitored daily, it is impossible to know in which phase each experiment was in its final, so, some of them were probably already in the death phase (A1 and A2 formulations), allowing the proliferation of heterotrophic bacteria and the release of nutrients back into the medium. According to this hypothesis,

PV group formulations only require more time for the microalgae to reach the death phase (>10 days). This is empirically supported by the observation of cultures during the experiments, where it was possible to verify that some experiments using A1 and A2 formulations showed some microalgae bleaching after a clear proliferation, an indication of the death phase (Pfendler et al., 2018).

Regarding PV-10A1 and PV-10A2 media, as no microalgae growth was detected, the values obtained are the result of bacterial growth alone. The increase in nitrates and phosphates concentration is not very significant when compared to A1 and A2 formulations, where microalgae proliferated. This may prove that without microalgae competition the bacteria managed to grow and the increase in NO_3^- and PO_4^{3-} is the result of the decomposition of the suspended material present. On the other hand, the results of COD and BOD_5 are due to a balance between the degradation of suspended material and the consumption of the dissolved organic nutrients by the bacteria, which increase and decrease both parameters, respectively. In these two formulations, the variation of COD and BOD_5 is not very significant when compared to the other tested conditions, which is related to the lack of microalgae growth.

The results of COD and BOD_5 for the other experiments are strictly related to two phenomena: the secretion of extracellular metabolites by microalgae and microalgae biomass degradation by bacteria after approaching death phase. It is known that microalgae growth alone, as promoted in this work through preferential culture conditions, is not efficient in remove COD and BOD_5 from the medium (Lee et al., 2019). This is because microalgae use CO_2 instead of organic carbon as a source of the same element, and release organic compounds, such as exopolysaccharides, increasing the COD level (Liu et al., 2016; Perera et al., 2022). That is the explanation for all removal rates being positive in the experiments where microalgae growth was detected. However, again, a great difference between the results from PV group and A1 and A2 formulations was recorded: the former presented far lower removal rates (i.e. higher increase in the concentration of both parameters). The higher microalgae growth obtained in PV formulations resulted in a higher secretion of external metabolites and therefore a greater increase in COD and BOD_5 levels. This high growth may also have fomented higher biomass degradation by bacteria, considering that the cultures were probably close to the death phase.

Nevertheless, the experimental segment of this work allowed to verify that microalgae, namely *Chlorella vulgaris*, can grow in leachate-based growth media, and

more significantly in green waste leachate based media, the target effluent to be treated. Regarding the removal of nitrates and phosphates, which are not well supported by the results, there is evidence that the defined methodology was not adequate enough to properly evaluate it. The removal of these two nutrients in particular from wastewaters by microalgae is more than proven for several conditions, reactor types and effluent sources, and it was not detected a motif for the proposed quasi-conventional system do not work at LIPOR I facilities with GWL. To overcome the limitations of the presented analysis and obtained results, future works should establish a plan of daily monitoring the microalgae concentration. This will make it possible to recognize the growth phases and therefore allow the study of the results with that valuable information and even adjust the duration of the experiments. Moreover, daily monitoring of the concentrations of nitrates and phosphates would also be a good improvement to the experimental approach, as it will allow knowing when there are variations in these parameters according to the microalgae growth curve. Concerning COD and BOD₅ parameters, it is expected that in a real-scale HRAP, where bacterial growth is also promoted, the increase in soluble organic compounds secreted by microalgae can be easily controlled by bacterial heterotrophic metabolism. In addition, it is expected that this activity will further promote the reduction of the concentration of these nutrients to values below the initial ones, treating the effluent. The more favorable conditions for the growth of microalgae tested in this work inhibited the bacterial action, not allowing a full proof-of-concept for the removal of these nutrients (COD and BOD₅). This was partially achieved with NO₃⁻ and PO₄³⁻ parameters, as already documented, where some formulations of the PV group allowed only partial removal.

6. Conclusions

In this work, an on-site microalgae-based bioremediation process was successfully designed and dimensioned to deal with a green waste leachate from LIPOR. For the reactor, a high rate algal pond system was suggested. For the harvesting step, microalga autoflocculation via magnesium hydroxide precipitation and centrifugation were considered. The whole system is estimated to produce 208 kg of dry microalgae biomass per year. For the treated effluent, a solar disinfection system using a compound parabolic collector was proposed to ensure the removal of pathogens demanded by regulation. According to the available and recent literature, the proposed system, which was summarized in a diagram, is capable of treating the target effluent.

Applications were explored for the resulting biomass and treated effluent. For the first one, the development of a soil-application product such as an organic soil improver with biostimulant properties was pointed out as the most suitable option. For the second, its use for irrigation or biodiversity promotion through the creation of blue infrastructures, as a network of small ponds, was explored. According to the legislation, its use for irrigation is already granted, even before bioremediation. The same is valid for the other two leachates produced at LIPOR I.

An economic assessment allowed to conclude that after two years of operation, the savings from avoiding the current treatment and transportation costs cover the investment costs. Afterwards, the process is sustainable, however, economically fragile since it presents a very low profit margin.

A proof-of-concept was tried to produce using a *Chlorella vulgaris* strain and the green waste leachate. The two landfill leachates were also assessed to verify whether the proposed system can be scaled up using other effluents produced at LIPOR's facilities. The experimental work allowed to verify that *Chlorella vulgaris* can grow in leachate-based growth media, and more significantly in green waste leachate based media, the target effluent to be treated. The higher growth rate obtained ($34.9 \text{ mg}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$) matches with the assumptions made in the process design for the final concentration of biomass ($0.25 \text{ g}\cdot\text{L}^{-1}$). Regarding the removal of nitrates, phosphates, COD and BOD₅, there are evidence that the defined methodology was not adequate enough to properly evaluate it. Suggestions for the adjustment of the methodology for future works were proposed. Nevertheless, in some experiments there was partial removal of nitrates (6%) and phosphates (11-21%), indicating the potential of the process for effluent remediation.

Phycoremediation processes present great potential in dealing with wastewaters, however, in most cases, a comprehensive study should be produced to prove that it will work in a given place, with different native or chosen microalgae species, for a given effluent. This is mainly caused by the still lack of understanding of these complex biological systems, which are the microalgae production systems, especially when associated with other organisms, such as in high rate algal ponds, where microalgae-bacterial consortia are key to their functioning and success.

This study was the first contribution to the simultaneous remediation and valorization of leachates, in particular green waste leachate, at LIPOR.

7. Recommendations for future work

As a first suggestion for future work in the development of a microalgae-based remediation process for the green waste leachate produced at LIPOR's facilities, the scale-up from the lab-scale assays for a medium-scale pilot reactor is recommended. Although the work here presented has shown that microalgae, specifically *Chlorella vulgaris*, may have potential to deal with this effluent and reduce its nutrient concentrations, a proof-of-concept under outdoor conditions (i.e., under rapid and abrupt changes in temperature, high susceptibility for contamination with other species and pathogens, sunlight and natural exposure period) is required to implement the designed system. Moreover, different microalgae species should be assessed to check if there is not a better alternative to deal with the leachate. Local wild and native strains should be considered, as these are probably the best adapted microalgae for an open system under local meteorological conditions.

To increase microalgae productivity, the feasibility of external supply of CO₂ should be assessed. The use of an intern continuous source of exhaust gas, which is significantly richer in CO₂ than atmospheric air, such as emissions from plants of biogas production/burning or waste incineration, is probably the most economic option. At LIPOR's facilities there is already an electric generator working with biogas from the sanitary landfill, however, its operation is intermittent. If LIPOR's gaseous waste streams were used to increase phycoremediation efficiency, several advantages arise: a more treated effluent is obtained, more CO₂ is retained in the biomass that would otherwise be emitted to the atmosphere, and more biomass is produced, which can be further valued.

The harvesting step should also be tested: first on a lab-scale, to avoid unnecessary large costs, and then on a pilot-scale. In this work, a harvesting process was proposed according to the state-of-the-art; however, a proof-of-concept should be produced to verify if it is suitable for this project. The efficiency of harvesting methods is highly dependent on the microalgae species and process-specific conditions. This may indicate that methods other than autoflocculation may be more suitable and therefore must be implemented. Moreover, the possibility of using only centrifugation to separate biomass from the broth should be assessed. Since the volume per batch is relatively low (15 m³), a single step harvesting process may be enough to reach a good separation without compromise the economic viability of the project by an abrupt increase in energy consumption.

Before emitting the treated effluent, either directly or indirectly to the river (Rio Tinto), to the sewage or through the irrigation system, a more comprehensive composition analysis should be carried out to verify that all parameters are below the emission limit values established by the legislation in force. If the option to indirectly emit to the river through a network of small ponds is implemented, a monitoring process should be initiated to evaluate the impact on the local diversity of fauna and flora around the same network. Such a study should focus on particular sensitive organisms (e.g. sensitive to contaminants). Discharge permits must be obtained. These are issued by the Direção Regional do Ambiente, according to the Decreto-Lei n.º 236/98.

Finally, and regarding the experimental segment of this work, a new proof-of-concept at the same scale here presented is recommended but with some adjustments to the methodology followed. These include the daily monitoring of biomass and nutrient concentrations. Other suggested changes are the filtration of the formulated media and its sterilization, to ensure that only microalgae and dissolved nutrients influence the results.

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Annex

Table I.1 - Emission limit values in wastewater discharge (Decreto-Lei n.º 236/98).

Parameter	Units	Emission limit values ⁽¹⁾
pH	Sørensen scale	6.9-9.0 ⁽²⁾
Temperature	°C	3 °C increase ⁽³⁾
BOD ₅	mg/L O ₂	40
COD	mg/L O ₂	150
TSS	mg/L	60
Aluminium	mg/L Al	10
Total iron	mg/L Fe	2.0
Total manganese	mg/L Mn	2.0
Smell	-	Not detectable at 1:20 dilution
Colour	-	Not visible at 1:20 dilution
Free residual chlorine	mg/L Cl ₂	0.5
Total residual chlorine	mg/L Cl ₂	1.0
Phenols	mg/L C ₆ H ₅ OH	0.5
Oils and fat	mg/L	15
Sulphides	mg/L S	1.0
Sulphites	mg/L SO ₃	1.0
Sulphates	mg/L SO ₄	2000
		10
Total phosphor	mg/L P	3 in waters that feed ponds and dams 0.5 in ponds and dams
Ammoniacal nitrogen	mg/L NH ₄	10
Total nitrogen	mg/L N	15
Nitrates	mg/L NO ₃	50
Aldehydes	mg/L	1.0
Total arsenic	mg/L As	1.0
Total lead	mg/L Pb	1.0
Total cadmium	mg/L Cd	0.2
Total chromium	mg/L Cr	2.0
Hexavalent chromium	mg/L Cr (VI)	0.1
Total copper	mg/L Cu	1.0
Total nickel	mg/L Ni	2.0
Total mercury	mg/L Hg	0.05
Total cyanides	mg/L CN	0.5
Mineral oils	mg/L	15
Detergents (sodium lauryl sulphate)	mg/L	2.0 ⁽⁴⁾⁽⁵⁾

(1) Emission limit value, understood as the monthly average, defined as the arithmetic average of the daily averages referring to the working days of a month, which must not be exceeded. The daily value, determined on the basis of a representative sample of wastewater discharged over a twenty-four-hour period, may not exceed the twice the monthly average value (the sample in a period of twenty-four hours must be composed taking into account the discharge regime of the wastewater produced).

(2) The average daily value may, at most, be in the range 5.0-10.0.

(3) Temperature of the receiving medium after the discharge of wastewater, measured 30 m downstream from the point of discharge, the average value being able to exceed the monthly average value of the second.

(4) The average daily amount may not exceed twice the average monthly amount.

(5) Value related to the discharge of the industrial unit for the production of HCH, extraction of lindane or, simultaneously, production of HCH and extraction of lindane.

Table I.2 - Quality of water intended for irrigation (Decreto-Lei n.º 236/98).

Parameter	Units	MRV	MAV
Aluminium (Al)	mg/L	5.0	20
Arsenic (As)	mg/L	0.10	10
Barium (Ba)	mg/L	1.0	
Beryllium (Be)	mg/L	0.5	1.0
Boron (B)	mg/L	0.3	3.75
Cadmium (Cd)	mg/L	0.01	0.05
Lead (Pb)	mg/L	5.0	20
Chlorides (Cl)	mg/L	70	-
Cobalt (Co)	mg/L	0.05	10
Copper (Cu)	mg/L	0.20	5.0
Total chromium (Cr)	mg/L	0.10	20
Tin (Sn)	mg/L	2.0	
Iron (Fe)	mg/L	5.0	
Fluorine (F)	mg/L	1.0	15
Lithium (Li)	mg/L	2.5	5.8
Manganese (Mn)	mg/L	0.20	10
Molybdenum (Mo)	mg/L	0.005	0.05
Nickel (Ni)	mg/L	0.5	2.0
Nitrates (NO ₃)	mg/L	50	
Electrical conductivity	dS/m	1	
Total dissolved solids	mg/L	640	
SAR ⁽¹⁾		8	
Selenium (Se)	mg/L	0.02	0.05
TSS	mg/L	60	
Sulphates (SO ₄)	mg/L	575	
Vanadium (Va)	mg/L	0.10	1.0
Zinc (Zn)	mg/L	2.0	10.0
pH	Sørensen scale	6.5-8.4	4.5-9.0
Faecal coliforms	/100 mL	100	
Intestinal parasite eggs	N/L		1

(1) The sodium adsorption ratio (SAR) is translated by the following equation, where the concentrations must be expressed in meq/l: $SAR = Na / [(Ca + Mg) / 2]^{1/2}$.

MRV - Maximum recommended value; MVA - Maximum value allowed.