# Life Cycle Assessment of microalgae systems for wastewater treatment and bioproducts recovery: natural pigments, biofertilizer and biogas

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## Abstract

The aim of this study was to assess the potential environmental impacts associated with microalgae systems for wastewater treatment and bioproducts recovery. In this sense, a Life Cycle Assessment was carried out evaluating two systems treating i) urban wastewater and ii) industrial wastewater (from a food industry), with the recovery of bioproducts (i.e. natural pigments and biofertilizer) and bioenergy (i.e. biogas). Additionally, both alternatives were compared to iii) a conventional system using a standard growth medium for microalgae cultivation in order to show the potential benefits of using wastewater compared to typical cultivation approaches. The results indicated that the system treating industrial wastewater with unialgal culture had lower environmental impacts than the system treating urban wastewater with mixed cultures. Bioproducts recovery from microalgae wastewater treatment systems can reduce the environmental impacts up to 5 times compared to a conventional system using a standard growth medium. This was mainly due to the lower chemicals consumption for microalgae cultivation. Food-industry effluent showed to be the most promising scenario for bioproducts recovery from microalgae treating wastewater, because of its better quality compared to urban wastewater which also allows the cultivation of a single microalgae species. In conclusion, microalgae wastewater treatment systems are a promising solution not only for wastewater treatment but also to boost the circular bioeconomy in the water sector through microalgae-based product recovery.

**Keywords**: Bioproducts; Environmental impact assessment; Life Cycle Assessment; Microalgae; Wastewater

# 1. Introduction

Microalgae have shown a great potential for the production of several bioproducts with a wide variety of applications such as biofuels and chemicals as well as food and feed (Christaki et al., 2015; Michalak and Chojnacka, 2015; Nagappan et al., 2021; Spolaore et al., 2006). The advantages of using microalgae are their high productivity, the possibility to grow on marginal land in fresh or saltwater, which can avoid competition with food crops, and the option of combining biomass growth with the treatment of waste streams (Clarens et al., 2011; Ahmad et al., 2022).

Natural pigments from microalgae, which are particularly strong dyes even at very low concentrations (parts per million), are now strongly demanded by the market as renewable natural colour enhancers for foods and feeds, which simultaneously provide certain health benefits (Christaki et al., 2015; Villaró et al., 2021). Among the pigments present in microalgae cells, the phycobiliproteins have important applications in the pharmaceutical, food and cosmetic industry due to their fluorescence properties (Cuellar-Bermudez et al., 2015; Qiang et al, 2021; Dagnino-Leone et al., 2022). Phycobiliproteins have been extracted and purified from several microalgae species, but commercial production is mainly from *Arthrospira* spp. (Spirulina) for phycocyanin, and *Porphyridium* spp. for phycoerythrin (Borowitzka, 2013; Christaki et al., 2015; Bayu et al., 2022). In particular, *A. platensis* (Spirulina) is widely chosen as a host for phycocyanin production merely because of its availability and favourable growing conditions rather than due to the particular qualities of its pigments (Eriksen, 2008; Chaiklahan et al., 2022). *A. platensis* (Spirulina) tolerates alkaline conditions and is grown at pH values up to 10.5, being among the few photoautotrophic microorganisms able to grow in open ponds without high risks of being out-competed by contaminating organisms (Richmond and Grobbelaar, 1986; Mona et al., 2021).

Both microalgae biomass and residual biomass generated from the production of natural pigments can be used for biogas and biofertilizer production (Solé-Bundó et al., 2017; Ramos-Suárez et al., 2020; Ammar et al., 2022). On the one hand, the high potential of microalgae to produce biofuels, such as biogas, has been intensively researched in the last decades (Iyovo et al., 2010; Arias et al., 2018; Solé-Bundó et al., 2019). In comparison to other biofuels like bioethanol or biodiesel, the process used to obtain biogas does not require complex extraction methods (Solé-Bundó et al., 2019). On the other hand, biofertilizers are obtained from the solid phase of the digestate and represent a more environmentally friendly alternative to synthetic fertilizers (Albuquerque et al., 2012).

Although the demand for natural pigments is increasing and microalgae are considered a potential candidate for natural pigments (e.g. phycobiliproteins) production, the requirement of huge quantities of water and chemicals (i.e. nutrients) in large scale systems leads to high costs and further hinders the production and commercialisation of these bioproducts. Microalgae cultivation using wastewater and/or recycled water has been recently explored as a potential solution to produce natural pigments (Acién et al., 2016; Delrue et al., 2016; Ho et al., 2018; K. Li et al., 2019; Jiang et al., 2022). In addition to this, the potential of applying anaerobic digestion at the end of the process to recover bioenergy from the residual biomass is interesting in environmental and economic terms (Ramos-Suárez et al., 2020).

Even though previous studies have shown the technical feasibility of such processes, little is still known regarding the environmental implications of using recycled water for natural pigment, biogas and biofertilizer recovery from microalgae. Evaluating the environmental performance of these processes is particularly important to support informed decision-making processes, as well as for identifying the main bottlenecks to be addressed during the scale-up towards sustainable industrial facilities (Pérez-López et al., 2017). It needs to be mentioned that other authors have carried out environmental performance analyses of microalgae cultivation using wastewater (Arashiro et al., 2018) and natural pigments recovered from microalgae systems with standard growth media (Papadaki et al., 2017). However, to the best of the authors' knowledge, there is still no study that analyses the environmental impacts of microalgae-based systems and bioproducts recovery using wastewater and including natural pigment recovery.

In light of the above, it is essential to better understand which are the environmental impacts of bioproducts recovery from microalgae-based systems treating wastewater and using standard grown medium. To this aim, this paper provides, for the first time, a comparative Life Cycle Assessment (LCA) of two microalgae-based systems for wastewater treatment and bioproducts recovery (i.e. natural pigments, biofertilizer and biogas): i) a high rate algal ponds (HRAPs) system treating urban wastewater followed by closed photobioreactors (PBRs) cultivating a mixed culture dominated by cyanobacteria; and ii) an up-flow anaerobic sludge blanket (UASB) reactor treating food-industry wastewater followed by HRAPs cultivating *A. platensis* (Spirulina). Moreover, both scenarios were compared to a conventional system (HRAPs) for bioproducts recovery using a standard growth medium. The main environmental burdens of each option were evaluated to compare their performances and to identify bottlenecks for up-scaling.

## 2. Materials and methods

### 2.1 Wastewater treatment systems description

The studied systems were hypothetical full-scale wastewater treatment plants based on extrapolation from pilot-scale studies (from 5 up to 600 m$^2$). The systems were designed to serve a population equivalent of 10,000 p.e. and treat a flow rate of 1,500 m$^3$/d. For the microalgae-based system treating urban wastewater (hereafter referred to as scenario UWW), the design parameters were based on experimental results obtained in lab-scale and pilot systems (5 $m^2$) located at the Universitat Politècnica de Catalunya-BarcelonaTech (UPC) (Barcelona, Spain) (García et al., 2006, 2000; Gutiérrez et al., 2016; Passos and Ferrer, 2014; Solé-Bundó et al., 2019, 2017). This scenario is a combination of HRAPs for urban wastewater treatment and PBRs for cyanobacteria biomass cultivation based on the system previously described in Arashiro et al. (2020b). The flow diagram of this case study is shown in Figure 1 (1) and the characteristics and design parameters are listed in Table 1. Firstly, it comprises a primary settler (hydraulic retention time (HRT)=2.5 h) followed by four HRAPs working in parallel, cultivating a mixed culture of green microalgae. From these units, wastewater goes through a secondary settler (HRT=3 h) where microalgal biomass is harvested and separated from wastewater. Part of the harvested microalgal biomass (2 and 10% on a dry weight basis in summer and winter, respectively) is recycled in order to enhance spontaneous flocculation (bioflocculation) and increase microalgae harvesting efficiency (Gutiérrez et al., 2016). The remaining harvested biomass is thickened (HRT=24 h) and co-digested with primary sludge (35 °C, 20 days). In this context, the HRT of each HRAP has to be modified over the year (8, 6 and 4 days) according to weather conditions (i.e. solar radiation and temperature) in order to accomplish wastewater treatment and meet effluent quality requirements for discharge (Arashiro et al., 2018; García et al., 2000; Gutiérrez et al., 2016). For this reason, it was considered that during the summer months (from May to July) only two HRAPs work in parallel (HRT=4 days), whereas all of them are operated during winter months (from November to April) (HRT=8 days). During the rest of the year (from August to October), the HRT is 6 days (3 HRAPs working in parallel). Secondly, the cultivation of cyanobacteria-dominated biomass is done in hybrid tubular PBRs, which are tubular horizontal semi-closed reactors, each one consisting of 2 lateral open tanks made from polypropylene connected through 16 low-density polyethylene tubes (García et al.. 2018). In this study, the design of the PBRs was based on a demo scale plant, which is described elsewhere (García et al., 2018; Uggetti et al., 2018). For that, most of the HRAP effluent is discharged into a surface water body, but part of it (6.5%) is used to support the cyanobacteria-dominated biomass growth. The secondary effluent is filtered (to avoid any possible grazer contamination) and used to dilute the centrate (the liquid part of digestate) from the microalgae anaerobic digestion unit. The effluent of the PBRs goes through a tertiary settler (HRT=3 h) where microalgal biomass is harvested and separated from wastewater that is discharged into a surface water body. The microalgae biomass is then centrifuged and the biomass paste is used for phycobiliproteins recovery, which is done through ultrasound extraction with phosphate buffer. The residual biomass (after extraction) is also used as a substrate for the anaerobic digester. The biogas produced is then converted into electricity and heat in a combined heat and power (CHP) unit, while the centrate is recirculated to the PBR and the solid part of the digestate is transported and reused in agriculture as biofertilizer.

For the microalgae-based system treating industrial wastewater (hereafter referred to as scenario IWW), the design parameters were based on data obtained by a company that produces plant-based food (located in Wevelgem, Belgium) and experimental results obtained in lab-scale systems at Ghent University (Kortrijk, Belgium) (Arashiro et al., 2020a). This scenario is a combination of a UASB reactor (2 $m^3$), to reduce the organic matter concentration of the wastewater, and HRAPs cultivating *A. platensis* (Spirulina). The flow diagram of this case study is shown in Figure 1(2) and the characteristics and design parameters are listed in Table 2. Firstly, the industrial wastewater goes through a drum sieve (0.5 mm) to remove the large particles. The wastewater is then treated in a UASB (HRT=30 h), from which the biogas produced is converted into electricity and heat through a CHP unit. The UASB effluent is filtered to remove suspended solids and the solids from both the UASB (digestate) and the filtration process (retained solids) are hypothetically transported and reused in agriculture as biofertilizer. After filtration, the wastewater is mixed with seawater to ensure enough salinity to cultivate Spirulina biomass in the HRAPs. The portion of the seawater was estimated in order to reach a similar concentration as the study described in (Arashiro et al., 2020a) (75% wastewater and 25% seawater, v/v). In this scenario, the HRT of each HRAP was also modified over the year (8, 6 and 4 days) assuming similar weather conditions to the first scenario (scenario UWW). The effluent from HRAPs goes through a secondary settler (HRT=3 h) where microalgal biomass is harvested and separated from the treated water. The microalgae biomass is then centrifuged and the biomass paste is used for natural pigments recovery, which is done through ultrasound extraction with phosphate buffer (Arashiro et al., 2020). The residual biomass (after extraction) is also used as a substrate for the UASB.

For reference purposes, the potential environmental impacts of the microalgae-based wastewater treatment systems were compared to those generated by a conventional microalgae cultivation system. For that purpose, the design of a typical facility for natural pigments production from *A. platensis* (Spirulina) using a standard growth medium (SGM) was considered, as described by Papadaki et al. (2017). The flow diagram of this study case (hereafter referred to as scenario SGM) is shown in Figure 1(3) and the characteristics and design parameters are listed in Table 3. It comprises HRAP systems to cultivate microalgae, followed by a centrifuge to recover the biomass paste, which is further used for the natural pigments recovery. As in the previous scenarios, an anaerobic digester is also considered to generate biogas (later converted into electricity and heat in a CHP unit) and the digestate is transported and reused in agriculture.

## 2.2 Life cycle assessment

The LCA was conducted following the ISO standards (ISO, 2006, 2000) in order to assess and quantify the potential environmental impacts of each scenario under study. The technical framework for LCA methodology consisted of four phases: 1) goal and scope definition; 2) inventory analysis; 3) impacts assessment; and 4) interpretation of the results (ISO, 2006). The following sub-sections describe the specific content of each phase.

## 2.3 Goal and scope definition

The goal of this study was to analyse and compare the potential environmental impacts associated with different microalgae-based systems for wastewater treatment and bioproducts (i.e. natural pigments, biogas and biofertilizer) recovery and to identify the vulnerable aspects in which the technologies studied can potentially improve in terms of environmental performance. To this aim, two configurations were compared:

a) an urban wastewater treatment system based on HRAPs followed by PBRs cultivating cyanobacteria-dominated biomass (scenario UWW);

b) an industrial wastewater (from a food company) treatment system based on a UASB reactor followed by HRAPs cultivating *A. platensis* (Spirulina) (scenario IWW).

The functional unit (FU) for this comparison was set as 1 m$^3$ of treated water since the main function of the technologies proposed is to treat wastewater (Arashiro et al., 2018). Additionally, both scenarios were compared to c) a conventional microalgae cultivation system using a standard growth medium (scenario SGM) in order to show their benefits compared to conventional cultivation systems. For the comparison of the three scenarios, the FU of 1 kg of microalgal biomass produced (i.e. kilogram of Total Suspended Solids (kgTSS)) was used (Pérez-López, 2017).

For this LCA study, cradle-to-grave boundaries comprised systems construction, operation and maintenance over a 20 years period (Bhatt et al., 2022; Garfí et al., 2017; Pérez-López et al., 2017; Rahman et al., 2016) (Figure 1). Input and output flow of materials (i.e. construction materials and chemicals) and energy resources (heat and electricity) were studied in detail for all scenarios. Direct greenhouse gas (GHG) emissions and $NH_3$ volatilization associated with wastewater treatment were also included in the boundaries. As treated water is discharged into the environment, direct emissions to water were also taken into account. The transportation (20 km) (Hospido et al., 2004), as well as direct emissions to soil (heavy metals) and direct GHG emissions, were accounted for digestate reuse in agriculture (as biofertilizer). The end-of-life of infrastructures and equipment were neglected since the impact would be marginal compared to the overall impact.

All the investigated scenarios generate bioproducts (i.e. natural pigments, biogas and biofertilizer), thus the system expansion method has been used following the ISO guidelines to consider the avoided production of conventional products (Guinée, 2002; ISO, 2006). This way, the avoided impact related to conventional products offsets the overall impact of the system (Collet et al., 2011; ISO, 2006; Sfez et al., 2015). Indeed, the digestate produced in anaerobic digesters were considered a substitute for chemical fertilizer (Coppens et al., 2016; Garcia-Gonzalez and Sommerfeld, 2016; Lamolinara et al., 2022; Solé-Bundó et al., 2017) and the natural pigments produced were considered a substitute for conventional pigments. Biogas cogeneration was also considered, with avoided burdens of using heat and electricity, instead of heat from natural gas and electricity supplied through the grid.

## 2.4 Inventory analysis

Inventory data for the investigated scenarios are summarized in Table 4, Table 5 and Table 6. The data regarding construction materials and operation for the urban wastewater and industrial wastewater treatment scenarios (scenarios UWW and IWW, respectively) were based on the detailed engineering designs performed in the frame of this study. Treated wastewater characteristics were estimated considering the removal efficiencies and experimental results obtained in previous studies.

As mentioned above, for the urban wastewater treatment scenario (scenario UWW), data for HRAPs were based on the pilot systems implemented at the Universitat Politècnica de Catalunya-BarcelonaTech (UPC) (5 $m^2$) (Gutiérrez et al., 2016) and data for the PBRs based on the demo scale system located at the Agròpolis experimental campus of UPC (600 $m^2$) (García et al., 2018; Uggetti et al., 2018). Biomass productivities in PBRs were estimated based on the biomass produced per nutrients removed observed by García et al. (2018) in those PBRs, but considering the influent of this study (secondary effluent and centrate, as shown in Arashiro et al. (2020b)). Heavy metals and nutrients (avoided nitrogen and phosphorus) content of the microalgae digestate was based on experimental results obtained in previous studies (Solé-Bundó et al., 2017). The natural pigments (phycobiliproteins) yields used in this scenario were also based on what was measured in the cyanobacteria-dominated biomass grown in secondary effluent and centrate described in Arashiro et al. (2020b).

For the industrial wastewater treatment scenario (scenario IWW), data for UASB were based on information provided by a company producing plant-based food (Wevelgem, Belgium), combined with operational aspects (e.g. sludge production) from the literature (Chang et al., 2008; Porwal et al., 2015; Yu, 2015). Data for HRAPs were based on the lab-scale systems operated at Ghent University (Kortrijk, Belgium), as described in Arashiro et al.

(2020a). Heavy metals and nutrients (avoided nitrogen and phosphorus) content of the digestate from the UASB were based on food digestate from the literature (Rigby and Smith, 2011). The natural pigments yields used in this scenario were based on the *A. platensis* (Spirulina) biomass grown in food-industry wastewater (A-75%WW) described in Arashiro et al. (2020a).

For the conventional microalgae cultivation system using a standard growth medium (scenario SGM), as mentioned above, data regarding the production of conventional pigments were gathered from the literature (Campbell et al., 2011; Collet et al., 2011; Papadaki et al., 2017). This scenario was included as a reference rather than for purposes of absolute comparison.

The data for the natural pigments extraction step in all scenarios were based on the detailed study carried out by Papadaki et al. (2017), considering the extraction of the wet paste with phosphate buffer (pH 7) using ultrasound. Energy and solvent needed for the extraction were considered, but construction materials were neglected, since no substantial data was found and because the impact would be minimal compared to the overall impact of operation, considering the 20 years period of this study. $NH_3$ volatilization in all scenarios was estimated through nitrogen mass balance. $NH_3$ and $N_2O$ emissions due to the application of digestate on agricultural land were calculated using emissions factors from the literature (Hospido et al., 2008; IPCC, 2006; Lundin et al., 2000). In this study, $CH_4$ emissions were not considered since anaerobic decompositions do not occur if liquid fertilizer is used and the climate is predominantly dry (IPCC, 2000; Lundin et al., 2000). In order to estimate electricity and heat production from biogas cogeneration in all scenarios, biogas production obtained in lab-scale experiments from previous studies was considered for mono and co-digestion (Passos et al., 2017; Solé-Bundó et al., 2019), and results presented in Arashiro et al. (2020b) were considered for biogas production from microalgae residual biomass.

Background data (i.e. data on construction materials, chemicals, energy production, avoided pigments, transportation and compost process) were obtained from the *Ecoinvent 3.7* database (Moreno Ruiz et al., 2020; Weidema et al., 2013).

## 2.5 Impact assessment

The environmental impacts associated with wastewater treatment systems coupled with microalgae-based products recovery under study were quantified using the software *SimaPro*$^{®}$ *9* ("PRé Sustainability," 2014). Potential environmental impacts were calculated according to the ReCiPe 2016 midpoint method (hierarchist approach) (Huijbregts et al., 2017). The selected method includes a series of impact categories, and the characterisation phase in this study was performed considering the following ones: Global Warming, Stratospheric Ozone Depletion, Terrestrial Acidification, Marine Eutrophication, Freshwater Eutrophication, Terrestrial Ecotoxicity, Human carcinogenic Toxicity, Mineral Resource Scarcity, Fossil Resource Scarcity and Fine Particulate Matter Formation. These impact categories were selected according to the most relevant environmental issues related to wastewater treatment and have been previously used for the evaluation of wastewater treatment and resources recovery (Corominas et al., 2013; Fang et al., 2016; Gallego et al., 2008; Garfí et al., 2017; Hospido et al., 2008). Normalisation was carried out in order to compare all the environmental impacts at the same scale. This provides information on the relative significance of the indicator results, allowing a fair comparison between the impacts estimated for each scenario (ISO, 2006). In this study, the European normalisation factors have been used (Europe ReCiPe H) (Huijbregts et al., 2017).

## 3. Results and discussion

### 3.1 Characterisation

The potential environmental impacts associated with the system treating urban wastewater (scenario UWW) and the system treating industrial wastewater (scenario IWW) are shown in Figure 2. On the other hand, Figure 3 shows the comparison between the aforementioned systems (scenarios UWW and IWW) and the conventional one using standard growth medium (scenario SGM).

Comparing the microalgae-based wastewater treatment systems proposed, the results indicated that the scenario treating urban wastewater (scenario UWW) had higher environmental impacts (from 1.2-fold to 2.4-fold) than the scenario treating industrial wastewater (scenario IWW) in 8 out of 10 impact categories (i.e. Global Warming, Stratospheric Ozone Depletion, Terrestrial Acidification, Freshwater Eutrophication, Terrestrial Ecotoxicity, Human carcinogenic Toxicity, Fossil Resource Scarcity and Fine Particulate Matter Formation).

For Global Warming, Stratospheric Ozone Depletion, Human carcinogenic Toxicity and Fossil Resource Scarcity impact categories, the main reasons for these results were the benefits generated from biogas cogeneration (Figure 2), which are higher in the latter (scenario IWW) compared to the former (scenario UWW). Indeed, the electricity and heat generated from biogas cogeneration in the scenario treating industrial wastewater (scenario IWW) were more than 3 times higher than in the scenario treating urban wastewater (scenario UWW), due to the much higher organic matter concentration in the industrial wastewater (2250 mg $O_2$/L) than in the urban wastewater (300 mg $O_2$/L), which would be further converted into biogas (Table 4 and Table 5). Hence, the electricity produced from biogas was equivalent to approximately

34% of the electricity consumption of the scenario treating industrial wastewater (scenario IWW), while only 11% for the scenario treating urban wastewater (scenario UWW).

Regarding Terrestrial Acidification and Fine Particulate Matter Formation impact categories, not only the offset by the biogas cogeneration favoured the scenario treating industrial wastewater (scenario IWW), but also the higher impact caused by the emissions to air (from $NH_3$ volatilisation) in the scenario treating urban wastewater (scenario UWW). Indeed, the average nitrogen emission from the HRAPs treating urban wastewater (scenario UWW) was higher than in the scenario treating industrial wastewater (scenario IWW), with 5.495 g N/m$^3$ of water against 1.080 g N/m$^3$ of water, respectively (Table 4 and Table 5). This was most probably related to the distinct inorganic nitrogen forms in both wastewaters. The major nitrogen form in the urban wastewater was ammonium, while in the industrial wastewater it was nitrate. The higher concentrations of ammonium caused higher ammonia volatilisation rates, as also suggested in previous studies (Alcántara et al., 2015; Jones, 2010; Li et al., 2022; Plouviez et al., 2019), leading to higher emissions to air observed in the scenario treating urban wastewater (scenario UWW) (Figure 2).

Concerning the Terrestrial Ecotoxicity impact category, a major contributor to the higher impacts in the scenario treating urban wastewater (scenario UWW) was the higher concentrations of heavy metals in the microalgae digestate than in the food-derived digestate (Table 4 and Table 5), as in accordance with Pismenskaya et al., (2022). Nevertheless, the heavy metals concentrations in all the microalgae digestates considered in this study were lower than the threshold established by the sludge European Directive 86/278/EEC (EEC, 1986) (Solé-Bundó et al., 2017).

For the Freshwater Eutrophication impact category, the scenario treating urban wastewater (scenario UWW) showed a higher environmental impact than the scenario treating industrial wastewater (scenario IWW), mostly as a result of emissions to water. This is explained by the higher total phosphorous concentrations in the effluent of HRAPs treating urban wastewater (scenario UWW), compared to industrial wastewater (scenario IWW) (Table 4 and Table 5). The difference between the effluent quality of the two systems is related not only to its source (one being urban and the other food wastewater) but also to the initial nutrients concentrations when they enter the systems (industrial wastewater with concentrations about 2-fold higher than urban wastewater, Table 1 and Table 2). Nevertheless, in any case, the effluent concentrations of phosphorus fulfill the discharge requirements set by the regulation.

The scenario treating urban wastewater (scenario UWW) shows better environmental performance than the scenario treating industrial wastewater (scenario IWW) in only 2 impact categories: Marine Eutrophication and Mineral Resource Scarcity. Regarding Marine Eutrophication, the latter (scenario IWW) showed significantly higher (by 3.74-fold) environmental impacts than the former (scenario UWW). This can be explained by the treated final effluent quality, in which the industrial wastewater had a higher total nitrogen concentration in the effluent (scenario IWW) (around 10 mg N/L) compared to the urban wastewater scenario (scenario UWW) (around 0.9) (Table 4 and Table 5). Concerning the Mineral Resource Scarcity impact category, the scenario treating industrial wastewater (scenario IWW) showed a slightly higher impact (only by 1.2-fold) than the scenario treating urban wastewater (scenario UWW), mostly due to the amount and type of construction materials required for the HRAPs. Indeed, the amount of steel needed in the first case (scenario IWW) is around 29% higher than the last (scenario UWW) (Table 4 and Table 5). This is related to the higher surface area of HRAPs estimated for this scenario (scenario IWW) (Table 1 and Table 2) since the industrial wastewater is mixed with seawater at a 75/25% (v/v%) ratio to ensure enough salinity level.

Electricity consumption was by far the most impacting aspect, in 5 impact categories (i.e. Global Warming, Stratospheric Ozone Depletion, Human carcinogenic Toxicity, Fine Particulate Matter Formation and Fossil Resource Scarcity), accounting for 43 to 81% of the overall impacts. Next, construction materials were the major contributor to the highest impacts (83 and 85%) in the Mineral Resource Scarcity impact category, but also a secondary contributor in 4 impact categories (i.e. Global Warming, Stratospheric Ozone Depletion, Human carcinogenic Toxicity, and Fossil Resource Scarcity), representing from 13 to 35% of the overall impacts. Subsequently, emissions to water through nutrients were the major contributor to the highest impacts in Freshwater Eutrophication potential (91% in scenario UWW and 84% in scenario IWW) and in Marine Eutrophication potential (72% in scenario UWW and 96% in scenario IWW). Emissions to the air of the scenario treating urban wastewater (scenario UWW) through $NH_3$ volatilisation from HRAPs were the main contributor to Terrestrial Acidification potential (accounting for 64% of the overall impacts) and secondary contributor to Fine Particulate Matter Formation and Marine Eutrophication potentials (39 and 18% of the overall impacts, respectively). Finally, digestate reuse in agriculture was the main contributor in the scenario treating urban wastewater (scenario UWW) for Terrestrial Ecotoxicity potential (accounting for 66% of the overall impacts, due to heavy metals concentrations) and a secondary contributor in the scenario treating industrial wastewater (scenario IWW) for Terrestrial Acidification (accounting for 24% of the overall impacts, due to nitrogen volatilisation).

Based on this, in order to improve the environmental performance of the microalgae-based systems studied, the following issues should still be studied: 1) increasing energy efficiency by optimising processes (e.g. natural pigments extraction, harvesting), maximising biogas production or integrating renewable sources to reduce impacts related to electricity consumption; 2) improving HRAP design to reduce construction materials consumption (e.g.

excavation instead of concrete structure); 3) improving nutrients removal efficiencies (e.g. installations in warmer regions); and 4) recovering heavy metals from digestate before application in agriculture.

The results shown in this study suggested the use of food-industry effluent (scenario IWW) as a more promising scenario for bioproducts recovery from microalgae treating wastewater mainly for the following reasons: 1) Cultivation system: several researchers have reported that HRAPs are more energetically self-sufficient and more environmentally sustainable than PBRs, especially in cases in which the heat and power requirement of the process can be provided by combusting the methane generated from the anaerobic digestion of the residual microalgae biomass (Moon, 2022; Stephenson et al., 2010; Shormeh Darko, 2022); 2) Microalgae biomass: to be deemed suitable for producing natural pigments commercially, microalgae strains have to meet various criteria, such as ease of culture, lack of toxicity, high nutritional value, and presence of digestible cell walls to make the pigments available (Christaki et al., 2015; Siddiki et al., 2022). Based on that, the most frequently used species are *Dunaliella salina*, *Haematococcus pluvialis*, *Chlorella* spp., *Muriellopsis* spp., *Scenedesmus* spp., *Arthrospira* spp. (Spirulina), and *Porphyridium* spp. (Borowitzka, 2013; Christaki et al., 2015; Eriksen, 2008; Ho et al., 2018; Patel et al., 2022; Spolaore et al., 2006). For this reason, cultivating a single species might be a better strategy than mixed cultures. This way, the cultivation parameters can be adjusted accordingly in order to maximise natural pigments recovery; 3) Risks of contamination and social acceptance: the application of the natural pigments recovered in the scenario treating urban wastewater (scenario UWW) is much more limited than in the scenario treating food-industry wastewater (scenario IWW). Indeed, urban wastewater usually contains a wider variety of contaminants (e.g. pathogens, heavy metals, micropollutants) than food-industry wastewater. Although the purity of the final product could be proved to be suitable according to the application of the natural pigments, the cultivation in urban wastewater could raise more concerns in terms of social acceptance and regulatory issues, which could hinder industrial-scale production. For this reason, the use of food-processing waste streams could be a more appropriate alternative for providing nutrients for microalgae biomass growth while ensuring no risks of contamination.

Comparing both scenarios with the conventional system for microalgae-based products production using a standard growth medium (scenario SGM), both systems investigated (scenarios UWW and IWW) showed better environmental performance. The scenario using standard growth medium (Scenario SGM) showed higher environmental impacts than the scenario treating urban wastewater (scenario UWW) in 6 out of 10 impact categories (from 1.7-fold to 3-fold higher) (i.e. Global Warming, Stratospheric Ozone Depletion, Terrestrial Ecotoxicity, Human carcinogenic Toxicity, Mineral Resource Scarcity and Fossil Resource Scarcity) (Figure 3). This was mainly due to the lower chemicals consumption for microalgae cultivation. On the other hand, it showed higher environmental impacts than the scenario treating industrial wastewater (scenario IWW) in 8 out of 10 impact categories (from 1.3-fold to 5.3-fold higher) (i.e. all impact categories except for Freshwater and Marine Eutrophication) (Figure 3). As expected, the main contributors to the higher impacts in the scenario using standard growth medium (scenario SGM) were electricity consumption and chemicals input, which represented from 82 to 99% of the overall impacts in all the impact categories evaluated. The only impact categories in which both wastewater systems (scenarios UWW and IWW) showed way higher environmental impacts than the scenario using standard growth medium (scenario SGM) were Marine Eutrophication (from 3.7 to 12-fold higher environmental impacts) and Freshwater Eutrophication (from 2 to 4-fold higher environmental impacts). Yet, it is important to note that these impacts were associated with the discharge of nutrients in the treated effluent, as previously explained. In the case of the scenario using a standard growth medium (scenario SGM), there were no discharges to water bodies, since the inventory was based on systems in which all nutrients are taken up by the microalgae by recycling the medium (Papadaki et al., 2017). However, the impacts related to nutrients discharge could be minimised in a full-scale plant, by optimising operational conditions, which could favour even more the use of wastewater for recovering bioproducts and bioenergy.

The results in this work are in accordance with previous research on microalgae and valuable compounds production. Ye et al. (2018) carried out a comparative LCA of industrial-scale production of Spirulina tablets and found out that the most impacting stage along the entire process was the cultivation, responsible for approximately 60% of the total impacts, followed by harvesting (1-20%) and tablets production (<10%). From the cultivation stage, the growth medium was the major contributor, accounting for 80% of the impacts due to the high nutrients needed for cultivation. In this context, extensive research has been done to identify the advantages and potential risks of either recycling growth medium or using waste streams in order to reduce costs and impacts of cultivation. However, the effects of recycling the medium reported in the literature are contradictory, with some studies revealing positive aspects of recycling (Ho et al., 2018; Y. Li et al., 2019; Wang et al., 2018) while others are highlighting inhibitory effects on biomass growth (Hadj-Romdhane et al., 2013; Loftus and Johnson, 2019). Therefore, the use of wastewater is a considerable option as it provides the necessary nutrients and environmental conditions required for the enhanced metabolite content of microalgae, while being a low-cost media and, thus, a better approach compared to the processing involved by using standard growth media (Alam and Wang, 2019; Cinq-Mars, 2022).

Finally, microalgae wastewater treatment systems are a promising solution not only for wastewater treatment but also for bioproducts recovery. Indeed, the use of wastewater can reduce the environmental impacts associated with the production of microalgae-based products (e.g. natural pigments and biofertilizer) and bioenergy (e.g. biogas), boosting the circular bioeconomy.

## 3.2 Normalisation

The normalised results showed that Freshwater Eutrophication, Marine Eutrophication, Terrestrial Acidification and Human carcinogenic Toxicity were the most significant impact categories for all the scenarios considered (Figure 4), which was in accordance with previous LCAs on wastewater treatment systems (Fang et al., 2016; Gallego et al., 2008; Hospido et al., 2004). The scenario treating industrial wastewater (scenario IWW) showed to be the solution with the lowest environmental impacts in 3 out of 4 of these impact categories (i.e. Freshwater Eutrophication, Terrestrial Acidification and Human carcinogenic Toxicity potentials). Regarding Marine Eutrophication, operational conditions could be addressed in order to optimise the nitrogen removal efficiency in such a system.

The results of the normalization confirmed that the scenario treating industrial wastewater (scenario IWW) is the solution for wastewater treatment and bioproducts recovery from microalgae with the lowest environmental impacts among the compared options.

## 4. Conclusions

The aim of this study was to compare the environmental impacts of two microalgae-based systems for wastewater treatment and bioproducts recovery (i.e. natural pigments, biofertilizer and biogas): i) a high rate algal ponds (HRAPs) system treating urban wastewater followed by closed photobioreactors (PBRs) cultivating a mixed culture dominated by cyanobacteria, and ii) an up-flow anaerobic sludge blanket (UASB) reactor treating food-industry wastewater followed by HRAPs cultivating *A. platensis* (Spirulina). For reference purposes, both scenarios were compared to a conventional system for microalgae cultivation using a standard growth medium.

Results indicated that the scenario treating industrial wastewater had lower environmental impacts than the scenario treating urban wastewater in 8 out of 10 impact categories (from 1.2-fold to 2.4-fold). This was mainly due to i) the higher amount of biogas generated from industrial wastewater which was converted into bioenergy (electricity and heat) compared to the urban wastewater; ii) the lower emissions to air (i.e. $NH_3$ volatilisation) associated with a lower ammonia concentration in industrial wastewater compared to urban wastewater; iii) the lower concentration of heavy metals in food-derived digestate compared to the urban one.

Comparing both scenarios with the conventional system for microalgae cultivation using a standard growth medium, both microalgae wastewater treatment systems investigated showed better environmental performance in most of the impact categories analysed (environmental impacts up to 5-fold lower). This was mainly due to the lower chemicals consumption for microalgae cultivation.

On the whole, food-industry effluent showed to be the most promising scenario for bioproducts recovery from microalgae treating wastewater mainly for the following reasons: i) microalgae cultivation in HRAP is more sustainable than PBRs (less energy and construction materials consumption); ii) cultivating a single species might be a better strategy than mixed cultures, since the cultivation parameters can be adjusted accordingly in order to maximise bioproducts recovery; iii) urban wastewater contains a wider variety of contaminants (e.g. pathogens, heavy metals, micropollutants) than food-industry wastewater which raise more concerns in terms of social acceptance and regulatory issues.

Finally, microalgae wastewater treatment systems are a promising solution not only for wastewater treatment but also for microalgae-based products recovery. Indeed, the use of wastewater can reduce the environmental impacts associated with the production of microalgae-based products (e.g. natural pigments and biofertilizer) and bioenergy (e.g. biogas) boosting the circular bioeconomy.

## Acknowledgments

This research was funded by the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie Grant Agreement No 676070 (SuPER-W). This communication reflects only the author's view and the Research Executive Agency of the EU is not responsible for any use that may be made of the information it contains. Authors acknowledge the AL4BIO project "RTI2018-099495-B-C21" (MCIU/AEI/FEDER, UE) and are grateful to the Government of Catalonia (Consolidated Research Group 2017 SGR 1029). Marianna Garfí is grateful to the MINECO (RYC-2016-20059).

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