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Life cycle assessment and economic analysis of bioplastics production from cyanobacteria

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A B S T R A C T

Cyanobacteria are interesting microorganisms to produce polyhydroxybutyrate (PHB), a biodegradable plastic similar to polypropylene. In this study, a Life Cycle Assessment and an economic analysis were carried out to assess the sustainability of the process. Different scenarios were analysed including different nutrient sources (i.e. wastewater and standard growth medium BG-11) and different PHB contents (from $15\%_{dcw}$ to $35\%_{dcw}$ and $50\%_{dcw}$). Regarding the environmental impacts, increasing the PHB content drastically reduced (between 67 and 75%) the environmental impacts of the process. Environmental impacts were mainly caused by construction materials and the use of chemicals (especially chloroform for PHB purification). The economic analysis showed that the minimum selling price was 135 € $kgPHB^{-1}$ (PHB content: $50\%_{dcw}$; productivity: 12.5 gPHB m^{-3} d^{-1}). A PHB productivity of 810 mg L^{-1} d^{-1} would be necessary to obtain a PHB selling price of 4 € $kgPHB^{-1}$ (i.e. PHB market price). To reach this productivity it would be necessary to improve the reactor design (lower Volume: Surface ratio) and modify the strains genetically.

1. Introduction

Polyhydroxybutyrate (PHB), is one of the most interesting bioplastics due to its biodegradability in many different environments (e.g. marine environments, freshwater, soil) and its similar properties to the ones of polypropylene [1,2]. PHB can be produced by many different prokaryotic microorganisms. Nevertheless, the most widespread PHB production is based on heterotrophic bacterial fermentation [3,4]. These microorganisms need organic carbon sources, which usually come from agri-food resources [3,4]. Another alternative is to produce PHB using cyanobacteria instead of heterotrophic microorganisms. Cyanobacteria, which are photosynthetic bacteria (commonly known as blue-green algae), have the advantage to synthetize PHB from CO_2 and sunlight. Moreover, compared with heterotrophic bacteria, they do not require oxygenation and do not need organic carbon sources. Additionally, in comparison to plants, cyanobacteria do not require arable land [3]. Therefore, they have been seen as a good candidate to achieve a more sustainable PHB production process. However, to the best of the authors' knowledge, there is still no study that analyses the environmental impacts of this process.

The production of PHB with cyanobacteria is usually done in two steps. First, cyanobacteria are grown using mainly N and P. Then, once they have grown, they are placed in a media without nutrients to stimulate the PHB production. In this context, the use of wastewater, instead of standard growth media, to cultivate cyanobacteria and produce PHB, has been seen as a good strategy to reduce production costs [5,6]. However, the use of wastewater to produce PHB is still a challenging process, mainly due to the difficulty to keep cyanobacteria dominant during long cultivation periods and non-sterile conditions [5].

Previous studies have used the Life Cycle Assessment (LCA) methodology to assess the environmental impacts of PHB production using heterotrophic microorganisms [7–10]. For instance, Walker S., et al. [7], analysed the environmental impacts of the production of different bioplastics and concluded that the PHB production using heterotrophic microorganisms consumes less energy than other bioplastics, it generates less eutrophication and climate change than polylactic acid and bio-

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polyethylene and performs similarly in terms of ozone depletion and terrestrial acidification than other polymers.

In light of the above, in this study, a full-scale plant (100 m^3) for the PHB production from cyanobacteria using vertical column closed photobioreactors has been designed based on biokinetic mathematical models and experimental results from the literature. The objective was to assess the potential environmental impacts of the process through the LCA methodology. The considered system consisted of 3 vertical column photobioreactors to grow cyanobacteria, and 97 accumulation reactors (same design as the growth reactors) to enhance PHB production. The growth reactors were continuously fed with a hydraulic retention time (HRT) of 6 days. This plant had a biomass productivity (based on model results) of 0.025 kg m^{-3} d^{-1}. Different scenarios were analysed, including different nutrient sources (i.e. wastewater and standard growth medium BG-11) and different PHB contents in the cyanobacteria biomass. Furthermore, an economic evaluation has been also carried out and the minimum productivity needed to achieve competitive costs has been calculated. Environmental impacts of the scenarios with improved productivity were also calculated.

2. Materials and methods

2.1. PHB production systems description

The cyanobacteria PHB production system was designed based on the extrapolation of lab-scale (up to 30 L) using *Synechocystis* sp. R2020 isolated from wastewater treatment systems as described in Rueda *et al.* (2020) [11]. Moreover, the mathematical model designed and calibrated for the aforementioned strain by Rueda and García, [12] was used to optimize the amount of nutrients needed and predict biomass productivity for this process. The system was considered to be placed outdoors in the South of Spain. It consisted of 3 main steps: (1) the cultivation and stimulation of PHB production, (2) biomass harvesting and (3) PHB extraction and purification (Fig. 1).

Cultivation was done in 100 vertical column photobioreactors of 1 m^3 each (total cultivation volume 100 m^3), made out of polymethacrylate reinforced with steel. Reactors had a diameter of 0.6 m and 4 m in height and a wall thickness of 1.6 cm. The reactor basement was made of 20 cm thick reinforced concrete (see appendix Fig. S1).

As mentioned above, the mathematical model described in Rueda and García, (2021) [12] was used to optimize the design of the system (e.g. choose the number of reactors in each stage, the amount of nutrients in the BG-11) and to calculate the biomass productivity. The PHB content considered in each scenario was based on experimental results found in the literature (see Section 2.2). Based on the optimal conditions obtained by the modelling results, the system contained two types of reactors: (1) growth reactors (3 units) and (2) accumulation reactors (97 units). The growth reactors were inoculated and fed continuously with a hydraulic retention time (HRT) of 6 days. In the scenarios using standard growth medium (BG-11), the influent content of nutrients (N, P) was 30 mgN L^{-1} and 6 mgP L^{-1}. These concentrations were optimized to maximize PHB productivity using the model described by Rueda and García, [12]. In the case of wastewater, it was hypothesized that it contained a similar amount of nutrients (e.g. secondary urban wastewater effluent) and that similar biomass productivities can be obtained.

The effluent of the 3 growth reactors was conveyed continuously to the 97 accumulation reactors, which were operated in batch to induce nutrients starvation and the subsequent accumulation of PHB [13–15]. The biomass productivity, predicted from the model, of the whole process (growth + accumulation), was 0.025 kg m^{-3} d^{-1}. Pure commercial CO_2 was bubbled in all the reactors on demand to maintain the pH between 8 and 9. The amount of CO_2 consumed by cyanobacteria was considered to be 1.88 kg $CO_2 \cdot kg_{dcw}^{-1}$. The CO_2 fixation efficiency (i.e. the amount of CO_2 uptaken by the cyanobacteria with respect to the total amount of CO_2 introduced in the reactor) was considered to be 80%.

The effluent from the accumulation reactors went to a centrifuge where the biomass was harvested. The exhausted BG-11 and the effluent wastewater were considered to contain negligible content of P or N.

In all the scenarios, a sequential process was designed to extract and purify the PHB based on a lab-scale procedure [16]. First, the biomass was mixed in a tank with NaClO (15%$_{v/v}$ in water) together with chloroform for 1 h at 38 °C to break the cells and dissolve the PHB. Then, the cell debris, the NaClO solution and the chloroform containing the PHB, were separated in a decanter centrifuge. The recovered chloroform phase, which contains the dissolved PHB, was concentrated in an evaporator and the chloroform was recirculated again to the mixing tank and reused in the next PHB extraction. Then the PHB was precipitated by mixing the chloroform phase with ice-cold methanol. The precipitated PHB was recovered by centrifugation and cleaned with water. Finally, the purified PHB was dried with a compressed air drier. More information on the PHB production plant design can be found in the supplementary materials (Fig. S1 and Table S1).

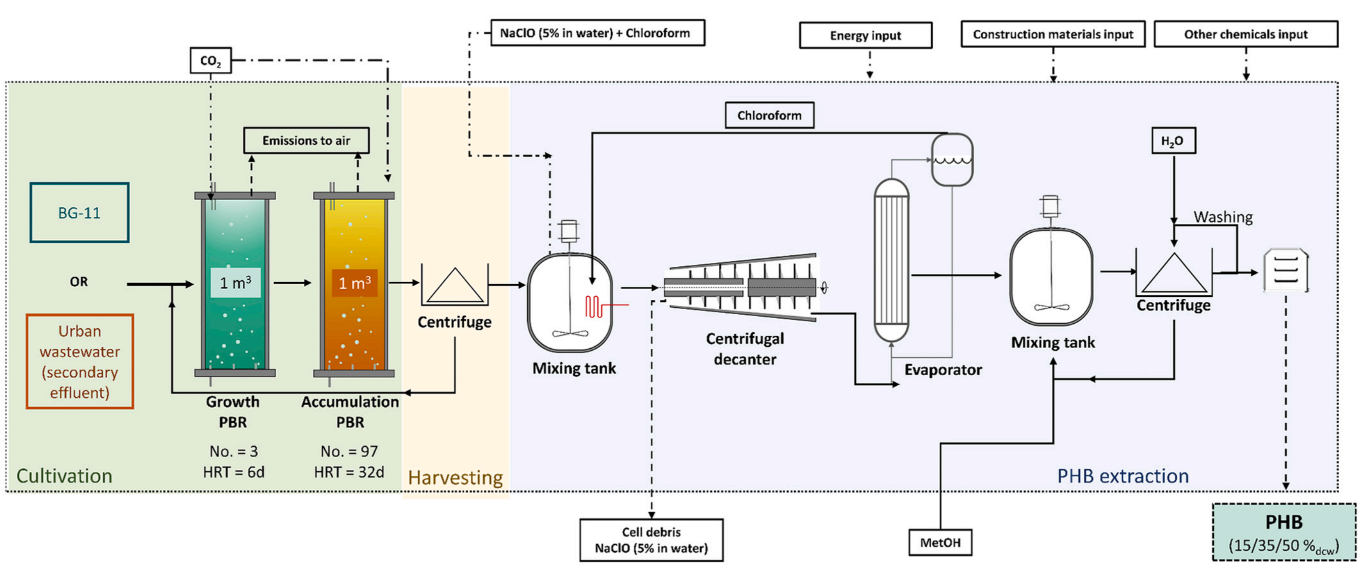

Fig. 1. Flow diagram of the cyanobacteria PHB production process considered in this study. *Abbreviations: BG-11: cultivation media for blue-green algae, PBR: photobioreactor; HRT: Hydraulic Retention Time; DCW: dry cell weight, No. is the number of reactors for each phase.*

2.2. Life cycle assessment

In this study, the LCA methodology was used to assess the potential environmental impact of PHB production from cyanobacteria. Throughout the following sections, the contents of the LCA phases (i.e. goal and scope definition, inventory analysis and impact assessment) are described in [17,18].

2.2.1. Goal and scope

The goal of the LCA was to assess the potential environmental impacts of PHB production from cyanobacteria. Different plausible PHB contents in the cyanobacteria biomass were evaluated, which are 15%$_{dcw}$, 35%$_{dcw}$ and 50%$_{dcw}$. For each content, the effect of using the standard growth medium BG-11 or wastewater as a source of nutrients was evaluated. These values were assumed based on previous experimental studies. For instance, Kamravamanesh, et al., and Rueda, et al., [19,20] obtained a PHB content of 16.4%$_{dcw}$ and 14%$_{dcw}$ respectively in lab-scale photobioreactors (1–3 L working volume), using a wild-type *Synechocystis* sp. and inorganic carbon as carbon source. Gracioso, et al., [21], reached a PHB content of 31.5%$_{dcw}$ in 250 mL photobioreactors with *Synechocystis* sp. by adding acetate. Koch, et al., [22] used genetically modified *Synechocystis* sp. PCC 6803 and obtained a PHB content of up to 63%$_{dcw}$ autotrophically. Similar results were observed when wastewater was used as cultivation media. Da Silva, et al., [23] obtained a PHB content of 10.6%$_{dcw}$ using *Spirulina* sp. LEB 18 cultivated in 1.5 L reactors using wastewater from PHB extraction. Krasaesueb, et al., [24] reached a PHB content up to 32.5%$_{dcw}$ with a genetically modified *Synechocystis* sp. PCC 6803 ΔSphU and shrimp aquaculture wastewater as a nutrient source in a 15 L photobioreactor. Bhati, et al., [25] obtained a PHB content of 65%$_{dcw}$ with *Nostoc muscorum* and poultry litter as a nutrient source in a 4 L reactor.

Additionally, the environmental impact of the process in the hypothetical case that a competitive PHB price (PHB market price of 4–15 $ kg^{-1} [26]) is achieved by increasing the PHB productivity was also evaluated. Thus, two additional hypothetical scenarios (called Improved Productivity scenarios) were included to estimate the environmental performance of a more economically competitive process.

In light of the above, the scenarios considered were as follows:

1) BG-11-15%: PHB production from cyanobacteria grown in standard growth medium (BG-11) with a PHB content in the biomass of 15%$_{dcw.}$
2) BG-11–35%: PHB production from cyanobacteria grown in standard growth medium (BG-11) with a PHB content in the biomass of 35%$_{dcw.}$
3) BG-11-50%: PHB production from cyanobacteria grown in standard growth medium (BG-11) with a PHB content in the biomass of 50%$_{dcw.}$
4) WW-15%: PHB production from cyanobacteria grown in treated urban wastewater with a PHB content in the biomass of 15%$_{dcw.}$
5) WW-35%: PHB production from cyanobacteria grown in treated urban wastewater with a PHB content in the biomass of 35%$_{dcw.}$
6) WW-50%: PHB production from cyanobacteria grown in treated urban wastewater with a PHB content in the biomass of 50%$_{dcw.}$
7) BG-11-Improved Productivity: PHB production from cyanobacteria grown in standard growth medium (BG-11) with the maximum PHB content obtained for cyanobacteria in literature with a genetically modified strain and photoheterotrophic growth conditions (81%$_{dcw}$) [22]. Biomass productivity is that one needed to have a Break-even price (based on a zero 20-years net present value) of 4 € kg $^{-1}$ (PHB market price) [6].
8) WW-Improved Productivity: PHB production from cyanobacteria grown in urban wastewater with the PHB content and productivity from scenario 7.

The functional unit used was 1 kg of PHB produced, since the main goal of the designed system was to produce PHB. The system boundaries (Fig. 2A) included the construction of the system and the operation and maintenance over 20 years. Direct emissions to air (CO_2 not consumed by the cyanobacteria, and NH_3 volatilized from wastewater) and to water were also considered. The cleaning operations required to maintain the plant operating were also taken into account. The end of life of the infrastructure and equipment were neglected as it is considered that the impact will be marginal in comparison to the overall impact [27,28].

2.2.2. Inventory analysis

Table 1 shows the summary of the inventory for the investigated scenarios. Data on construction materials and operation were taken from the engineering designs specified in Table S1 and from the manufacturer specifications provided by local suppliers.

The energy required for the PHB production process (heating, cooling, mixing, pumping etc.) was considered to be electrical energy (electricity generation mix in Spain [29]). The energy consumption of the equipment was estimated according to manufacturer specifications (Table 1).

In the scenarios using standard growth medium, the needed BG-11 used during the cultivation was prepared by mixing deionized freshwater with the chemicals of the BG-11 (the amount of N and P needed was obtained from the mathematical model) (Table 1). In all the scenarios, the amount of chemicals needed for the PHB extraction and purification was estimated considering results from lab-scale experiments (Table 1) [16]. It was also considered that the chemicals used for PHB extraction (chloroform and methanol) were reused in the process. It should be noted that there is no data available considering how many times the solvents can be reused. Therefore, it was assumed that solvents are replaced once per year. Moreover, 10% of solvent losses during the PHB extraction process were also assumed.

Regarding the emissions to air during the cultivation, it was considered that 20% of the CO_2 injected was lost and released into the atmosphere in all scenarios. The amount of CO_2 consumed by the culture was 1.8 kg of CO_2 per kg of dry biomass [30]. In the scenarios using wastewater, NH_3 volatilization was estimated through nitrogen mass balances [27]. In all the scenarios, emissions to water were considered to be the wastewater generated during the cleaning and PHB purification processes.

The background data (i.e. materials used for construction, chemicals production, energy production) were obtained from Ecoinvent 3.8 database [31].

2.2.3. Impact assessment

The potential environmental impacts generated by the PHB production from cyanobacteria were calculated using the SimaPro® software [30]. The ReCiPe 2016 Midpoint (H) methodology was used [32]. In this study, characterization phase considered the following impact categories: Global warming, Stratospheric Ozone Depletion, Fine Particulate Matter Formation, Terrestrial Acidification, Freshwater Eutrophication, Marine Eutrophication, Mineral resource scarcity and Fossil resource scarcity. These impact categories were selected as they have been commonly considered in previous evaluations of PHB production systems [7,10,33]. Normalization of the environmental impacts was done to fairly compare all the environmental impacts between the different scenarios. To this end, the European normalization factors were used [32].

2.3. Economic analysis

The aim of the economic analysis was to assess the economic feasibility of PHB production from cyanobacteria and to understand how much should the process productivity be increased to reach an economically competitive PHB production process using cyanobacteria.

Total investment, operation and maintenance costs were calculated and compared for all the scenarios described in Section 2.2.1. Capital

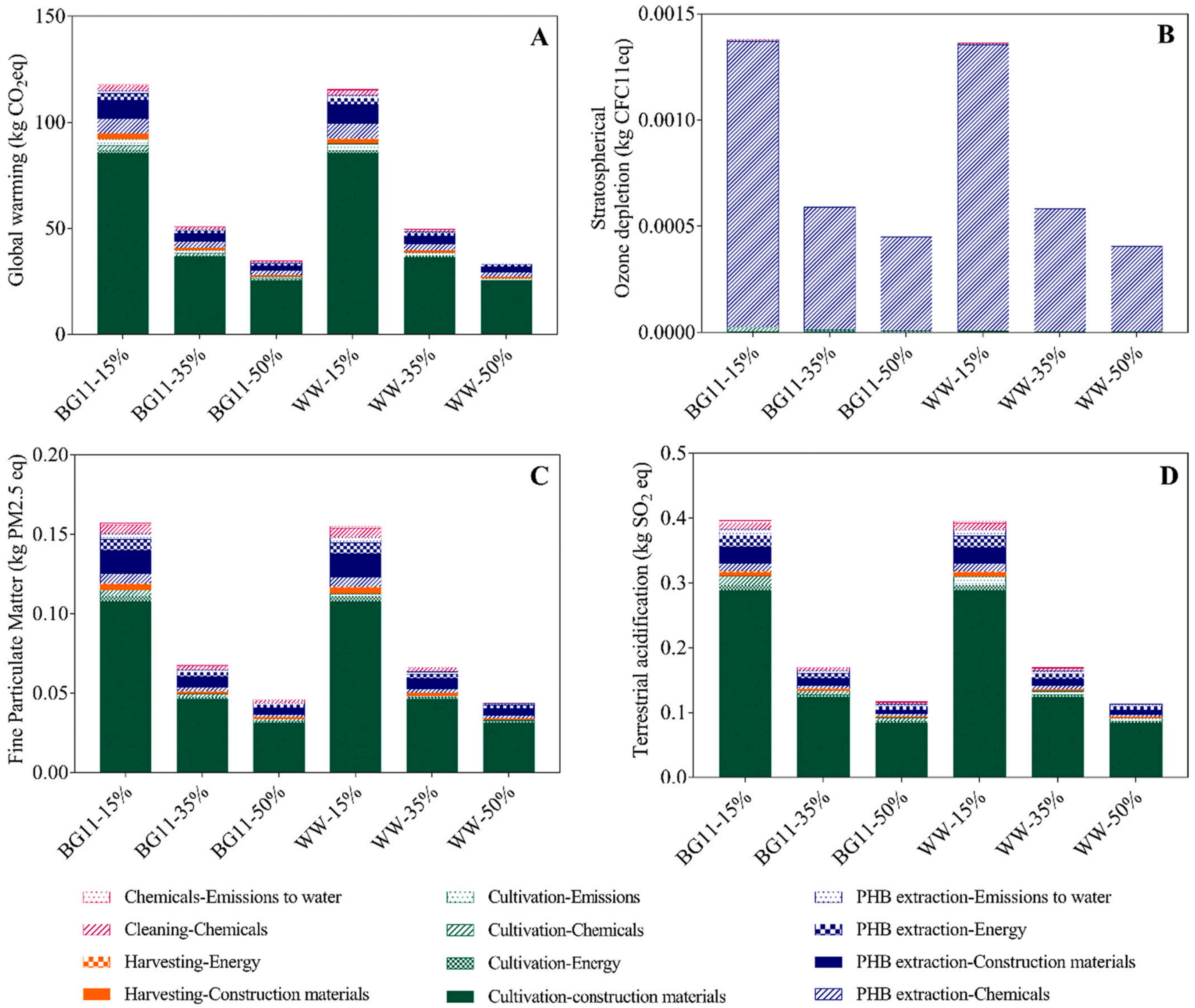

Fig. 2. Potential environmental impacts to produce 1 kg of PHB for the different scenarios considered in this study. (A) correspond to the impact category of Global Warming, (B) to Stratospheric Ozone Depletion, (C) to Fine Particulate Matter, (D) Terrestrial Acidification, (E) Freshwater Eutrophication, (F) Marine Eutrophication, (G) Mineral Resource Scarcity, (H) Fossil Fuel Scarcity.

Note: Scenarios: BG-11-15% PHB: PHB production from cyanobacteria grown in BG-11 with a PHB content of 15%$_{dcw}$; BG-11–30%: PHB production from cyanobacteria grown in BG-11 with a PHB content of 30%$_{dcw}$; BG-11-50%: PHB production from cyanobacteria grown in BG-11 with a PHB content of 50%$_{dcw}$; WW-15%: PHB production from cyanobacteria grown in urban wastewater with a PHB content of 15%$_{dcw}$; WW-35%: PHB production from cyanobacteria grown in urban wastewater with a PHB content of 35%$_{dcw}$; WW-50%: PHB production from cyanobacteria grown in urban wastewater with a PHB content of 50%$_{dcw}$.

costs included the major equipment costs (MEC), installation, instrumentation and control, construction expenses (including engineering and supervision of the construction), contractor's fee and contingency costs. The major equipment costs were provided by local suppliers. Lang factors, obtained from [34], were used to estimate the capital investment, by multiplying the MEC by specific factors. On the other hand, the operation and maintenance costs included the costs associated with energy and chemicals consumption, labour costs and maintenance. In all scenarios, chemical costs were assumed to be provided by local suppliers. The amount of chemicals needed in each scenario was calculated from mass balances. Electricity costs were calculated as the product of the total power consumption of the equipment and the average electricity costs in 2021 reported by [35] in Spain for household consumers (<5000 kWh/year). The number of employees was assumed to be one part-time operator (20% of standard workweek hours), one part-time supervisor (2% of standard workweek hours) and one part-time supervisor (2% of standard workweek hours) [34]. Personnel salary was assumed to be 30,000 € year^{-1} for the operator, 40,000 € year^{-1} for the supervisor and 65,000 € year^{-1} for the plant manager [34]. According to the Spanish regulations, 23.6% of the Employer's contribution has been also considered [36]. Maintenance costs were assumed to be 4% of the MEC costs [34] (Table 2).

Moreover, the PHB breakeven selling price was calculated based on a zero 20 years net present value (NPV) in each scenario considered. A discount rate of 8% was used for the cash flow analysis.

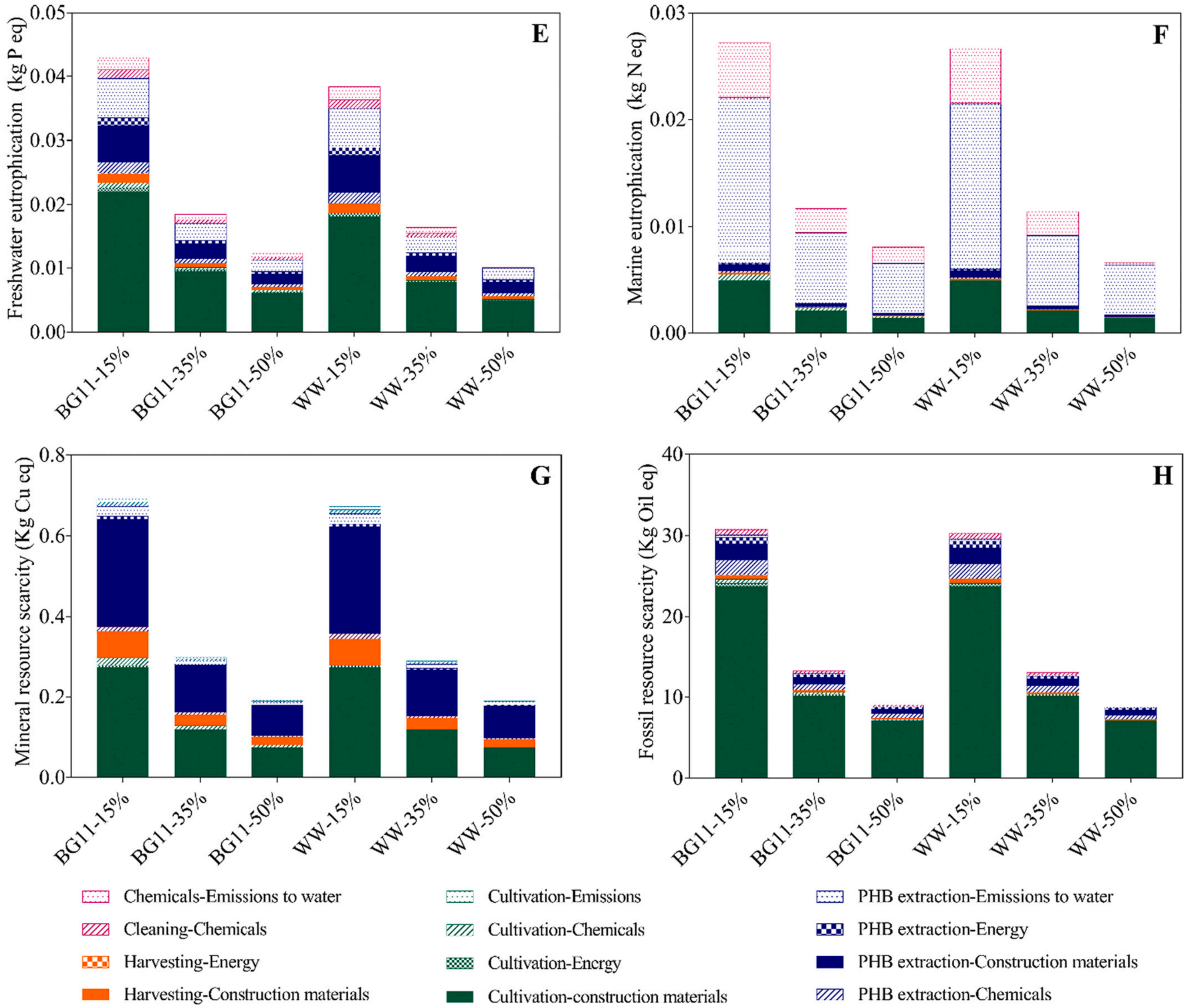

Fig. 2. (*continued*).

3. Results and discussion

3.1. Life cycle assessment

3.1.1. Characterization

Fig. 2 shows the potential environmental impacts associated with each scenario. Comparing the scenarios using standard growth medium with the scenarios using wastewater, it can be concluded that the use of wastewater slightly (2–19%) reduced the environmental impact in all the impact categories. Nevertheless, the highest reduction in the environmental impacts was produced when the PHB content in the biomass was increased. Indeed, an increase from 15% to 50% of PHB content in the biomass, reduced the environmental impacts by up to75%. These results are in accordance with the findings of Nitkiewicz, et al., [10] who compared the heterotrophic bacteria production of PHB using different carbon sources (e.g. raw vegetable oil, glycerol from biodiesel production or used oil) and discovered that the increase in the biopolymer content by 10% was the most environmentally beneficial change.

Construction materials, chemicals used for PHB extraction and the emissions to water had the highest contribution to the overall impacts in all the scenarios considered. In the case of the Global Warming impact category (Fig. 2A), the construction materials contributed to 72–75% of the overall impact in all the scenarios. Similar results were obtained for the impact categories of Fine Particulate Matter, Terrestrial Acidification, Freshwater Eutrophication, Mineral Resource Scarcity and Fossil Resource Scarcity (Fig. 2C, D and H), where the construction materials accounted for 61–92% of the overall impact in all the scenarios. The Marine Eutrophication impact category was mostly influenced by the emissions to water during PHB extraction (57–71% of the total impact) (Fig. 2F) in all the scenarios. Stratospherical Ozone Depletion was highly influenced by the use of chemicals during PHB extraction (97–99% of the total impact) (Fig. 2B) in all the scenarios considered. This impact was mainly attributed to the use of chloroform during the PHB extraction. This is in accordance with previous studies which showed that the use of chemicals for the purification of PHB from heterotopic bacteria was one of the main contributors to the overall environmental impact [10,37]. These results suggested that the amount of solvents used should be reduced and more sustainable alternatives for PHB recovery should be found. Several PHB extraction methods have been developed for heterotrophic bacteria, such as the use of alternative solvents like butyl acetate or 1,3-dioxolane, the digestion with alkaline or acid chemicals, super critical CO_2, enzymatic processes or mechanical processes

Table 1
Summary of the inventory for the different scenarios of PHB production from cyanobacteria. Values are referred to the functional unit (1 kg of PHB).

	Base Scenarios						Future Technology Scenarios		Unit
	BG-11 15% PHB	BG-11 30% PHB	BG-11 50% PHB	WW 15% PHB	WW 30% PHB	WW 50% PHB	BG-11-Improved Productivity	WW- Improved Productivity	
	Inputs								
Construction materials									
Photobioreactor (cultivation)									
Concrete (infrastructure)	0.011	0.005	0.003	0.011	0.005	0.003	$5.07 \cdot 10^{-5}$	$5.07 \cdot 10^{-5}$	kg/kgPHB
Steel (reinforcement)	0.274	0.117	0.082	0.274	0.117	0.082	0.0013	0.0013	kg/kgPHB
Steel (reactor structure)	1.381	0.592	0.414	1.381	0.592	0.414	0.0128	0.0128	kg/kgPHB
Polymethyl methacrylate (reactors)	6.119	2.623	1.836	6.119	2.623	1.836	0.056	0.056	kg/kgPHB
Centrifuge (harvesting)									
Stainless steel	0.506	0.217	0.152	0.506	0.217	0.152	0.0023	0.0023	kg/kgPHB
Mixing tank 1 (PHB extraction)									
Stainless steel	0.205	0.088	0.061	0.205	0.088	0.061	0.00094	0.00094	kg/kgPHB
Centrifugal decanter (PHB extraction)									
Stainless steel	0.785	0.337	0.236	0.785	0.337	0.236	0.0036	0.0036	kg/kgPHB
Evaporator (PHB extraction)									
Stainless steel	0.046	0.157	0.046	0.046	0.157	0.046	0.0017	0.0017	kg/kgPHB
Mixing tank 2 (PHB extraction)									
Stainless steel	0.205	0.088	0.061	0.205	0.088	0.061	0.00095	0.00095	kg/kgPHB
Centrifuge 2 (PHB extraction)									
Stainless steel	0.506	0.217	0.152	0.506	0.217	0.152	0.0023	0.0023	kg/kgPHB
Dryer (PHB extraction)									
Stainless steel	0.005	0.002	0.012	0.005	0.002	0.012	$2.53 \cdot 10^{-5}$	$2.53 \cdot 10^{-5}$	kg/kgPHB
Operation									
Energy consumption									
Photobioreactor (cultivation)									
Pumping	2.533	1.086	0.760	2.533	1.086	0.760	0.012	0.012	kWh/kgPHB
Compressor	2.000	0.857	0.600	2.000	0.857	0.600	0.009	0.009	kWh/kgPHB
Centrifuge (harvesting)									
Centrifugation	0.671	0.287	0.201	0.671	0.287	0.201	0.003	0.003	kWh/kgPHB
Pumping	0.329	0.141	0.099	0.329	0.141	0.099	0.001	0.001	kWh/kgPHB
Mixing tank 1 (PHB extraction)									
Pumping	0.132	0.056	0.039	0.132	0.056	0.039	0.0007	0.0007	kWh/kgPHB
Mixing	2.959	1.268	0.888	2.959	1.268	0.888	0.0137	0.0137	kWh/kgPHB
Heating	1.614	0.692	0.484	1.614	0.692	0.484	0.0074	0.0074	kWh/kgPHB
Centrifugal decanter (PHB extraction)									
Pumping	0.132	0.056	0.039	0.132	0.056	0.039	0.0006	0.0006	kWh/kgPHB
Centrifuge	0.710	0.304	0.213	0.710	0.304	0.213	0.0033	0.0033	kWh/kgPHB
Evaporator (PHB extraction)									
Evaporation	0.789	0.338	0.237	0.789	0.338	0.237	0.0036	0.0036	kWh/kgPHB
Pumping	0.132	0.056	0.039	0.132	0.056	0.039	0.0061	0.0061	kWh/kgPHB
Mixing tank 2 (PHB extraction)									
Mixing	0.074	0.032	0.022	0.074	0.032	0.022	0.0003	0.0003	kWh/kgPHB
Pumping	2.271	0.973	0.681	2.271	0.973	0.681	0.0105	0.0105	kWh/kgPHB
Cooling	0.066	0.028	0.020	0.066	0.028	0.020	0.0003	0.0003	kWh/kgPHB
Centrifuge 2 (PHB extraction)									
Pumping	0.132	0.056	0.039	0.132	0.056	0.039	0.0006	0.0006	kWh/kgPHB
Centrifuge	0.335	0.144	0.101	0.335	0.144	0.101	0.0015	0.0015	

(*continued on next page*)

Table 1 (*continued*)

	Base Scenarios						Future Technology Scenarios		Unit
	BG-11 15% PHB	BG-11 30% PHB	BG-11 50% PHB	WW 15% PHB	WW 30% PHB	WW 50% PHB	BG-11-Improved Productivity	WW- Improved Productivity	
									kWh/ kgPHB
Dryer (PHB extraction)	0.039	0.017	0.012	0.039	0.017	0.012	0.0002	0.0002	kWh/ kgPHB
Total energy consumption	14.917	6.393	4.475	14.917	6.393	4.475	0.043	0.043	kWh/ kgPHB
Chemicals									
Photobioreactor (cultivation)									
Clean water	1.33	0.57	0.4	–	–	–	0.0062	–	m^3/ kgPHB
$NaNO_3$	$2.43{\cdot}10^{-1}$	$1.04{\cdot}10^{-1}$	$7.28{\cdot}10^{-2}$	–	–	–	0.0337	–	kg/kgPHB
$MgSO_4{\cdot}7H_2O$	$1.00{\cdot}10^{-2}$	$4.28{\cdot}10^{-3}$	$3.00{\cdot}10^{-3}$	–	–	–	$4.63 \cdot 10^{-5}$	–	kg/kgPHB
Citric acid	$8.00{\cdot}10^{-4}$	$3.4{\cdot}10^{-4}$	$2.40{\cdot}10^{-4}$	–	–	–	$3.7 \cdot 10^{-6}$	–	kg/kgPHB
K_2HPO_4	$4.49{\cdot}10^{-2}$	$1.9{\cdot}10^{-2}$	$1.35{\cdot}10^{-2}$	–	–	–	0.002	–	kg/kgPHB
Ammonium ferric citrate green	$8.00{\cdot}10^{-4}$	$3.4{\cdot}10^{-4}$	$2.40{\cdot}10^{-4}$	–	–	–	$3.7 \cdot 10^{-6}$	–	kg/kgPHB
$CaCl \cdot 2H_2O$	$4.80{\cdot}10^{-3}$	$2.06{\cdot}10^{-3}$	$1.44{\cdot}10^{-3}$	–	–	–	$2.2 \cdot 10^{-5}$	–	kg/kgPHB
$EDTANa_2$	$1.33{\cdot}10^{-4}$	$5.71{\cdot}10^{-5}$	$4.00{\cdot}10^{-5}$	–	–	–	$6.2 \cdot 10^{-7}$	–	kg/kgPHB
$NaCO_3$	$2.67{\cdot}10^{-3}$	$1.14{\cdot}10^{-3}$	$8.00{\cdot}10^{-4}$	–	–	–	$1.23 \cdot 10^{-5}$	–	kg/kgPHB
CO_2	15.670	6.714	4.700	15.670	6.714	4.700	0.58	0.58	kg/kgPHB
Mixing tank 1 (PHB extraction)									
Chloroform	1.497	0.641	0.449	1.497	0.641	0.449	0.277	0.277	kg/kgPHB
NaClO solution 15%	0.342	0.146	0.102	0.342	0.146	0.102	0.063	0.063	kg/kgPHB
Mixing tank 2 (PHB extraction)									
Methanol	0.794	0.340	0.238	0.794	0.340	0.238	0.1469	0.1469	kg/kgPHB
Centrifuge 2 (PHB extraction)									
Water (PHB cleaning)	60.000	60.000	60.000	60.000	60.000	60.000	60	60	L/kgPHB
Cleaning									
NaClO	1.008	0.432	0.302	1.008	0.432	0.302	0.0047	0.0047	kg/kgPHB
Water	0.029	0.376	0.263	0.029	0.376	0.263	0.0041	0.0041	m^3/ kgPHB
	Outputs								
Emissions to Water									
PHB extraction									
NaClO and oxidized organic matter	2.630	1.127	0.789	2.630	1.127	0.789	0.0122	0.0122	m^3/ kgPHB
Cleaning									
NaClO	0.877	0.376	0.263	0.877	0.376	0.263	0.0041	0.0041	m^3/ kgPHB
Emissions to Air									
Photobioreactor (cultivation)									
NH_3	0.000	0.000	0.000	0.007	0.003	0.002	0.000	$3.09 \cdot 10^{-5}$	kg/kgPHB
CO_2	3.130	1.343	0.940	3.130	1.343	0.940	0.58	0.58	kg/kgPHB

Note: Scenarios: BG-11-15% PHB: PHB production from cyanobacteria grown in BG-11 with a PHB content of 15%$_{dcw}$; BG-11–30%: PHB production from cyanobacteria grown in BG-11 with a PHB content of 30%$_{dcw}$; BG-11-50%: PHB production from cyanobacteria grown in BG-11 with a PHB content of 50%$_{dcw}$; WW-15%: PHB production from cyanobacteria grown in urban wastewater with a PHB content of 15%$_{dcw}$; WW-35%: PHB production from cyanobacteria grown in urban wastewater with a PHB content of 35%$_{dcw}$; WW-50%: PHB production from cyanobacteria grown in urban wastewater with a PHB content of 50%$_{dcw}$.; BG-11-Improved productivity: PHB production from cyanobacteria grown in BG-11 with a PHB content of 81%$_{dcw}$ and a biomass productivity to obtain a Break-even price of 4 € kg $^{-1}$. WW - Improved productivity: PHB production from cyanobacteria grown in urban wastewater with a PHB productivity from BG-11-Improved productivity scenario.
Abbreviations: BG-11: cultivation media for blue-green algae; HRT: Hydraulic Retention Time; WW: wastewater.

[38–40]. However, these methods have been hardly tested for cyanobacteria, which can have a more thick and more resistant cell wall than heterotrophic bacteria [41].

Considering the results obtained in this study, it can be concluded that if similar PHB percentages are achieved using BG-11 and wastewater, the use of wastewater as a source of nutrients would only slightly improve the environmental impact of the process. However, considering that the use of wastewater as a source of nutrients is usually more challenging, it is predictable that a lower PHB will be accumulated using wastewater, and therefore similar or even worse performance in terms of environmental impact is probable. Moreover, although polymer thermal properties are not modified by the use of wastewater as a nutrient source [1,2], wastewater may affect other polymer properties (e.g. polymer size) that would affect the marketable price of cyanobacterial PHB. Results also evidenced the importance of increasing PHB productivity. Reactor design should be also improved to reduce the amount of materials used. Further investigation is also required to study more environmentally friendly solvents used in the PHB extraction.

3.1.2. Normalization

The normalised results (Fig. S3) show that Freshwater Eutrophication was the most significant impact category for all the scenarios considered, followed by Fossil Resource Scarcity and Stratospheric ozone depletion. This was in accordance with previous LCA studies using

Table 2
Procedures to estimate the production costs of PHB from cyanobacteria.

		Description	Assumption	Price
Fixed Capital investment	**Direct Costs (DC)**	**Major equipment costs (MEC)**		
		Cultivation		
		Concrete		3094 €
		Steel (reinforcement)		997 €
		Steel (plate)		5027 €
		Polymethyl methacrylate (reactors)		41,880 €
		Harvesting		
		Centrifuge		6205 €
		PHB Extraction		
		Mixing tank 1		3000 €
		Decanter Centrifuge		25,000 €
		Evaporator		49,900 €
		Mixing tank 2		3000 €
		Centrifuge		18,500 €
		Evaporator		950 €
		Installation costs	47% MEC	74,050 €
		Instrumentation and control	35% MEC	55,144 €
	Indirect costs (IC)	**Construction expenses**	15% MEC	23,633 €
		Engineering and Supervision	10% DC	28,675 €
	Other costs	**Contractor's fee and contingency costs**	20% (DC + IC)	67,811 €
OPEX	**Electricity** [1]		Calculated from equipment consumption and average electricity costs in 2021 in Spain for household consumers	0.1647 € kwh^{-1}
	Labour			9912.7 € $year^{-1}$
	Operator		Salary of 1 worker (20% of standard workweek hours)	6000 € $year^{-1}$
	Supervisor		Salary of 1 worker (2% of standard workweek hours)	800 € $year^{-1}$
	Plant manager		Salary of 1 worker (2% of standard workweek hours)	1300 € $year^{-1}$
	Employer's contribution		23.6% of the labour costs	1892.7 € $year^{-1}$
	Maintenance		4% MEC	6302 € $year^{-1}$
	Chemicals		Calculated from mass balances	
	Cultivation			
	CO_2			
	$NaNO_3$		Only considered in scenarios using BG-11	29.8 € kg^{-1}
	$MgSO_4 \cdot 7H_2O$			45 € kg^{-1}
	Citric acid			54 € kg^{-1}
	K_2HPO_4			80.7 € kg^{-1}
	Ammonium ferric citrate green			36 € kg^{-1}
	$CaCl\ 2H_2O$			42.1 € kg^{-1}
	$EDTANa_2$			120 € kg^{-1}
	$NaCO_3$			23 € kg^{-1}
	PHB extraction			
	Water			2.7 € kg^{-1}
	NaClO			0.6 € kg^{-1}
	Chloroform			41.8 € kg^{-1}
	Methanol			7.9 € kg^{-1}

[1] Considering the electricity price rise in 2022, the electricity costs considered here will increase by 57% (from 0.1647 € kwh^{-1} in 2021 to 0.2579 € kwh^{-1} in the first semester of 2022).

cyanobacteria to treat wastewater and produce bioproducts (pigments, biofertilizers, biogas) [27,42]. However, in the previous studies the impact on Freshwater Eutrophication was attributed to the emissions to water caused by wastewater discharge. On the contrary, in all the scenarios of the present study, this impact category was mainly influenced by the manufacturing of construction materials (Fig. 2E).

Normalization results confirmed that in the most significant impact categories the most environmentally friendly scenarios were those with a higher amount of PHB in the biomass.

3.2. Economic analysis

Table 3 shows the PHB production costs for each scenario. The total capital cost of the system was 406,868 € for all scenarios (Table 2). The most expensive part of the systems was the major equipment (157,554 €, 39% of the total capital investment costs). The equipment with the higher costs were the photobioreactors (50,999 €, 13% of the total capital investment costs) and the evaporator (49,900 €, 12% of the total capital investment costs). After major equipment, the installation costs (74,050 €, 18% of the total capital investment costs) and the instrumentation costs (55,144 €, 14% of the total capital investment costs) were the categories that contributed the most to the capital costs. These

Table 3
Specific PHB production costs for the different scenarios.

	Units	BG-11 15% PHB	BG-11 30% PHB	BG-11 50% PHB	WW 15% PHB	WW 30% PHB	WW 50% PHB	BG-11 Improved productivity	WW Improved productivity
Fixed Capital costs									
Direct Costs	€ kg^{-1}	104.7	44.9	31.4	104.7	44.9	31.4	0.48	0.48
Indirect Costs	€ kg^{-1}	19.1	8.19	5.7	19.1	8.19	5.7	0.09	0.09
Other Costs	€ kg^{-1}	24.7	10.6	7.4	24.7	10.6	7.4	0.1	0.1
Operational costs									
Electricity[1]	€ kg^{-1}	2.5	1.1	0.7	2.5	1.1	0.7	0.01	0.01
Raw materials	€ kg^{-1}	24.9	10.7	7.6	9.6	4.2	3.0	2.12	0.93
Labour	€ kg^{-1}	73.1	31.3	21.9	73.1	31.3	21.9	0.34	0.34
Maintenance	€ kg^{-1}	46.0	19.7	13.8	46.0	19.7	13.8	0.21	0.21
PHB Productivity	gPHB d^{-1} m^{-3}	3.75	8.75	12.5	3.75	8.75	12.5	810	810
Breakeven PHB selling price	€ Kg^{-1}	449.2	192.6	134.9	433.9	186.1	130.3	4.1	2.9

Note: Scenarios: BG-11-15% PHB: PHB production from cyanobacteria grown in BG-11 with a PHB content of 15%$_{dcw}$; BG-11–30%: PHB production from cyanobacteria grown in BG-11 with a PHB content of 30%$_{dcw}$; BG-11-50%: PHB production from cyanobacteria grown in BG-11 with a PHB content of 50%$_{dcw}$; WW-15%: PHB production from cyanobacteria grown in urban wastewater with a PHB content of 15%$_{dcw}$; WW-35%: PHB production from cyanobacteria grown in urban wastewater with a PHB content of 35%$_{dcw}$; WW-50%: PHB production from cyanobacteria grown in urban wastewater with a PHB content of 50%$_{dcw}$.; BG-11-Improved productivity: PHB production from cyanobacteria grown in BG-11 with a PHB content of 81%$_{dcw}$ and a biomass productivity to obtain a Break-even price of 4 € kg^{-1}. WW - Improved productivity: PHB production from cyanobacteria grown in urban wastewater with a PHB productivity from BG-11-Improved productivity scenario.

[1] *If the energy prices of the first semester of 2022 (0.2579 € Kwh^{-1}) were considered, the electricity cost would increase by 57%. However, the rise in electricity prices would not significantly affect the PHB production costs.*

results were similar to the ones obtained by [6,43], who reported that the cost of the cultivation equipment has the highest contribution to the total capital costs. However, PHB extraction equipment costs had a much lower contribution in previous studies (< 10% of the total capital costs [6,43] compared to 25% of the total capital costs in this study). These differences may be attributed to the extraction method used in this study and the smaller size of the system considered.

Regarding the operational and maintenance costs, in all the scenarios the labour and maintenance expenses were the highest operation costs (26% and 16% of the total operational and maintenance costs, respectively). These results were in contradiction with previous studies on cyanobacteria PHB production costs, where labour accounted for less than 4% of the production costs [6,43]. Nevertheless, other studies on microalgae biomass production predicted a similar contribution. For instance, Acién, et al., [44] obtained a labour contribution of a 51.6%. Differences between studies may be attributed to the number of workers and the salary considered. For example, Acién, et al., [44] considered 3 workers for a production surface of 0.04 ha, while Panuschka, et al., [43] considered only 1 worker for 0.45 ha. Moreover, the labour requirements and the system size are not linear [34]. Therefore, the bigger the system the lower the ratio between the number of workers and the system surface.

Fig. 3A shows the effect of varying the PHB content from 15 to 50%$_{dcw}$ on the PHB breakeven price. The increase in the PHB content strongly decreases the PHB breakeven prices from 449 € $kgPHB^{-1}$ to 135 € $kgPHB^{-1}$. On the other hand, very similar results are obtained when wastewater or BG-11 are used as nutrients source.

These results showed the necessity to increase PHB productivity to make this process competitive. To the best of the authors' knowledge, the maximum PHB productivity obtained has been 59 mg L^{-1} d^{-1} [19]. Considering this productivity, a breakeven selling price of 38.5 € $kgPHB^{-1}$ was obtained (Fig. 3B). This is still 9.6 folds higher than PHB current market price. According to our calculations, a PHB productivity of 810 mg L^{-1} d^{-1} would be necessary to obtain a PHB selling price of 4 € $kgPHB^{-1}$ (PHB market price) (Fig. 3B). If this productivity is reached, the use of wastewater as a nutrient source would reduce the PHB price by 29% (2.9 € $kgPHB^{-1}$) compared to the standard grow medium.

Slightly lower PHB production costs were obtained previously using

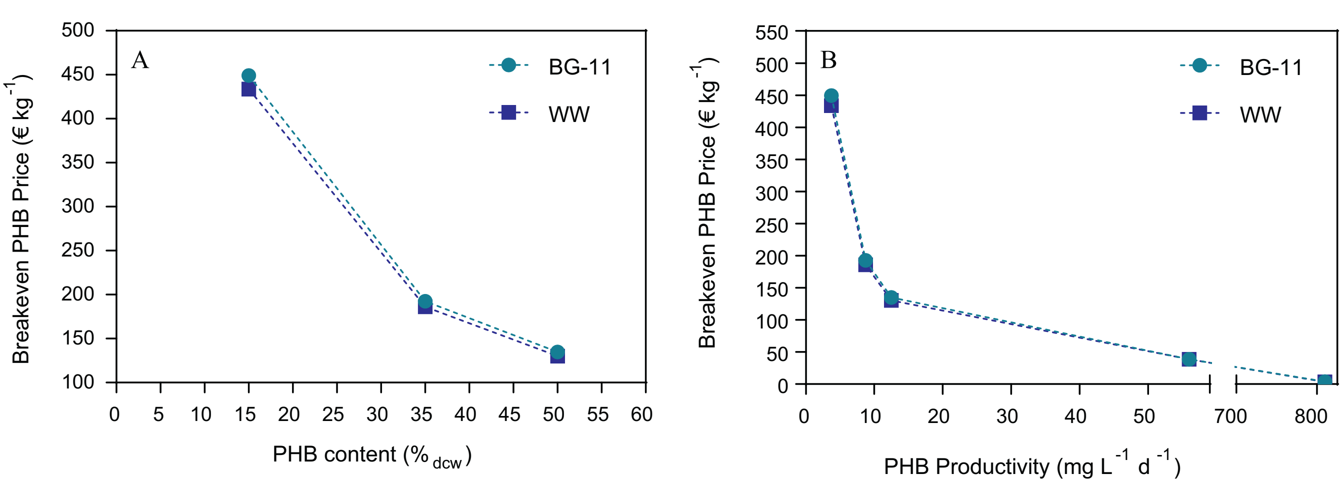

Fig. 3. Effect of PHB content in the biomass (A) and PHB productivity (B) on the minimum PHB selling price based on a zero 20 years net present value (NPV) in scenarios using BG-11 or wastewater as a nutrient source. A biomass productivity of 25 mg L^{-1} d^{-1} was assumed in graph A.

other cultivation systems. For instance, Panuschka, et al., [43], found that the minimum selling price for PHB was 24 € kg^{-1} using a thin-layer-photobioreactor and a 60% PHB content. Price, et al., [6] predicted a minimum PHB selling price of 18.3 € kg^{-1} using an open raceway pond producing 10,000 tons per year. Moreover, they observed that the application of other strategies (e.g. pigment extraction, the use of wastewater as a nutrient source, use of flocculants, use of solar power, the digestion of residual biomass, the use of deep ripening ponds, the use of CO_2 from anaerobic digestion) reduced the PHB producing price up to 7.7 € kg^{-1}. Differences between studies may be attributed to the type of reactor used (thin-layer, open raceway pond or vertical column), the design scale (4500 m^2, 182 ha or 150 m^2) and the PHB productivity considered (23.1 g m^{-2} d^{-1}, 15 g m^{-2} d^{-1} or 8.3 g m^{-2} d^{-1}) [6,43].

As previously stated, the results obtained in this study clearly showed the need to increase the PHB content and biomass productivity. Recent studies achieved up to $81\%_{dcw}$ of PHB using genetically modified microorganisms and photoheterotrophic growth conditions [22]. Regarding biomass productivity, the maximum theoretical biomass productivity (i.e. considering that all the cells in the reactor receive all the photons they can uptake) is 77 g m^{-2} d^{-1} (photosynthetic efficiency of 8–10%) [45]. Considering that the Volume:Surface ratio of the reactors used in this study was 666 L m^{-2}, a maximum theoretical biomass productivity of 116 mgVSS L^{-1} d^{-1} would be possible, which will be lower than the previously calculated productivity (1000 mgVSS L^{-1} d^{-1}) needed to reduce the PHB production costs to 4 € kg^{-1}. However, if reactors had a lower Volume:Surface ratio, higher productivities could be obtained. For instance, Panuschka, et al., [43], used a thin-layer reactor with a Volume:Surface ratio of 0.01 m^3 m^{-2}. In that case, the maximum theoretical biomass productivity would be 7700 mgVSS L^{-1} d^{-1}. It can be therefore concluded that using appropriate reactors (low Volume:Surface ratio) and genetically improved strains would enhance the economic feasibility of PHB production from cyanobacteria. Another possible strategy to reduce PHB production costs and environmental impacts would be to produce PHB in a biorefinery concept and recover other bioproducts of industrial interest, such as pigments, lipids, or exopolysaccharides. For instance, Price, *et al.* (2022) [6] observed that the production of pigments together with PHB would reduce the PHB selling price from 18 $ kg^{-1} to 13.9 $ kg^{-1}. However, experimental studies on the production of different bioproducts together with PHB using cyanobacteria are still very scarce [46].

3.2.1. *Environmental impacts of improved and economically feasible scenarios*

To assess the effect of increased productivity on the environmental impact, the LCA was carried out considering two additional scenarios using high productivities and BG-11 or wastewater (Fig. S4), as described in Section 2.2.1. In these improved scenarios, the environmental impacts were strongly reduced (57–97%) in all impact categories compared to the previously considered scenarios. As for the previous analysis, the use of wastewater as a nutrient source slightly reduced (1–19%) the environmental impacts in both scenarios.

In both improved scenarios, the chemicals needed for cyanobacteria cultivation contributed the most to the overall environmental impact in all the impact categories except for Stratospheric Ozone Depletion. This was mainly attributed to the use of commercial CO_2. Therefore, if flue CO_2 would be used, the environmental impacts of this process could be further reduced. Similarly to previous scenarios, in the Stratospheric Ozone Depletion impact category, the environmental impact was mainly caused by the use of chemicals for PHB extraction in both scenarios.

All in all, PHB production from cyanobacteria are competitive in most of the impact categories (see supplementary materials). However, process improvements are required, especially in PHB purification, photobioreactor design and PHB productivity.

4. Conclusions

The environmental analysis, carried out using the Life Cycle Assessment methodology, showed that increasing the PHB content in the biomass drastically reduce (between 67 and 75%) the environmental impacts of the process. On the other hand, using wastewater instead of standard growth medium slightly reduced the environmental impacts (up to 20%). Environmental impacts were mainly caused by construction materials and the use of chemicals (especially chloroform for PHB purification).

The results of the economic evaluation showed that not only PHB content influenced the minimum PHB selling price, but also the biomass productivity. A PHB productivity of 810 mg L^{-1} d^{-1} would be necessary to obtain a PHB selling price of 4 € $kgPHB^{-1}$ (PHB market price). If this productivity is obtained, the use of wastewater instead of standard growth medium will further reduce the PHB price to 2.9 € $kgPHB^{-1}$. Using a well-designed reactor (low Volume:Surface ratio) and genetically improved strains would enhance the economic feasibility of PHB production from cyanobacteria.

CRediT authorship contribution statement

Estel Rueda: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Visualization. **Vincenzo Senatore:** Conceptualization, Data curation, Methodology, Formal analysis, Investigation, Writing – original draft, Visualization. **Tiziano Zarra:** Writing – review & editing, Supervision. **Vincenzo Naddeo:** Writing – review & editing, Supervision. **Joan García:** Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Marianna Garfí:** Resources, Conceptualization, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.susmat.2023.e00579.

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