Algal Research 64 (2022) 102698

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Heterotrophic vs autotrophic production of microalgae: Bringing some light into the everlasting cost controversy

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ARTICLE INFO

Keywords:
Techno-economic analysis
Microalgae
Autotrophic
Heterotrophic
Production cost

ABSTRACT

Heterotrophic or autotrophic? This is the continuous question the industry faces when microalgae production is the endeavor. Surprisingly, nowadays specialists have not reached a consensus on which is the most economical option. The current work analyses costs for heterotrophic and autotrophic cultivation of microalgae at an industrial scale. Heterotrophic cultivation of microalgae results in a production cost of 4.00 €·kg^{-1} of dry weight as a centrifuged paste. This is within the range of autotrophic costs, but still above the production cost in some photobioreactors. The study also identifies the current limitations on the technology and studies the effect on the cost of overcoming these. Once achieved, the advances in the process could result in a heterotrophic production cost reduced to 1.08 €·kg^{-1}. Autotrophic cultivation seems competitive with heterotrophic production. It is time to leap forward in the autotrophic production scale to achieve the critical reduction in production cost.

1. Introduction

Despite the variety of trophic modes in microalgae metabolism, autotrophic and heterotrophic cultivation are the main strategies in the commercial production of microalgae. Heterotrophic production relies on organic compounds —glucose or even waste sugars— like energy and carbon sources, while autotrophic cultures use light and CO_2 respectively.

The comparison of both strategies brings a number of facts favoring heterotrophic production. According to Scaife et al. [1], 40 g·l^{-1} is the maximum cell density reached in an autotrophic culture, using a thin layer system, which achieved biomass productivity of 3.3 g·l^{-1}d^{-1}. Other studies have shown productivities ten times greater in microalgae grown heterotrophically [2,3]. This shows that heterotrophic cultures can achieve biomass productivities exceeding in one order of magnitude than those obtained in autotrophy. This difference becomes even more pronounced when lipid productivities are compared. Heterotrophic growth enables high cell densities that can exceed 75 g·l^{-1} as they are not light-limited [4]. This seems like an overwhelming value when compared to typical concentrations in photosynthetic production (0.5–4 g·l^{-1}; [5,6]). Moreover, recent studies have shown that the hypothesized inability to produce light-induced metabolites in heterotrophic cultures is now refuted. It has been demonstrated that the synthesis of certain pigments by wild strains is possible in the darkness, although exclusively done at a lab-scale [7,8]. On top of that, there is a whole legacy of knowledge from conventional fermentation to take advantage of, as heterotrophic microalgae production does not require a customized fermenter differing from state-of-the-art systems [4,9].

These listed benefits may lead us to envision heterotrophy as the golden option for the microalgal industry. The market has revealed quite the opposite. In 2018, the global algae market was valued at 717 M$, with more than 80% from cultivation in open ponds and a limited impact from cultivation in closed fermenters [10]. While about 110 sites for autotrophic production are found in the area of Asia-Pacific [11], there is a short list of large players in heterotrophic production, such as DSM, Corbion, or BASF.

Is this prevalence explained by costs? It could be the reason, as profit is a primary driver in the industry. However, we are far from a consensus about the most economic production mode. While some authors claimed heterotrophic production as the most cost-viable method [12,13], there

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https://doi.org/10.1016/j.algal.2022.102698
Received 10 September 2021; Received in revised form 18 February 2022; Accepted 31 March 2022

is also a movement that strongly encouraged the use of photosynthetic microalgae as the best option due to economic advantages [2,14]. Albeit apparently none of these opinions were based on an impartial comparison arising from a techno-economic assessment.

The scientific community has produced a plethora of results concerning both autotrophic and heterotrophic microalgae production costs. Nevertheless, abundance is not always synonymous with agreement and methodologies differ. While some case studies have shown production costs reaching more than 100 €·kg^{-1}, others have put forward economic feasibility on biofuels production [15–17]. The production cost has been studied under different scenarios both for heterotrophic [16,18–21] and autotrophic production. Nevertheless, a clear and direct comparison is missing.

The current work thoroughly analyses costs for heterotrophic cultivation of microalgae. Our study follows a similar procedure as the one we previously developed to explore the costs of autotrophic production [22]. These cost studies on heterotrophic and autotrophic production follow analogous methodologies, and they both share some of the fundamental points, such as location or biomass capacity. These facts indicate that a reliable comparison can be performed. Firstly, this study assesses the production cost of a base case for heterotrophic production of microalgae. This is followed by a critical comparison to the autotrophic production, which enables a discussion on the production strategies. Finally, the outlook for heterotrophy is evaluated by performing a sensitivity analysis to reveal its potential.

2. Materials and methods

2.1. Basis

The biomass capacity of the projected facility located in Spain is 6094-ton·year^{-1} measured as dry weight. Both the location and production capacity are identical to our Article *Towards industrial products from microalgae* for Flat Panels [22]. Under this overarching background, our cost assessment with analogous methodology allows a fair comparison of both production strategies (Fig. 1).

We keep the dry weight of microalgae as centrifuged paste as the functional unit for comparison. This paste can yield different products after downstream processing, which has been covered in our previous work for microalgae grown autotrophically [22].

Chlorella spp. is used as a cell factory. This is the most studied heterotrophic microalga and its performance exceeds most of the other microalgae under these conditions. The methodology and model used for *Chlorella* spp. could be applied to other species, as long as the specific inputs are changed. A scheme of the process is depicted in Fig. 2. We considered a batch process with biomass productivity of 4.81 g·l^{-1}·d^{-1} (this is an average from 23 experiments on *Chlorella* spp. in glucose, which ranged between 31.86 and 0.15 g·l^{-1}·d^{-1}) (Supplementary Table 1). The differences on productivities are mainly due to non-uniform culture conditions and volumes among the studies.

Glucose is used as the substrate, at a cost of 0.44 €·kg^{-1}, and associated mineral nutrients [23,24]. This is the preferred organic source [14], with a growth yield for heterotrophic *Chlorella* spp. of 0.35 g biomass·g sugar^{-1} [23].

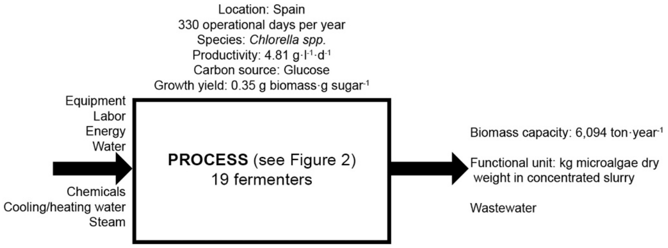

Fig. 1. System boundaries, main inputs, and outputs used in the study.

The cost of water for the industry is the result of a variable cost (2.4 €·m^{-3}) and a fixed cost (101 €·month^{-1}). This information is the average of three locations in Spain (Cádiz, Seville, and Catalonia), provided by municipal water management companies. After harvesting, this water is treated before discharge at a cost of 0.43 €·m^{-3} [25]. Sodium hydroxide and nitric acid are required for the cleaning process, being their cost 0.69 and 0.93 €·kg^{-1} respectively (see Annex). The cost of NaClO for cleaning is 0.26 €·l^{-1} at a concentration of 100 ppm free chlorine per liter. The cost of energy is 0.122 €·kWh^{-1} [22].

330 days of operation per year are considered, as in a similar study on fermentation [26]. 20 batches per fermenter take place per year, with a length of 16.5 days per batch (this length includes cleaning, sterilization, and downtime). This results in a total working volume of fermenters of 4.692 m^3.

2.2. Fermenters

The volume results in 19 cylindrical fermenters of 302 m^3 made of Stainless Steel 316, with a diameter of 5.77 m and a height of 11.5 m. The working volume of the fermenters is 83% of its maximum capacity (250.7 m^3) [26]. Its cost, as well as the cost of other major equipment, are shown in Table 1.

The seed fermenters are used to inoculate the fermenters, using a volume ratio to upscale 1:10. A number of 0.44 seed fermenters are required per fermenter [27]. This results in a total of 9 seed fermenters of each capacity (25.1, 2.5, and 0.2 m^3 working volume).

The energy for agitation in the seed fermenters is 0.5 kW·m^{-3} [28] and the power consumed in the fermenters is 260 kW per fermenter [27].

A building to harbor the fermenters, seed fermenters, and all the additional processes (equipment, pipes, cleaning in place…) is required for this facility. The estimated surface for this is 1 ha, calculated as 6 times the area occupied by the fermenters, assuming they occupy a space equal to 1.5 times their diameter.

2.3. Labor

The direct labor cost was calculated by estimating 19 full-time employees (fte), resulting from 1 plant manager, 3 supervisors, and 15 operators. This number of employees ensures three operators by shift in three shifts per day. For comparison: Gapes assumes a minimum of 10 fte for a 4500 ton·year^{-1} butanol production facility [29]; Tabernero et al. considered 4 workers by shift for microalgae heterotrophic culture at a capacity of 10,000-ton biodiesel·year^{-1} [19]; our previous study on autotrophic production assumed 32 workers for an identical production (6094-ton·year^{-1}) and a much larger area —100 ha [22].

Salaries are identical to those from Ruiz et al. [22], being 60,839 €·year^{-1}, 39,046 €·year^{-1}, 27,241 €·year^{-1} for the plant manager, supervisor, and operator respectively. The workforce cost is increased a 23.6% from the employer's contribution and an extra 20% is finally applied for labor supervision activities [22].

2.4. Equipment

The number and capacity of the equipment are calculated from mass balances. The values originating the main calculations are the annual productivity to achieve, the biomass productivity, the number of batches, and operational days per year. These data provide the major flows, which originate the rest of mass balances. To be conservative, the capacities of equipment are 11% greater than the calculated capacity —in this way equipment will never operate above 90% of its maximum design capacity.

The time required to fill the fermenters when a batch starts is 22 h [27]. This defines the capacity of the feeding pumps, as the volume of the fermenter is known.

The time required to process a whole fermenter in the centrifuge is 4

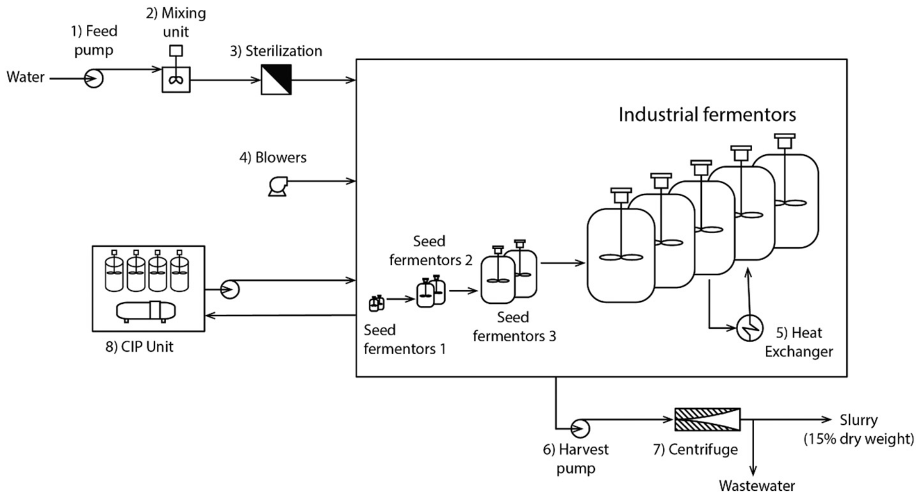

Fig. 2. Scheme of the process. Water is pumped, enriched in nutrients (glucose and minerals), and filter sterilized to fill the fermenters. Agitators and blowers provide culture mixing and oxygen respectively in the fermenters. Heat exchangers integrated into the systems allow maintenance of temperature within the desired range. Harvesting is performed using a combination of pump and centrifuge, which results in a concentrated slurry (15% dry weight). A cleaning in place (CIP) unit cleans and sterilizes the fermenters after each batch, ensuring hygienic conditions in the process.

Table 1
List of major equipment. When data was not from 2020, the effect of inflation was considered in these prices, being expressed for the same base year (2020). The source for this inflation data was Harmonized Indices of Consumer Prices (HICP) database from Eurostat, using data for consumption of "all-items" in Spain, with 2015 as the reference year.

Equipment	capacity	$€\cdot unit^{-1}$	Power	REF.
Fermenter[a]	250.7 m^3	397,453	–	[27]
Agitator fermenter	*Fermenter*	575,588	Average: 260 kW	
Seed fermenter 3[b]	25.1 m^3	94,674	–	
Seed fermenter 2[b]	2.5 m^3	57,063	–	
Seed fermenter 1[b]	0.2 m^3	4565	–	
Blower	2499 $m^3\cdot h^{-1}$	11,616	11.15 kW	[22]
Pump	2 $m^3\cdot h^{-1}$	473	0.18 kW	
Pump	4 $m^3\cdot h^{-1}$	1075	0.4 kW	
Centrifuge	65 $m^3\cdot h^{-1}$	309,673	55 kW	
Mixing unit	4 m^3	228,532	2.07 kW	
Sterilization unit (cascade filters)	5.99 $m^3\cdot h^{-1}$	17,311[c]	–	
Cleaning in place Unit	53,500 m^3[d]	417,798[c]	Described in Material and Methods	[27]

[a] Cooling coil included.
[b] Cooling jackets and agitator included.
[c] Calculated from: CostB = CostA · (SizeB/SizeA)$^{\hat{}0.85}$.
[d] Capacity of vessels to clean.

h, which in combination with the volume of the fermenter provides, in turn, the capacity of the centrifuges.

The aeration rate in the fermenter is 0.7 vvm (volume of air per volume of reactor and minute) [30]. The total volume of the fermenters and this aeration rate are used to calculate the capacity of the blowers.

The residence time in the mixing unit to prepare the culture medium is 30 s [31]. The volume of this unit is then obtained from the calculated feeding flow mentioned in this section.

The temperature control and cleaning in place were also integrated into the model and are described in the Annex.

2.5. Capital (CAPEX) and operational costs (OPEX)

Our analysis specifically customizes the standard methodology applied on cost assessment from industry to our specific process. We apply the modified method of Lang Factors [32], which originated in 1948 [33]. The original methodology has been updated and improved and is widely used in the field of chemical engineering [34–37]. According to AACE International, this type of Capital Cost Estimate typically has an accuracy of ±10–30% [32]. Specific Lang factors multiply the major equipment cost, obtaining different items in cost, which integrate the capital investment (Table 2). These are identical to those used in the similar study on microalgae production used as a reference [22]. The Fixed Capital Investment is amortized (spread out) over the lifetime of the facility (Table 2) to establish a yearly cost. To do this we apply the Straight-Line Depreciation Method, where an equal amount of depreciation is charged each year, considering a salvage value of the equipment of zero. Property tax, insurance, and cost of the site are added to the depreciation and interest to form the CAPEX. The site is rented at a cost of 2.35 $€\cdot m^{-2}\cdot month^{-1}$, averaged from the rental cost of 10 industrial units in the south of Spain (www.idealista.com). The assessment does not consider the commercialization of products; hence we did not include any income tax.

The currency has been converted to euro (€) when the prices were in US dollars ($). The euro to US dollar exchange rate used is 1€ = 1.1354

Table 2
Factors applied to calculate CAPEX and OPEX of the facility.

Fixed capital investment	Direct Capital Costs	Major Equipment Cost (MEC)	-
		Installation costs	20% MEC
		Instrumentation and control	15% MEC
		Piping	20% MEC
		Electrical	10% MEC
		Buildings	23% MEC
		Land improvements	12% MEC
		Service facilities	20% MEC
	Others	Contractor's fee	5% Direct cost
		Contingency (Major Equipment)	15% Direct cost + 15% Indirect cost
	Indirect Capital Costs	Construction expenses	10% Direct cost
		Engineering and supervision	30% MEC
		Lifetime of the facility	15 years
		Interest rate	8% fixed capital investment
		Property tax	1% Depreciation + 1% Interest rate
		Insurance	0.6% Depreciation + 0.6% Interest rate
		Maintenance	4% MEC
		Operating supplies	0.4% Electricity + 0.4% Raw materials + 0.4% Utilities
		Contingencies	15% Raw materials + 15% Utilities
		Overheads	55% Labor + 55% Maintenance

US$ —the average rate in December for 2020.

Operational Costs (OPEX) are calculated as described in Materials and methods and the Annex. However, maintenance, operating supplies, contingencies in operation, and overheads are indirectly calculated as described in [22] (Table 2). Contingency is a factor included to cover unforeseen circumstances in operation (Table 2). Since industrial microalgae production is not yet completely established, the process may be prone to eventualities. As a safeguard, we used a factor of 15% of direct and indirect costs for contingencies (Table 2), the highest value within the recommended range for chemical engineering [38].

2.6. Assumptions used in the sensitivity analysis

The abovementioned methodology and assumptions establish the base case (case 0 in Fig. 3). This represents the original projection, based on the currently achievable inputs. We also studied other projections, originating from the base case, as sensitivity analysis. The following changes were performed to the base case to study the effect of future improvements in the process (see "3.3 Techno-economic analysis: future potential"). They were adopted independently from each other, excepting the last case (Case 5. Future scenario), where these improvements are combined.

Case 1. Increased productivity: volumetric productivity increased from 4.81 to 31.86 g·l^{-1}·d^{-1} [2]; duration of the batch was 7 days, with 49 batches per year to attain a maximum biomass concentration of 120 g·l^{-1} cell dry weight. This is the same biomass concentration experimentally attained in a 5 m^3 fermenter [2]. All other inputs remained identical to the base case.

Case 2. Energy savings in agitation: energy required to stir seed fermenters is reduced from 0.5 to 0.375 kW·m^{-3} and power in the fermenters from 260 to 195 kW per fermenter. This assumption is based on studies demonstrating that energy savings between 25 and 66% can be achieved in the agitation [39,40]. All other inputs remained identical to the base case.

Case 3. Increased sugar conversion efficiency: the yield on glucose in microalgae was increased from the original 0.35 to 0.62 g biomass·g sugar^{-1} [41,42]. All other inputs remained identical to the base case.

Case 4. Use of molasses as substrate: cost of sugars reduced from 0.44 €·kg^{-1} from glucose to 0.106 €·kg^{-1} [24]. All other inputs remained identical to the base case.

Case 5. Future scenario: Combination from changes commented in Cases 1–4 simultaneously. All other inputs remained identical to the base case.

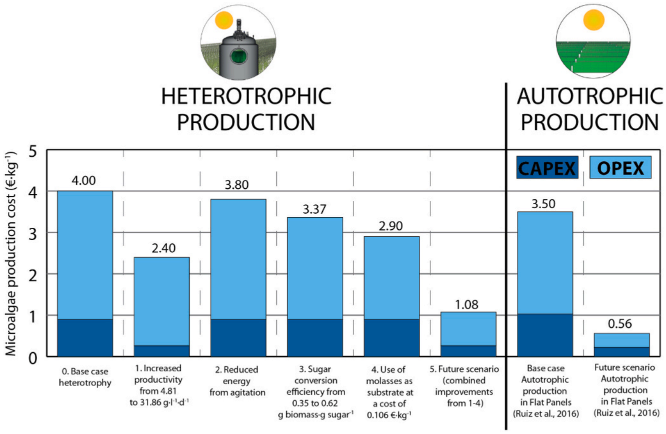

Fig. 3. Projected biomass production costs (cultivation and harvesting) for current scenarios and the future projection for the south of Spain. Costs as the sum of CAPEX and OPEX.

3. Results and discussion

3.1. Techno-economic analysis: heterotrophic production of microalgae in the current scenario

The first scenario to analyze is the base case, i.e. the projection of the model based on assumptions or inputs achievable or considered as more reasonable for production in the immediate future. These assumptions are variables like species, volumetric productivity, or source of organic carbon. The techno-economic analysis brings a biomass cost for the base case in heterotrophic production of 4.00 €·kg^{-1} dry weight for a centrifuged biomass (Fig. 3). Although results may be very dependent on location, production capacity, or other assumptions, this projected value falls between costs estimated by other authors; previous studies projected costs for heterotrophic production between 1.45 €·kg^{-1}, from optimistic key assumptions like productivity to a less promising 11.28 €·kg^{-1} [20,21].

Our previous work [22], showed that a production facility of 100 ha consisting of flat panels reactors in Spain would produce 6094 tons of microalgae per year (as dry weight). The production cost for the resulting centrifuged paste of microalgae was 3.50 €·kg^{-1} dry weight, with a potential reduction to 0.56 €·kg^{-1} during the next decade (costs updated to the year 2020 based on Harmonized Consumer Price Index in Spain). After comparing the production cost from our heterotrophic base case (4.00 €·kg^{-1}) and the base case of autotrophic production in flat panels (3.50 €·kg^{-1} [22]), the latter appears as the most economic option (Fig. 3). However, it depends on the production system, as our previous study also reveals that photosynthetic production in tubular photobioreactors or open ponds can reach a cost above 5.20 €·kg^{-1} [22]. Thus, although we can claim that autotrophic culture in flat panels would be cheaper than heterotrophic production, this is not always the most expensive option.

Nineteen fermenters, with a working volume of 250.7 m^3 each, are then required for this base case. As a reference, large-scale production of heterotrophic microalgae was already done in the '90s, in fermenters with capacities up to 150 m^3 [14]. Nowadays, Solazyme, Roquette, and Corbion have fermentation tanks with even greater capacities. Needless to say, the surface required is far below 100 ha in heterotrophic production, which is considered in the cost calculation.

Our analysis of required investments reveals that, despite the large difference in the type of reactors and area occupied in heterotrophic and autotrophic productions, the investment is remarkably similar in both base cases. While autotrophic production in flat panels requires 90.6 M€ [22], fermentation involves the investment of 76.4 M€ (for the annual capacity in both cases of 6094 tons of microalgae) (Table 3). Production systems in heterotrophy (i.e. fermenters, seed fermenters, and their internal agitators) are the most expensive equipment, generating 92% of Major Equipment Cost. Other authors also identified capital investment for microalgal fermentation mainly attributed to reactors [43]. On the contrary, autotrophic production in flat panels involves a reduced cost from the production systems themselves (27% of Major Equipment Cost) and a greater amount due to additional equipment (blowers, centrifuges…).

As commented, the investments are comparable in both production alternatives; nonetheless, the biomass production cost is higher in heterotrophy. At this point, the relevant effect of OPEX in heterotrophic cultivation becomes apparent. A closer look at the production cost shows that from the calculated 4.00 €·kg^{-1}, 0.89 €·kg^{-1} is CAPEX (22%) and 3.11 €·kg^{-1} OPEX (78%) (Fig. 3). These percentages were 29% and 71% for CAPEX and OPEX in autotrophic production, respectively. In the base case for heterotrophy, OPEX itself is almost as high as the total production cost in autotrophy, which is striking. The majority of costs due to OPEX are fundamentally from electricity and the organic carbon source (Table 3), a result shared with other authors studying heterotrophy from microalgae [21]. Glucose and energy contribute to 78% of OPEX, with 1.26 €·kg^{-1} and 1.16 €·kg^{-1} respectively. Despite requiring

Table 3
Economic results from projections 1–5 on heterotrophic production. All cases are for facilities producing 6094 tons of microalgae per year. The values in the cost breakdown are the percentages of the results in Fig. 3.

Case	Initial investment (M€)	Biomass production cost breakdown (%)									
		Capital cost	Raw materials	Utilities	Energy	Labor	Wastewater treatment	Maintenance	Operating supplies	General plant overheads	Other contingencies
0. Base case	76.4	22.3	32.5	<0.1	28.9	3.5	0.2	3.6	0.2	3.9	4.9
1. Productivity 31.86 $g\cdot l^{-1}\cdot d^{-1}$	22.6	11.0	53.5	0.3	15.0	5.8	0.1	1.8	0.3	4.2	8.1
2. Reduced energy from agitation	76.4	23.5	34.2	<0.1	25.2	3.6	0.2	3.8	0.2	4.1	5.1
3. Sugar conversion efficiency 0.62 g biomass·g sugar^{-1}	76.4	26.6	22.4	<0.1	34.3	4.1	0.2	4.3	0.2	4.6	3.4
4. Molasses as substrate	76.4	30.8	11.9	<0.1	39.9	4.8	0.2	4.9	0.2	5.3	1.8
5. Future scenario (combined Cases 1-4)	22.6	24.5	18.1	0.1	28.0	12.8	0.3	3.9	0.2	9.2	2.7

the addition of CO_2 and nutrients, the contribution of these raw materials is not much higher than 0.4 $€\cdot kg^{-1}$ in the autotrophic strategy. A detailed analysis of energy consumption shows that almost 70% is due to agitation of the culture; a common point with autotrophy [22]. The next key factors in energy usage are sterilization of the vessels by pressurized steam and aeration to supply oxygen. Centrifugation only demands 0.1% of energy requirements in heterotrophy, as cell densities after batch cultures are high, reaching 65 $g\cdot l^{-1}$.

Harvesting is a relevant factor in autotrophic production, with 5 to 23% of cultivation cost due to centrifugation [22]. However, the denser cultures reduce this contribution to 3.2% in heterotrophic production.

Our study also revealed that, although water use could be an issue from an environmental perspective, the contribution to the total cost is below 1.5%. An operation aimed at recycling the water would not have a relevant effect on the biomass production cost.

3.2. Pigments and oils: product richness matters

To date, major fields of commercial production of microalgae include whole biomass, but also oils and pigments [44]. Up to this point, we have simply analyzed biomass production cost, regardless of its composition. Despite a thorough analysis of the cost of products being out of the scope of our study, we can make some statements on the issue. Firstly, although autotrophy results in cheaper biomass production, these costs for autotrophy and heterotrophy do not differ remarkably; both values are in the same order of magnitude. Secondly, the richness of oils and pigments in the biomass is notably different within these culture strategies, being autotrophic microalgae richer in pigments and heterotrophic richer in oils. Hence, it indicates a lower production cost of pigments under autotrophy and a more favorable cost for the production of oils in heterotrophic conditions. This statement agrees with the current industrial production of pigments and oils from microalgae, which are mainly performed in photobioreactors and fermenters, respectively.

3.3. Techno-economic analysis: future potential

Developments of this, still to expand technology, may ensure a lower production cost than the initially established in our base case. The following projections in our model provide a closer look into the future perspective of the heterotrophic production of microalgae.

Productivity is the most influential aspect in total costs, as the impact of its increase results in a direct cost decrease. There are strategies that can substantially increase biomass productivity. Kim et al. [45] demonstrated a significant increase in the biomass productivity of heterotrophic cultivation of Chlorella sp. HS2 from the culture medium optimization and phosphorus feeding strategy. The use of mixed carbon sources has revealed benefits in microalgae production [18], as also shown in other organisms due to the stimulation of reductive metabolism [46]. The original biomass productivity of 4.81 $g\cdot l^{-1}\cdot d^{-1}$ adopted for our base case is not a daring assumption. Indeed, values more than sixteen times greater were reported in 200 l volume [3]. In case we assumed in our projection a productivity of 31.86 $g\cdot l^{-1}\cdot d^{-1}$, as the greatest value from Supplementary Table 1, the effect would be relevant. This is a volumetric productivity increase of 6.6 times when compared to the initial base case. In this case, the productivity of the facility is also fixed at 6094-ton·year^{-1} to establish a comparable scenario to our previous study on autotrophy. Therefore, this greater volumetric productivity firstly results in a lower volume of fermenters. Achieving 31.86 $g\cdot l^{-1}\cdot d^{-1}$ for productivity results in a potential cost decrease from the original 4.00 $€\cdot kg^{-1}$ to 2.40 $€\cdot kg^{-1}$ (Fig. 3). Under this assumption, the cost of glucose becomes the most important factor, accounting for 52.5% of total costs.

There are claims that productivities up to 100 $g\cdot l^{-1}\cdot d^{-1}$ are feasible. To the best of our knowledge, only one study has demonstrated a productivity in the order of 80 $g\cdot l^{-1}\cdot d^{-1}$ [3]. We have not included these high values in our projections, as consistently attaining those values currently seems overambitious. As a matter of fact, such achievement would only drop total costs at around 2 $€\cdot kg^{-1}$, being the reduction hampered by the cost of glucose, as it would still represent 63% of the costs.

As commented, the cost structure in the base case showed an intense OPEX from glucose and energy. Both of these factors can improve in the next future, with more efficient systems, a greater glucose conversion, or alternative sources of organic carbon.

The stirring of tank reactors is a main factor in the power consumption in process industries, a fact that we also found in this work. Different studies have demonstrated that there is a margin for improvement. Proper engineering or novel impeller design can achieve energy savings between 25 and 66% [39,40]. Assuming a 25% lower energy consumption in agitation as the only change, the cost could reduce in 0.20 $€\cdot kg^{-1}$, reaching production cost of 3.80 $€\cdot kg^{-1}$ (Fig. 3).

Typical biomass yields on glucose in microalgae are below 0.5 g biomass·g sugar^{-1} [14], and our base case considered a value of 0.35. However, greater levels of sugar conversion efficiency can be achieved. Assuming a sugar-to-biomass conversion of 0.62 g biomass·g sugar^{-1}, as shown in previous works for *Chlorella* spp. [41,42], a reduction to 3.37 $€\cdot kg^{-1}$ could be achieved (Fig. 3). The effect of more efficient use of glucose greatly reduces the effect of the high cost of this substrate.

Previous studies pointed at glucose as a relevant reason behind the costs of microalgal heterotrophic cultures [14]. This is something we also identified. Glucose, at a cost of 0.44 $€·kg^{-1}$ represents itself 31% of total production cost (1.26 $€·kg^{-1}$ biomass). Some industrial by-products rich in the organic matter could be a low-cost substitution for this glucose. Molasses from sugar refineries are a substrate meeting criteria for production media and used in all sorts of fermentation processes, resulting even appropriate for production of specialty products [47]. This by-product is also a suitable source of fermentable sugars to produce microalgal biomass, as confirmed by the promising results from several studies [43,48,49]. Besides, similar sugar-to-biomass conversions are reported for glucose and molasses, with values between 0.45 and 0.54 g biomass·g $sugar^{-1}$ for molasses [43,49]. This allows us to safely maintain in our projection for molasses as carbon source the original biomass yield used for glucose (0.35 g biomass·g $sugar^{-1}$). Molasses, at a cost of 0.106 $€·kg^{-1}$ [24] would drop production costs from 4.00 $€·kg^{-1}$ to 2.90 $€·kg^{-1}$ of biomass (Fig. 3). Yan et al. [43] found a halved production cost of lipids when glucose was replaced with molasses.

So far in this section, the changes were independent, studying the effect of each individual change. However, the higher productivity, a more efficient impeller, and the use of cheaper sugars with greater biomass conversion could take place simultaneously in the culture. Hence, the future scenario could bring a combination of all the above mentioned achievements. All of these improvements occurring in the cultivation represent a pronounced reduction in production cost, achieving 1.08 $€·kg^{-1}$ (Fig. 3). This is a similar cost to the production of baker's yeast [23]. In our previous study, autotrophic production also showed a substantial potential cost reduction once expected future achievements were implemented, dropping to 0.56 $€·kg^{-1}$ [22] (Fig. 3).

3.4. *Tackling the current challenges*

Despite the inherited knowledge from classical commercial fermentation, which could support heterotrophic microalgae production, it has not been widely applied to microalgae. Certainly, some practical limitations are restraining its development:

The number of microalgal species able to grow heterotrophically is restricted. Although metabolic pathways for carbohydrates are present in microalgae, the inability to transport sugars is a major reason for the obligate autotrophy [50]. Genetic engineering has demonstrated being a tool to overcome this trophic burden, as it allowed some obligate photoautotrophic species to thrive on sugars in darkness. Expression of sugar transporters in obligate autotrophs can accomplish this trophic conversion in microalgae [51,52]. Nevertheless, the inability to absorb organic compounds may not be the only reason for obligate phototrophy, since some phototrophs can uptake sugars [50]. It could be species-dependent, but lesions in central metabolism possibly explain some cases [53]. Therefore, the development of cell lines able to import carbohydrates is not a universal trigger towards heterotrophy. In this regard, further development of heterotrophy involves still controversial genetic engineering.

Unquestionably, biological pollution is an existing constraint at large-scale cultivation, taking research an active part to tackle it and benefit industrial production [54]. Keeping a large photobioreactor sterilized and free of bacterial and fungal contamination in long-term operation is practically out of reach nowadays. Autotrophic culture media are formulated with the absence or minimum organic matter, limiting the growth of other organisms. However, in practice, a dense culture in mass cultivation in photobioreactors is not virtually free of organic matter, as released organic compounds or debris will be present. Oppositely, the culture media used to produce heterotrophic microalgae is rich in sugars at all stages. Heterotrophic cultures present high concentrations of soluble organic compounds, which requires extreme caution to avoid other microorganisms colonizing the fermenter.

In many cases, the commercialization of intracellular compounds is the driving force towards industrialization. Light-induced and other metabolites are reduced in darkness, placing heterotrophy at a disadvantage in some business cases. This may even result in a direct exclusion of this option at the initial conception stage.

A more specific issue on heterotrophic production is the inhibitory effect on the growth of high organic substrate concentrations in the medium. Microalgae present a low affinity for soluble organic matter in the medium. Therefore, relatively high concentrations of soluble organic carbon favor growth. Nevertheless, substrate inhibition is a common problem in commercial production [14]. As this is more pronounced in batch cultivation, culture systems like fed-batch cultivation, where carbon is strategically supplied to maintain its levels below noxious concentrations, can minimize this issue. Other culture systems like continuous cultivation, perfusion, and perfusion-bleeding can also remove toxic metabolites generated by the cells [14]. Still, these are less explored cultivation modes. Adaptive laboratory evolution may also relieve the toxic effect of high concentrations of organic substrates during heterotrophic cultivation. This technique progressively adapts microalgae to challenging conditions, resulting in mutations that improve the genotype. This has been shown to increase tolerance to sugars in microalgae [55].

3.5. *The bottom line*

Despite the short gap in terms of microalgae production cost, worldwide autotrophic production prevails over cultivation in darkness. Indeed, this is a complicated issue, where financial reasons must be just one more added cause for producers to opt for (sun) light-based cultivation.

Autotrophic production of microalgae is the classic approach and the most used at an industrial level. Production in open ponds offers a significantly simpler solution when we take into consideration quality standards and strict GMP to meet in fermentation. This may be the turning point for small companies considering both alternatives. Additionally, autotrophy lacks dependence on a source of organic carbon — potentially used as a human food source — and consequently, results in a shorter route from solar energy to products.

As a result of the wide experience on fermentation, gained along decades, breakthroughs in the process itself or fermenter design are currently limited. Microalgae production in heterotrophy shares most of its foundation with fermentation. Nevertheless, a quantum improvement is still needed to vanquish the described issues, which limit industrial production. Metabolic engineering could potentially act as a trigger for this, rendering a robust microalga strain able to consume cheap sources of organic carbon, while being rich in interesting metabolites, and all this must come without giving up on high productivities.

4. Conclusions

The results show a cost for heterotrophic production of microalgae at an industrial scale of 4.00 $€·kg^{-1}$ dry weight as a centrifuged paste. This is a comparable cost to autotrophic production. OPEX represent 78% of total costs in heterotrophic cultivation, mainly due to the contribution of energy and glucose. There is potential to reduce the total cost to 1.08 $€·kg^{-1}$. Microalgae need a leap forward in production scale to become a competitive novel feedstock for biobased products. A facility producing thousands of tons of biomass per year could benefit from the economy of scale, overcoming most cost restraints.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2022.102698.

CRediT authorship contribution statement

Individual contributions to the paper using the relevant CRediT roles are the following:

- **Jesús Ruiz**: Conceptualization; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing – original draft.
- **René H. Wijffels**: Conceptualization; Funding acquisition; Writing – review & editing.
- **Manuel Dominguez**: Formal analysis; Methodology; Writing – review & editing.
- **Maria J. Barbosa**: Conceptualization; Funding acquisition; Supervision; Writing – review & editing.

Data and materials availability

All data are available in the main text.

Funding

This work was supported by the project titled MAGNIFICENT - "Microalgae As a Green source from Nutritional Ingredients for Food/Feed and Ingredients for Cosmetics by cost effective New Technologies", funded by Bio-based Industries Joint Technology Initiative under H2020 of the European Research Council [project ID: 745754].

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.

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