

Research Paper

Scale-up of Astaxanthin Production from *Haematococcus pluvialis*

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ABSTRACT: Astaxanthin is a valuable ketocarotenoid with exceptionally strong antioxidant activity, widely applied in nutraceutical. pharmaceutical, aquaculture, and food industries. Among natural sources, Haematococcus pluvialis is regarded as the most efficient producer due to its ability to accumulate high levels of Astaxanthin under stress conditions. This study aims to scale up Astaxanthin production from laboratory to industrial scale to achieve 10 kg of purified product. The process design consisted of six main stages: cultivation, harvesting, pretreatment, extraction, purification, and recovery. Laboratory data were integrated with literature references to construct a reliable industrial-scale model. This research adopts a hybrid approach, in which laboratory experimental data for extraction and purification are integrated with literature-based modelling to construct a reliable industrial-scale process design. Microwave-assisted extraction (MAE) was employed, vielding a recovery rate of 57.42%, while purification via column chromatography achieved an efficiency of 85.95%. Mass and energy balances indicated that approximately 405.32 kg of dried microalgae, equivalent to 1,945.35 kg of wet biomass, are required to obtain 10 kg of Astaxanthin. The scale-up process revealed that cultivation, harvesting, and solvent recovery are the most energy-intensive stages, suggesting the need for optimisation of operating conditions and recycling strategies. Despite these challenges, the results demonstrate the technical feasibility of large-scale Astaxanthin production and provide essential data for process design, cost estimation, and sustainability assessment. Overall, this research contributes to bridging laboratory findings with industrial applications and supports future commercialisation of Astaxanthin to meet increasing global demand for high-value natural antioxidants.

Keywords: Astaxanthin; Haematococcus pluvialis; scale-up; microwave assisted extraction; column chromatography

1. INTRODUCTION

Astaxanthin is a high-value ketocarotenoid compound with exceptionally strong antioxidant activity [1], surpassing that of vitamin E [2] and vitamin C [3], [4]. As a result, it offers numerous health benefits, including anti-inflammatory properties, as well as support for eye, heart, nerve, and immune system health, and even cancer prevention [5]-[7]. Due to its safety and the stability of its red pigment, Astaxanthin is also widely used as a colouring agent in aquaculture [8] and the food industry [9].

Among various natural sources, the microalga Haematococcus pluvialis (H. pluvialis) is recognised as the most effective natural producer of Astaxanthin, capable of accumulating up to 4% of its dry cell weight under stress conditions [10]. Microalgae have the advantage of a shorter harvesting time, and higher growth rate and biomass productivity compared to other plants, making them a promising candidate for further development [11], [12].

Due to its benefits and wide applications [13]. The demand for Astaxanthin has increased rapidly [14], [15], reaching approximately USD 800 million in 2022 [16]. Astaxanthin is expected to become a potential solution for various future human health problems [17].

Although extensive research has been conducted at the laboratory scale to optimise Astaxanthin production, scaling up to the pilot plant level is a crucial step before industrial application. This stage is essential to evaluate process performance under more realistic conditions [18], [19]. The successful scale-up of Astaxanthin production from H. pluvialis is key to bridging laboratory research with sustainable and economically viable industrial-scale production. This study aims to scale up Astaxanthin production to 10 kg,

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starting from cultivation, harvesting, and extraction, and ending with purification, in order to provide scale-up data for the industrial production of this antioxidant compound.

2. METHODOLOGY

Astaxanthin production consists of five stages: cultivation, harvesting, pretreatment, extraction, and purification. This study employs a hybrid methodology combining experimental work and modelling. Laboratory experiments were conducted for the extraction and purification stages, while cultivation, harvesting, and large-scale mass and energy balances were developed using literature data and process modelling. The data for the cultivation to harvesting stages were based on [20], while the data for pretreatment, extraction, and purification referred to studies by [21] and [22].

Several key assumptions were applied during the scale-up calculations to ensure consistency between laboratory data and industrial-scale modelling, including:

- (1) The composition of *H. pluvialis* biomass and its astaxanthin content remain constant regardless of scale;
- (2) Mass and energy requirements scale linearly based on production capacity;
- (3) Solvent recovery efficiencies follow industrial data, specifically 80% for acetone and 99.7% for chromatography eluents;
- (4) Due to the unavailability of astaxanthin solubility data in acetone, the solubility of lutein—another carotenoid with a similar structure—was used as a proxy; and
- (5) No additional material losses occur during transfer, equipment handling, or operational downtime beyond those quantified in the purification step.

3. RESULTS AND DISCUSSIONS

The scale-up process was carried out to produce 10 kg of Astaxanthin. The scale-up calculations in this study were performed under several assumptions, including constant biomass characteristics, linear scalability of process requirements, solvent recovery efficiencies based on industrial benchmarks, and the use of lutein solubility data as an approximation for astaxanthin in acetone. Figure 1 shows the Astaxanthin production scheme based on [20].

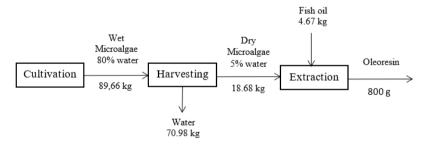


Figure 1. Schematic diagram of the Astaxanthin production process in the form of oleoresin based on [20].

Figure 2 illustrates the production process scheme for 10 kg of Astaxanthin.

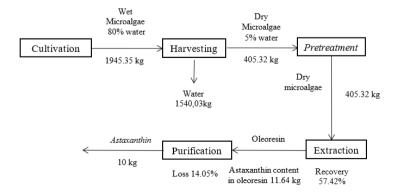


Figure 2. Process flow diagram for the production of 10 kg of Astaxanthin.

In the production process of 10 kg of Astaxanthin, the extraction was carried out using the Microwave Assisted Extraction (MAE) method, resulting in a recovery rate of 57.42%. Following the extraction, the purification process was conducted using column chromatography. The amount of Astaxanthin retained on the silica gel was 14.05%, consistent with the findings reported by [23].

As shown in Figure 1, the cultivation and harvesting process conducted by [20] yielded 18.68 kg of dried microalgae. In contrast, the scale-up process to produce 10 kg of Astaxanthin required 405.32 kg of dried microalgae. The cultivation and harvesting stages were based on the data from [20], with all process components scaled up by a factor of 21.70. This factor was obtained by dividing the amount of dried microalgae required to produce 10 kg of Astaxanthin (405.32 kg) by the dried microalgae yield reported by [20].

3.1. Scale-up of the cultivation process

The cultivation process refers to the data from [20], which involved the production of 800 grams of carotenoids in the form of oleoresin with an Astaxanthin content of 10%. The cultivation process consists of two stages: the growth phase and the stress phase. According to [20], to obtain *H. pluvialis* microalgae with an Astaxanthin content of 4–5%, specific nutrients are required to support algal growth. In addition, this process also generates waste, as presented in Table 1.

Table 1. Input and output materials in the cultivation process

	Input (kg)	Waste (kg)
Poliamide	0.117	
$NaNO_3$	4.465	0.089
K_2HPO_4	0.912	0.019
KH_2PO_4	0.404	0.009
CaCl ₂	0.291	0.004
$MgSO_4$	1.347	0.019
NaCl	0.119	0.002
$C_6H_8O_7$	0.029	$4x10^{-4}$
$C_6H_{5+4y}Fe_xN_yO_7$	0.029	$4x10^{-4}$
Na_2CO_3	0.478	$6.7x10^{-3}$
$C_{10}H_{16}N_2O_8$	0.026	$4x10^{-4}$
H_3BO_3	0.014	$2x10^{-4}$
$ZnSO_4$	0.001	$1x10^{-5}$
CuSO ₄	$4x10^{-4}$	$5x10^{-6}$
$Co(NO_3)_2$	$2x10^{-4}$	$3x10^{-6}$
FeCl ₃	$2.8x10^{-3}$	$3.9x10^{-5}$
$ZnCl_2$	1×10^{-4}	$2x10^{-6}$
$CoCl_2$	1×10^{-4}	$1x10^{-6}$
$MnCl_2$	$9.8x10^{-3}$	1.38x10 ⁻⁴
Na_2MoO_4	$1.2x10^{-3}$	1.6×10^{-5}
River water	2.786 m^3	
Inoculum	0.017	
Air	434.72 t	434.72 t
CO_2	0.26 t	0.24 t

The material requirements for cultivation presented in Table 1 were used as the basis for scaling up to obtain 89.66 kg of wet microalgae with 80% moisture content. The results of the scale-up calculations for

the cultivation process to produce 1,945.35 kg of wet microalgae with 80% moisture content are shown in Table 2.

Table 2. Scale-up of Input and Output Materials for the Cultivation Process

	Cultiv	ation (Growth an	d Stress Phas	es)	
	800 g Carotenoid Production [20]		10 kg Astaxanthin Production		Production
	Input (kg)	Waste (kg)	Input (kg)	Waste (kg)	Next process (kg)
Polyamide	0.117		2.537		
$NaNO_3$	4.465	0.089	96.893	1.933	
K_2HPO_4	0.912	0.019	19.793	0.421	
KH_2PO_4	0.404	0.009	8.769	0.187	
CaCl ₂	0.291	0.004	6.323	0.089	
$MgSO_4$	1.347	0.019	29.236	0.410	
NaCl	0.119	0.002	2.591	0.037	
$C_6H_8O_7$	0.029	$4x10^{-4}$	0.623	0.009	
$C_6H_{5+4y}Fe_xN_yO_7$	0.029	$4x10^{-4}$	0.623	0.009	
Na_2CO_3	0.478	0.007	10.368	0.145	
$C_{10}H_{16}N_2O_8$	0.026	$4x10^{-4}$	0.571	0.009	
H_3BO_3	0.014	$2x10^{-4}$	0.297	0.004	
$ZnSO_4$	0.001	$1x10^{-5}$	0.024	2.17x10 ⁻⁴	
CuSO ₄	$4x10^{-4}$	$5x10^{-6}$	0.009	$1.09x10^{-4}$	
$Co(NO_3)_2$	$2x10^{-4}$	$3x10^{-6}$	0.004	6.5×10^{-5}	
FeCl ₃	0.003	$3.9x10^{-5}$	0.061	8.46x10 ⁻⁴	
$ZnCl_2$	$1x10^{-4}$	$2x10^{-6}$	0.002	$4.3x10^{-5}$	
CoCl ₂	1×10^{-4}	$1x10^{-6}$	0.002	$2.2x10^{-5}$	
$MnCl_2$	0.010	1.38x10 ⁻⁴	0.213	$3x10^{-3}$	
Na_2MoO_4	0.001	1.6×10^{-5}	0.026	3.47x10 ⁻⁴	
River water	2.786 m^3		60.456 m^3		
Inoculum	0.017		0.369		
Air	434.72 t	434.72 t	9433.424 t	9433.424 t	
CO_2	0.260 t	0.24 t	5.642 t	5.208 t	
H. Pluvialis 80%					1945.35

The energy requirements for the cultivation process are presented in Table 3.

Table 3. Energy Requirements for the Cultivation Process According to [20].

Type of process	Energy, kWh	
Type of process	800g Carotenoid Production [20]	
Reactor cleaning process		
Ozonated water circulation energy	0.12	
Reverse osmosis filtration	7.71	
UV filtration	0.35	
Cultivation		
1. Growth Phase		
CO ₂ supply	48.11	
Mixing	48.11	
Lighting in the photobioreactor	769.72	
2. Stress Phase		

Type of process	Energy, kWh	
Type of process	800g Carotenoid Production [20]	
CO ₂ supply	48.11	
Mixing	48.11	

The scaled-up energy requirements for the production of 10 kg of Astaxanthin are presented in Table 4.

Table 4. Scaled-Up Energy Requirements for the Cultivation Process

Type of pueces	Energy, kWh		
Type of process	800g Carotenoid [20]	10 kg Astaxanthin	
Reactor cleaning process			
Ozonated water circulation energy	0.12	2.60	
Reverse osmosis filtration	7.71	167.31	
UV filtration	0.35	7.60	
Cultivation			
1. Growth Phase			
CO ₂ supply	48.11	1,043.99	
Mixing	48.11	1,043.99	
Lighting in the photobioreactor	769.72	16,702.92	
2. Stress Phase			
CO ₂ supply	48.11	1,043.99	
Mixing	48.11	1,043.99	

3.2. Scale-Up of the Harvesting Process

In the harvesting process, the moisture content of the microalgae is reduced from 80% to 5%. No additional materials are used in this process; only energy is required to decrease the water content in the microalgae. Table 5 presents the energy requirements for the harvesting process according to [20].

Table 5. Energy Requirements for the Harvesting Process According to [20].

Type of process	Energy, kWh
Type of process —	800 g Carotenoid Production [20]
Centrifugation	1.50
Spray drying	82.70

The scaled-up energy requirements for the harvesting process are presented in Table 6.

Table 6. Scaled-Up Energy Requirements for the Harvesting Process

Type of nyones	Energy, kWh 800 g Carotenoid [20] 10 kg Astaxanthin	
Type of process		
Sentrifugation	1.50	32.55
Spray dryer	82.70	1,794.59

3.3. Scale-Up of the Pretreatment Process

The pretreatment was carried out by mixing microalgae with 4N HCl solution prior to extraction. The ratio of microalgae to 4N HCl solution used was 1:10 w/v (grams/mL), at a temperature of 70°C for 2 minutes.

The scaled-up requirement of 4N HCl for the pretreatment of 405.32 kg of microalgae was 4,053.20 L, with an additional 20% make-up volume of 810.64 L. The required amount of 37% HCl was 270.21 L,

which was mixed with 540.43 L of distilled water. The energy required in the pretreatment process includes energy for heating, stirring, centrifugation, and spray drying to dry the microalgae prior to extraction. The energy used for heating is calculated using Equation (1).

$$Q=m.c.\Delta T.$$
 (1)

with:

Q = Energy required for heating, J.

m = Heated mass (g), 4,053.20 L HCl or 3,553.52 kg or 3,553,520 g.

c = specific heat HCl 4N $(J/g.^{\circ}C)$, 4.18 $J/g.^{\circ}C$.

 ΔT = temperature increase (°C), 40°C (70°C – 30°C).

From the calculation, the value of Q was obtained 594,148,544 J or 170 kWh.

The energy used for stirring and centrifugation was scaled up based on data from [20]. Stirring 1,000 L or 1,000 kg for 8 days, operating continuously for 24 hours per day, requires 48.11 kWh of energy. Therefore, stirring 405.32 kg of microalgae with 4,053.20 L or 4,441.90 kg of 4N HCl for 2 minutes requires 0.04 kWh.

The energy required for centrifugation during the harvesting process is 1.50 kWh to separate 1,540.03 kg or 1,540.03 L of water. Therefore, to separate 4,053.20 L of 4N HCl, an energy of 3.95 kWh is needed. The energy for the spray dryer is used to dry the microalgae after centrifugation, ensuring that the microalgae entering the extraction process are completely dry. This energy is equivalent to the energy required to evaporate 20% of the total amount of 4N HCl used. The required energy is calculated using Equation (2).

$$Q = m.H$$
(2)

with:

Q = The energy required to evaporate 4N HCl (cal).

m = The mass of 4N HCl evaporated (g).

H = Latent heat of 4N HCl (cal/g).

Since latent heat data is only available for 12N HCl, Equation (2) is calculated by summing the energy required to evaporate 12N HCl and the energy needed to evaporate the amount of water added to obtain 4N HCl, as shown in Equation (3). A total of 810.64 L of 4N HCl consists of 270.21 L of 12N HCl, equivalent to 324.25 kg or 324,250 g (assuming the density of 37% HCl is 1200 kg/m³), and 540.43 L of water, equivalent to 538.81 kg or 538,810 g.

$$Q = Q_{HC112N} + Q_{H2O}$$
 (3)

$$Q = (m.H)_{HC112N} + (m.H)_{H2O}...(4)$$

with:

 $H_{HCL12N} = 103.12 \text{ cal/g}; \quad H_{H2O} = 556.40 \text{ cal/g}$

 $Q = 324250g \times 103.12cal/g + 538810 g \times 556.40 cal/g$

Q = 33436660 cal + 299794160.5 cal

Q = 38.86 kWh + 348.42 kWh

Q = 387.28 kWh

The energy required during the pretreatment process of microalgae prior to the extraction stage for the production of 10 kg of Astaxanthin is presented in Table 7.

Table 7. Energy Requirements in the Pretreatment Process

Process	Energy, kWh
Heating 70°C	170.00
Mixing	0.04
Centrifugation	3.95
Spray dryer	387.28

3.4. Scale-Up of the Extraction Process

In the laboratory-scale study, the extraction process achieved a recovery of 57.42% Astaxanthin by extracting 2.5 grams of dried microalgae powder in 250 mL of acetone. The study by [24] also reported that the use of acetone as a solvent in the extraction of Astaxanthin from *H. pluvialis* resulted in a higher Astaxanthin yield compared to methanol. For the industrial-scale production of Astaxanthin, the scale-up process refers to the solubility data of Astaxanthin in acetone. However, since solubility data of Astaxanthin in acetone is not available in previous literature, it is approximated using the solubility data of lutein in acetone. Lutein also belongs to the carotenoid group and has a molecular structure similar to that of Astaxanthin. The solubility of lutein in acetone is reported to be 800 mg/L or 0.8 g/L [25].

The use of acetone as a solvent can be recycled up to 80% of the total amount used [26]. The scale-up of the extraction process is carried out using an industrial-scale microwave extractor with a capacity of 100 L, a power requirement of 30 kW, and a frequency of 915 MHz. In a single extraction cycle, 1.5 kg of microalgae can be extracted using 93.75 L of acetone. Therefore, to extract 405.32 kg of microalgae containing 5% Astaxanthin (equivalent to 20.27 kg), a total of 270 extraction cycles are required, with a total acetone requirement of 5,067.50 L. The extraction yields 11.64 kg of Astaxanthin, which is still mixed with other carotenoid compounds in the form of oleoresin. Table 8 presents the results of the scale-up of the extraction process from the laboratory scale in this study.

Table 8. Scale-Up of Materials in the Extraction Process for the Production of 10 kg Astaxanthin.

	Laboratory scale	Scale up
Microalgae	2.5 g	405.32 kg
Acetone	250 mL	5067.50 L

In the extraction process, the required energy consists of the energy for microwave-assisted extraction and the energy for solvent recovery. A single extraction process lasting 5 minutes requires 2.5 kWh of energy; therefore, 270 extraction cycles require a total of 675 kWh. The energy required for solvent recovery, amounting to 80% of the total solvent used or 20,270 L (equivalent to 15,918,436 g), can be calculated using Equation (5).

$$Q = m.H$$
(5)

with:

Q = The energy required to evaporate acetone (cal).

m = The mass of evaporated acetone (g), 15,918,436 g.

H = Latent heat of acetone (cal/g), 23.42 cal/g.

then:

Q = 15,918,436 g x 23.42 cal/g

Q = 372,809,771 cal

Q = 433.29 kWh

The scale-up of energy requirements based on experimental data for the extraction process in the production of 10 kg of Astaxanthin is presented in Table 9.

Table 9. Scale-Up of Energy Requirements in the Extraction Process

	Energy requirement, kWh
Extraction	675
Solvent recovery	433.29

3.5. Scale-Up of the Purification Process

In the laboratory-scale of purification process, 5 mL of oleoresin containing 33.41 mg/L of Astaxanthin was purified using column chromatography with an eluent consisting of a mixture of *n*-hexane and acetone in volumes ranging from 369.23 to 519.00 mL. The column used had a volume of 0.1413 L. The amount of purified Astaxanthin obtained ranged from 132.65 to 140.60 micrograms. Therefore, it can be concluded that the eluent requirement per gram of Astaxanthin product is 2,783.70 to 3,627.31 mL/g of product or 2,783.70 to 3,627.31 L/kg of product.

The results of this study differ significantly from the specifications of industrial-scale purification equipment from Novasep Inc. According to [27], the purification equipment from Novasep Inc. requires 1,200 L of eluent per kilogram of product, and the eluent can be recycled up to 99.7%. Therefore, to better approximate industrial-scale conditions, data from an actual industrial unit Novasep Inc.'s LPLC Prochrom®-Bio Lp-1600 was used.

To obtain 10 kg of Astaxanthin product, the purification process requires 12,000 L of eluent, of which 11,640 L can be recycled, and 36 L is needed as make-up solvent. The make-up solvent consists of 27 L of *n*-hexane and 9 L of acetone. This type of column chromatography has a maximum bed volume specification of 580 L. The chromatography column is filled with adsorbents, which may include silica gel, aluminum oxide, zeolite, activated carbon, and others. The scale-up data for the purification process are presented in Table 10.

Table 10. Scale-Up of the Purification Process for the Production of 10 kg of Astaxanthin.

	Laboratory scale	Scale up
Eluent	369.23-510 mL	12,000 mL
Bed Volume	0.1413 L	580 L
Product	132.64-140.60 μg	10 kg

The energy used in the purification process is solely for the recovery of the eluent. The amount of eluent recycled is 11,640 L, consisting of 8,730 L of *n*-hexane (equivalent to 5,718,150 g) and 2,910 L of acetone (equivalent to 2,281,440 g). The energy used is calculated using Equation (6).

$$Q = (m.H)_{hexane} + (m.H)_{acetone}...(6)$$

with:

Q = Energy for eluent recycling (cal)

 m_{hexane} = The mass of evaporated *n*-hexane (g), 5,718,150 g

 H_{hexane} = Latent heat of *n*-hexane (cal/g), 23.42 cal/g

 $m_{acetone}$ = The mass of evaporate acetone (g), 2,281,440 g

 $H_{acetone}$ = Latent heat of acetone (cal/g), 36.13 cal/g

As a result, it was found that:

Q = (5,718,150 g x 23.42 cal/g) + (2,281,440 g x 36.13 cal/g)

= 133,919,070 cal + 82,428,430 cal

= 216,347,500 cal

= 251.44 kWh

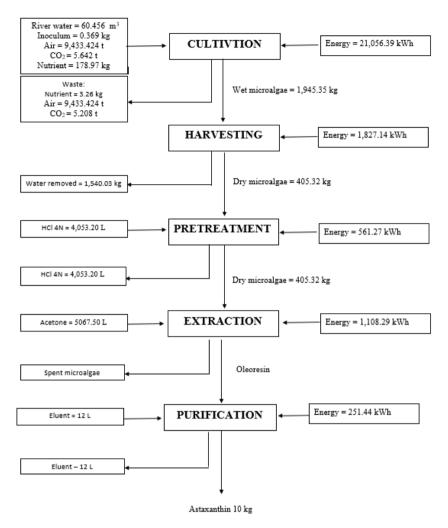


Figure 3. Overall mass and energy flow diagram for the production of 10 kg Astaxanthin.

3.6. Comparative Analysis with Existing Scale-up Studies

Our scale-up model highlights that cultivation is the most energy-intensive stage (16,702.92 kWh for lighting). This aligns with the broader consensus in LCA studies, such as [28], which reports that photobioreactors (PBR) generally exhibit a higher cumulative energy demand (CED) compared to open raceway ponds due to the requirement for artificial illumination and mixing. However, despite the higher energy cost, PBRs are often preferred for *Haematococcus pluvialis* to strictly control culture conditions and prevent contamination, which is critical for achieving the high Astaxanthin content (4%) assumed in this study.

Regarding the downstream process, our use of Microwave-Assisted Extraction (MAE) offers a rapid recovery route. While our model calculates an energy consumption of 675 kWh for extraction, it is important to benchmark this against Supercritical CO₂ (SC-CO₂) extraction, a common industrial standard. A study by [29] indicates that while SC-CO₂ eliminates the use of organic solvents, it requires significant energy for high-pressure compression. By using MAE with an optimised solvent recovery system (97% efficiency in purification), our process attempts to balance energy speed with solvent sustainability, addressing the environmental concerns typically associated with solvent-based extraction methods [30], [31].

3.7. Economic and Sustainability Implications

The scale-up results also indicate several economic and sustainability implications. From an economic perspective, the high energy demand—approximately 2,480.45 kWh per kg of astaxanthin—is one of the main contributors to operational cost, particularly for cultivation lighting, drying, and solvent evaporation. Solvent consumption, especially acetone in the extraction stage, further adds to both material cost and the need for

efficient solvent recovery systems. Improving biomass productivity, optimising photobioreactor design, or integrating waste-heat sources could significantly reduce per-unit production cost.

From a sustainability standpoint, the dominance of electricity-driven processes means that the environmental footprint is strongly linked to the electricity mix. Using low-carbon or renewable energy sources would substantially reduce life-cycle impacts, as supported by previous LCA studies. Likewise, increasing solvent recycling rates and selecting extraction methods with lower solvent requirements can minimise emissions and waste generation. Therefore, while the process is technically feasible at a large scale, economic viability and sustainability performance can be improved through energy optimisation, renewable integration, and solvent management strategies.

4. CONCLUSION

This study successfully scaled up the production process of Astaxanthin from the microalga *Haematococcus pluvialis* to yield 10 kg of pure product. The production process consists of six main stages: cultivation, harvesting, pretreatment, extraction, purification, and recovery—each designed based on laboratory data and relevant literature. The extraction process employed Microwave-Assisted Extraction (MAE) with a recovery rate of 57.42%, while purification was carried out using column chromatography, resulting in high purification efficiency with a loss rate of 14.05%. Based on the calculation of material and energy requirements, the production of 10 kg of Astaxanthin requires approximately 405.32 kg of dried microalgae, equivalent to 1,945.35 kg of wet biomass. The process also involves significant energy consumption, particularly during the cultivation, drying, and solvent recovery stages. The results of this study provide a technical overview and detailed calculations as a critical foundation for planning sustainable and economically feasible industrial-scale Astaxanthin production.

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