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Commercial astaxanthin production derived by green alga *Haematococcus pluvialis*: A microalgae process model and a techno-economic assessment all through production line

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ABSTRACT

The freshwater green microalgal strain *Haematococcus pluvialis* is the richest source for the production of astaxanthin. Astaxanthin is member of the xanthophyll family of carotenoids and constitutes the highest value product derived by microalgae. So far, algal astaxanthin amounts to <1% of the global market, since the synthetic alternative involves lower production costs. In this study, the technical and economic performance throughout large scale astaxanthin production, for two European cities (Livadeia, Greece and Amsterdam, the Netherlands), is investigated. The techno-economic assessment was facilitated by creating a theoretical process model, which simulated all phases of the production process. A hybrid system for photoautotrophic cultivation comprised by a photobioreactor (PBR) fence and a raceway pond complex was assumed for the 'green' and the 'red stage' respectively. The area covered by each cultivation system was assumed as 1 ha. The technical part included the mass-energy flows associated with the production process. The most important mass inflow refers to freshwater. More specifically, 63,526 m^3/year and 23,793 m^3/year are needed for the production of 426 kg/year and 143 kg/year astaxanthin in Livadeia and Amsterdam respectively. Regarding total energy needs, they were calculated at 751.2 MWh/year and 396.5 MWh/year for the Greek and the Dutch city respectively. With respect to the economic performance, a Profit and Loss (P&L) analysis was conducted applying three scenarios (worst-, base- and best case). Determining CAPEX and annual OPEX, the return of investment (ROI) for different market prices of astaxanthin was calculated. It was found that only in Livadeia high economic viability can be achieved for all market prices. The costs per kilogram of natural astaxanthin for Livadeia and Amsterdam were calculated at €1536/kg_{ASTAX} and €6403/kg_{ASTAX} respectively (best case scenario), rendering natural astaxanthin unable to compete with the synthetic alternative (€880/kg_{ASTAX}) yet, at least for feeding purposes.

1. Introduction

As global population and consequently energy demand increase over time the introduction and commercialization of renewable sources of energy becomes a critical issue. Microalgal biomass as feedstock for bio-energy production is an attractive alternative to bio-energy derived from terrestrial plant utilization [58]. Nonetheless, microalgae cultivation solely for bio-energy generation purposes seems not yet to be economically feasible [8,17,49,80]. Therefore, other applications of microalgae have been investigated. Microalgae, cultivated under specific stress conditions, can accumulate, along with the lipids and carbohydrates, considerable amount of secondary metabolites, whose industrial exploitation strongly enhances a bio-based economy [57].

Among these metabolites, the carotenoid pigment astaxanthin is considered to be one of the most valuable algal compounds with a wide range of applications in the food, feed, cosmetics and pharmaceutical sector [8,14,49,78]. Astaxanthin ($C_{40}H_{52}O_4$, 3,3′-dihydroxy-β,β′-carotene-4,4′-dione) is a member of the xanthophyll family of carotenoids and is ubiquitous in fresh/saltwater [57,111]. It is a substance best known for giving the pinkish-red hue to the flesh of salmonids (salmons and trouts), shrimps, lobsters and crayfishes, while it displays a central role for their immune-system and positively impacts their fertility [49]. From the nutritional point of view, astaxanthin is considered as the most powerful antioxidant in the nature, serving the role of a highly efficient scavenger of free radicals build up within the human body [49,67]. Astaxanthin is a substance that protects the skin against UV-induced photo-oxidation and it is used for anti-tumor therapies and prevention - treatment of neural damage interrelated with age-related macular degeneration, Alzheimer and Parkinson diseases [14,110]. Furthermore, it is considered as a natural superfood destined to enhance

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athletic performance by increasing stamina and reducing the time of muscle recovery [13].

Nowadays, the market value of astaxanthin varies usually from $2500–7000/kg, while its global market potential was estimated at 280 metric tons and was valued at $447 million in 2014 [8,49,43,61,78]. Of this market, more than 95% refers to synthetically derived astaxanthin, since it involves lower production costs (around $1000/kg) than the algal alternative, which accounts to < 1% of the commercialized quantity [49,52,78]. Synthetic astaxanthin is produced from petrochemical sources, which raises the issues of food safety (potential toxicity in the final product), pollution, and sustainability [52,61]. In fact, to date, synthetic astaxanthin can only be used as an additive to fish feed for pigmentation purposes and has not been approved for direct human consumption in food or supplements [52]. Thus, as society, nowadays, stimulates a transition towards 'green solutions' and natural products, while global market is estimated to exceed $1.5 billion by 2020, algae-derived astaxanthin seems to be gaining potential in the market [68,78].

Hitherto, there is scarce scientific research on the performance and viability of large scale astaxanthin production lines exploiting microalgae [52,78]. Most publications focus on the different ways to optimize technologies on laboratory-pilot scale without assessing commercialization, and/or if natural astaxanthin could compete with the synthetic alternative in the forthcoming years. This is the knowledge gap that this paper aims to fill. Furthermore, this study could also be of use for those who investigate commercialization of other microalgae products, such as biofuels.

2. Methodology

In this study a process model was created simulating large scale production of natural astaxanthin. The model calculates areal biomass-astaxanthin productivity and constituted the benchmark in order to determine the theoretical mass and energy flows all through the three phases (cultivation, harvesting, extraction) of the production process as well as to assess economic performance of such ventures.

2.1. Process model

2.1.1. Description

Microalgae cultivation constitutes the most important phase within the process. A successful cultivation results in a 'healthy' highly concentrated algal broth, which can further be processed for the recovery of the desired metabolites. Thus, the core of the process model refers to microalgae cultivation. In this paper, modeling cultivation phase is based mostly on previous attempts to simulate algae growth theoretically [44,46,89,90]. There are different nutritional modes to cultivate microalgae. In this paper, natural photoautotrophic metabolism is investigated. It involves the use of sunlight as energy source and inorganic Carbon as the Carbon source for the formation of biochemical energy through photosynthesis [41]. Before being captured by an algal cell to be metabolized, incident light is subjected to various inefficiencies and loss mechanisms. Further parameters that vigorously affect algal productivity refer to temperature and nutrients uptake (see Section 3.1).

After cultivation phase, harvesting and extraction phases take place. The goal of these phases revolves around the dewatering of the 'wet' biomass and the recovery of the pigment. There is an abundance of methods that can be employed during harvesting and extraction. A comparative research was conducted to result in the most appropriate combination of methods for the production of astaxanthin [75]. These methods were introduced into the process model in the form of recovery efficiencies (see Sections 3.2 and 3.3).

2.1.2. Regional scenarios

In photoautotrophic metabolism algae cells proliferation depends directly on the levels of solar irradiance, and significantly high or low values may result in an adverse impact on algal biomass productivity and the desired metabolite accumulation. Therefore, regional scenarios are necessary. In this paper, two European cities, Livadeia, Greece (38°43′33″ N/22° 86′67″ E) and Amsterdam, the Netherlands (52°36′67″ N, 4°90′00″ E), are chosen for investigation. The main reason of this choice was to delineate fluctuations regarding astaxanthin productivity in two locations from the same climatic zone (temperate zone) but with significantly different latitude. As main model input, detailed climate data (irradiance and temperature data) throughout a calendar year (2014) were used in order to determine biomass productivity in the two cities, from which astaxanthin is derived. For Livadeia, ETHER, a company focused on photovoltaic parks and the National Observatory of Athens (NOA) provided the appropriate climate data. For Amsterdam, the climate data were derived by the official website of Royal Netherlands Meteorological Institute (in Dutch Koninklijk Nederlands Meteorologisch Instituut-KNMI).

2.2. Mass-energy flows

In this theoretical study, the mass and energy flows all through production process were calculated, using the annual biomass-astaxanthin productivities as benchmarks. The mass flows refer to the in- and outflows of the different substrates, while the energy flows refer to the direct energy consumption of equipment within the system boundaries (see Sections 4.2 and 4.3).

2.3. Economic performance

With regard to the economic evaluation, a Profit and Loss (P&L) analysis was conducted applying three scenarios (worst-, base- and best case). The costs throughout the production chain of astaxanthin referred to the capital- (CAPEX) and operational expenditures (OPEX). CAPEX included equipment costs and fixed capital costs, while the project lifetime is assumed as 10 years. OPEX refers to all costs in order the production line to operate, and was derived from cost analysis based on the mass-energy flows associated with the different systems that build the bio-refinery along with labor, maintenance and insurance. The profits were determined by the market prices for astaxanthin and residual biomass. The P&L analysis resulted in a financial statement that calculated the return of investment (ROI) for the selected locations. The costs per kg of astaxanthin were calculated as well.

Fig. 1 summarizes the methodology that was followed, delineating all stages of the research and including the key parameters of the process model as well.

3. Construction of the process model

3.1. Cultivation phase

3.1.1. Species and culture system

There are several microalgae strains that are reported as potential feedstock to produce astaxanthin, such as *Chlorella* sp., *Chlorococcum* sp. and *Scenedesmus* sp. [22,56,79]. Nevertheless, the accumulation of astaxanthin inside *Haematococcus pluvialis* cells exceeds any other known microalgae species (up to 4% of dry biomass) and thus it is the most preferred one for large scale natural astaxanthin production [112].

H. pluvialis is a freshwater strain of green microalgae with a very unique life, which is divided in two stages [10]: The first refers to a green, motile vegetative stage, in which the microalgal cells continuously divide and proliferate, synthesizing chlorophyll. During this stage full nutrient medium and moderate light intensity, temperature and pH are required [3,10,24]. The second refers to a red, non-motile resting stage, in which cell division stops and chlorophyll levels do not fluctuate, resulting in a continuous increase of astaxanthin content and cellular dry weight. The inhibition of cell proliferation and, thus, the accumulation of astaxanthin are triggered, when microalgal cells experience nutrient starvation. Further adverse environmental conditions involve high light intensity, high temperature and salt stress [9,39,57].

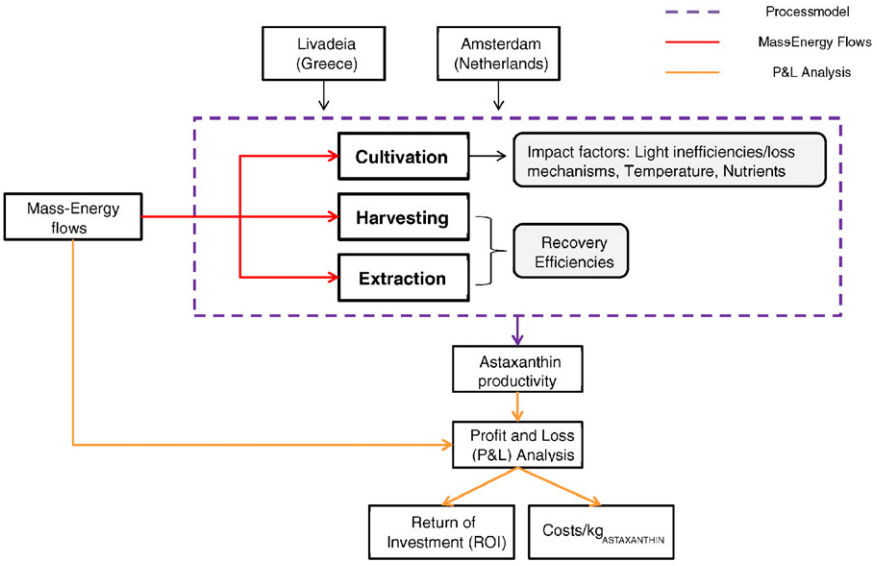

Fig. 1. Flow chart of the methods.

Due to such discrepancies, it is reported that the two stages should be separated into different cultivation systems: First producing green biomass under optimal growth conditions ('green stage') and next exposing algal cells into the abovementioned stress environmental conditions to induce astaxanthin accumulation ('red stage') [3,10,72]. Therefore, this paper investigated the performance of a hybrid culture system in continuous mode, which consists of a horizontal tubular photobioreactor fence and a complex of open raceway ponds. It was assumed that each system covered an area of 1 ha respectively. In terms of economic feasibility and high astaxanthin yields, this combination of systems is deemed to be the most suitable [8,52].

A 15-stage horizontal tubular PBR fence was used for the 'green stage', since controllable environmental and nutritional conditions that can be achieved in the PBR, facilitate optimal growth [15,63]. The tubes were made of polycarbonate (ideal for locations with temperature variations), while their diameter was chosen at 0.05 m (average value of the proposed range of depth (<0.1 m)) [15,42]. Agitation, mixing of the substrates and prevention of biomass sedimentation in the tubes is succeeded by maintaining continuous turbulent flow. This can be accomplished by using a mechanical pump or a gentler airlift system [11,12,88]. In this paper, an airlift system was assumed, since, besides agitation and mixing, the exchange of CO_2 and O_2 between the liquid medium and aeration gas can be achieved as well [1,15].

The raceway pond complex was selected for the 'red stage', principally in order to offset high construction and operation costs associated with cultivation in a PBR (3–10 times higher) [63]. A depth of 0.3 m was chosen as input for the model (average value of the proposed range of depth (0.1–0.5 m)), while submerged aerators were assumed to enhance CO_2 uptake [11,47].

3.1.2. Parameters affecting microalgae growth

Sunlight is the ultimate source of energy in photoautotrophic microalgae cultivation. Oxygenic photosynthesis in microalgae cultivation can be expressed as a reaction driven by light energy (harvested by chlorophyll molecules), in which Carbon dioxide, water and nutrients are converted to algal biomass (mainly carbohydrates), Oxygen and Hydrogen cations [82]. Although the wavelength range of solar irradiation is very broad, microalgae can utilize only a fraction of it, which is called Photosynthetically Active Radiation (PAR). PAR ranges between 400 nm–750 nm, which is basically the spectral pattern of visible light and corresponds to approximately 40–45% of the total light spectrum [42,73,82]. According to the quantum theory, light energy is delivered in the form of separated packages called *light quanta* or *photons*, which are the tools to drive photosynthesis [108]. Light intensity can be expressed as the number of photons that strike a flat surface per unit of time (μmol m^{-2} s^{-1}). This rhythm is called Photon Flux Density (PFD) [45]. Nevertheless, light intensity is usually measured in units of power per area (Wm^{-2} or Jm^{-2} s^{-1}). As photosynthesis is a quantum process, a conversion factor between μmol m^{-2} s^{-1} and Wm^{-2} is needed. Integrating Einstein's law ($E = N * h\frac{c}{\lambda}$) it is found that for PAR, the values of the photon flux density conversion factor range from 4.5–5.14 μmol m^{-2} s^{-1} per Wm^{-2} [45,82]. From the total amount of light impinging upon the surface of water, one fraction is reflected as a function of Frenzel's law [73]. The reflection losses values involve a fraction of around 10% and 12% for the raceway pond and the tubular PBR respectively [6,76]. The rest fraction of solar irradiation enters the water and it can be either absorbed by water and substances dissolved in it or it can penetrate an algal cell [73]. As light permeates in the deeper layers, its intensity attenuates. This intensity attenuation is a function (Beer-Lambert law) of the incident irradiation (I_0) on the surface, a constant X called attenuation coefficient (range 0.15 m^{-1} to 0.6 m^{-1}) and the depth of the culture system (Z) [45,73]. Another significant factor in microalgae growth refers to the photosynthetic efficiency. The gross photosynthetic efficiency amounts to ~27%. However, various inefficiencies and loss mechanisms (such as respiratory CO_2 losses and photo-utilization efficiency) decrease this number, resulting in a maximum theoretical efficiency of ~20% [73,90]. Incident light is also affected from land efficiency, which amounts to 98% in optimal conditions [90].

For optimal cell proliferation (i.e. 'green stage') of *H. pluvialis*, a temperature of 20 °C and a saturation intensity of 250 μmol m^{-2} s^{-1} are proposed [34]. Saturation intensity refers to the light intensity value, above which (slightly greater) irreversible damage of the parts in algae cells that are responsible for photosynthesis occurs, leading to a reduction of the biomass growth rate [83]. This phenomenon is called photoinhibition. For the accumulation of astaxanthin in the intracellular environment (i.e. 'red stage') the stress environmental conditions of increased light intensity and temperature are required. At 27 °C *H. pluvialis* has demonstrated the highest astaxanthin production, while the saturation intensity at these temperature levels corresponds to 500 μmol m^{-2} s^{-1} [27,34].

Like all photosynthetic organisms, microalgae (in photoautotrophic nutritional mode) need CO_2 as a Carbon source, which will be converted into chemical energy inside the algal cell [42,101]. Microalgae can capture CO_2 mainly from three different sources: 1) Atmospheric CO_2; 2)

CO_2 included in gas emissions from industrial processes (e.g. flue- and flaring gases); and 3) fixed CO_2 in the form of soluble carbonates (e.g., $NaHCO_3$ and Na_2CO_3) [11]. For high biomass productivity, a CO_2 concentration that exceeds 2.2 mg/l is required [46]. Based on the average chemical composition of algal biomass ($CH_{1.83}O_{0.48}N_{0.11}$), approximately 1.8 tons of CO_2 are needed in order to harvest 1 ton of algal biomass [15,42,101]. In oxygenic photosynthesis the uptake of CO_2 results in the production of molecular Oxygen, which doesn't facilitate microalgae growth. In fact, dissolved Oxygen is labeled as a 'waste' product, which at higher concentrations than air saturation values can obstruct the capture of CO_2 and cause photo-oxidative damage to algal cells, affecting consequently algal growth [64]. This is a significant issue for microalgae especially for cultivation in PBRs, where algal broth is isolated in a closed environment and Oxygen cannot escape easily. The molecular Oxygen limit, above which microalgae suffer, ranges from 25–40 mg/l of water [46,102]. In this paper, it was assumed that CO_2 needs were facilitated by supplying the hybrid system with flue gases. In both cities extensive industrial activity takes place emitting considerable amount of flue gases, which could be exploited to meet CO_2 needs during production. Regarding concentrations, the average values of CO_2 and O_2 existing in flue gas were taken into account; namely, 10% v/v for CO_2 (range is 5%–15%) and 3% v/v for O_2 (range is 2.5–3.5%) [7,101].

As for acidity, in a hybrid system comprising by a tubular PBR and a raceway pond complex, Li et al. [52] propose for the tubular PBR ('green stage') a pH of 7.5, while for the raceway pond ('red stage') increased pH of 8.0 maintained by controlled addition of CO_2.

3.1.3. Growth medium and cell composition

Besides light absorption and CO_2 supply, the growth medium needs a sufficient supply of macro- and micronutrients, which will serve the role of fertilizers. These fertilizers are inoculated in the culture system in the form of chemical compounds, synthesizing the so called 'initial medium recipe' [32]. Since the hybrid system, consists of a tubular PBR and a raceway pond complex, this paper adopted the 'initial medium recipe' proposed by Li et al. [52], who used in their study the same hybrid system for cultivation. It contains 10 mM KNO_3, 2 mM Na_2HPO_4, 0.5 mM $CaCl_2$, 0.5 mM $MgSO_4$, 2 mM $NaHCO_3$, 50 μM H_3BO_3, 50 μM *EDTA*, 10 μM $MnCl_2$, 5 μM $FeCl_3$, 2 μM Na_2MnO_4, 1.5 μM $NaVO_3$, 0.8 μM $ZnSO_4$, 0.4 μM $CuSO_4$, 0.2 μM $CoCl_2$, 10 μg/l *biotin*, 1 μg/l *vitamin* B_{12}. Using the different atomic masses, the elemental composition of the initial recipe as well as the respective weight percentages can be calculated (see Table 1).

In order to maintain optimal growth during the 'green stage', nitrate concentrations up to 10 mM are required [33]. Encystement of the green cells and astaxanthin accumulation are induced by nutrient deprivation along with high light intensity and temperature. It is reported that nitrate concentrations in the growth medium during 'red stage' should vary between 2.4 mM and 6.6 mM [30,71]. Since the 'initial medium recipe' contains 10 mM KNO_3 during the 'green stage', a potassium nitrate concentration of 6.6 mM was adopted for the 'red stage'. This value corresponds to 66% of the initial nitrate concentration. It was assumed that the rest chemical compounds of the medium recipe would be deprived to this quota during 'red stage'. In that way, we determined the elemental composition of the aplanospores, which is not mentioned in the literature yet but is needed for calculation of the mass balances. Regarding 'green stage' the elemental biomass composition is similar to the ones of most green microalgae strains and is already known (see Table 2).

Table 1
Composition of the initial medium recipe.

Element	Weight (grams/l)	Weight (%)
Oxygen (O)	0.745	48.32
Potassium (K)	0.39	25.29
Nitrogen (N)	0.141	9.14
Sodium (Na)	0.138	8.95
Phosphorus (P)	0.062	4.02
Carbon (C)	0.03	1.95
Sulfur (S)	0.016	1.04
Magnesium (Mg)	0.012	0.78
Hydrogen (H)	0.005	0.32
Chlorine (Cl)	0.0013	0.08
Manganese (Mn)	0.00066	0.04
Boron (B)	0.00055	0.04
Iron (Fe)	0.00028	0.02
Vanadium (V)	0.000077	0.00
Zinc (Zn)	0.000052	0.00
Copper (Cu)	0.000025	0.00
Cobalt (Co)	0.000012	0.00

Table 2
General elemental microalgae biomass composition after 'green' and 'red stage' (adjusted information from [23,30,33,52,82]).

Element	'Green stage' weight (%)	'Red stage' weight (%)
Carbon (C)	45.90	53.30
Oxygen (O)	29.00	30.10
Hydrogen (H)	8.20	5.40
Nitrogen (N)	8.10	5.40
Potassium (K)	4.90	3.20
Phosphorus (P)	1.20	0.80
Sulfur (S)	0.80	0.50
Iron (Fe)	0.70	0.50
Sodium (Na)	0.60	0.40
Magnesium (Mg)	0.60	0.40
Chlorine (Cl)	0.00	0.00
Manganese (Mn)	0.00	0.00
Boron (B)	0.00	0.00
Vanadium (V)	0.00	0.00
Zinc (Zn)	0.00	0.00
Copper (Cu)	0.00	0.00
Cobalt (Co)	0.00	0.00

3.2. Harvesting phase

Harvesting of algal biomass constitutes a critical part within the production line, since it usually represents 20–30% of the total production costs [5,11,16,58,65,81]. High harvesting costs are due to various algal features that make harvesting of algal biomass difficult: 1) Low microalgae cell densities in the broth (typically mass concentrations are in the range of 0.3–5 g/l); 2) the small size of most algal cells (typically in the range of 2–40 μm); 3) the negatively charged algal cell surface that results in a stable dispersed state of the algal suspension; and 4) the fast growth rates of microalgae, which require frequent harvesting compared to terrestrial plants [5,11,21,51,62]. To date, there is hardly a harvesting method that is economically viable and energy efficient simultaneously [5,62]. There are two methodologies to follow in the harvesting process: 1) A two-step approach, where the algal suspension is primarily thickened to slurry consisting of 2–7% of the total suspended solids (TSS). Afterwards, the slurry is further dewatered to a cake comprising of 15–25% TSS; 2) a single-step approach, where thickening and dewatering processes are merged [5,11,94]. Microalgae harvesting currently involves mechanical (gravity sedimentation, flotation, filtration and centrifugation), chemical (coagulation/flocculation), biological (bio-flocculation) and to a lesser extent, electrical based methods (electrophoresis) [5,94].

In this paper, a two-step approach employing disk-stack centrifugation at 13,000 g and gravity sedimentation as a preliminary step (settling) was selected for the process model. Centrifugation is the most energy intensive and consequently expensive method, limiting its applicability only for the recovery of high-value products, such as highly unsaturated fatty acids, pigments for pharmaceuticals and cosmetics and high-value nutritional metabolites [5]. Nonetheless, its operational times are rapid, it can be applied to the majority of algal strains and it is not associated with cell composition changes and contamination. Especially, when combined with gravity sedimentation (preliminary step) it is considered as the most reliable process [5,66,81,86]. Furthermore,

disk-stack centrifugation constitutes an ideal method for species with size between 3–30 μm and suspended solids in the broth between 0.02–0.05% [62]. Considering the size of a *H. pluvialis* cell (i.e. 20 μm) as well as the calculated concentration of suspended solids in the 'red stage' (i.e. 0.035%, see Section 4.2)[1] in association with the high TSS concentration of disk-stack centrifugation (15% combined with gravity sedimentation), this method is ideal for an astaxanthin production line [37,42,65,94,109]. Implementing disk-stack centrifugation at 13,000 g, a biomass recovery efficiency that exceeds 95% can be achieved [40].

3.3. Extraction phase

One of the main obstacles to fully taking advantage of astaxanthin and channel it into the market refers to the ability to successfully and efficiently extract the pigment from the algal cake. The major component of the tough outer (exine) walls of plant spores and pollen grains is called sporopollenin. *H. pluvialis* cells are characterized by a thick sporopollenin wall, which impedes astaxanthin extraction from the intracellular part [59]. Extraction phase can be divided into three main processes: 1) Cell disruption; 2) dehydration; and 3) recovery of the desired metabolite [70]. In this study, bead milling was chosen in order to disrupt the cell. This method is most effective and energy wise, when biomass concentration after harvesting in the algal cake is between 100 and 200 g/l [35].[2] After algal cell walls have been disrupted, biomass must be further processed rapidly, or it can be spoiled within few hours. Thus, dehydration is a process applied prior to recovery of the desired metabolite, in order to extend the shelf-life of the algal biomass [58,65]. Spray drying has been labeled as the most appropriate method to dry high-value microlagal products [11,62]. The dry biomass (in powder) recovery efficiency of this method exceeds 95% [50]. After spray drying, the moisture content in 'red' biomass corresponds to 5% [78]. By the time the cell wall is disrupted and the biomass is fully dried, the intracellular content is protected only by the thin cell membrane and the recovery of the desired product is possible. Supercritical fluid extraction (SFE) is a modern and a widely accepted method to recover high value metabolites from microalgae that are destined for the pharmaceutical and nutraceutical sector [60,91]. The main principle behind this method is the utilization of supercritical fluid, whose physicochemical properties are between those of a liquid and a gas [63]. Carbon dioxide is considered as an ideal compound for this process, since its critical temperature and pressure (31.1 °C and 7.4 MPa) are relatively low compared to others, while due to its gaseous behavior in room temperature it can be easily removed after recovery [19,60,84]. Several studies have reported experiments on supercritical CO_2 extraction for the recovery of astaxanthin from *H. pluvialis*. In this study, we assumed that *H. pluvialis* cells were subjected into supercritical CO_2 extraction at a temperature of 60 °C and a pressure of 30 MPa, while using ethanol (9.4%) as a co-solvent. This is a method proposed by Valderrama et al. [95] resulting in an astaxanthin recovery efficiency of 97%. The waste product (i.e. residual biomass) after supercritical CO_2 extraction is a light brown powder rich in chemical compounds that can be used as a high quality bio-fertilizer [78].

Usually, the final product is a semi-solid extract called oleoresin, which comprises by 10–20% astaxanthin. However, most academic papers assume that both synthetic and algal astaxanthin can be isolated from petrochemicals and biomass as a pure substance (95–100%) respectively. The same assumption was made in this paper in order our methods and results to be adjustable and comparable to reference values. Assuming total isolation of the carotenoid from biomass, it can also be assumed that the algal residue can be channeled as a whole into the market.

3.4. Schematic view of the process model

The central idea behind the creation of the model is to translate incident solar irradiation into biomass productivity using the Higher Heating Value (HHV). The amount of sunlight absorbed is the energy stored in the biomass [73]. The HHV is the amount of energy (i.e. heat) released during the combustion of a specified amount of a substance. In case of microalgae biomass, HHV could be considered as the solar energy metabolized by the primary compositions (lipids, carbohydrates and proteins), translated into heat, which is released when one unit of biomass is burned in a device, such as a combustion boiler. In other words, dividing solar energy uptake (in MJ) with the HHV (in MJ/kg), the biomass productivity can be calculated. Depending on the concentration of the primary compositions HHV can be determined [90]. This paper assumed a chemical composition with 40% lipids, 40% carbohydrates and 20% proteins for both stages [54,90]. Each fraction has a specific calorific value called Lower Heating Value (LHV), see Table 3 [90]. Using the LHV of each composition as well as their fraction in the microalgae cell, HHV can be calculated using the following formula:

$$HHV = f_L{*}LHV_L + f_C{*}LHV_C + f_P{*}LHV_P \quad (1)$$

Cultivation constitutes the most important part of the model, since algae growth is the most complex process to be modeled. Thus, we developed a model that shows the following two features: The first illustrates seasonal fluctuation of the annual 'wet' biomass productivity after cultivation; and the second determines the annual astaxanthin yield after employing harvesting and extraction for the selected locations.

'Wet' biomass productivity is the dry biomass prior to harvesting and extraction. It constitutes in principle, the amount of dry biomass existing in the broth during cultivation phase. Besides incident solar irradiation that is subjected to various inefficiencies and loss mechanisms, the model takes into account the impact of the temperature into the system. Furthermore, the impact of nutrients on optimal algae growth and astaxanthin accumulation as well as the equalization of the broth's volume between the two culture systems were also considered. 'Wet' biomass productivity can be eventually calculated as follows:

$$PROD_{WET} = \frac{\eta_{DISTRIBUTION}{*}REFL * PAR * PE * L_{EFF}{*}T_{EFFECT}{*}F_{NUTRIENTS}{*}F_{EQ} * SUN}{HHV} \quad (2)$$

Determining the astaxanthin yield that can be achieved in the selected locations, 'wet' productivity has to be subjected into the different recovery efficiencies (RE) associated with the harvesting and extraction phases. Last but not least, an average astaxanthin concentration of 2.5% (range is 1–4%) existed in the dry biomass, is assumed (see Fig. 2) [52,55,57,112]. Consequently, the astaxanthin yield can be calculated as follows:

$$\text{Astaxanthin yield} = PROD_{WET}{*}RE_{CENTR}{*}RE_{BEAD}{*}RE_{SPRAY}{*}RE_{CO2}{*}\%C_{ASTAX} \quad (3)$$

The description of each abbreviation depicted in formulas 2 and 3 can be found in Table 4.

Table 3
Microalgae biomass fractions, net calorific values and HHV (adjusted by [54,90]).

Composition	Fraction (f)	LHV (MJ/kg)	HHV (MJ/kg)
Lipids (L)	40%	38.3	
Carbohydrates (C)	40%	13	23.6
Proteins (P)	20%	15.5	

[1] The calculated concentration of TSS prior disk-stack centrifugation (i.e. 0.035% or 0.35 g/l) resembles significantly with the respected value (0.4 g/l) mentioned by Li et al. [52]), who conducted actual experiments using the same combination of cultivation systems.

[2] The concentration after gravity sedimentation and disk-stack centrifugation amounts to 15% or 150 g/l.

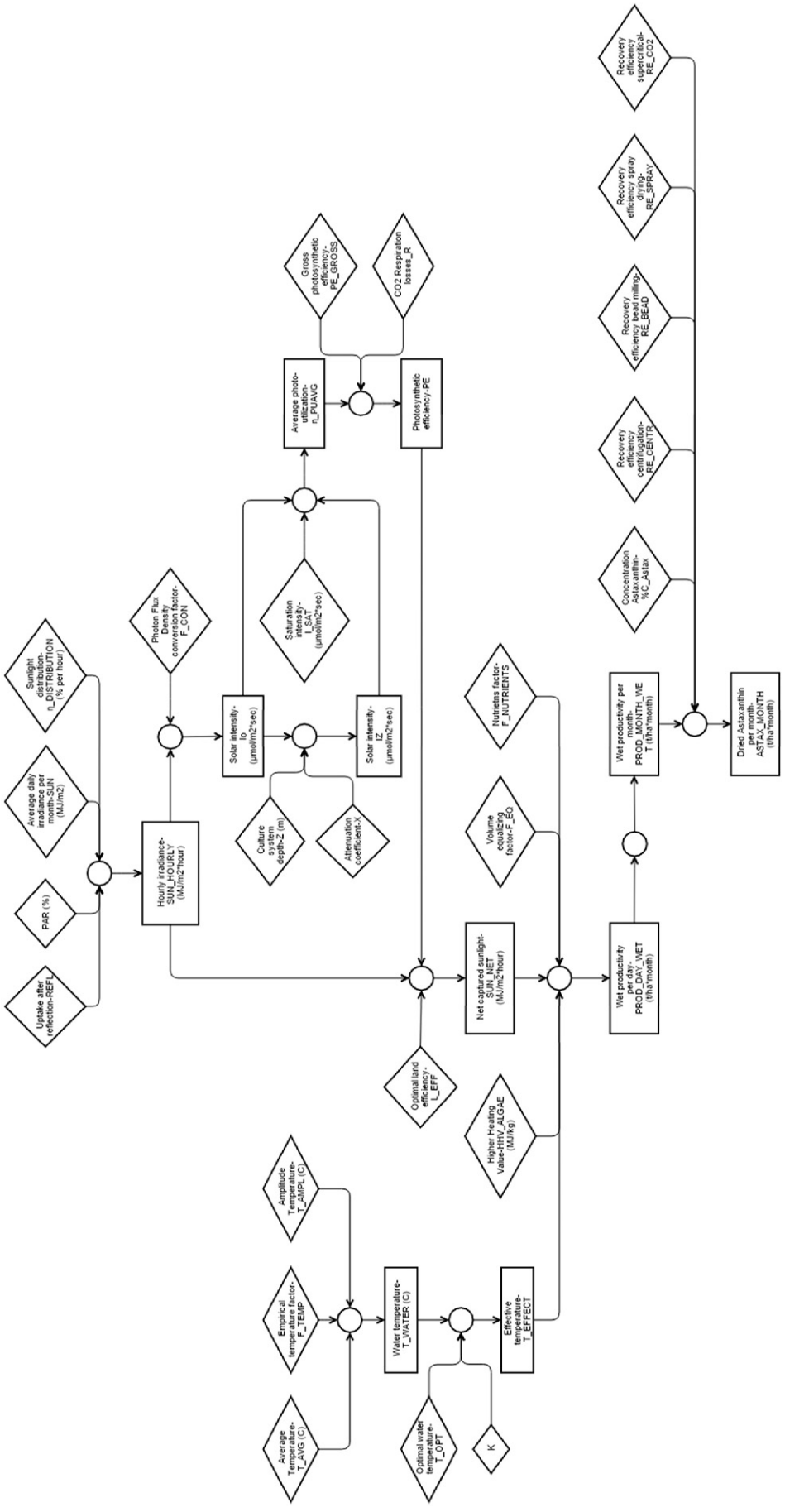

Fig. 2. Flow chart of the microalgae process model.

Table 4
Process model input.

Definition	Abbreviation	Value		Source
		Horizontal tubular PBR ('green stage')	Raceway pond ('red stage')	
Average daily irradiance/month	SUN			[74,48]
Distribution of sunlight (per hour)	$\eta_{DISTRIBUTION}$			[74]
Photon Flux Density conversion factor[a]	F_{CON}	$4.82 * 10^6$ μmol/m^2 s/MJ/m^2 s		[45,82]
Uptake after reflection	REFL	88%	90%	[6,76]
Percentage PAR[b]	PAR	43%		[42,73,82]
Culture system depth	Z	0.05 m	0.3 m	[15,47]
Attenuation coefficient[c]	X	0.38 m^{-1}		[45,73]
Saturation intensity	I_{SAT}	250 μmol/m^2 s	500 μmol/m^2 s	[34]
Gross photosynthetic efficiency	PE_{GROSS}	27%		[73]
CO_2 respiration losses	R	30%		[113]
Average temperature	T_{AVG}			[69,48]
Temperature amplitude	T_{AMPL}			[69,48]
Water temperature factor	F_{TEMP}	0.9		[89]
Optimal water temperature	T_{OPT}	20 °C	27 °C	[34]
Effective temperature factor	K	0.007		[44]
Factor nutrients	$F_{NUTRIENTS}$	1.00	0.66	[−]
Volume equalization factor	F_{EQ}	15	1	[−]
Higher heating value	HHV_{ALGAE}	23.6 MJ/kg		(see Table 3)
Optimal land efficiency	L_{EFF}	98%		[90]
Biomass recovery efficiency disk-stack centrifugation[d]	RE_{CENTR}	[−]	98%	[40]
Biomass recovery efficiency bead milling[d]	RE_{BEAD}	[−]	100%	[−]
Biomass recovery efficiency spray drying[d]	RE_{SPRAY}	[−]	98%	[50]
Astaxanthin recovery efficiency supercritical CO_2 extraction[d]	RE_{CO2}	[−]	97%	[95]
Astaxanthin concentration in biomass	$\%C_{ASTAX}$	[−]	2.5%	[112]
Hourly irradiance	SUN_{HOURLY}	$= REFL * SUN * \eta_{DISTRIBUTION} * PAR$		[−]
Solar intensity on pond/reactor top-surface	I_0	$=(SUN_{HOURLY}/3600) * F_{CON}$		[−]
Solar intensity on pond/reactor in depth Z	I_Z	$= I_0 * e^{-X * Z}$		[73]
Photo-utilization efficiency on surface (Bush equation)	η_{PUS}	$=(I_{SAT}/I_0) * [(\ln(I_0/I_{SAT}) + 1)]$		[90]
Photo-utilization efficiency in depth Z (Bush equation)	η_{PUZ}	$=(I_{SAT}/I_Z) * [(\ln(I_Z/I_{SAT}) + 1)]$		[90]
Average photo-utilization efficiency	η_{PUAVG}	$=(\eta_{PUS} + \eta_{PUZ})/2$		[−]
Photosynthetic efficiency	PE	$= PE_{GROSS} * \eta_{PUAVG} * (1 - R)$		[89,90]
Net captured sunlight per hour	SUN_{NET}	$= SUN_{HOURLY} * PE * L_{EFF}$		[90]
Water temperature	T_{WATER}	$= F_{TEMP} * [T_{AVG} - T_{AMPL} * \cos(2\pi * hour/24)]$		[89]
Effective temperature	T_{EFFECT}	$= e\hat{}[-K * (T_{WATER} - T_{OPT})^2]$		[44]
Wet Productivity (g/m^2/day)	$PROD_{DAY\_WET}$	$= 1000 * \Sigma(T_{EFFECT} * F_{NUTRIENTS} * F_{EQ} * SUN_{NET})/HHV_{ALGAE}$		[−]
Wet productivity (t/ha/month)	$PROD_{MONTH\_WET}$	$= 0.3 * PROD_{DAY}$		[−]
Astaxanthin yield (t/month)	$ASTAX_{MONTH}$	$= PROD_{MONTH\_WET} * RE_{CENTR} * RE_{BEAD} * RE_{SPRAY} * RE_{CO2} * \%C_{ASTAX}$		[−]

[a] Average value of the conversion factor (4.5–5.14 μmol m^{-2} s^{-1}).
[b] Average value of PAR (40–45%).
[c] Average value of the attenuation coefficient (0.15–0.6 m^{-1}).
[d] Average values for the recovery efficiencies during harvesting and extraction phases.

4. Results

4.1. Biomass productivity and astaxanthin yield

The model ran two times, one for the 'green' and one for the 'red stage'. For optimal growth in the 'green stage', solar intensity on surface and in depth Z should be below saturation point, since exceeding this point results in photoinhibition and consequently to a reduction of the biomass growth rate. Therefore, it is assumed that all through 'green stage', solar intensities values above 250 μmol m^{-2} s^{-1} do not contribute to 'green' biomass production and were not taken into account. This decision goes in line with various studies such as the one from Domínguez-Bocanegra et al. [25] and Zhekisheva et al. [112], who state that maximum growth of *H. pluvialis* has been obtained under continuous illumination of 177 and 75 μmol m^{-2} s^{-1} respectively. On the other hand, in the 'red stage', the adverse condition of high solar intensities is needed to inhibit cell proliferation and induce astaxanthin accumulation. Thus, the values of solar intensity on surface and in depth Z should exceed the saturation point. Several papers go along with this statement, such as the one from Dragoş et al. [26] and from Garcia-Malea et al. [32], who stressed *H. pluvialis* cells under 630 μmol m^{-2} s^{-1} 1 and 350–2500 μmol m^{-2} s^{-1} for intracellular astaxanthin accumulation respectively. Therefore, all through 'red stage', solar intensities under 500 μmol m^{-2} s^{-1} do not contribute to astaxanthin accumulation and were not taken into account.

The annual 'wet' biomass productivities for Livadeia and Amsterdam, during the 'green stage', amount to 45.71 t/ha/year and 31.33 t/ha/year respectively. On the other hand, during 'red stage', taking into account that inhibition of algae growth leads to the death and sedimentation of a significant amount of cells a noteworthy decrease of 'red' biomass productivity can be noticed [39,52]. In fact, the 'red' biomass productivity for Livadeia amounted to 18.28 t/ha/year, while the one for Amsterdam reached 6.15 t/ha/year. Applying the recovery efficiencies during harvesting and extraction phases and taking into account the intracellular astaxanthin concentration (i.e. 2.5%) the monthly astaxanthin yield can be calculated (see Fig. 3).

Highest monthly differences in astaxanthin yield between the selected locations can be observed during summer. This is because solar intensity values for Amsterdam did not surpass saturation point, above which astaxanthin is accumulated, to a great extent as occurred in Livadeia. The annual astaxanthin yields for Livadeia and Amsterdam were calculated 426 kg/year and 143 kg/year respectively. Li et al. [52] conducted a biennial production of astaxanthin on a pilot scale, cultivating *H. pluvialis* using the same systems all through production line as assumed in this paper. The pilot facility was established in Shenzhen, China, a city located at 22°32′00″ N/114°8′00″ E, with an annual average temperature of 22 °C,

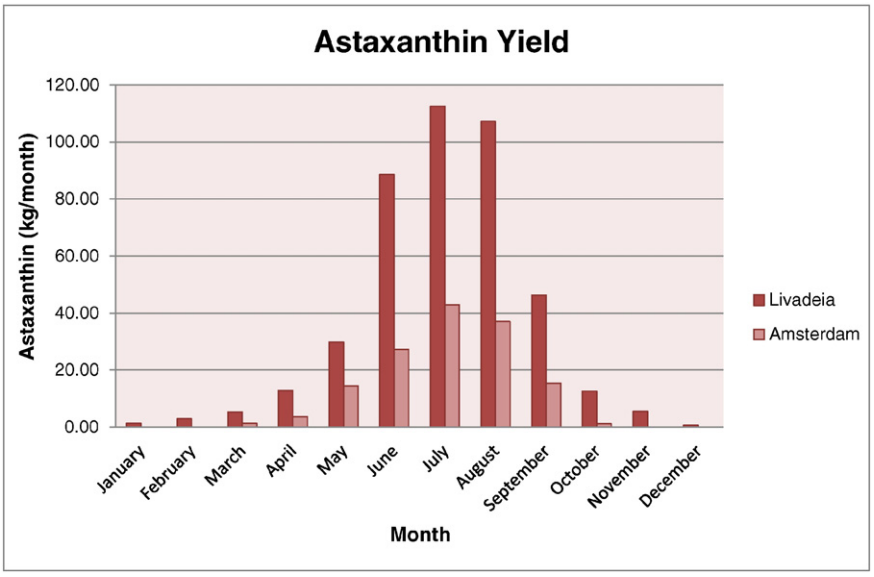

Fig. 3. Monthly astaxanthin yield.

2200 h/year sunshine time and about 5000 MJ/m^2 annual solar radiation [36,52]. Based on the estimated process parameters, Li et al. [52] scaled up the pilot operation and estimated that an astaxanthin production of 900 kg/2 ha/year at 2.5% could be achieved. In order to validate the process model, we run the model for Shenzhen, considering solar radiation and temperature data for the Chinese city. The process model calculated a theoretical astaxanthin productivity of 926 kg/2 ha/year. It is evident that for the same location, a remarkable resemblance between the theoretical value and the one from an actual facility occurs.

4.1.1. Sensitivity analysis

Sensitivity analysis was conducted in order to identify which parameters used in the process model are critical for the calculation of astaxanthin productivity. Parameters analyzed in this study include irradiance, temperature, photon flux density, attenuation coefficient, water and effective temperature factors, and HHV. Figs. 4 and 5 present the results of the sensitivity analysis for the selected locations. Each curve indicates the change in astaxanthin productivity associated with the variation of a parameter (range of variation is between −30% and 30%), while all other parameters are kept unaltered. It is clear that for both locations temperature is the most sensitive parameter. This is the reason, why large algal astaxanthin production facilities are not established on sites that experience only high incident solar radiation (e.g. on high altitudes with little cloud cover), but in combination with high average temperatures as well (e.g. Cyanotech in Hawaii and Algatechnologies in Israel) [4,20]. Further parameters that significantly affect astaxanthin production calculation refer to the empirical factors for water and effective temperatures (see Table 4). These factors are approximated by Sukenik et al. [89] and James & Boriah [44] in their theoretical models on algal growth, meaning that the level of uncertainty is high. This paper did not considered uncertainties for all data used in the process model. However, a detailed examination of these uncertainties may be valuable in the future for other purposes.

4.2. Mass balances

The 'initial medium recipe' comprises by different macro- and micronutrients. The demand for the macronutrients (KNO_3, Na_2HPO_4, $NaHCO_3$, $MgSO_4$) was calculated separately, while the demand of micronutrients was calculated as a whole. For the 'green stage', the medium renewal rate mentioned by Li et al. [52] for Shenzhen (25%) was adopted for Livadeia, since both cities experience similar solar radiation in annual basis. Considering that nutrients' uptake by algal cells and solar intensity have a proportionate linear behavior, the medium renewal rate for Amsterdam was calculated at 18% [18,104]. Regarding red stage the medium renewal rate was decreased applying the nutrient

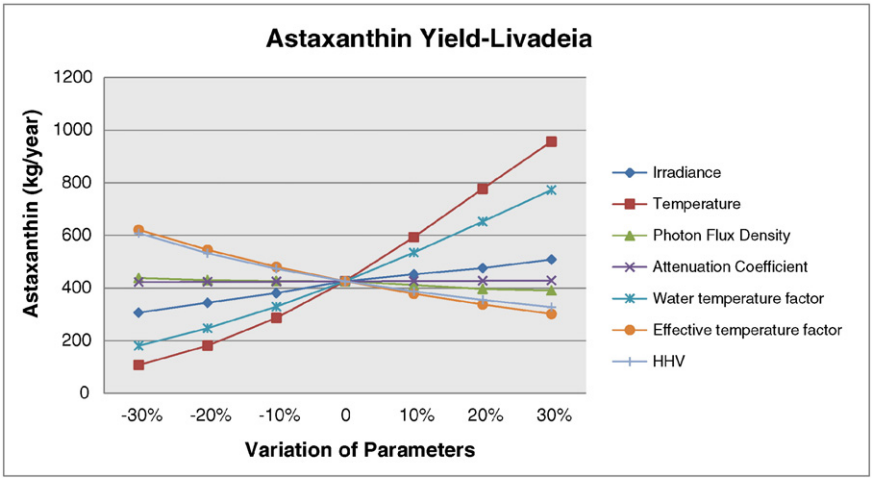

Fig. 4. Annual astaxanthin productivity relative to different parameters variations (Livadeia).

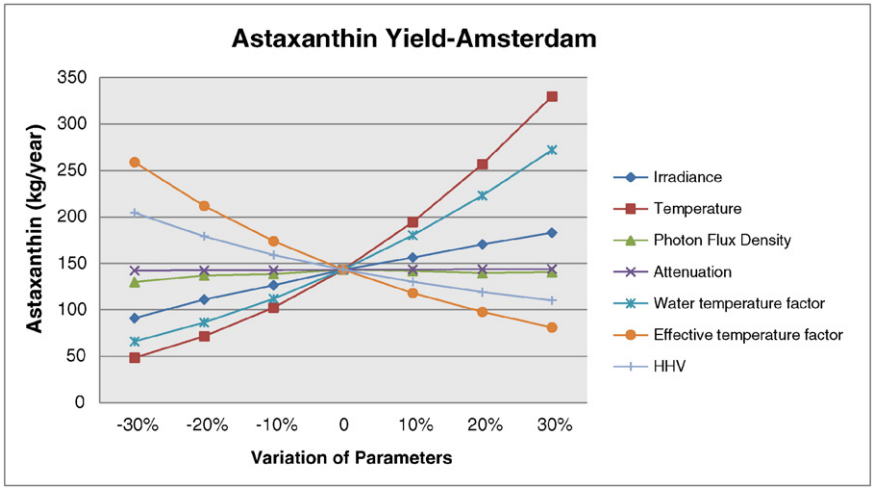

Fig. 5. Annual astaxanthin productivity relative to different parameters variations (Amsterdam).

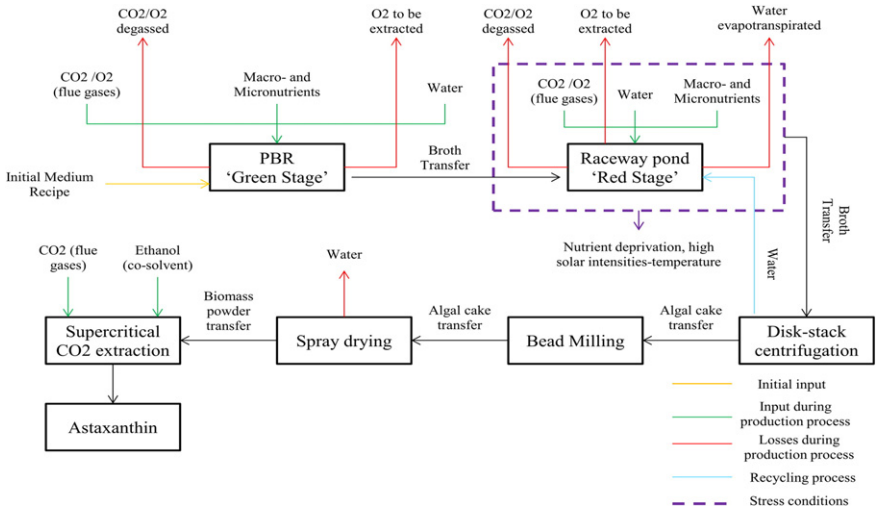

Fig. 6. Flow chart of the mass in- and outflows throughout the astaxanthin production process.

deprivation factor (0.66). The utilization efficiency of nutrients amounts to 75% for raceway ponds, while for the horizontal tubular PBR, the respective value equals to 90% [106,107]. The operation days correspond to 360 in an annual basis.

In this paper, the general rule for CO_2 demand is implemented: In order to produce 1 ton of algal biomass, 1.8 tons of CO_2 are needed. CO_2 utilization efficiency amounts to 75% for the horizontal tubular PBR and 35% for the raceway pond [2,87,103]. The rest is degassed (25% and 65% respectively). Using the densities of CO_2 (1.84 kg/m^3) and O_2 (1.33 kg/m^3) as well as concentrations in flue gases, the amount of O_2 (existed in flue gases) entering the hybrid system was calculated as well. CO_2 uptake during photosynthesis results in production of molecular Oxygen, which inhibits cell proliferation at high concentrations (see Section 3.1.2). Removing the excess molecular Oxygen (labeled as waste product) is imperative in order to ensure continuous algal growth. The produced molecular Oxygen was calculated using the generic algal stoichiometry during oxygenic photosynthesis.[3] The molecular Oxygen limit, above which microalgae suffer, ranges from 25–40 mg/l of water. Using an average value of this range (i.e. 32 mg/l of water), the suffering limit as well as the amount of molecular Oxygen to be extracted were determined (see Fig. 6 and Table 5). In tubular PBR this process is accomplished in the degassing zone, while in raceway ponds it happens naturally since raceways are open to the atmosphere. An aspect that was not taken into account refers to de-oxygenation due to respiration in dark conditions.

The second inflow of CO_2 in the production line refers to supercritical CO_2 extraction (CO_2 enhanced with ethanol as co-solvent at 9.4%) during extraction phase. Using data from Valderrama et al. [95], a trend-line was created in order to calculate the amount of solvent per kg of feed (i.e. dried 'red' biomass) at 2.5% astaxanthin content. The solvent/feed ratio (kg/kg) was calculated at 20.12.

Algae require considerable amounts of water in order to grow and thrive. The organisms themselves are 80–85% water (cellular water) [66]. Besides water incorporated in the algal cell, most algae grow in aqueous suspension. The suspended solids are proven to be marginal. Typically miroalgal biomass varies from 0.02–0.05% in raceway ponds and between 0.1%–0.5% in tubular PBRs [42,109]. Taking into account the average of these values (i.e. 0.035%–0.35 g/l and 0.3%–3 g/l respectively) as well as evapotranspiration losses, the water demand was calculated. No evapotranspiration losses were assumed for the tubular PBR, since it is a closed system. Regarding harvesting phase, considering the fraction of suspended solids in the algal cake (i.e. 15%) after applying disk-stack

[3] Photosynthesis in microalgae is similar to that in green plants. It is a reaction driven by light energy, in which Carbon dioxide, water and nutrients are converted to algal biomass, Oxygen and Hydrogen cations [46]: $CO_2 + 0.71H_2O + 0.12NH_4^+ \xrightarrow{\text{Photosynthesis}} \{CH_{1.78}O_{0.36}N_{0.12}\} + 1.18O_2 + 0.12H^+$. Using the molecular masses the correlation between moles and actual masses can be determined.

Table 5
Mass balances during production process.

Mass balances				
(t/ha/year)	Livadeia		Amsterdam	
	Horizontal tubular PBR ('green stage')	Raceway pond ('red stage')	Horizontal tubular PBR ('green stage')	Raceway Pond ('red stage')
	Cultivation phase			
'Wet' biomass productivity	45.71	18.28	31.33	6.15
KNO_3	4.3	10.7	2.1	2.2
Na_2HPO_4	1.2	3	0.5	0.6
$NaHCO_3$	0.7	1.8	0.4	0.4
$MgSO_4$	0.3	0.6	0.1	0.1
Micronutrients	0.1	0.2	0.04	0.05
CO_2 demand (from flue gases)	109.1	90.6	74.9	30.9
CO_2 degassed	27.3	58.9	18.7	20.1
O_2 inoculated (from flue gases)	23.6	19.6	16.2	6.7
O_2 degassed	5.9	12.7	4.1	4.4
Photosynthetic molecular O_2	70.2	27.2	48.2	9.3
Molecular O_2 suffering limit in growth medium	0.5	2	0.3	0.8
O_2 to be extracted	69.7	25.2	47.9	8.5
Broth	15,237	63,544	10,443	23,800
Water	15,191	63,526	10,412	23,793
Water evapotranspirated	0	11,316	0	6228
	Harvesting phase			
Biomass in algal cake	0	17.91	0	6.03
Water in cake after centrifugation	0	101	0	34
Water removal employing centrifugation	0	63,425	0	23,759
Water recycling	0	47,569	0	17,819
	Extraction phase			
Dry biomass	0	17.56	0	5.91
Water in the powder after spray drying	0	0.9	0	0.3
Water removal after spray drying	0	130.1	0	43.7
CO_2 demand supercritical CO_2 extraction	0	320.1	0	107.7
Ethanol (9.4%) as co-solvent supercritical CO_2 extraction	0	33.2	0	11.2

centrifugation, the water in the cake was determined. In order to realize a water-intensive process such as algal cultivation, a sustainable production pathway (water management on irrigation systems) must be established [96,97]. Thus, water recycling after harvesting phase is considered. This strategy not only decreases water demand but improves financial figures as well. However, recycled water may be of lower quality (due to toxic agents) decreasing microalgae growth rate [31]. As during 'green stage' optimal cell proliferation is desired, this paper examines water recycling only for the 'red stage'. Water recycling occurs after harvesting phase and the average recycling efficiency has been estimated at 75% [38]. The moisture content in the 'red' biomass, after implementing spray drying, amounts to 5% [78].

4.3. Energy requirements

Energy consumption involves power consumption for the different operations of the production line. Literature energy values were adjusted to the boundaries of the bio-refinery and considering the biomass-astaxanthin productivities along with mass balances as benchmark values, the annual energy requirements of the production line were determined (see Table 6).

The tubular PBR fence and the raceway pond use a different system (i.e. airlift system and paddlewheels respectively) for continuous turbulent flow (24 h) in order to ensure homogenization of the culture. The mixing/circulation in the airlift system, is associated with a power consumption of 170 (W/m^3_{BROTH}) [1]. On the other hand, a power consumption of 18–54 kWh/ha/day is proposed for the paddlewheel [46]. An average value of 36 kWh/ha/day was adopted in this paper. The energy needs to introduce flue gases in the hybrid system can be expressed as the energy requirements of CO_2 capture and compression from flue gases. The energy needs of CO_2 capture and compression from flue gases at 13% v/v amounts to 0.2 KWh/kg_{CO2} [53]. In this study, this reference value was assumed for both the airlift system and the submerged aerators. Molecular Oxygen produced during photosynthesis hampers microalgae growth and, thus, it has to be removed. In the raceway pond, dissolved Oxygen, is removed naturally, since the pond is open to the atmosphere. On the other hand, in tubular PBRs, Oxygen is separated from liquid in a degassing zone and blown off through an exhaust of the airlift system [1]. The annual power consumption for the degassing zone in a tubular PBR with an airlift system has been calculated from Jonker & Faaij [46] at 47 MWh/ha. Water pumps are employed in order to fill and maintain the water levels in the hybrid system all through cultivation phase. In this study, it is assumed that the pumps introducing water into the hybrid system or transporting the broth (including water recycling) are of the same energy intensity [85]: 0.09 kWh/m^3. Furthermore, it is imperative the tubular PBR be cooled down in order to ensure microalgae's health during 'green stage'. Power consumption for PBR cooling corresponds to 270 MWh/ha/year as Li et al. [52] have reported for Shenzhen. This value was assumed for Livadeia, which experiences similar environmental conditions. For Amsterdam the respective value was calculated proportionately at 162 MWh/ha/year. Cooling in the raceway pond is achieved by evaporation [15].

For the harvesting phase gravity sedimentation and disk-stack centrifugation at 13,000 g was selected, resulting in an algal cake of 15%

Table 6
Energy requirements during production process.

Energy requirements				
Annual power consumption (MWh)	Livadeia		Amsterdam	
	Horizontal tubular PBR ('green stage')	Raceway pond ('red stage')	Horizontal tubular PBR ('green stage')	Raceway pond ('red stage')
	Cultivation phase			
Mixing/Circulation	62.1	13	42.6	13
Flue gases supply	21.8	18.1	15	6.2
O_2 removal	47	0	47	0
Water/broth pumping	2.7	10.1	1.9	3.3
Cooling	270	–	162	–
Total cultivation phase	444.8		291	
	Harvesting phase			
Disk-stack centrifugation	63.5		23.8	
	Extraction phase			
Bead milling	114.6		38.6	
Spray drying	44		14.8	
Supercritical CO_2 extraction	84.3		28.3	
Total extraction phase	242.9		81.7	
Grand total energy needs	751.2		396.5	

TSS. Molina Grima et al. [65] have reported a power consumption of 1 kWh/m^3_{BROTH} using a Westfalia self-cleaning disk stack centrifuge. No energy requirements are reported for gravity sedimentation.

For the recovery of astaxanthin bead milling, spray drying and supercritical CO_2 extraction were employed. Razon & Tan [80] have reported a power consumption range between 2.8 and 10 kWh per kg of algal cake for bead milling. An average value (6.4 kWh/kg) was assumed. Regarding energy requirements during spray drying and supercritical CO_2 extraction enhanced with ethanol at 9.4% as co-solvent, Pérez-López et al. [78] have calculated a value of 82.7 kWh and 158.25 kWh per 0.8 kg of astaxanthin as the end product respectively. These values were adjusted to our production calculated by the process model.

4.4. Economic performance

In this study, the economic performance all through production line involves a profit and loss (P&L) analysis. A P&L analysis is a financial statement that summarizes the revenues, costs and expenses incurred during a specific period of time. A significant aspect regarding astaxanthin production revolves around the reduction of production costs. Thus, three scenarios were created in order to assess cost efficiency. The worst case scenario did not involve any cost reduction policies. The base case scenario is the one described in the methodology and involved water recycling and CO_2 exploitation derived by flue gases (see Section 4.2). Flue gases are a waste product that is associated with significant pollution issues. A company that exploits CO_2 instead of being emitted to the atmosphere would not pay for the raw material but only for distribution, decreasing consequently production costs. The best case scenario refers to the base case scenario in conjunction with the use of solar panels (panel specifications: 250 W, 15% conversion efficiency, 1.65 m^2) in order to meet the total energy needs. It should be pointed out that one of the biggest astaxanthin producers worldwide, Algatechnologies from Israel, uses solar power as the primary source of energy for the facility's operation [4]. In this paper CAPEX and OPEX for the base case scenario are demonstrated (see Tables 7 and 8). Literature values for CAPEX and OPEX were either selected as reported or adjusted to the different outcomes [75]. The costs associated with industrial power for the worst and base case scenarios amounts to €0.13/kWh and €0.089/kWh for Livadeia and Amsterdam respectively [28]. The costs of water for industrial purposes in all scenarios amount to €0.81/m^3 and €1.00/m^3 for Livadeia and Amsterdam respecrively [29,100]. The labor was assumed to include fifteen workers, four supervisors and two marketing experts at two shifts per day. The salary of the personnel was adjusted to the basic wages of each expertise for the selected locations [77,92]. When needed costs in $US were translated into Euros, using the exchange rate for September 2015. This amounts to 0.88 (€/$US) [105].

The scenario analysis resulted in three different production costs (including depreciation)[4] per kg of astaxanthin for the selected locations. For Livadeia production costs amount to €1857/kg_{ASTAX}, €1725/kg_{ASTAX} and €1536/kg_{ASTAX} for the worst-, base- and best case scenario respectively. The respective values for Amsterdam were calculated at €6723/kg_{ASTAX}, €6571/kg_{ASTAX} and €6403/kg_{ASTAX}. Stimulating sustainable development (best case scenario) within the boundaries of the system, production costs were reduced by 18% and 5% for Livadeia and Amsterdam respectively. Li et al. [52] have also conducted a financial analysis for large scale photoautotrophic astaxanthin production in

Table 7
Capital expenditures of the bio-refinery (base case scenario).

CAPEX (€/ha)	Livadeia		Amsterdam	
	Tubular PBR	Raceway pond	Tubular PBR	Raceway pond
		Equipment costs		
Medium supply station	21,120	[−]	21,120	[−]
Medium feed pumps	23,060	[−]	23,060	[−]
Medium filter unit	15,840	[−]	15,840	[−]
Photobioreactors	633,600	[−]	633,600	[−]
Airlift system	37,400	[−]	37,400	[−]
Raceway pond	[−]	17,700	[−]	17,700
Paddlewheel	[−]	7000	[−]	7000
CO_2 supply system	2640	3900	2640	3900
CO_2 storage tank	35,200		35,200	
Sedimentation tank	10,000		10,000	
Disk-stack centrifuge	58,000		50,000	
Centrifuge feed pump	11,390		4340	
Harvest biomass conveyor belt	12,500		12,500	
Harvesting storage tank	17,600		17,600	
Bead miller	60,000		60,000	
Spray dryer	26,400		26,400	
Supercritical CO_2 extraction facility	85,000		85,000	
Packaging line	20,000		20,000	
Laboratory equipment	80,000		80,000	
Total equipment costs	1,178,350		1,163,300	
		Fixed capital costs		
Land acquisition	25,000	25,000	45,000	45,000
Land preparation	1000	1000	1000	1000
Piping	232,670	9100	232,670	9100
Electrical	77,550	3030	77,550	3030
Buildings	114,060	3030	114,060	3030
Installation	228,100	6070	228,100	6070
Instrumentation and control	77,550	6070	77,550	6070
Engineering & Supervision	199,930		199,930	
Total fixed capital costs	1,009,160		1,049,160	
Contractor's fee[a]	218,750		221,250	
Grand total CAPEX	2,406,260		2,433,710	

[a] The contractor's fee is usually estimated at 10% of the total equipment and fixed capital costs.

Table 8
Annual operational expenses during the production process (base case scenario).

OPEX (€/year)	Livadeia		Amsterdam	
	Tubular PBR	Raceway pond	Tubular PBR	Raceway pond
		Cultivation phase		
KNO_3	1890	4710	930	970
Na_2HPO_4	850	2110	350	420
$NaHCO_3$	150	400	90	90
$MgSO_4$	30	60	10	10
Micronutrients	180	350	70	90
CO_2 distribution (flue gases)	5000		5000	
Water (including recycling)	12,300	580	10,410	0
Power mixing/circulation	8070	1690	3790	1160
Power flue gases supply	2830	2350	1340	550
Power O_2 removal	6110	0	4180	0
Power water pumping	350	1310	170	290
Power cooling	35,100	0	14,420	0
		Harvesting phase		
Power disk-stack centrifugation	8250		2120	
		Extraction phase		
Power bead milling	14,900		3440	
Power spray drying	5720		1320	
Power supercritical CO_2 extraction	10,960		2520	
Workers	153,000		337,500	
Supervisors	120,000		180,000	
Marketing (salespersons)	45,000		75,000	
Maintenance	20,000		20,000	
Insurance	15,000		15,000	
Power laboratory & Buildings	5000		5000	
Other repairs	10,000		10,000	
Total OPEX	494,250		696,240	

[4] Depreciation for the worst- and base case scenario is the same, since CAPEX does not change. Regarding best case scenario depreciation was increased due to the solar panels. Depreciation was calculated for a time span of 10 years, meaning 10% annually.

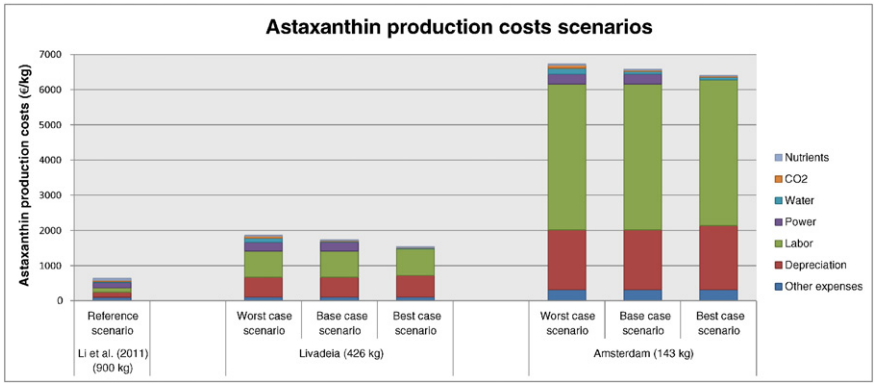

Fig. 7. Scenarios analysis regarding astaxanthin production costs.

Table 9
P&L statement for Livadeia (base case scenario).

Price(€)/kg	astaxanthin	1408	1760	2640	3520	4400	5280	6160
kg	astaxanthin	426	426	426	426	426	426	426
Price(€)/kg	biomass	45	45	45	45	45	45	45
kg	biomass	17,130	17,130	17,130	17,130	17,130	17,130	17,130
Gross revenue		1,370,658	1,520,610	1,895,490	2,270,370	2,645,250	3,020,130	3,395,010
VAT (23%)		315,251	349,740	435,963	522,185	608,408	694,630	780,852
Total revenues		1,055,407	1,170,870	1,459,527	1,748,185	2,036,843	2,325,500	2,614,158
OPEX		494,250	494,250	494,250	494,250	494,250	494,250	494,250
EBITDA		561,157	676,620	965,277	1,253,935	1,542,593	1,831,250	2,119,908
Depreciation (10%)		240,626	240,626	240,626	240,626	240,626	240,626	240,626
EBIT		320,531	435,994	724,651	1,013,309	1,301,967	1,590,624	1,879,282
Interest expense debt								
Interest income on cash								
EBT		320,531	435,994	724,651	1,013,309	1,301,967	1,590,624	1,879,282
Tax (29%)		92,954	126,438	210,149	293,860	377,570	461,281	544,992
EAT		227,577	309,556	514,502	719,449	924,396	1,129,343	1,334,290
CAD		468,203	550,182	755,128	960,075	1,165,022	1,369,969	1,574,916
CAPEX		2,406,260	2,406,260	2,406,260	2,406,260	2,406,260	2,406,260	2,406,260
ROI		19.46%	22.86%	31.38%	39.90%	48.42%	56.93%	65.45%

Shenzhen. They have calculated a value of $718/kg$_{ASTAX}$ or €632/kg$_{ASTAX}$ (reference scenario). It is evident that production costs per kg astaxanhin in Shenzhen are less than half compared to the respective ones in Livadeia. This can be attributed to two factors: 1) Astaxanthin productivity in Shenzen is double than the one calculated for Livadeia (see Section 4.1); 2) Labor, land and utility costs in China are significantly lower than the ones from Western countries. For instance, labor costs (considering the same number of people) in Shenzhen were calculated by Li et al. [52] at €112,000/year, while for Livadeia and Amsterdam they amount to €318,000/year and €592,500/year respectively. Western countries are unable to compete the friendly environment in terms of productions costs that China can offer to the industries. In Fig. 7 the scenario analysis is portrayed, presenting the impact of the different categories that compose production costs.

The cornerstone of the P&L statement refers to the return of investment (ROI) for the selected locations (see Tables 9 and 10, base case scenario is presented). ROI reflects the potential of a microalgae production company to offset the CAPEX, and constitutes a valuable tool to assess its viability from a business point of view. The formula to calculate ROI is given below:

$$\mathrm{ROI} = \frac{\mathrm{CAD}}{\mathrm{CAPEX}} * 100\% \quad (4)$$

In order to assess in depth the economic viability of a potential company, the return of investment (ROI) was calculated for the three scenarios and for different market prices of pure astaxanthin (i.e. 1600,[5] 2000, 3000, 4000, 5000, 6000 and 7000 ($/kg$_{ASTAX}$), see Section 1). Furthermore, besides channeling astaxanthin into the market, it is assumed that the residual biomass could be exploited from an economic point of view as well. The residual biomass could be channeled to the market for bio-fertilizer purposes. An average market price of the biomass for bio-fertilizers (€30–60/kg) was assumed. This price amounted to €45/kg$_{BIOMASS}$.

CAD refers to the cash available for distribution to shareholders and is the subtraction between EBITDA and corporate taxes. EBITDA is one of the most important indicators of a company's financial performance. EBITDA is essentially the net income before interest, taxes, depreciation, and amortization, and can be used to analyze and compare profitability between companies and industries because it eliminates the effects of financing and accounting decisions. In this paper, it was assumed that no loan was taken, but an external investor provided the company with the capital needed. Therefore, no amortization, interest expense debt and interest income on cash are taken into account.

ROI for Livadeia was calculated at 17.8–63.79%, 19.46–65.45% and 20.83–62.93% for the worst-, base- and best case scenario. The respective values for Livadeia are −14.55–7.51%, −13.65–8.4% and −11.24–9.43%. Comparing the different values of ROI for the selected locations, it can be noticed that only in Livadeia a potential microalgae company is viable for the whole range of astaxanthin market prices, including

[5] Average market price of both synthetic and natural astaxanthin in 2014 ($447 million for 280 metric tons).

Table 10
P&L statement for Amsterdam (base case scenario).

Price(€)/kg	Astaxanthin	1408	1760	2640	3520	4400	5280	6160
kg	Astaxanthin	143	143	143	143	143	143	143
Price(€)/kg	Biomass	45	45	45	45	45	45	45
kg	Biomass	5763	5763	5763	5763	5763	5763	5763
Gross revenue		460,679	511,015	636,855	762,695	888,535	1,014,375	1,140,215
VAT (21%)		96,743	107,313	133,740	160,166	186,592	213,019	239,445
Total revenues		363,936	403,702	503,115	602,529	701,943	801,356	900,770
OPEX		696,240	696,240	696,240	696,240	696,240	696,240	696,240
EBITDA		−332,304	−292,538	−193,125	−93,711	5703	105,116	204,530
Depreciation (10%)		243,371	243,371	243,371	243,371	243,371	243,371	243,371
EBIT		−575,675	−535,909	−436,496	−337,082	−237,668	−138,255	−38,841
Interest expense debt								
Interest income on cash								
EBT		−575,675	−535,909	−436,496	−337,082	−237,668	−138,255	−38,841
Tax (25%)[a]		0	0	0	0	0	0	0
EAT		−575,675	−535,909	−436,496	−337,082	−237,668	−138,255	−38,841
CAD		−332,304	−292,538	−193,125	−93,711	5703	105,116	204,530
CAPEX		2,433,710	2,433,710	2,433,710	2,433,710	2,433,710	2,433,710	2,433,710
ROI		−13.65%	−12.02%	−7.94%	−3.85%	0.23%	4.32%	8.40%

[a] For negative earnings before taxes (EBT) taxes are zero.

residual biomass exploitation from the economic point of view as well (see Fig. 8). On the other hand, poor ROI in Amsterdam can be mainly attributed to the lower biomass productivity, which in turn resulted in a lower astaxanthin yield compared to the one of Livadeia. Under a specific threshold of astaxanthin yield, viability of the company cannot be achieved for all market prices. We agree that in Amsterdam natural photoautotrophic cultivation is not an attractive option for commercial astaxanthin production. Economic viability for the whole range of astaxanthin market prices could be either achieved if solar radiation and temperature are further enhanced, using for instance artificial illumination and temperature control devices or by switching to heterotrophic/mixotrophic metabolism. Both alternatives will result in significantly higher operational costs. However, they could be offset by the higher astaxanthin productivity, which will result in higher revenues. Thus, enhanced cultivation with artificial illumination for one or even both stages or switching to another metabolism that does not require sunlight, needs further investigation.

Costs for synthetic astaxanthin have been estimated at $1000/$kg_{ASTAX}$ or €880/kg_{ASTAX} [52]. Although high economic viability is proven for Livadeia, it can be noticed that the annual costs per kg of natural astaxanthin are approximately double as much the synthetic alternative, even for the best case scenario (i.e. €1536/kg_{ASTAX}). This means that for facilities established in the EU, natural astaxanthin cannot compete the synthetic one yet, at least as an additive to fish feed for pigmentation. Algae-derived astaxanthin is attractive in the commercial point of view only as a food supplement.

5. Conclusions and discussion

This paper examined three aspects of large scale natural astaxanthin production using the algal strain *H. pluvialis*: 1) the construction of a theoretical process model that calculates astaxanthin production employing natural photoautotrophic metabolism; 2) the mass-energy flows associated with the production process; and 3) economic performance of a potential company that wants to be established in the market, creating a facility in the EU. The model calculated the annual astaxanthin yield for two European cities of the same climatic zone (temperate) but with different environmental regimes, assuming a hybrid system (covering an area of 2 ha) for cultivation. The yield was calculated at 426 kg/year and 143 kg/year for Livadeia and Amsterdam respectively. Consequently, equator countries are more suitable for astaxanthin production. This statement is also enhanced by the locations, where the most important natural astaxanthin producers have built their facilities for photoautotrophic cultivation. Cyanotech has its facilities in Hawaii, while Algatechnologies in the south part of Israel [4,20]. Validity of the model was assessed by running the model for an established facility in Shenzhen, China, using environmental data for the Chinese city. It was found that theoretical and actual astaxanthin

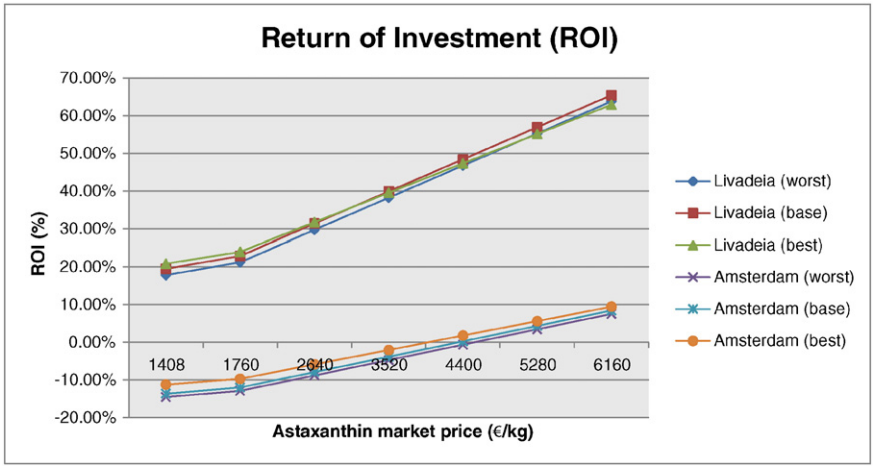

Fig. 8. Development of ROI for the selected locations as function of the different astaxanthin market prices.

yield resemble significantly. Therefore, we agree that the process model can be rendered as a valid tool for biomass productivity calculation of every microalgal strain, if adjustments on model inputs (e.g. modifying saturation intensities) are made.

Based on the biomass productivity calculated by the process model, the mass- and by extension energy flows all through production process were calculated. Regarding mass flows, it is evident that microalgae cultivation is associated with high fresh water consumption. In fact, it was calculated that for Livadeia and Amsterdam a supply of 63,526 m^3/year and 23,793 m^3/year is required. These values correspond to the water volume of 20 and 8 Olympic pools respectively in an annual basis. This constitutes significant water consumption, especially if we take into account that usual large scale microalgae facilities are bigger than 10 ha. In order to decrease water footprint, this paper implemented water recycling for the 'red stage', resulting in significant water- and consequently production costs savings in an annual basis. However, inevitable water loss during the production process may result in waterlogging and increased salinity in agricultural fields as Valipour [96] claims. Thus, irrigation management associated with water-intensive agriculture activities, such as microalgae cultivation are of particular significance [93,98,99].

A detailed discussion on the energy requirements all through production process is presented as well. Tubular PBR cooling is the most energy-intensive process throughout the production line for both locations (270 MWh/ha/year and 162 MWh/ha/year for Livadeia and Amsterdam respectively). This confirms the rule of thumb, that cultivation in PBRs is associated with the highest costs during the production process [63]. Furthermore, this study did not include indirect energy consumption. Any energy consumption is labeled as 'indirect' if the actual burning of fossil fuels or consumption of the energy is off-site. An investigation on this technical aspect would provide a rounded view of all energy needs that accompany a microalgae facility.

Regarding economic performance, a Profit and Loss (P&L) analysis was conducted. The P&L statement ended with the return of investment (ROI) for the different market prices of astaxanthin applying three scenarios. Stimulating eco-friendly manufacturing within the boundaries of the production process by employing water recycling and exploitation of flue gases CO_2 as well as solar power to satisfy the total energy needs, production costs dropped by 18% and 5% for Livadeia and Amsterdam respectively. Nevertheless, it was found that only in Livadeia viability of a microalgae company would be ensured, for the whole range of market prices. This highlights the significance of a warm climate, when cultivating microalgae photoautotrophically. The P&L analysis assesses the economic performance only for the first year. However, projections of this performance show the actual potential of the company in the global market. Thus, detailed long-run business forecasts would constitute a valuable asset for future viability of microalgae ventures. Furthermore, even if high economic potential is proven for Livadeia, costs per kg of astaxanthin for all scenarios could not compete adequately the synthetic alternative (€880/kg) in the feeding sector. The more expensive natural astaxanthin would result in overpriced fishes in the market, which is not desirable by the public especially in a period of recession. On the other side, most consumers probably do not realize that most of the salmonids, shrimps, lobsters and crayfishes found in the supermarkets nowadays are farmed introducing astaxanthin derived by petrochemicals. As long as public stays uninformed on the advantages of natural astaxanthin over the synthetic one, natural astaxanthin is condemned to remain at <1% of the global market. Therefore, future policies should support research and marketing initiatives regarding natural astaxanthin, in order to educate public on the beneficial properties of the most powerful antioxidant, gaining the place it deserves in the market.

In retrospect, natural astaxanthin production derived by *H. pluvialis* that is cultivated in sites characterized by high solar radiation and high temperatures is an attractive venture for dietary supplements and cosmetics. Nonetheless, for the EU natural astaxanthin is not a competitive alternative to synthetic one for aquaculture yet. Looking ahead, as society stimulates a transition towards 'green' solutions and taking into account that the global market is estimated to skyrocket in the forthcoming years, it is worth investigating further the different possibilities to produce this carrotenoid naturally at lower costs; for instance, focusing in energy efficiency within the different production stages and considering other types of renewable energy.

Potential domination of natural astaxanthin over the synthetic alternative will offer high quality fisheries that metabolize this pigment in their nutrition and will expand the applications in the pharmaceutical/nutraceutical/cosmetics sector as well.

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