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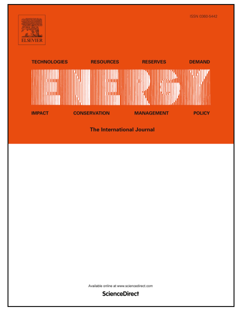

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Optimization of an Integrated Algae-based Biorefinery for the Production of Biodiesel, Astaxanthin and PHB

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ABSTRACT

In this work, we address the optimal design of an integrated microalgae-based biorefinery through the formulation of a mixed integer nonlinear programming model for the production of biodiesel and potential high-added value products. Main bioproducts are poly(hydroxybutyrate) (PHB) and astaxanthin. A combined heat and power cycle to transform biogas generated by the anaerobic digestion of waste streams is also included in the superstructure. Mass and energy balances are formulated for the biorefinery. Different alternatives for PHB extraction are taken into account. The anaerobic digestion model accounts for detailed composition of the different feed streams. Detailed capital cost models for process equipment are formulated and implemented in GAMS to maximize net present value (NPV). Results show that the production of astaxanthin and PHB provides a way to make biodiesel production economically feasible. Open pond and surfactant-chelate are selected for microalgae cultivation and PHB extraction method, respectively. Biodiesel price can be reduced to $0.48 due to incomes from astaxanthin and PHB sales. Also, an economic sensitivity

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analysis is performed. Further comparison between domestic and international cost conditions is carried out, showing higher NPV in the domestic case.

1. INTRODUCTION

The economic and environmental impact of biodiesel production from microalgae has been increasingly addressed in recent publications. Chisti[1] carries out a detailed analysis supporting the idea of microalgae as important sources for the provision of worldwide transport fuel requirements. He concludes that lowering the production cost will make microalgae biodiesel economically competitive. Davis et al.[2] examine two different systems, open pond (OP) and closed tubular photobioreactor (TPBR) using Aspen Plus simulation software, aiming at the establishment of baseline economics for these two pathways. Gebreslassie et al.[3] propose a multi-objective mixed-integer nonlinear programming model that simultaneously maximizes the net present value (NPV) and minimizes the global warming potential. Gong and You[4] address global optimization for a large-scale algae plant considering economic and environmental criteria. Based on a superstructure that includes several processing routes, they solve a multiobjective mixed integer nonlinear programming (MINLP) problem optimizing simultaneously the unit cost and the unit global warming potential (GWP) for the production of biodiesel or renewable diesel. Yan et al.[5] present a review on biotechnological preparation of biodiesel using algae based oils as feedstock for biodiesel production. Furthermore Pinedo et al.[6] address the optimization of a microalgae based biorefinery including economic aspects, complementing with safety analysis to move forward on sustainable processes, determining the same process and solvent selection that satisfies both the economical optimization and safety criteria.

More recently, the concept of integrated microalgae based biorefinery has been explored. A few authors analyze the use of microalgae biomass not only for biodiesel production but also for the production of value-added products, to improve economic aspects. Martín and Grossmann[7] propose an MINLP problem to optimize the production of methanol from glycerol, which is a byproduct in the oil transesterification reaction to biodiesel. The integrated process has an operational cost of \$0.16/L, \$0.05/L higher than the one that uses methanol from non-renewable sources, involving a compromise between costs and environmental issues. Sawaengsak et al.[8] conclude that it is even possible to get higher profit by integrating the production of Omega–3 fatty acids (value added product from microalgae biomass), but this is not enough to turn NPV to positive due to high capital and operating production costs.

García and You[9] develop a bioconversion network to determine the most cost-effective and environmentally sustainable process pathway for the productions of biofuels by an optimization model. Čuček et al.[10] developed a multi-period synthesis and optimization model to maximize the sustainably viable utilization of resources taking into account the competition between fuels and food production. Their results, basing the capital costs calculations on the six-tenth rule, show that switchgrass and algae are promising raw materials for producing biofuels. Gnansounou and Raman[11] perform life cycle assessment (LCA) for the production of biodiesel using Simapro 7.3.3, proteins for animal feed and succinic acid using algae as a feed stock. They observe a substantial impact reduction when comparing with conventional diesel production, soy protein and succinic acid system. Kokossis et al.[12] propose a mathematical model for the maximization of the economic potential including investments costs as exponential relationship with capacity, mass and energy balances. Two different scenarios were considered for the application of the proposed model;

however they not include biodiesel production. The first one was a lignocellulosic biorefinery, and the second one was a CO_2 microalgae biorefinery using the halophytic alga *Dunaliella* for the production of high value nutraceutical, β-carotene and glycerol.[13,14] Ahn et al.[15] implement a deterministic model for microalgae to biodiesel supply chain network considering naphtha and power as co-products. They use annualized fixed capital cost of the refineries as a function of contributions factors. Basing their study on Korea biodiesel market they reach a biodiesel production cost of 1.78 $/kg biodiesel.

Gong and You[16] propose a superstructure for a microalgae based biorefinery to produce of biodiesel and by-products as hydrogen, propylene glycol, glycerol-tert-butyl ether, and poly-3-hydroxybutyrate through multiobjective optimization where the co-production of glycerol-tert-butyl ether reduces the biodiesel production cost. Rizwan et al.[17] formulate an MINLP model including different technologies for the production of biofuels from microalgae. Different objective functions are formulated for the production of biodiesel, glycerol, bio-oil, bioethanol and biogas: Maximization of product yield and maximization of utilities. Negative economic results are achieved for the proposed technologies. Cheali et al.[18] propose an alternative scheme of hydrothermal liquefaction and transesterification with acid (H_2SO_4) or KOH, by the optimization of a superstructure for the production of biodiesel, glycerol, gasoline and co-products (fertilizer, animal feed, biogas and bioethanol). Lee et al.[19] carry out a review on recent progress of microalgae-based biofuels (biodiesel, bioethanol and bio-oil), emphasizing the importance of the integrated biorefinery as a way to reduce the production cost for microalgae based biofuels.

Even though, recent technologies include enzyme catalyst and supercritical transesterification[20], biodiesel is commercially produced from oil transesterification with methanol catalyzed by an acid or base solution, producing glycerol as a by-product in a 1/10 glycerol to biodiesel weight ratio[21]. Glycerol is mostly used for pharmaceutical products, food and cosmetics. However, its overproduction due to increasing biodiesel production, makes glycerol supply higher than demand. According to REN21 Renewables[22] the amount of biodiesel increased from 10.49 billion liters in 2007 to 29.75 billion liters in 2014. Glycerol can be used by microorganisms (i.e. *Cupriavidus necator, Bacillus* sp., *Alcaligenes latus*) as carbon substrate for the production of biopolymer PHB which is an alternative to fossil fuel based polymers as it has similar properties[23] and important applications such as drug delivery systems[24,25,26], food packaging[37] and biomedicine[28], among others. Current PHB production is based on substrates that are expensive or compete with human food and the cost of the raw material can reach up to 50% of the production cost.

Aiming at the integrated biorefinery concept[29,30], astaxanthin is a high value bioproduct that can be obtained from a few species of microalgae. Astaxanthin, a natural ketocarotenoid that is a secondary metabolite in microalgae, is a powerful antioxidant with application in nutraceuticals, pharmaceuticals, cosmetics and food industries. It is approved by The United States Food and Drug Administration (US FDA) as a food additive for aquaculture industry and as a dietary supplement.[31] According to a new report by Global Industry Analysts Inc.,[32] global carotenoids market reaches $1.3 billion by 2017 driven by the growing demand of natural food products and natural colorants. From a bioengineering point of view, it can be attractive to maximize both astaxanthin and oil production from microalgae species like *Haematococcus pluvialis,* as their accumulation takes place manipulating the same culture variables: nutrient depletion and high irradiances.[33]

In this work, we propose a mixed integer nonlinear programming model for the economic optimization of an integrated algae-based biorefinery, using detailed equipment cost capital correlations. It includes the biodiesel production of 43,800 t/year, potentially integrated to biopolymer and carotenoid production, as high-added value products, as well as a combined heat and power cycle. The objective function is the net present value (NPV) and numerical results show that the inclusion of high added-value products makes biodiesel production from microalgae economically attractive. We provide a comparison considering different process alternatives and market prices with international and domestic values. The following items remark the novelty of this work:

a) Detailed equipment design and cost correlations (Ulrich[34]) are used for most of the equipment, while most published work related to optimization models for integrated biorefineries follow the sixth-tenths rule or similar calculation.

b) Different PHB extraction technologies are embedded within a superstructure and the optimal alternative is selected by solving the resulting MINLP problem while Posada et al.[35] analyzed several extraction technologies for PHB production by simulation of different process schemes.

c) The use of waste generated in the production process of biodiesel, has been addressed in several papers and its importance has been highlighted. Anaerobic digestion is one of the most used alternatives,[36] but detailed design of this step has not been developed in optimization models for integrated biorefineries. In this paper, each feed stream to the anaerobic digester is characterized by its chemical composition to ensure the correct operation of AD being the final composition of the feed stream an optimization result.

d) Couple biodiesel to astaxanthin production, in order to increase the contribution of renewable fuels into the energy matrix making use of the installed facilities. Astaxanthin is a high value bioproduct produced in an industrial scale and with growing demand. Research on astaxanthin production is mainly based on experimental work.[37]

2. PROCESS DESCRIPTION

We formulate an integrated biorefinery superstructure for biodiesel production and high value-added products based on microalgae as it is shown in Fig. 1. It includes potential production of biopolymers (PHB) and food supplements (astaxanthin). Main processes are described below.

< Insert Figure 1 >

2.1 Biodiesel Production Process

2.1.1. Microalgae cultivation

Microalgae cultivation has basic requirements that include carbon dioxide as carbon source, macro and micronutrients (mainly phosphorous and nitrogen) and light source. Flue gas from a power-plant can be used as carbon dioxide source for microalgae cultivation, making the integration process attractive to be aligned to the objectives of the Kyoto Protocol[38].

Culture growing systems can be mainly classified into open and closed systems. Open ponds (OPs) have the advantage of being less expensive than tubular photobioreactors (TPBRs), but they have higher land requirement. Davis[2] reports that TPBRs are twice as expensive as OPs per liter of lipid extracted: \$2.25/L and \$4.78/L for OPs and TPBRs, respectively. Even though the productivity in TPBRs could be greater than in OPs, 0.41 g/L day against 0.125 g/L day (open pond depth = 0.12 m), TPBRs are also more energy intensive[3].

In this work, we consider two alternatives for *Haematococcus pluvialis* (lipid 18.3%, carbohydrate 50.4% and protein 31.3%) cultivation: a TPBR and an OP. The carbon dioxide feed stream is purified flue gas from a thermoelectric plant that is located near the area where the biodiesel plant can be installed. Carbon dioxide is also provided by an anaerobic digestion process and a combined heat and power unit (CHP). A nutrient recycle stream coming from the anaerobic digester (AD) is also fed to the cultivation system and a makeup stream is required to achieve nutrients requirement[39].

2.1.2. Harvesting and Dewatering

Algal slurry has a low concentration of algae biomass (around 0.202 g per 1000 g of water), so large amounts of water must be removed to obtain a concentrated stream of algae biomass. Solid-liquid separation processes include sedimentation and flotation as well as filtration and screening. Results provided by Gebreslassie et al.[3] show that the filtration technology returns a concentrated product (around 40% dry solids) and even though it requires important maintenance, its implementation allows the highest NPV of the configuration proposed.

In this work, we consider a settling tank, a filtration unit and a dryer as major equipment for harvesting and dewatering.

2.1.3. Lipid Extraction

Lipid extraction can be carried out either by mechanical or chemical methods. Expeller/press and ultrasonic assisted extraction are mechanical methods, whereas solvent oil extraction and supercritical fluid extraction method are chemical methods.

The use of mechanical press generally is energy intensive for the algae drying process are most commonly used.

In this work we consider hexane extraction where hexane makeup can be reduced due to the addition of a hexane recovery section[40]. Main products from the lipid extraction step include a lipid rich stream and an oil cake, which can be sent to the anaerobic digester in order to produce biogas and further electrical and thermal energy.

2.1.4. Oil transesterification

Biodiesel is produced by transesterification of vegetable fatty oils with low molecular weight alcohols, generally methanol. Several catalysts such as acid, alkali or even enzymes are commonly used for biodiesel production being the second ones most preferred in commercial processes due to their shorter reaction time. Biodiesel yield is dependent on the operating temperature, methanol-to-algal oil feed ratio, and the amount of catalyst[41].

In this work, we consider transesterification of algae oil using methanol and sodium methoxide catalyst.

2.1.5. Glycerol purification and methanol recovery

A purification step is required to remove impurities from glycerol, such as methanol, fats, soaps, catalyst, ash and water, resulting from the biodiesel production process. For this purpose, the glycerol enriched stream (22%) is fed into a stripper for methanol removal where superheated steam is used as heat source. The saturated methanol vapour is sent to a distillation column, where 91.7 % is separated from water and is recycled back to the transesterification reactor after a condensation step with a 99.9 % of purity[35].

The stripper bottoms stream is sent to a neutralization reactor where an acid solution of hydrochloric acid (HCl) is added. Residual catalyst (sodium methoxide) reacts with the acid to form methanol and salts. Soaps react with HCl to form free fatty acids (FFAs) and sodium chloride (NaCl). FFAs and other impurities such as ash or salts are removed from main stream in a decanter and an 80% weight glycerol stream is obtained.

In this paper we analyze, not only the possibility of selling glycerol as a final product but also, to use it as substrate for an anaerobic digester and as carbon source in PHB production process.

2.2 Anaerobic Digestion Process and Combined Heat and Power Unit

2.2.1. Anaerobic Digestion Process

Within the context of microalgae biodiesel biorefinery, the production of biogas by the anaerobic digestion of the microalgae cake, mainly composed of carbohydrates and proteins, is considered. The products of the anaerobic digestion are biogas and digestate, which can be used as fertilizer. It is technically feasible to integrate anaerobic digestion process and microalgae production step leading to an improvement on the energy balance of the process (Ledda et al.[42]). Carbon-to-nitrogen biomass ratio content is one of the main variables in the AD. Acceptable C/N ratios are between 20/1 and 30/1[43]. The unbalanced nutrients of microalgae sludge with low C/N ratios, around 4/1 and 6/1, are regarded as an important limitation to the AD operation. One method to avoid potential inhibition of the anaerobic digestion is to adjust low feedstock C/N ratios by adding high carbon content materials. In that sense, the addition of waste paper, which has C/N ratios ranging from 173/1 to more than 1000/1, in algal sludge feedstock, is a reasonable alternative to achieve a balanced C/N ratio. Yen and Brune[44] show that adding 50% (based on volatile solid) of waste paper in algal sludge feedstock increase methane production rate and suggest an optimum C/N ratio for co-digestion of algal sludge and wastepaper was in the range of 20/1 to 25/1. Amon et al.[45], Fountoulakis et al.[46] and Ehimen et al.[47] show that the incorporation of glycerol to other substrates (sewage sludge, manure and residues from microalgae biodiesel process) improves methane conversion yield, achieving a maximum conversion value, after which higher glycerol concentrations inhibit methanogenesis.

In this work, we consider the anaerobic digestion of process waste streams (microalgae oil cake from lipid extraction and cell residual material from PHB extraction) to obtain biogas (60% methane and 40% carbon dioxide[48]) and a digestate, sold as fertilizer. Potential substrates in the model include sludge from water treatment plant, waste paper and glycerol to improve carbon-to-nitrogen ratio. The addition of glycerol to the anaerobic digester enhances methane yield, but its inclusion and amount is an optimization result.

2.2.2. Combined Heat and Power Unit

The combined heat and power (CHP) system provides electricity and heat using methane as feedstock. In a heat engine, heat from a hot fluid is used to carry out mechanical work. Combined heat and power has three main stages: power generation, heat recovery and heat use.

In this work, the distribution of energy through the CHP combustion is considered: 32% power, 55% heat and 13% loss.

2.3 PHB Production Process

2.3.1. Fermentation

The biotechnological production of PHB is carried out in a fermentation stage where bacteria produce PHB by limiting essential nutrients for growth, such as nitrogen or phosphorus, while a stream of carbon source (glycerol) is fed in excess., We consider PHB production by the Gram-negative bacteria *Cupriavidus necator* due to its high productivity of about 1.52 g PHB/L h[49] and its use at industrial level. Before entering the fermentation step, the purified glycerol is diluted to a concentration of approximately 249 g/L[50]. Then, it is sent to a sterilization step where glycerol temperature and pressure is increased in order to reach the conditions required by the chosen strain. At this point, two fermentation vessels are needed. In the first one, cell growth is maintained without nutrient limitation aiming at increasing of cell biomass while the second fermentation tank is used to carry out PHB production. In this fermenter, an essential nutrient is limited to allow an efficient PHB synthesis by the microorganisms. The residence times are 21 h and 22.5 h, respectively[35].

2.3.2. PHB extraction

PHB is extracted from the bacterial cytoplasm to isolate the polymer from the cell residual material which is fed to anaerobic digester to contribute with energy generation. At industrial scale, this is the most important step in PHB production as it determines the biopolymer selling price. The appropriate selection of the extraction method is crucial for the process economic viability.

Among several extraction methods described in literature, surfactant-chelate digestion are presented as a promising alternative due to the low environmental pollution and the high product quality, together with the requirements of low quantities of chemicals requirements. Surfactant and chelate to dry biomass ratios of 0.0075 and 0.01 have been reported[51]. Also, solvent extraction could be an encouraging option because it allows the use of PHB in medical applications by the elimination of endotoxins produced by Gram-negative bacteria and it does not degrade the biopolymer, which can be obtained with high purity.

In this work, we include two extraction alternatives in the PHB process superstructure: chemical and solvent extraction, respectively.

In the first case, the addition of chelate and surfactant to the PHB stream produces a destabilization in bacteria outer membrane by the formation of complexes with divalent cations[51]. These induced changes in the outer membrane cause a destabilization in the inner membrane as well, leading to the microorganism disruption and the extraction of a higher purity biopolymer. The disrupted cell mass of the microorganisms is separated by centrifugation and sent to the anaerobic digester. Surfactants and chelates are eliminated in a decantation step.

In the second case, after cellular lysis, the biopolymer extraction is carried out by solvent addition, diethyl-succinate (DES). A mass polymer to solvent ratio of 1/20 is used. A second centrifugation step is required for the separation of residual cell mass, which is fed into de anaerobic digester. After cooling, a mixture of PHB and water is obtained, enabling the solvent recovery.

The product stream in both process alternatives is sent to a spray drier, where PHB is purified to a final humidity content of 0.1%[52].

2.4 Astaxanthin Production Process

Haematococcus pluvialis can both accumulate lipids and astaxanthin. Part of the microalgae cultivation system for the production of biomass can be destinated to astaxanthin accumulation.

In this work, after dewatering, part of the algae biomass is dried in a spray dryer and then cracked by a bed airflow pulveriser to obtain the final product[53], which has around 2.5% astaxanthin concentration.

3. Mathematical Model

An MINLP model with nonlinear and nonconvex constraints is formulated and solved using the global optimization solver BARON[54] to optimize the integrated biorefinery design for the production of 43,800 t/year of biodiesel.

3.1. Mass Balances

Mass balances are formulated for each non-reactive unit (θ) in the integrated biorefinery superstructure shown in Fig. 2 as follows:

$$\sum_{k\in K} f_{\theta,j}^{k} = \sum_{r\in R} f_{r,j}^{\theta} \qquad \forall j \in J \tag{1}$$

Where:

$f_{\theta,j}^{k}$: Mass flowrate of component j from inlet stream k to unit θ [kg j/day]

$f_{r,j}^{\theta}$: Mass flowrate of component j from θ to outlet stream r [kg j/day]

Mass balances for each reactive unit (θ') are described by Eq. (2):

$$f_{r,j}^{\theta'} = \sum_{k\in K} f_{\theta',j}^{k} + \sum_{h\in H} \xi_{j,s_h} \cdot M_j / M_{s_h} \cdot C_{s_h} \cdot \sum_{k\in K} f_{\theta',s_h}^{k} \qquad \forall j \in J \tag{2}$$

Where:

$f_{r,j}^{\theta'}$: Mass flowrate of component j from θ' to outlet stream r [*kg j/day*]

$f_{\theta',j}^{k}$: Mass flowrate of component j from inlet stream k to unit θ' [*kg j/day*]

ξ_{j,s_h}: Stoichiometric coefficient between component j and component s_h [*kmolj/kmol* s_h]

s_h : Limiting reactant for reaction h

M_j: Molecular weight of component j [*kg j/kmol j*]

M_{s_h}: Molecular weight of component s_h [*kg s_h/kmol s_h*]

C_{s_h}: Limiting reactant conversion for reaction h

f_{θ,s_h}^{k} : Mass flowrate of component s_h from inlet stream k to unit θ *[kg s_h/day]*

< Insert Figure 2 >

Detailed mass balances and model parameters for the Anaerobic Digestion Process, PHB Fermentation and PHB Extraction Processes are described below.

3.2. Energy balances

Linear relationships are assumed for energy consumption, as follows:

$$EC_\theta = ECR_\theta \cdot m_\theta \qquad (3)$$

Where EC_θ corresponds to energy consumption in unit θ in *kWh/day*, ECR_θ is energy consumption ratio per unit of mass flowrate relative to unit θ, in kWh/kg, and m_θ is the mass flowrate relative to unit θ in *kg/day*. Energy consumption in OP and TPBR, ECR_{OP} and ECR_{TPBR} respectively, are calculated as function of the corresponding reactor volumes. Decanters used for PHB extraction are considered as gravity separators following Ulrich[34], hence no power requirements are computed for them. Main parameters are listed in Table 1.

< Insert Table 1 >

3.3. Integer and Mixed Integer constraints

Potential units in the proposed superstructure (Fig. 2) are associated to binary variables. Two binary variables, y_1 and y_2, are used to model the selection of microalgae cultivation technology corresponding to OP and TPBR, respectively. Eq. (4) guarantees that only one of the proposed microalgae cultivation technologies can be selected:

$$\sum_{i=1}^{2} y_i = 1 \tag{4}$$

The amount of glycerol produced by the transesterification reaction of microalgae oil can be sold as a final product, fed into the anaerobic digester to improve methane yield and/or used as carbon source for the production of PHB. In the case that any amount of glycerol is used for PHB production (y_3 =1), the PHB extraction method is associated to y_4 and y_5, which correspond to surfactant-chelate extraction and solvent extraction technologies, respectively. Eqs. (5) and (6) ensure the selection of only one extraction method if y_3 is equal to one. Eqs (7) and (8) ensure that no PHB extraction technology is necessary if the PHB production process is not selected (y_3 =0).

$$y_3 \leq y_4 + y_5 \tag{5}$$

$$y_3 + y_4 + y_5 \leq 2 \tag{6}$$

$$y_4 \leq y_3 \tag{7}$$

$$y_5 \leq y_3 \tag{8}$$

Big M constraints[61] are formulated for potential units in the superstructure, as follows:

$$F_{j,i}{}^{s} - My_i \leq 0 \quad \forall j \in J, i \in I, s \in S \tag{9}$$

Where $F_{j,i}{}^{s}$ corresponds to mass flowrate of component j in stream s and M is a parameter large enough that when y_i=1, the constraint becomes redundant, otherwise, if y_i=0, the mass flowrate is enforced to be null, resulting in the non-existence of the unit related with $F_{j,i}{}^{s}$.

3.4. Design and economic constraints

Detailed equipment design and cost equations for decanters (DC1-6), conditioner tank (CT), steam dryer of biomass sludge (DRY), hexane recovery unit (HR), transesterification reactor (TRANS), washing column (WC), biodiesel purification splitter (SPL), methanol-glycerol stripper (STR), distillation column to recovery methanol (DIS), neutralization reactor used in the glycerol purification step (NEUTR), steam dryer where PHB is purified to a final humidity content (SD2), centrifuges involved in the PHB extraction step (CN1-3), cellular lyses homogenizer (HOM) and fermenters involved in the PHB production process (BR1, BR2, RC1, RC2) are given in the Supporting Information.

Capital cost for filter press (FP), lipid extractor (LE), tubular photobioreactor (TPBR), open pond (OP), anaerobic digester (AD), combined heat and power unit (CHP) and spray dyer (SD1) and pulverization unit (PAS) from astaxanthin production process have been calculated using the six-tenths rule.

$$C_{eq4,1} = I_{CE1}/I_{CE2} \, . \, C_{eq4,2} . \left(Q_{eq4,1}/Q_{eq4,2}\right)^{\gamma} \tag{10}$$

Where $C_{eq4,1}$and $C_{eq4,2}$ are the purchase equipment costs of each equipment in the actual year and in the year of reference, respectively, in $\$$. I_{CE1} and I_{CE2} are the chemical engineering cost indices for the year of interest and reference. $Q_{eq4,1}$ and $Q_{eq4,2}$ correspond to equipment capacities and γ is the size exponent (0.6, for most cases).

3.5. Inequality constraints

As it is shown in Table 2, produced carbon dioxide is set to be less than the amount of required carbon dioxide stream for biomass generation to ensure global emissions reduction. The upper bound for the carbon dioxide stream is given by the amount produced in a mid-size thermoelectric plant. Astaxanthin production is limited by current market demands in Latin America[62] considering an overproduction of 33%, which can be directed to other international markets. An upper bound for the waste paper stream that is fed to the anaerobic digester is assumed to be the total amount of the domestic recycled paper from a city whose population is nearly 400,000 inhabitants (as it is the case for Bahía Blanca, Argentina). Upper bounds for the sludge stream fed to the anaerobic digester are given by available quantities from a wastewater treatment plant located next to the same city. For both raw materials, no cost is considered in the economic assessment. To ensure an optimal operation of the anaerobic digester, the C/N ratio is set between 20 and 25[44], which has not been taken into account in previous related work.

< Insert Table 2 >

3.6. Objective function

Net present value is used as the objective function to be maximized for the economic analysis.

$$NPV = -Inv + a^{-1}\left(Rev - C_{manuf} - C_{rawmat} - C_{utilit}\right) \tag{11}$$

Where NPV corresponds to net present value in $\$$, which measures economic performance of the integrated microalgae-based biorefinery. Inv is the total capital investment cost and is the sum of fixed capital ($F_{capital}$), working capital ($W_{capital}$) and land cost (C_{land}).

Fixed capital is the actual equipment cost ($C_{equipment}$) considering a contingency and fee factor (α = 1.18) and a grass-roots plant factor (β = 1.3) if the biorefinery is a new plant. Working capital is assumed as recommended by Ulrich[34] to be 10% of fixed capital.

$$F_{capital} = C_{equipment} \cdot \alpha \cdot \beta \tag{12}$$

a is the annuity and it has been calculated for a project life (n) of 15 years and 10% interest rate $i\%$.

$$a = i\%. (1+i)^n / ((1+i)^n - 1) \tag{13}$$

Rev represents the revenues from selling products and by-products in $/ year as is calculated as:

$$Rev = 365 \cdot \sum_p pr_p \cdot m_p + 365 \cdot INC_{prod} \cdot m_{bd} \tag{14}$$

$$\forall\, p \in \{biod, phb, ast, glyc, fer\}$$

Where pr_p is the selling price in *$/kg*, m_p is the daily production of each product or by-product in *kg/day*, INC_{prod} corresponds to biodiesel production incentives in *$/kg biodiesel* and m_{bd} is the total amount of biodiesel produced per day.

Manufacturing cost (C_{manuf}), in *$/year*, is given by the sum of different operating expenses such as operating and supervisory labor (O_{labor} and S_{labor} respectively), maintenance and repairs ($M_{repairs}$), operating supplies ($O_{supplies}$), laboratory charges ($L_{charges}$), plant overhead ($P_{overhead}$), local taxes (L_{taxes}) and insurances (Ins), as follows:

$$C_{manuf} = O_{labor} + S_{labor} + M_{repairs} + O_{supplies} + L_{charges} + P_{overhead} + L_{taxes} + Ins \quad (15)$$

Operating labor is related to the attention that it is required by an operator in order to run the equipment, $O_{requirements}$ from Ulrich[34]. Considering that an operating slot (O_{slot}) requires five people and an annual operator salary that can be estimated since it is assumed that $salary$ ($salary$ = 41,600 $/year [34]) increases at a rate of δ = 0.03 per year, operating labor can calculated as:

$$O_{labor} = O_{requirements} \cdot O_{slot} \cdot salary \cdot (1+\delta)^{(\omega-2003)} \quad (16)$$

Where ω is the current year.

S_{labor}, $M_{repairs}$, $O_{supplies}$, $L_{charges}$, $P_{overhead}$, L_{taxes} and Ins are calculated by Eq. 17-23 with X_{sup}, X_{oper} and X_{lab} equal to 0.1, X_{main}= 0.02, X_{over} = 0.5 and X_{tax} and X_{ins} equal to 0.01.

$$S_{labor} = X_{sup}.\, O_{labor} \quad (17)$$

$$M_{repairs} = X_{main}.\, F_{capital} \quad (18)$$

$$O_{supplies} = X_{oper}.\, M_{repairs} \quad (19)$$

$$L_{charges} = X_{lab}.\, O_{labor} \quad (20)$$

$$P_{overhead} = X_{over}.\left(O_{labor} + S_{labor} + M_{repairs}\right) \quad (21)$$

$$L_{taxes} = X_{tax}.\, F_{capital} \quad (22)$$

$$Ins = X_{ins}.\, F_{capital} \quad (23)$$

C_{rawmat} represents the raw materials cost in $/year and it is calculated as:

$$C_{rawmat} = 365 \sum_j pr_r \cdot m_r \quad (24)$$

$$\forall\, r \in \{met, hex, nit, pot, smo, hcl, srf, che, des\}$$

where pr_r is the purchase price in *\$/kg*, m_r is the daily requirement of raw material in *kg/day*. Raw materials cost (pr_r) are shown in Table 3 together with selling prices (pr_p) and other costs.

< Insert Table 3 >

Finally, in Eq. (25) C_{utilit} is the cost associated to utilities, which includes water and energy (thermal and electric).

$$C_{utilit} = 365 \cdot \left[\left(HE_{cons} - HE_{prod} \right) \cdot pr_{HE} + \left(EE_{cons} - EE_{prod} \right) \cdot pr_{EE} + Wt_{cons} \cdot pr_{wt} \right] \qquad (25)$$

Where HE_{cons} and EE_{cons} corresponds to the consumed thermal energy and the consumed electric energy, respectively, expressed in *kWh/day*. HE_{prod} and EE_{prod} represents the thermal and electric energy, respectively, expressed in *kWh/day* and generated in the process due to the inclusion of the anaerobic digester and further CHP unit. Wt_{cons} corresponds to the total consumed water in the integrated biorefinery expressed in *kg/day*, whereas pr_{HE}, pr_{EE} and pr_{wt} are the prices of thermal energy, electric energy and water, respectively (Table 3). To analyze the inclusion of value added products into the microalgae based biorefinery, biodiesel production cost relative to co-products revenues ($Cprod_{rev\_coprod}$) are calculated as follow (and will be referred to as biodiesel production cost).

$$Cprod_{rev\_coprod} = \left(C_{manuf} + C_{rawmat} + C_{utilit} + C_{capital} - Rev_{coprod} \right) / (365 \cdot m_{bd}) \qquad (26)$$

$$C_{capital} = Inv \cdot a \qquad (27)$$

Where Rev_{coprod} in *\$/year* includes co-products revenues (PHB, fertilizer) and astaxanthin, as well as biodiesel production incentives, when applied. $C_{capital}$ is the annualized capital cost in *\$/year* calculated as the product of total capital investment cost (Inv) for the annuity (a).

3.7. Sensitivity Analysis

In order to evaluate the impact over the objective function considering the uncertainty values of certain parameters, a sensitivity analysis is performed.

4. Results and Discussion

The resulting MINLP model for the production of 43,800 t/year of biodiesel has 3,582 continuous variables, 5 discrete variables and 2,870 constraints. The model is implemented in GAMS[69] and solved with the global optimization solver BARON[54]. CPU time is 28.926 s. Our model size is similar to the one reported by Gebreslassie et al.[3] for an algae-based hydrocarbon biorefinery. Numerical results for the maximization of NPV show that the optimal configuration includes biodiesel, PHB and astaxanthin production processes. For algae growth, an open pond (OP) is selected $(y_1 = 1)$. Surfactant-chelate digestion is selected as PHB extraction method $(y_4 = 1)$. Figure 3 shows optimal values for the main streams.

< Insert Figure 3 >

Anaerobic digestion of the cell residual material from the PHB extraction, oil cake, waste paper and sludge, provides thermal and electric energy to the biorefinery process by biogas production. Biogas is supplied to a combined heat and power cycle (CHP) where electricity and useful thermal energy in a single, integrated system are generated, thus increasing efficiency from separate processes from 45% to 80%. The biodigestion of the mentioned substrates ($CN_{\text{Anaerobic Digestion Ratio}}$ = 21.789) leads to the generation of 4.51×10^{8} kWh of thermal energy (HE_{prod}) and 2.62×10^{8} kWh of electrical energy

(EE_{prod}), complementing and contributing to the microalgae biorefinery as Ubando et al.[70] concluded in their optimization work. Even though it represents 0.05% of total energy requirements, the anaerobic digester and combined heat and power cycle provide a sustainable way to manage waste streams from the biorefinery itself and nearby plants. Solid residues obtained in the anaerobic digester (2.6×10^4 t/year) are sold as fertilizers.

PHB production level is indirectly determined by the biodiesel production as it is associated to glycerol availability, which is obtained as a by-product of the transesterification reaction. Not only glycerol can be fed as raw material for the PHB production process (as Ray et al.[71], Garlapati et al.[72] and Moreno et al.[73] propose in their work), but it can be sold as a final product[74] and it can also be incorporated into the anaerobic digester, to improve methane production, as it was demonstrated by Ehimen et al.[47] and Zhang et al.[75]. In the optimal configuration, the total amount of produced glycerol is sent to the PHB production process, allowing the production of 8.07×10^2 t/year of PHB. This value is similar to the production of a commercial scale plant for PHB. Current PHB plants in Brazil and Japon produce 50 t/year and 1,000 t/year respectively as it is mentioned by Chanprateep in his review[76]. PHB can be used as raw material for bag and bottle production as is the case of PHBottle project which receives funding from the European Union's Seventh Framework Programme. Its main goal is the development of biodegradable material for packaging and non-packaging uses by fermentation of juice processing wastewater[77]. Due to domestic reutilization of these bags for dumping garbage and a strategic separation, by means of waste classification, the use of this biopolymer can be an attractive option. Feeding the biodigester with the domestic rubbish and the used PHB bags could be an interesting alternative, as well. The incorporation of an anaerobic digester allows the reduction of the environmental impact due to minimization of the consumption of non-renewable resources and waste disposal.

Based on the international considered market values for raw materials and products (Table 3), the net present value is 174.02 $MM, corresponding to the optimal configuration, which selects biodiesel (open pond as microalgae cultivation techonology), astaxanthin and PHB production processes (surfact-chelate extraction method). For this case, biodiesel production cost, calculated as Eq. (26), is $0.48/kg biodiesel. Gebreslassie et al.[3] consider biodiesel price of $3.07/kg, higher than the current unit price, to obtain a positive NPV. In that case, the optimal process (NPV of 540.5 $MM), takes into account TPBRs for algae growth with a production of 141,028 t of biodiesel per year, and a production cost of $1.95/kg. This result shows that even when NPV is higher than the obtained in our work, it is due to the size of the production plant. Also, it is shown that biodiesel production cost is higher, when no value-added products are included in the superstructure. Gong and You[16] consider the addition of potential high value-added products to the superstructure for a microalga based biorefinery to produce biodiesel. Optimization results show that co-production of glycerol-tert-butyl ether reduces biodiesel production cost to 0.87 $/kg of biodiesel. In that case, even though biodiesel production cost has been reduced, it cannot reach a competitive value as compared with the values obtained in this work.

Main components of the biodiesel production cost ($Cprod_{rev\_coprod}$) and its contribution are shown in Fig. 4. Revenues (Rev_{coprod}) include astaxanthin and by-products sales (PHB and fertilizer) as well as biodiesel production incentives ($0.3/kg biodiesel). As the major contribution to the production cost is due to utilities (C_{utilit}), Table 6 shows the distribution of energy consumption in the biorefinery for the annual production of astaxanthin, biodiesel and PHB. The biodiesel production process accounts for main energy consumption (approximately 95% of total) and Table 4 also shows the breakdown of each step contribution. Harvesting and dewatering represent the step with major energy consumption (74% of total energy consumption), which is mainly due to the large amount of water that must be removed from algae biomass prior to oil extraction due to the selection of OP technology as Sanchez et al.[78] claimed. This result is in agreement with published work[79]. Gebreslassie et al.[3] conclude that the dewatering section is the main power consumer, ranging from 50 to 70% of total power consumption. Even though water consumption is 16 times higher than that required by tubular photobioreactors[55], the optimal configuration includes open pond as algae cultivation technology. This is due to the fact that TPBR technology involves high energy consumption. We have additionally simulated the TPBR scheme for the sake of comparison with the OP option and it requires 536.85 kWh/kg biodiesel considering a functioning of 12 h without LED lightening against 85.20 kWh/kg biodiesel in the open pond case (algae cultivation, harvesting and dewatering sections are considered). This value is higher than the one reported by Martín and Grossmann[80] (2.75 kWh/kg biodiesel) because we are considering current commercial dewatering technologies.

< Insert Table 4 >

< Insert Figure 4 >

Capital cost ($C_{capital}$) is also an important item in the biodiesel production cost in Fig 4. It is worth mentioning that this figure shows the contribution of the different variables involved in the calculation of $Cprod_{rev\_coprod}$ by Eq. (26). In this sense, revenues are below the costs because is a negative term in the biodiesel production cost equation. Table 5 shows investment cost distribution between main sections of the integrated biorefinery. The biodiesel production process from microalgae accounts for the highest investment cost and its breakdown is also shown in Table 5. It can be seen that algae growth and harvesting and dewatering constitute the steps that require higher capital costs (199.09 $MM and 55.37 $MM, respectively). To further analyze the benefits of an integrated biorefinery, we have solved two additional MINLP problems. In the first one (MINLP_A) we fix astaxanthin production equal to zero. In the second one (MINLP_B), astaxanthin production is set equal to zero and glycerol sent to PHB production process is set to zero, too ($y_3 = 0$), leading to the alternatives of selling glycerol as a by-product and/or using it as raw material for the anaerobic digestion process.

< Insert Table 5 >

Numerical results for MINLP_A determine a negative NPV of -462.09 $MM, 365.54% lower than the optimal configuration, giving a biodiesel production cost of $2.39/kg biodiesel, 499.37% higher than the optimal solution for the original MINLP where biodiesel, astaxanthin and PHB are produced. Furthermore, MINLP_B numerical results show that the corresponding NPV in this case is even lower than the obtained for MINLP_A (-481.79 $MM) and biodiesel production cost is even higher ($2.42/kg biodiesel), as compared to the optimal case. Fig. 5 shows NPV and distribution of biodiesel production cost for each case. Process schemes comparison emphasizes the need for integrated biorefineries to ensure economical process feasibility.

< Insert Figure 5 >

A sensitivity analysis was performed in order to assess the influence of several parameters variations over the objective function (NPV) [70], namely, methanol recovery in glycerol purification section; lipid content; PHB productivity; energy parameters for dryer and TPBR in harvesting and dewatering section; and selling prices of biodiesel, astaxanthin and PHB. It is worth mentioning that every parameter variation is applied to the entire superstructure.

As it is shown in Figure 6, the most sensitive parameters are the lipid content and astaxanthin price. Moreover, the NPV is considerably sensitive to changes in the energy parameter referred to the dewatering section and biodiesel price. On the other hand, PHB price and PHB productivity have a similar sensitivity but little influence on the objective function. For instance, a 10% increase in PHB price or PHB productivity, represents nearly a 2% variation in the objective function. Methanol recovery proves to be the least sensitive parameter; a change of 10% only produces an impact on the NPV of nearly 0.5%. Finally, it can be seen that changes on ECR_{TPBR} have no effect on the NPV because the model keeps selecting the OP alternative for algae growth.

Sensitivity analysis allows establishing the aspects where efforts should focus in order to obtain higher benefits with the present integrated biorefinery. Even though two of the four main sensitive parameters depend on market situation (astaxanthin and biodiesel selling price), there are also process aspects such as drying technologies improvements in dewatering section, which can generate a positive impact on the integrated biorefinery. Furthermore, the influence of microalgal lipid content over the NPV indicates the importance of including actual and exact data for the biological aspects of the model.

< Insert Figure 6 >

Finally, we analyze the implementation of the proposed integrated biorefinery considering domestic conditions in Argentina. Main differences include electric energy cost, \$0.05/kWh[81] against \$0.0722/kWh, land cost, \$0.0295/m^2 [82] against \$0.3/m^2

considering non-arable land for open ponds and biodiesel price, 0.77 $/kg[83] against 0.98 $/kg. In the domestic case and to reflect current situation in Argentina, we do not include incentives for biodiesel production. Optimal NPV is 115.06 $MM and the production cost for biodiesel is $0.44 kg biodiesel, showing that the installation of an integrated biorefinery in Argentina is also an attractive alternative (Fig. 7). Even when extended areas are required for ponds installation, it is only an 8%[84] of the soybean cultivated area, required to ensure the same volume of biodiesel (43,800 t/year), thus using non-arable areas and not competing with food crops in concordance with Maranduba et al. [85].

< Insert Figure 7 >

In this work, we develop a model considering only the most promising technologies reported in literature. Also, potential value-added products to satisfy local demands are included, focusing on the concept of integrated biorefineries, where the generation of these products is a key aspect to get positive economic results. In this microalgae-based biorefinery, several products as biodiesel, glycerol, astaxanthin and PHB can be obtained. The decision of which by-products are taken into account as well as the location of the biorefinery are important issues that must be considered to ensure economic feasibility. Numerical results also show that astaxanthin production is required to make NPV positive and biodiesel production cost competitive, being relevant to the economic feasibility of the biorefinery. The installation of a local microalgae-based biorefinery has been explored and provides also promising results.

5. Conclusions

We propose an MINLP model for the economical optimization of a microalgae-based biorefinery for the production of biodiesel, PHB from glycerol and astaxanthin as potential value-added bioproducts. The superstructure includes two alternatives for the microalgae cultivation technology (OP and TPBR), three alternatives for the use of glycerol (final product, submit to PHB production process and added as substrate to the anaerobic digester) and two alternatives for PHB extraction method (solvent extraction, surfactant-chelate extraction).

Numerical results show that the use of open ponds for microalgae cultivation, the production of astaxanthin, as well as the use of glycerol as carbon source for PHB production, constitute an economically attractive alternative for biodiesel production. NPV for this optimal configuration is 174.02$MM, rendering a biodiesel production cost of $0.48/kg biodiesel mainly due to the inclusion of astaxanthin revenues.

The sensitivity analysis points out the aspects that should be addressed to achieve higher profit on the integrated biorefinery. Regardless the market issues, there exist process aspects such as improvement in dewatering technology which can lead to generate a positive impact on the microalgae based biorefinery.

A global optimum has been determined for the MINLP problem representing an integrated biorefinery. Current work focusses on formulating a multi-objective MINLP to simultaneously address environmental and economic objective functions.

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Supporting Information

Mass balances for Anaerobic Digestion Process and PHB Production Process are included in the supporting information as well as detailed equipment design and cost equations.

Nomenclature

Components:

al	algae
ast	astaxanthin
biod	biodiesel
carb	carbohydrate
cdo	carbon dioxide
ch4	methane
che	chelate
crm	cell residual material
des	diethyl-succinate
fer	fertilizer
ffas	free fatty acids
glyc	glycerol
hcl	chloride acid
hex	hexane
lip	lipid
met	methanol
micr	microorganisms
nacl	sodium chloride
nit	nitrogen
phb	poly(hydroxybutyrate)
pot	phosphorous
prot	protein
sld	sludge
smo	sodium methoxide
srf	surfactant
wt	water
wp	waste paper

Streams:

air	air
ao	algae oil
ap	aqueous phase
apt	algae paste
as	algae sludge
ax	astaxanthin
bd	biodiesel
bg	biogas
crms	cell residual material
chs	conditioner
dsst	diethyl-succinate
ep	ester phase
epo	electric power
fert	fertilizer

fg	flue gas
glyp1-3	purified glycerol
gp	glycerol phase
hcls	chloride acid
hpo	heat power
hr	recycled hexane
me	methanol
mh	hexane makeup
mnt	nutrients makeup
mwr	water makeup
oc	oil cake
pb	phb
rcdo	recycled carbon dioxide
rme	recycled methanol
rnt	recycled nutrients
rwr	recycled water
rwr1-2	recycled water
sch	surfactant and chelate
slds	waste water treatment plant sludge
smo	sodium methoxide
stm1-2	steam
wps	waste paper
wr1-3	water
wst1-8	waste stream
wv1-3	water vapor

Non-reactive units:

CN1-3	centrifuge
CT	conditioner tank
DC1-6	decanter
DIS	distillation column
DRY	dryer
FP	filter press
HR	hexane recovery unit
LE	lipid extractor
PAS	pulverizer
SD1-2	spray dryer
SPL	splitter
STR	stripper
WC	washing column

Reactive Units:

AD	anaerobic digester
BR1-2	fermenter
CHP	combined heat power unit
HOM	homogenizer
NEUTR	neutralization reactor
OP	open pond
RC1-2	reactor
TRANS	transesterification reactor
TPBR	tubular photobioreactor

Mass balance:

$f_{\theta,j}^{k}$	mass flowrate of component j from inlet stream k to unit θ in *kg j/day*

$f_{r,j}^{\theta}$ mass flowrate of component j from θ to outlet stream r in *kg j/day*
J set of components indexed by j
$f_{r,j}^{\theta'}$ mass flowrate of component j from θ' to outlet stream r in *kg j/day*
$f_{\theta',j}^{k}$ mass flowrate of component j from inlet stream k to unit θ' in *kg j/day*
ξ_{j,s_h} stoichiometric coefficient between component j and component s_h in *kmolj/kmols*$_h$
s_h limiting reactant for reaction h
M_j molecular weight of component j in *kg j/kmol j*
M_{s_h} molecular weight of component s_h in *kg* s_h*/kmol* s_h
C_{s_h} limiting reactant conversion for reaction h
f_{θ,s_h}^{k} mass flowrate of component s_h from inlet stream k to unit θ in *kg*s_h*/day*

Energy balance:
EC_θ energy consumption in unit θ in *kWh/day*
ECR_θ energy consumption ratio per unit of mass flowrate relative to unit θ in *kWh/kg*
m_θ mass flowrate relative to unit θ in *kg/day*
θ set of process units including OP, TPBR, DC1, FP, LE, HR, TRANS, DC2, DC3, DRY, AD, SD1, PAS, STR, BR1, RC2, CN1, SD2, HOM, CN2, CN3 and RC2
y_i binary variables with i from 1 to 5, representing OP microalgae cultivation technology, TPBR microalgae cultivation technology, glycerol as raw material for PHB production, surfactant-chelate PHB extraction method and solvent PHB extraction method, respectively.
$F_{j,i}{}^{s}$ mass flowrate of component j in stream s in *kg j/day*
M parameter associated to Big M constraints
I set of technology alternatives for microalgae cultivation and PHB extraction indexed by i
S set of streams indexed by s

Equipment design and economic equations:
$C_{eq4,1}$ purchase equipment costs of each equipment in the actual year in *$*
$C_{eq4,2}$ purchase equipment costs of each equipment in the year of reference in *$*
I_{CE1} chemical engineering cost indices for the year of interest
I_{CE2} chemical engineering cost indices for the year of reference
$Q_{eq4,1-2}$ equipment capacities
γ size exponent
Inv total capital investment cost in *$*
$F_{capital}$ fixed capital in *$*
$W_{capital}$ working capital in *$*
C_{land} land cost in *$*
$C_{equipment}$ equipment cost in *$*
α contingency and fee factor
β grass-roots plant factor
a annuity
n project life
$i\%$ interest rate
Rev revenues from selling products and by-products in *$/ year*
pr_p selling price in *$/kg*
m_p daily production of each product or by-product in *kg/day*
P set of products and by-products indexed by p
INC_{prod} biodiesel production incentives in *$/kg biodiesel*
m_{bd} total amount of biodiesel produced per day in *kg biodiesel/day*
C_{manuf} manufacturing cost in *$/year*

O_{labor} operating labor in *\$/year*
S_{labor} supervisory labor in *\$/year*
$M_{repairs}$ maintenance and repairs in *\$/year*
$O_{supplies}$ operating supplies in *\$/year*
$L_{charges}$ laboratory charges in *\$/year*
$P_{overhead}$ plant overhead in *\$/year*
L_{taxes} local taxes in *\$/year*
Ins insurances in *\$/year*
$O_{requirements}$ attention that it is required by an operator in order to run the equipment in *operator/shift*
O_{slot} operating slot in *shift*
$salary$ annual operator salary in *\$/(operator.year)*
δ salary increment ratio
ω current year
X_{sup} ratio between supervisory labor cost and operating labor
X_{main} ratio between maintenance and repairs cost and fixed capital
X_{oper} ratio between operating supplies cost and maintenance and repairs
X_{lab} ratio between laboratory charges cost and operating labor
X_{over} ratio between plant overhead cost and operating labor, supervisory labor and maintenance and repairs
X_{tax} ratio between local taxes cost and fixed capital
X_{ins} ratio between insurances cost and fixed capital
C_{rawmat} raw materials cost in \$/year
pr_r purchase price of raw material in *\$/kg*
m_r daily requirement of raw material in *kg/day*
R set of raw materials indexed by r
C_{utilit} utilities cost in \$/year
HE_{cons} consumed thermal energy in *kWh/day*
EE_{cons} consumed electric energy in *kWh/day*
HE_{prod} generated thermal energy in *kWh/day*
EE_{prod} generated electric energy in *kWh/day*
Wt_{cons} consumed water in *kg/day*
pr_{HE} purchase price of thermal energy in *\$/kWh*
pr_{EE} purchase price of electric energy in *\$/kWh*
pr_{wt} purchase price of water in *\$/kg*
$Cprod_{rev\_coprod}$ biodiesel production cost relative to co-products revenues in *\$/kg biodiesel*
$C_{capital}$ annualized capital cost in *\$/year*
Rev_{coprod} co-products revenues in *\$/year*
MINLP_A mixed integer nonlinear programming model where astaxanthin production was set equal to zero
MINLP_B mixed integer nonlinear programming model where astaxanthin production is set equal to zero and glycerol sent to PHB production process is set to zero, too
MINLP_international mixed integer nonlinear programming model with international considerations
MINLP_domestic mixed integer nonlinear programming model with domestic (Argentina) considerations

Abbreviations:
GWP global warming potential
LCA life cycle assessment
MINLP mixed integer nonlinear programming
NPV net present value in *\$*

US FDA The United States Food and Drug Administration
PHB poly(hydroxybutyrate)

References

(1) Chisti, Y. *Biotechnol. Adv.* **2007**, *25*, 294-306.

(2) Davis, R.; Aden, A.; Pienkos, P. T. *Appl. Energy* **2011**, *88*, 3524-3531.

(3) Gebreslassie, B. H.; Waymire R.; You F. *AIChE J.* **2013**, *59*, 1599-1621.

(4) Gong, J.; You, F. *AIChE J.* **2014**, *60 (9)*, 3195-3210.

(5) Yan Y., Li X., Wang G., Gui X., Li G.,Su F., Wang X., Liu T. *Appl. Energy* **2014**, *113*, 1614-1631.

(6) Pinedo, J.; García Prieto, C.V.; D'alessandro, A.A.; Ibáñez, R.; Tonelli, S.; Diaz, M.S.; Irabien, A.. *Chemical Engineering Research and Design.* **2016**, *107*, 117-125.

(7) Martín, M.; Grossmann, I. E. *Environ. Prog. Sustainable Energy* **2013**, *32*, 891-901.

(8) Sawaengsak, W.; Silalrtruksa, T.; Bangviwat, A.; Gheewala, S. H. *Energy Sustainable Dev*. **2014**, *18*, 67-74.

(9) García D. J., You, F. *AIChE J.* **2014**, *61 (2)*, 530-554.

(10) Čuček L., Martín M., Grossmann I.E., Kravanja Z. *Comput. Chem. Eng.* **2014**, *66*, 57–70.

(11) Gnansounou E., Raman J. K. *Appl. Energy.* **2016**, *161*, 300–308.

(12) Kokossis A.C., Tsakalova M., Pyrgakis K. *Comput. Chem. Eng*. **2015**, *81*, 40–56.

(13) Harvey P., Bailey D., Ben-Amotz A., Verdelho V., Harris G., Rooke D., Hoekstra H., Goacher P., Crespi J., Reinhardt G., Martinelli L., Schroder D., Pipe R., Igl-Schmid N., Kokossis A., Perrson K. *New Biotechnol.* **2014**, *31*, 14-15.

(14) Psycha M., Pyrgakis K., Kokossis A.C. *Comput. -Aided Chem. Eng.* **2014**, *33*, 1543-1548.

(15) Ahn Y. C., Lee I. B., Lee K. H., Han J. H. *Appl. Energy.* **2015**, *154*, 528–542

(16) Gong, J.; You, F. *ACS Sustainable Chem. Eng.* **2015**, *3*, 82-96.

(17) Rizwan, M.; Lee, J. H.; Gani, R. *Appl. Energy*. **2015**, *150*, 69-79.

(18) Cheali P., Vivion A., Gernaey K.V., Sin G. *12th International Symposium on Process Systems Engineering and 25th European Symposium on Computer Aided Process Engineering.* 2015.

(19) Lee O.K., Seong D.H., Lee C.G., Lee E. Y. *J. Ind. Eng. Chem.* **2015**, *29*, 24–31.

(20) Diaz, M. S.; Espinosa, S.; Brignole, E. A. *Energy Fuels* **2009**, *23*, 5587-5595.

(21) Ashby, R.D.; Solaiman D.K.Y.; Foglia T.A. *J. Polym.Environ.* **2004**, *12*, 105-112.

(22) REN21 Renewables, 2015. Global Status report. REN21, Secrétariat Paris, France.

(23) Chatzidoukasa, C.; Penlogloub, G.; Kiparissidesa, C. *Biochem. Eng. J.* **2013**, *71*, 72-80.

(24) Michalak, M.; Marek, A. A.; Zawadiak, J.; Kawalec, M.; Kurcok, P. *Eur. Polym. J.* **2013**, *49*, 4149-4156.

(25) Rodriguez-Contreras, A.; Koller, M.; Miranda de Sousa Dias, M.; Calafell-Monfort, M.; Marqués-Calvo, M. S. *J. Appl. Microbiol.* **2013**, *114 (5)*, 1378-1387.

(26) Fernandes, J. C.; Correia, D. M.; Botelho, G.; Padrao, J.; Dourado, F.; Ribeiro, C.; Lanceros-Méndes, S.; Sencadas, V. *Polym. Test.* **2014**, *34*, 64-71.

(27) Arrieta, M. P.; López, J.; Hernández, A.; Rayón, E. *Eur. Polym. J*. **2013**, *50*, 255-270.

(28) Jin, Y. X.; Shi, L. H.; Kawata, Y. *Bioresour. Technol.* **2013**, *140*, 73-79.

(29) Moncada, J.; El-Halwagi, M. M.; Cardona, C. A. *Bioresour. Technol.* **2013**, 135, 533-543.

(30) Abdelaziz, O. Y.; Gadalla, M. A.; El-Halwagi, M. M.; Ashour, F. H. *Bioresour. Technol.* **2015**, 181, 321-329.

(31) Guerin M.; Huntley, M. E.; Olaizola, M. *Trends Biotechnol.* **2003**, *21*, 210–216.

(32) Global Industry Analysts Inc. 2013, MCP-1700: 389 pp.

(33) Huntley, M. E.; Redalje, D.G. *Mitig. Adapt. Strategies Glob. Chang*. **2007**, *12*, 573-608.

(34) Ulrich, G. D. A guide to Chemical Engineering Process Design and Economics; Jonh Wiley and Sons: New York, 1984.

(35) Posada, J. A.; Naranjo, J.M.; López, J. A.; Higuita J. C.; Cardona C. A. *Process Biochem*. **2011**, *46(1)*, 310-317.

(36) Meyer, M. A.; Weiss, A. *Energy* **2014**, 78, 84-93.

(37) Damiani M.C., Popovich C.A., Constenla D., Leonardi P.I. *Bioresour Technol.* **2010**, *101(18)*, 7201.

(38) Kyoto Protocol. Available online at <http://www.kyotoprotocol.com/resource/kpspan.pdf> (accessed July 2015).

(39) Manganaro, J. L.; Lawal, A. *Energy Fuels* **2016**, *30*, 3130-3139.

(40) Ferreira, A. F.; Soares Dias, A. P. Silva, C. M.; Costa, M. *Energy* **2016**, 92, 365-372.

(41) Tapasvi D, Wiesenborn D, Gustafson C. *Trans ASAE.* **2005**, *48(6)*, 2215–2221.

(42) Ledda C., Schievano A., Scaglia B., Rossoni M., Acién Fernéndez F.G., Adani F. *J. Cleaner Prod.* **2016**, *112*, 103-112.

(43) Parkin, J. F.; Owen, W. F. *J. Environ. Eng. Div*. **1986**, *122*,867-920.

(44) Yen, H. W.; Brune, D. E. *Bioresour. Technol.* **2007**, *98*, 130-134.

(45) Amon, T.; Amon, B.; Kryvoruchko, V.;Bodiroza, V.; Pötsch, E.; Zollitsch, W.. *Int. Congr. Ser.* **1993**, 217-220.

(46) Fountoulakis, M. S.; Petousi, I.; Manios, T. *Waste Manag*. **2010**, *30*, 1849-1853.

(47) Ehimen, E. A.; Sun, Z. F.; Carrington, C. G.; Birch, E. J.; Eaton-Rye, J. J. *Appl. Energy*. **2011**, 88, 3454-3463.

(48) Yan, C.; Zhang, L.; Luo, X.; Zheng, Z. *Energy* **2012**, 69, 419-126.

(49) Cavalheiro, J. M. B. T.; Catarina, M. M. D.; Grandfils, C.; Fonseca, M. M. R. *Process Biochem*. **2009**, *44*, 509-515.

(50) Mothes, G.; Schnorpfeil, C.; Ackermann, C. J. U. *Eng. Life Sci.* **2007**, *7*, 475-479.

(51) Jacquel, N.; Lo, C-W.; Wei, Y-H.; Wu, H-S.; Wang, S. S. Isolation and Purification of Bacterial Poly(3-hydroxyalcanoates). *Biochem. Eng. J*. **2008**, *39*, 15-27.

(52) Harding, K. G.; Dennis, J. S.; Blottnitz, H.; Harrison, S. T. L.. *J. Biotechnol.* **2007**, *130*, 57-66.

(53) Li, J.; Zhu, D.; Niu, J.; Shen, S.; Wang, G. *Biotechnol. Adv*. **2011**, *29*, 568-574.

(54) Sahinidis, N. V., BARON 14.4.0: Global Optimization of Mixed-Integer Nonlinear Programs, User's manual, **2014**.

(55) Jorquera, O.; Kiperstok, A.; Sales, E. A.; Embiruçu, M.; Ghirardi, M. L. *Bioresour. Technol*. **2010**, *101*, 1406-1413.

(56) Sierra, E.; Acien, F. G., Fernandez, J. M.; Garcia, J. L.; Gonzalez, C.; Molina, E. *Chem. Eng. J*. **2008**, 138, 136-147.

(57) Shelef, G. S.; Sukenik, A.; Green, M. Golden, CO: Solar Energy Research Institute SERI, 1984.

(58) Elsayed, M. A.; Matthews, R.; Mortimer, N. D. Report for Resources Research Unit Sheffield Hallam University: United Kingdom, March 2003.

(59) Morken, J.; Sapci, Z.; Strømme J. E. T. *Energy Policy* **2013**, *60*, 98-105.

(60) Sander, K.; Murthy, G. S. *Int. J. Life Assess.* **2010**, 15, 704-714.

(61) Grossmann, I. E.; Ruiz, J. P. Mixed Integer Nonlinear Programming; Springer: New York, **2012**.

(62) Rivas P.G.Universidad de Chile, Facultad de Ciencias Físicas y Matemáticas, Departamento de Ingeniería industrial, Santiago de Chile, Noviembre 2007.

(63) Martín, M.; Grossmann, I. *Ind. Eng. Chem. Res.* **2014**, *53 (28)*, 11371-11383.

(64) Alibaba.com. International Prices, 2015. Available online at <http://www.alibaba.com/> (accessed June 2015)

(65) Eia.gov. Energy Information Administration, 2015. Available online at <http:// http://www.eia.gov//> (accessed June 2015)

(66) Energy.gov.
Available online at < http://www.afdc.energy.gov/fuels/biodiesel.html//> (accessed March 2016)

(67) Oilgae.com. Available online at <http://www.oilgae.com/> (accessed June 2015)

(68) Thinkkentucky.com. Available online at <http://www. www.thinkkentucky.com/kyedc/pdfs/kybusinc.pdf />

(69) Brooke, A.; Kendrick, D.; Meeraus, A.; Raman, R.; GAMS- A User's Guide, GAMS Development Corporation: Washington, 2013.

(70) Ubando, A. T.; Culaba, A. B.; Aviso, K. B.; Tan, R. R.; Cuello, J. L.; Ng, D. K. S.; El-Halwagi, M. M. *Journal of Cleaner Production* **2016**, 136, 183-196.

(71) Ray, S.; Prajapati, V.; Patel, K.; Trivedi, U. *Int. J. Biol. Macromol*. **2016**, 86, 741-749.

(72) Garlapati, V. K.; Shankar, U.; Budhiraja, A. *Biotechnol. Reports* **2016**, 9, 9-14.

(73) Moreno, P.; Yañez, C.; Medeiros Cardozo, N. S.; Escalante, H.; Combariza, M. Y.; Guzman, C. *New Biotechnol*. **2015**, 32, 682-689.

(74) Galadima, A. Muraza, O. *Energy* **2014**, 78, 72-83.

(75) Zhang, F.; Zhang, Y.; Chen, Y.; Dai, K.; van Loosdrecht, M. C. M.; Zeng, R. J. *Appl. Energy* **2015**, 148, 326-333.

(76) Chanprateep, S. *J. Biosci. Bioeng*. **2010**, 110, 621-632.

(77) Phbottle.eu. PHBOTTLE project. Available online at < http://www.phbottle.eu/ > (accessed February 2016)

(78) Sánchez, E.; Ojeda, K.; El-Halwagi, M.; Kafarov, V. *Chemical Engineering Journal* **2011**, 176-177, 211-216.

(79) National Algal Biofuels Technology Roadmap, 2010. Available online at <http:// www1.eere.energy.gov/bioenergy/pdfs/algal_biofuels_roadmap.pdf. />

(80) Martín, M.; Grossmann, I. E. *Ind. Eng. Chem. Res.* **2012**, *51* (23), 7998–8014.

(81) Weforum.org. Electric energy cost, 2015. Available online at <http://www3.weforum.org/docs/WEF_EN_NEA_Report_2014.pdf /> (accessed June 2015)

(82) Agroads.com.ar. Domestic land cost, 2015. Available online at <http://www.agroads.com.ar/> (accessed June 2015)

(83) Energia.gob.ar. Biodiesel price, 2016. Available online at <http://www.energia.gob.ar/home/> (accessed March 2016)

(84) Bolsadecereales.com. Soybean cultivation area, 2015. Available online at <http://www.bolsadecereales.com/> (accessed October 2016)

(85) Maranduba, H. L.; Robra, S.; Nascimento, I. A.; Cruz, R. S.; Rodrigues, L. B.; Almeida Neto, J. A. *Energy* **2016**, 115, 888-895.

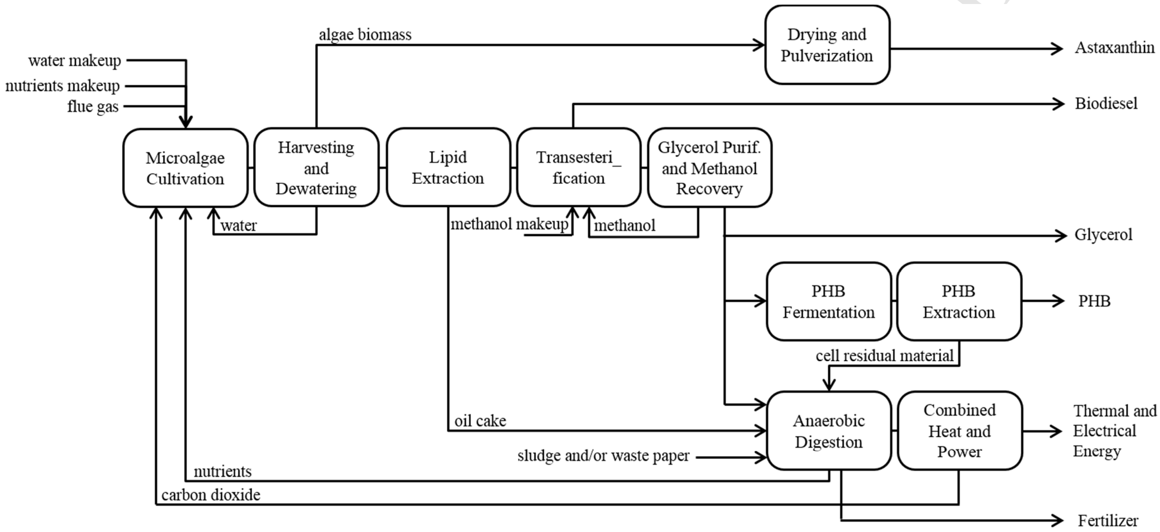

Figure 1. Overview of major processing steps of an integrated microalgae-based biorefinery

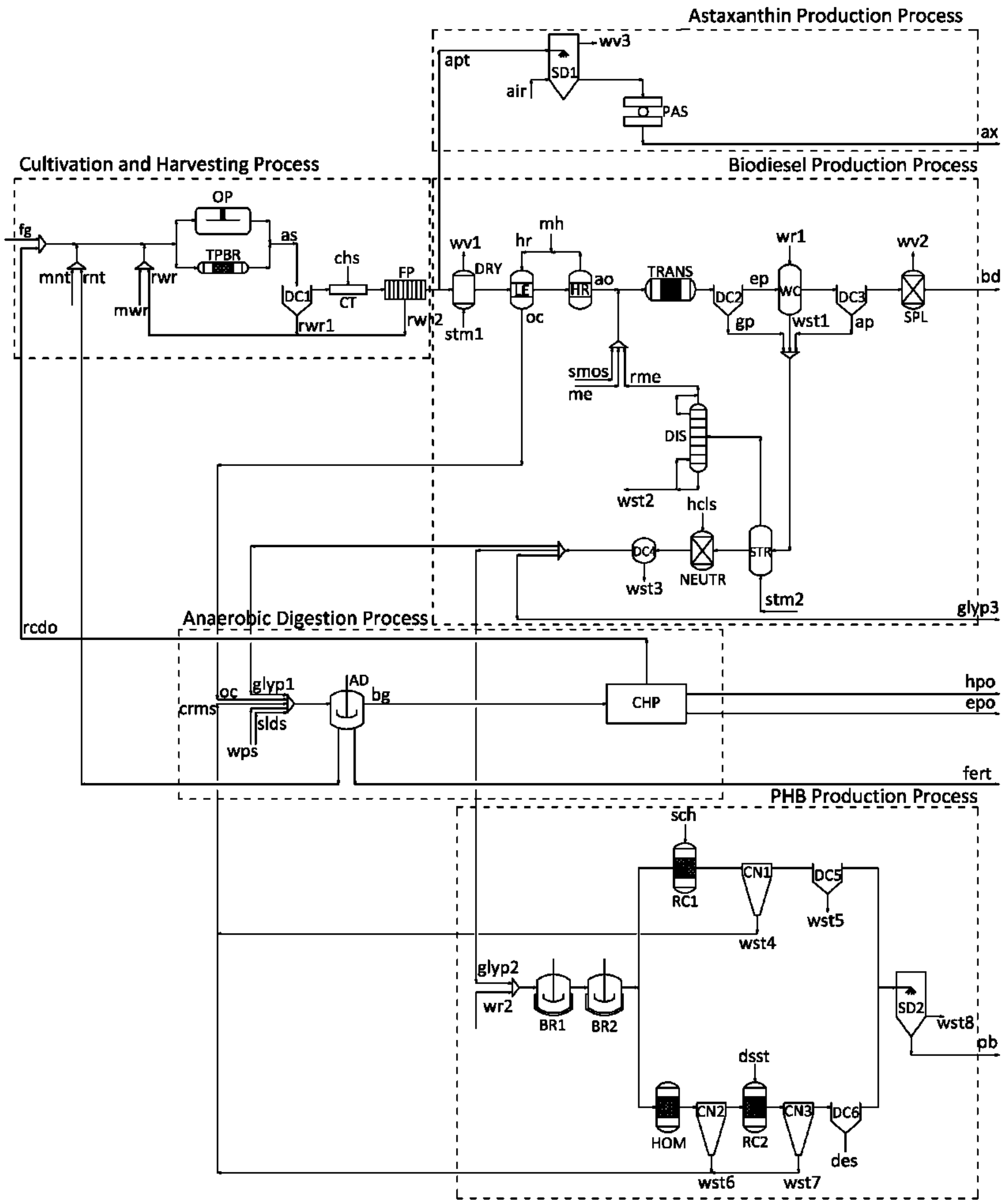

Figure 2. Integrated microalgae-based biorefinery superstructure

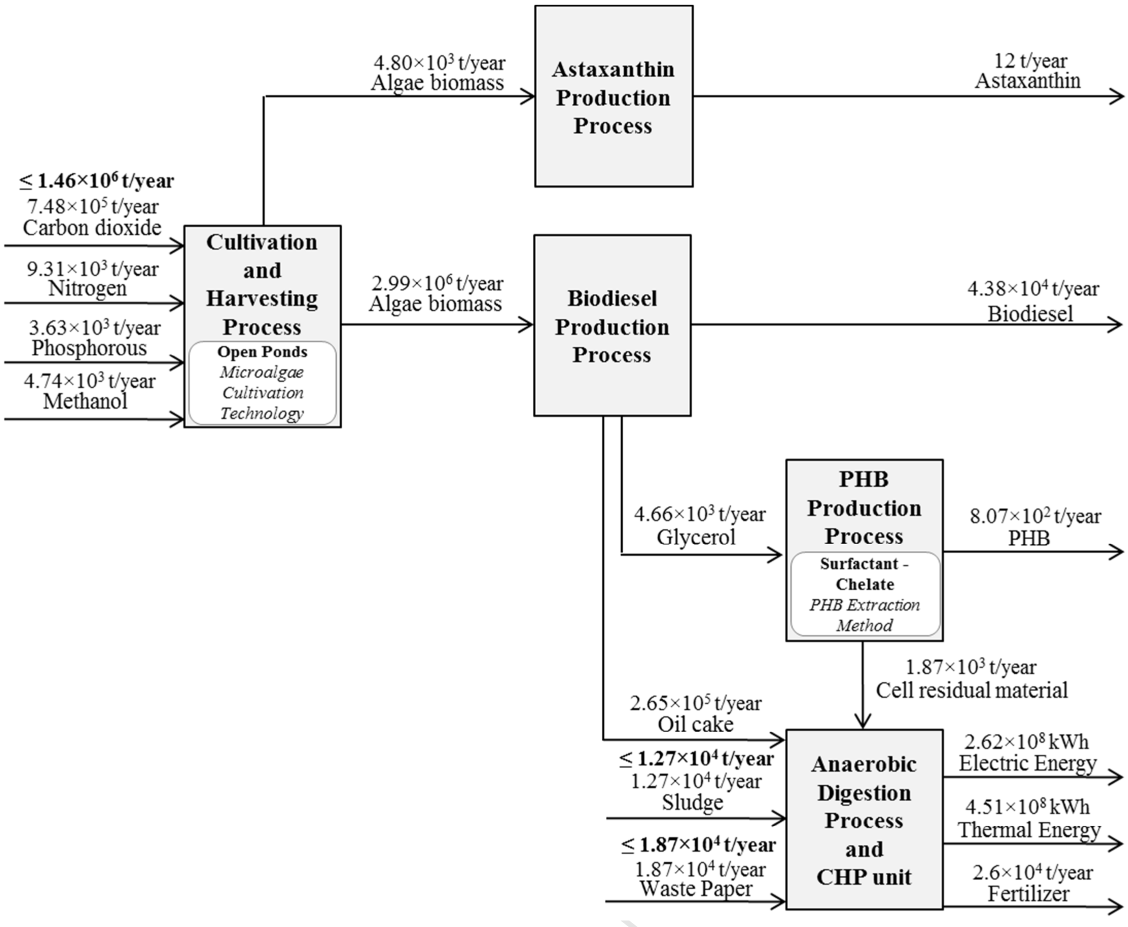

Figure 3. Microalgae based biorefinery optimization results.

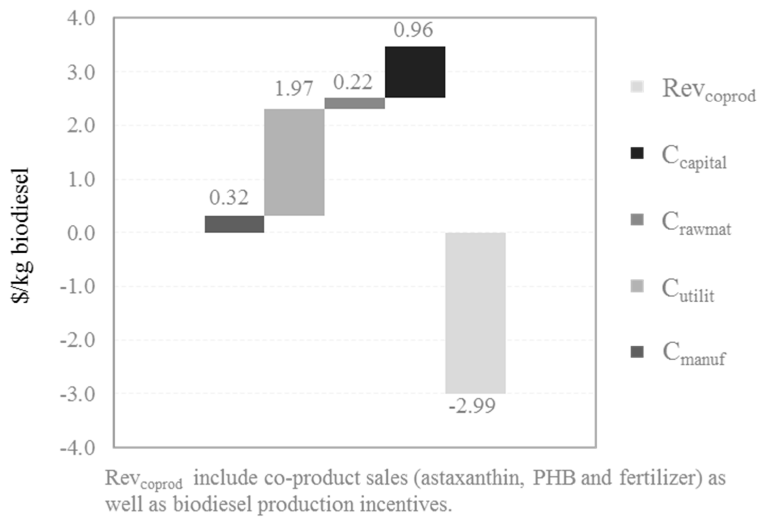

Figure 4. Biodiesel production cost distribution

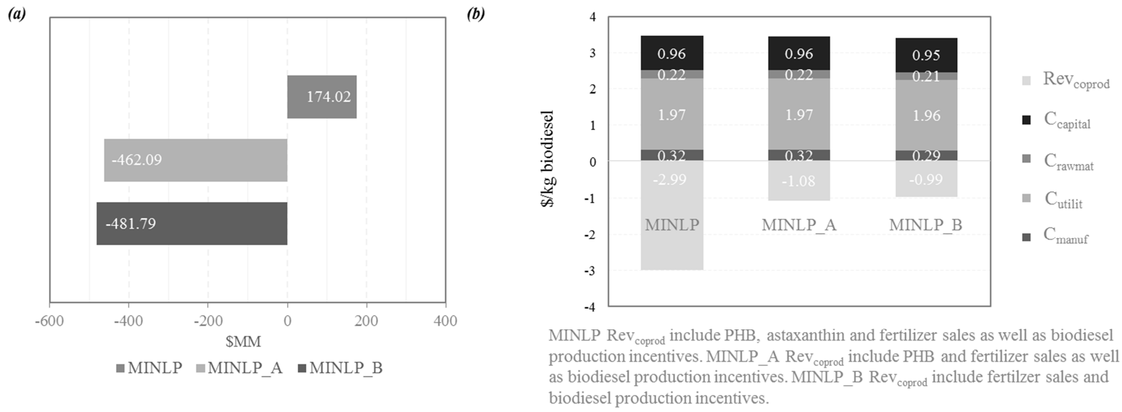

Figure 5. Comparison between MINLP, MINLP_A and MINLP_B for ***a)*** Net present value and ***b)*** Biodiesel production cost distribution.

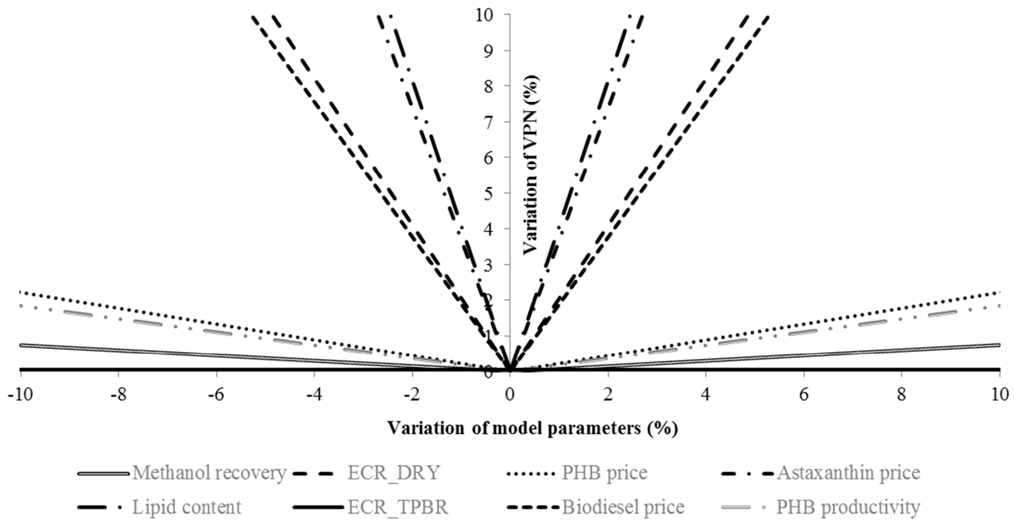

Figure 6. Sensitivity analysis of the integrated biorefinery

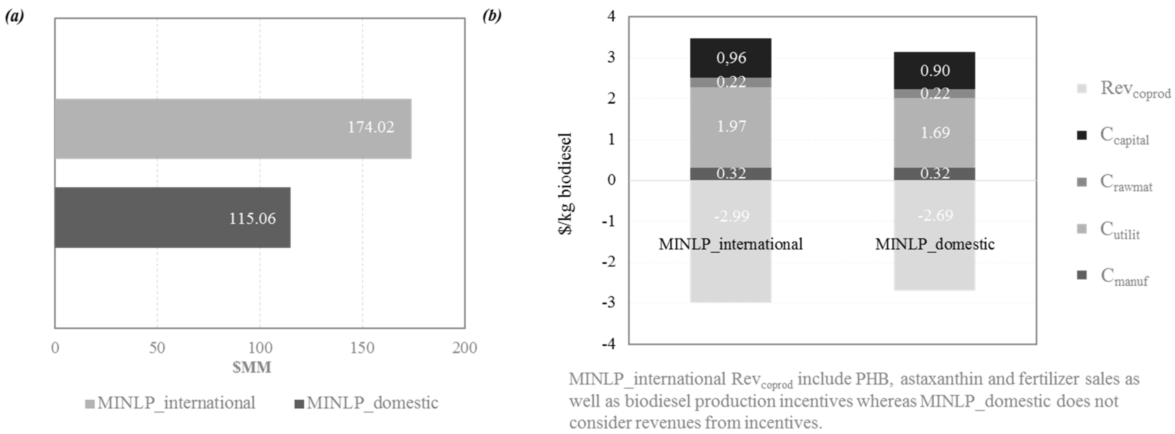

Figure 7. Comparison between MINLP_international and MINLP_domestic for ***a)*** Net present value and ***b)*** Biodiesel production cost distribution.

Table 1. Main energy consumption parameters

Energy Consumption Ratio	*Value*	*Unit*	*References*
ECR_{OP}	0.089	kWh/m^3	55
ECR_{TPBR}	24	kWh/m^3	56
ECR_{DC1}	0.06251	kWh/kg algae	4
ECR_{FP}	0.00088	kWh/kg water	57
ECR_{LE-HR}	0.581	kWh/kg algae oil	58
ECR_{TRANS}	0.299	kWh/kg biodiesel	59
ECR_{DC2}	0.0275	kWh/kg biodiesel	41
ECR_{DC3}	0.0315	kWh/kg biodiesel	41
ECR_{DRY}	0.988	kWh/kg water	60
ECR_{AD}	1.140	kWh/kg biogas	59
ECR_{SD1}	1.93	kWh/kg water	53
ECR_{PAS}	3.5	kWh/kg powder	53
ECR_{STR}	1.386	kWh/kg methanol	35
ECR_{BR1}	0.985	kWh/kg glycerol	35
ECR_{RC1}	0.716	kWh/kg PHB	35
ECR_{CN1}	13.548	kWh/kg PHB	35
ECR_{SD2}	1.144	kWh/kg water	35
ECR_{HOM}	2.842	kWh/kg PHB	35
ECR_{CN2}	4.748	kWh/kg PHB	35
ECR_{CN3}	7.14	kWh/kg PHB	35
ECR_{RC2}	1.48	kWh/kg PHB	35

Table 2. Model main constraints

Variable	*Upper Bound*
$CO_{2\ Produced}$ - $CO_{2\ Algae\ Cultivation\ requirements}$	0.01 t/year
$CO_{2\ Algae\ Cultivation\ Feedflow\ fromThermoelectric}$	1.46×10^6 t/year
Astaxanthin $_{\mathbf{Production}}$	12 t/year
$WastePaper_{AnaerobicDigestionFeedflow}$	1.87×10^4 t/year
$Sludge_{AnaerobicDigestionFeedflow}$	1.27×10^4 t/year
- $C/N_{\ Anaerobic\ Digestion\ Ratio}$	- 20
$C/N_{\ Anaerobic\ Digestion\ Ratio}$	25

Table 3. Prices and costs

Items	***Value***	***Reference***
Raw materials		
Hexane ($/kg)	0.41	63
Nutrient ($/kg)	0.367	63
Methanol ($/kg)	0.286	3
Sodium methoxide ($/kg)	0.98	3
Hydrochloric acid ($/kg)	0.208	64
Diethylsuccinate($/kg)	3	64
Surfactant ($/kg)	1	64
Chelate ($/kg)	1	64
Utilities		
Electric Energy ($/kWh)	0.0722	65
Thermal Energy ($/kWh)	0.015841	65
Water ($/kg)	0.000007	3
Selling Prices		
Biodiesel ($/kg)	0.98	66
Astaxanthin ($/kg)	7000	67
Glycerol ($/kg)	0.2574	3
Fertilizer ($/kg)	1.115	64
PHB ($/kg)	6.25	35
Other		
Land ($/m^2)	0.3	16
Incentives ($/kg)	0.3	68

Table 4. Annual energy consumption distribution

Section	*KWh*	*Percentage*
Biodiesel Production Process	3.79×10^{9}	94.82%
Microalgae Cultivation	7.78×10^{8}	19.46%
Harvesting and Dewatering	2.95×10^{9}	73.99%
Lipid Extraction	3.14×10^{7}	0.79%
Transesterification	1.31×10^{7}	0.33%
Glycerol purif. and methanol recov.	9.69×10^{6}	0.25%
Anaerobic Digestion Process	1.78×10^{8}	4.47%
PHB Production Process	1.80×10^{7}	0.46%
Astaxanthin Production Process	1.01×10^{7}	0.25%

Table 5. Total investment cost distribution

Section	*$MM*	*Percentage*
Biodiesel Production Process	261.36	82.21%
Microalgae Cultivation	199.09	62.61%
Harvesting and Dewatering	55.37	17.41%
Lipid Extraction	0.72	0.23%
Transesterification	2.79	0.88%
Glycerol purif. and methanol recov.	3.44	1.08%
Anaerobic Digestion Process	54.68	17.20%
PHB Production Process	0.81	0.25%
Astaxanthin Production Process	1.07	0.34%

Highlights

- Design and optimization of integrated microalgae-based biorefinery.
- Astaxanthin as high-value added co-product makes project economically attractive.
- Reduction of biodiesel price from \$2.42/kg to \$0.48/kg.
- Economic sensitivity analysis is performed.
- International and domestic case scenarios are considered.