Contents lists available at ScienceDirect

Algal Research



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

Multi-scenario energy-economic evaluation for a biorefinery based on microalgae biomass with application of anaerobic digestion



Cristián P. Bravo-Fritz^{a,*}, César A. Sáez-Navarrete^a, Leandro A. Herrera-Zeppelin^b, Felipe Varas-Concha^a

^a Chemical and Bioprocess Engineering Department, Engineering Faculty, Pontificia Universidad Católica de Chile, Av. Vicuña Mackenna 4860, Macul, 7820436 Santiago, Chile ^b Chemical Engineering and Biotechnology Department, Universidad de Chile, Beauchef 851, 8370456 Santiago, Chile

ARTICLE INFO

Article history: Received 9 November 2015 Received in revised form 5 March 2016 Accepted 12 March 2016 Available online 2 April 2016

Keywords: Techno-economic Energy Sensitivity analysis Critical variables Biodiesel Biogas

SUMMARY

Microalgae are a source of biomass that has aroused the interest of the bioenergy industry due to its sustainability potential and maximum use of different abundant natural resources. This research proposes an energy-economic evaluation model for 11 scenarios for a biorefinery based on microalgae biomass, including a final stage of anaerobic digestion. Furthermore, it allows for comparisons between different scales of production, farming technologies and microalgae species, in line with latest industry information. Results are displayed by means of economic (NPV) and energy (EROI) indicators. Almost all the scenarios evaluated returned negative economic profitability, except for the extraction and commercialization of concentrated proteins (the PE scenario with protein sales of US\$3/kg). In order to guide future research and investment in microalgae projects, a sensitivity analysis was conducted into the critical variables of the overall process. An optimistic context, led by the increase of the percentage of biomass lipids, allows a minimum biodiesel selling price to be reached which is close to the international value of fossil diesel (US\$1/L) for scenarios in which this biofuel is commercialized.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Global energy consumption has continued to rise in recent years, primarily driven by the economic development and opening up of emerging nations (Brazil, Russia, India and China). Projections from the Energy Information Administration (EIA) postulate that the current energy consumption of non-OECD (Organization for Economic Cooperation and Development) nations will almost double by 2040, resulting in a global consumption increase from 529 QBTU (QBTU = Quadrillion British Thermal Unit, 10^{15} BTU) (in 2012) to 820 QBTU (projection to 2040) [1]. Accordingly, one of the main challenges of the 21st century will be finding sustainable energy sources capable of sustaining the projected energy scenario, as well as the lifestyle of contemporary society.

Over the last decade, the energy sector has been maintained through the exploitation of fossil-based resources rich in carbon, whether derived from petroleum, natural gas or coal. The percentage of the global energy matrix represented by the sum of the aforementioned sources comes to 86%, which in addition has undergone no variation over the last ten years [1].

A direct consequence of dependence on fossil fuels is the emission of combustion gases into the atmosphere, primarily in the form of CO₂. These emissions exceed the natural rate with which the planet's ecosystems capture and fix this compound, resulting in a large accumulation of

* Corresponding author. E-mail address: ctbravo@uc.cl (C.P. Bravo-Fritz). CO_2 in the atmosphere [2,3]. This accumulation has strengthened the natural greenhouse effect of the Earth, raising the average temperature of the planet in the process [4,5].

Regardless of the results or consequences arising from the emission of greenhouse gases (GHG), there are two concepts which provide a certain amount of security in regard to an uncertain future: prevention and resource diversification.

For the energy sector, renewable energy surpassed 7.5% of global energy consumption in 2002, and 9% in 2012. This increase is largely the result of the greater participation of Non-Conventional Renewable Energy (NCRE), which has tripled in generation capacity, essentially via the development of wind, solar and biomass energy [1].

Motivation behind this research lies specifically in the field of bioenergy. Traditional forms of bioenergy relate to electricity generated from the direct combustion of biomass or biogas, stemming from their anaerobic degradation, as well as the use of liquid biofuels which entirely or partially displace those derived from petroleum [6,7]. Among the liquid fuels, ethanol is usually produced via the fermentation of raw material rich in glycosides (or carbohydrates), such as corn and sugar cane [8,9]. Alternatively, biodiesel is obtained through the esterification and transesterification of used oils and oleaginous products obtained from the farming of soybean, rapeseed, palm oil and other different seeds [8,10]. However, there is a less conventional alternative to consider and evaluate within the bioenergy industry: biomass obtained from microalgae.

While research in the field of microalgae has increased over the last decade, it dates back to the mid-19th century, when isolated microalgae

were cultured in laboratory conditions [11]. Subsequently, towards the end of the 1970s the US Department of Energy created a division called the Aquatic Species Programme (ASP), which remained active until 1996. The aim of this programme was to study the economic feasibility, the scaling to pilot and industrial scales and the application of different technologies for the farming and processing of microalgae for bioenergy purposes [12]. Currently, industrial-scale farming is restricted to the production of feed for the aquaculture industry, or as a source of highvalue metabolites (proteins, special oils or antioxidant pigments) which are of interest to the pharmaceutical industry [13].

The production of bioenergy from microalgae reached only pilot level, due to its high operational and related capital costs. Diverse research groups have conducted evaluations into the cost of producing biofuel from microalgae, determining that the minimum selling price of biodiesel should be around US\$4/L, in order to ensure the sustainable economic development of the industry (see Fig. 1) [14-19]. This far exceeds the US\$0.94/L of diesel, as per its global average price at the beginning of 2015 [20].

Economic evaluation results of microalgae biodiesel demonstrated that its production on an industrial scale will only become economically viable through the generation of products with a higher commercial value than that of traditional fuels. This provides greater relevance to the biorefinery concept. Biorefineries are chemical plants or factories which integrate the concept of "zero waste", in which all biomass fractions (proteins, glycosides and lipids) are utilized to generate different types of products and energy [10,21,22].

Generally, microalgae biomass can have different bioenergy uses [23]. Consequently, it is necessary to evaluate and understand the multiple configurations of the processes which make up a biorefinery. This will facilitate the development of a sustainable industrial-scale design.

In conceptual models in which a biorefinery is described, anaerobic digestion (AD) usually emerges as a stage of final biomass recovery, supplementary to the main process, and which enables its transformation into energy [24]. For example, if the objective is to produce biodiesel, the AD should be undertaken at the end of the process, by degrading the glycerol and residual glycosides and proteins as well as all cell debris. In other cases, when the main objective is to produce electricity, only a single operating unit of energy recovery is usually evaluated, either through the generation of biogas with AD or via direct combustion [25].

Over the last decade, the AD of numerous species of microalgae have undergone experimentation in order to determine: a) the empirical biogas production yields based on a fraction or the total amount of processed biomass; b) special and restricted cases associated with the use of microalgae; and c) the operational parameters (hydraulic retention time [HRT], temperature, and mixing speed, among others) that optimize the AD [24,26]. There is currently only limited evidence relating to microalgae AD plants on a pilot scale [27].

Biogas production yield from microalgae biomass can be determined experimentally or through theoretical procedures. These allow estimates to be devised for the amount and composition of the biogas, based on a particular residue with a known elemental chemical formula (CHONS). A stoichiometry formula was proposed by Buswell and Mueller in 1952 [28], as follows:

$$C_{c}H_{h}O_{o}N_{n}S_{s} + \left(\frac{4c-h-2o+3n+2s}{4}\right)H_{2}O \rightarrow \left(\frac{4c+h-2o-3n-2s}{8}\right)CH_{4} + \left(\frac{4c-h+2o+3n+2s}{8}\right)CO_{2} + nNH_{3} + sH_{2}S.$$

It should be noted that this theoretical formula usually overestimates the production of biogas by approximately 40% [27]. Nevertheless, the formula remains useful to identify what changes occur inside the digesters, as well as helping to generate a first dimensioning of the overall process.

AD begins with the hydrolysis of complex polymers or macromolecules (glycosides, proteins and lipids [for elemental chemical formulas see Table 1]) and proceeds towards simpler, lower molecular weight compounds. Consequently, in order to determine the elemental chemical formula of the biomass for biogas production, the particular characterization of the residue (in terms of its percentages of the three aforementioned macromolecules) can be used with the CHONS formula [27]. Typically, microalgae biomass is usually characterized in the same way. This approach would allow for other theoretical yields of biogas generation to be devised, as well as their comparisons with the reported experimental productivities. Table 2 was devised in this way. It shows the elemental chemical formula of different microalgae and provides evidence that the Buswell and Mueller (1952) [28] formula overestimates real biogas production.

Prior to addressing the design and economic evaluation of microalgae AD, consideration should be made and care taken regarding three possible inhibitory variables in the process, specific to the characteristics of the



Fig. 1. Estimates of the minimum selling price per litter of microalgae biodiesel (OP = open ponds; PBRs = photobioreactors; Hybrid = OP + PBRs).

Table 1
Stoichiometry and biogas production yields, proposed by numerous studies, given the degradation of the main macromolecules.
Source: Own research conducted from Refs. [24], [27], [29] and [30].

Complex polymer	Stoichiometry formula	Buswell & Mueller (1952) Feinberg (1984) [28] [99]		Weiβbach (2009) [100]	VDI guideline 4630 (2006) [101]		
		(Methane) and biogas, in Nm ³ /kgSV					
Lipids	$C_{50}H_{87}O_6$	(0.98) 1.43	(0.82) 1.37	(0.96) 1.35	(1.0) 1.39		
Proteins	$C_{184}H_{350}O_{86}N_{33}S$	(0.52) 1.10	(0.39) 0.65	(0.40) 0.78	(0.48) 0.8		
Glycosides	$(C_6H_{11}O_5)_n$	(0.41) 0.83	(0.30) 0.50	(0.40) 0.79	(0.38) 0.75		

particular microalgae: 1) both the composition of the cell wall and the specific metabolism of the treated species can help the microalgae survive anaerobic reactor conditions [21]; 2) the protein content (nitrogen) of the microalgae, in conjunction with the pH of the reactor, will determine the balance between the ammonia (NH₃) and ammonium ion (NH₄⁺), whereby the former is toxic to the methanogenic microorganisms and inhibitory to the process in general [29,31]; and 3) the sodium ion (Na⁺) present in the moisture of marine biomass species may also be an inhibiting factor in anaerobic microflora [29,32].

Fortunately, there are alternatives which help to avoid or reduce the inhibitory effect of each one of these microalgae characteristics. To prevent the cells remaining intact in the digesters, a cell disruption stage can be used to favour the bioavailability of macromolecules to the microorganisms responsible for hydrolysis [21,26]. Similarly, the problem of nitrogen can be overcome with pre-treatments that alter the C/N ratio of the treated biomass [24,29]; be it by extracting proteins from the microalgae or adding other carbon-rich compounds for degrading (co-digestion with paper or agricultural waste), prior to AD [27]. Finally, inhibition by NaCl can be rectified by prior and gradual acclimatization of the anaerobic inoculum to greater concentrations of Na⁺, before feeding them with marine microalgae. Alternatively, this can be done by utilizing natural environment inoculum, more tolerant to salts, such as microbial consortia from sea beds or estuaries [29].

The pre-treatment applied to microalgae serves to prevent AD inhibition, increasing the exposure of cell content (biodegradability), and as a result can optimize or increase biogas generation. There are numerous pre-treatment alternatives, including mechanical, thermal, chemical, biological, and electrical methods, as well as by means of irradiation, in addition to combinations thereof [33]. However, under the biorefinery scenario, biomass arrives highly processed at the final stage of AD, meaning that the aforementioned inhibitory factors might no longer be so relevant in the biogas production stage.

Consensus is still lacking as to what is the best pre-treatment to apply to microalgae AD. This area requires further research in order to broaden knowledge regarding a greater number of microalgae species and technology combinations [34]. However, analysis of technoeconomic evaluations, such as the one undertaken in this paper, may help to ensure the early detection of certain alternatives. By means of process engineering and the evaluation of industrial upscaling, feasibility comparisons can be made of the different processing options, prior to the investment of time and resources at the laboratory level in search of new technologies. This research aims to compile sufficient information to generate an energy-economic evaluation model to help the design of processes that consider different species of microalgae and pre-treatment technologies for power generation and by-products, including AD, with main product generation achieved through biomass processing. Furthermore, the aim is to uncover critical variables for establishing the economic profitability of the process by means of conducting a sensitivity analysis, and to present the effective potential of AD applied to a biorefinery.

2. Methodology

To develop the energy-economic evaluation, the research process began by reviewing pre-existing research into the technical and economic production of biodiesel and biogas with microalgae via AD [19, 25,34-41]. There are significant differences between the studies reviewed, at both the level of design of the microalgae production process, as well as the results obtained (economic, energy and associated CO₂e emissions).

Given the large number of options and variables of the processes reviewed in the literature, the model begins with the definition of the particular project undergoing evaluation, in which each of the main stages of the process are outlined step-by-step (as shown in Fig. 2).

The model will evaluate the processes using different marine species, from their production as inoculum at laboratory level, followed by obtaining a specific culture medium, which will be used during scaling or bulk production of the microalgae. Subsequently, the biomass will be subjected to the harvesting stage to obtain a concentrated moist sludge intended for the different pre-treatment alternatives due for evaluation. Finally, the processing of the biomass will be evaluated, both via AD as well as additional unitary operations which help to obtain energy and other marketable by-products.

2.1. Technical aspects of the model

2.1.1. Evaluated microalgae and inoculum preparation (laboratory)

The model proposed in this research will help in the evaluation of the production of different marine species of microalgae, with information originally pertaining to *Tetraselmis sp.* [42–44] and *Isochrysis sp.* [43, 45,46]. To evaluate a new species, data on its biomass productivity during farming and its elemental composition in terms of the percentage of lipids, proteins and glycosides will be required. The farming of

Table 2

Theoretical and real biogas production yields based on microalgae biomass. Source: Own research conducted from [24].

Microalgae species	Stoichiometry formula	Buswell & Mueller (1952) Experimental [28]		Exp/theoretical ratio	
		(Methane) and biogas, in Nm ³ /kg	SV	%	
Scenedesmus sp.	$C_{192}H_{348}O_{62}N_{26}P_2S$	(0.64) 1.08	(0.27) 0.44	(42%) 41%	
Chlorella sp.	$C_{287}H_{543}O_{120}N_{33}S$	(0.59) 1.12	(0.35) 0.49	(59%) 44%	
Arthrospira maxima	$C_{281}H_{485}O_{167}N_{51}P_2S$	(0.43) 1.03	(0.29) 0.48	(67%) 47%	
Chlamydomonas reinhardii	$C_{10}H_{16}O_6N$	(0.47) 1.00	(0.39) 0.59	(82%) 44%	
Microalgae average	$C_{212}H_{399}O_{91}N_{33}S$	(0.55) 1.13	=		

The average microalgae cited consists of 25% lipids, 50% proteins and 25% glycosides.



Fig. 2. General outline of the production and processing of microalgae through a biorefinery, according to the evaluation model of the current study.

freshwater algae could also be evaluated. Naturally, this would necessitate the modelling and collection of information relating to the preparation and provision of the specific culture medium required by the respective microalgae. Furthermore, the model considers the construction and operation of a laboratory responsible for maintaining a microalgae culture library, as well as the preparation of inoculum for initiating farming and conducting related research.

2.1.2. Culture medium

The proposed model includes the associated costs of filtering and pumping, from coastal areas to the farming facilities, followed by UV disinfection and storage stages. Specifically, it takes account of the production of f/2 culture medium, given its applicability to a large number of marine species, mixing the sea water with key nutrients for the development of the microalgae [11]. To estimate the daily flow of sea water for reuse, consideration is taken of losses from natural evaporation during farming, in accordance with local environmental parameters, as well as losses from remaining moisture following the harvest stage.

2.1.3. Farming

As stated by Zamalloa et al. (2011), the farming of microalgae has been studied extensively over the last 40 years. This has enabled numerous technical and economic aspects to be addressed regarding photobioreactors (PBRs) and raceway ponds (RWPs) [34,47–49]. This model considers the sole use of RWPs (174.60; 582.00 and 1746.00 m³, depending on scale, small; medium and large, respectively) and flat-plate PBRs (0.44 m³, independent of scale) for farming, as well as a combination of both technologies, known as a hybrid system. The maximum achievable concentration of microalgae was defined to be 1 g/L and 5 g/L for RWPs and PBRs [47], respectively. The PBRs are considered to be under a constant aeration system of 0.25 L_{air}/L_{PBR}/min [47,50,51], while the RWPs would be stirred by paddle wheels, with the fluid reaching a speed of 0.2 m/s [11,47]. The number of farming days for each technology would be determined in accordance with the biomass productivity (in g/L/day) of each species evaluated, with each bioreactor beginning from an initial concentration of 0.1 g/L [52,53], until the moment of harvest when maximum concentration is reached. This system defines a batch production, which in turn is intended to reduce the risk of contaminating the culture.

The amount of microalgae being produced determines the total overall size of the plant. The model delivers three pre-established scales: small, medium and large (greater than 2, 55 and 300 dry weight ton/ day, respectively), and allows the user to enter their own biomass dimension for production. These scales were defined on the basis of proposals made by Drapcho et al. (2008) for industrial biodiesel plants [54]. Therefore, the model sets an approximation regarding the microalgae biomass required for sustaining such scales.

Regarding the location of the production plants, work undertaken by Bravo-Fritz et al. (2015) is used as a reference point. That research outlined potential microalgae farming sites in Chile which assure a minimum level of biomass productivity [55].

2.1.4. Harvesting

Harvesting, concentration or dehydration are terms which usually refer to the process of microalgae cell extraction from aqueous medium at the final stages of culturing. This study models harvesting as a process with five sequential stages: pumping from PBR or RWP; fast mixing to rapidly disperse a flocculation reagent (first flocculation step); slow mixing to allow flocks to form (second flocculation step); sedimentation; and centrifugation [56–58] (see Appendix A). These steps allow the biomass concentration to be increased by two orders of magnitude.

The culture medium recovered through the different stages of harvesting is recirculated [30,59] towards the UV disinfection stage, where it is mixed with the fresh sea water to form new f/2. This

operational configuration requires measures to be sought for counteracting the accumulation of salinity in the medium caused by natural evaporation and recycling. Certain alternatives for evaluation include the use of a purge flow directed towards drying ponds or the use of fog collector to dilute the salts.

2.1.5. Scenarios for evaluation (pre-treatments)

Microalgae sludge, following the centrifugation of the harvest (75– 85% moisture), is intended for the multiple alternatives of evaluated pre-treatment. These are described briefly as follows (for additional information please refer to the Appendix A, in which further examples are provided, such as that expressed in Fig. 3, where each stage icon links to the parameters necessary for the design of the technical, economic and energy sizing component:

- 1) Baseline (BL): This alternative provides a comparison framework with all pre-treatment alternatives, in terms of traditional industry practice. This requires no pre-treatment technologies.
- 2) Direct anaerobic digestion (DAD) [36]: This alternative evaluates the direct movement of microalgae sludge to AD. In reality, this alternative is not related to pre-treatment technologies either. However, it is included in this section as a comparison scenario regarding other alternatives (just like the BL scenario).

- 3) Protein extraction (PE) [60–62]: As mentioned in the Introduction, this pre-treatment alternative meets the need to change the C/N ratio of the microalgae biomass prior to AD, in order to prevent inhibitions due NH₃⁺ accumulation. The proteins are separated by liquid extraction and subsequently decanted by changing the pH. Development requires stages of cell disruption, phase separation (solubilized proteins from cell *debris* [residue]), pH change reactors, phase separation (hydrophilic protein fraction) and dehydration.
- 4) Lipid extraction (LE) [40]: This alternative is evaluated as a result of Harun et al. (2011) study. They argue that it is possible to obtain more energy from microalgae by taking advantage of lipids to produce biodiesel and the rest of the biomass to generate biogas [25]. This is possible by considering the stages of ultrasound disruption, solvent extraction of lipids, phase separation (hydrophobic fraction from cell *debris*) and subsequent flash distillation of lipids. The glycerol produced in the subsequent transesterification of lipids is sent to AD.
- 5) In situ transesterification (TiS) [24,63–69] (see Fig. 3): In recent years of the microalgae research, it has been stated that in situ transesterification could favour the economic feasibility of the microalgae bioenergy industry (allowing for the reduction of production costs by skipping some drying stages) [13,64]. However, techno-economic evaluations of its scaling to an industrial scale



Fig. 3. Conceptual diagram of in situ transesterification with its respective flow and relevant operating units.

have still not been performed. Furthermore, it has been demonstrated that the production of biogas by means of the AD of TiS waste is possible [68], which is why its incorporation is an interesting pretreatment alternative. In literature, two types of TiS are mentioned, defined on the basis of the initial moisture of the biomass (dry and wet TiS), and both require different unit operations. To decide which of these to evaluate, a preliminary and parallel technoeconomic analysis was conducted. Using state-of-the-art techniques, this analysis determined that the wet TiS is the more expensive alternative (seven times the capital and operational costs). As Fig. 3 shows, the stages comprising the dry TiS [66] include an initial dehydration of the post-centrifuge biomass, acid esterification, phase separation (filtration of cell debris), phase separation with solvent extraction (hydrophobic fraction of the hydrophilic); hydrophilic route: phase separation (glycerol from water and sulphuric acid) and subsequent flash distillation; and hydrophobic route: dehydration with anhydrous sodium sulphate, phase separation (filtration) and hexane flash distillation.

- 6) Co-digestion (CoD) [24,27,63,70–72]: As in the case of PE, the CoD was mentioned in the Introduction as an alternative to adjust the C/N ratio of the material in the AD. Due to the high protein content of the microalgae, sludge should be mixed with co-substrates rich in carbon, such as paper waste [70], switchgrass [71], glycerol [63], and soybean [72], for example. The mixing ratio of microalgae and co-substrate will be determined by the C/N ratio desired for AD. The model uses an optimum C/N ratio of 22.5 for mixing paper waste, since according to the research conducted by Yen & Brune (2007), this value should be between 20 and 25 [70]. Regarding the CoD all that is required for the paper waste is to be triturated and mixed with the post-centrifuge sludge prior to being sent to AD. The CoD is an alternative which can be combined with additional pre-treatments.
- 7) Microalgae rupture or Cell Disruption (CDisr) [24,27,73–75]: To prevent possible microalgae cell survival in the digesters and to increase exposure of the cell contents, pre-treating the sludge with a cell disruption technology was proposed for increasing the generation of biogas, specifically with ultrasound, operating at 30 MJ/m³ of treated sludge [76,77]. This value is assumed as constant for all species of microalgae evaluated, and recognizes that, in fact, it is necessary to have information regarding the energy consumption of the cell disruption for each species, according to its physiological characteristics. Samson & Leduy (1983) have compared ultrasound disruption with thermochemical disruption, concluding that the former is more effective and less toxic for anaerobic microflora [73]. Despite the aforementioned, in recent years the use of thermochemical disruption has achieved positive results in relation to biogas generation [74,75]. This scenario is similar to direct AD, except that the digesters are preceded by a cell disruption stage.

Since CoD is not an exclusive route for biomass, this recovery alternative can be combined with the other aforementioned pre-treatments. Accordingly, the following four evaluation scenarios are applicable:

- 8) Cell disruption followed by co-digestion (CDisr + CoD).
- 9) Protein extraction followed by co-digestion (PE + CoD).
- 10) Lipid extraction followed by co-digestion (LE + CoD).
- 11) In situ transesterification followed by co-digestion (TiS + CoD).

2.1.6. Processing

The pre-treatments will generate two distinguishable biomass fractions: one directed towards AD, and the other which is used for obtaining additional products with commercial value. The latter is the one which drives processing, with the aim of obtaining biodiesel, fishmeal for animals in the aquaculture industry and microalgae protein supplements. Only the BL, PE, LE, PE + CoD and LE + CoD scenarios are associated with biomass processing.

- BL: This includes stages of dehydration (bringing biomass to 5% moisture), cell disruption (ball mills) and phase separation (lipids from cell *debris*). Subsequently, the lipids are sent to transesterification processes to form methyl esters (biodiesel), while the cell *debris* is sent to packing to be sold as animal feed.
- LE: Only extracted lipids are processed and sent to the transesterification process to produce biodiesel.
- PE: Proteins must be neutralized, dehydrated once more (4–5% moisture) and sent to packing to comply with the marketing standards of protein supplements. This type of product is similar to the supplements obtained from soybean and milk (casein).
- Transesterification [78,79]: Lipids or triglycerides are submitted to a process of traditional alkaline transesterification to produce methyl esters (biodiesel or FAME). The process consists of different stages of pre-mixing with reagents (MeOH and NaOH), a transesterification reactor with stirring and controlled temperature, flash distillation, phase separation (glycerol and biodiesel), a neutralization reactor and biodiesel washing with water, phase separation or dehydration and biodiesel storage.
- *Packing*: This consists of bagging and subsequent assembly of pallets for commercialization.

2.1.7. Anaerobic digestion

The AD process is modelled as a wet fermentation technology with scaling capacity, capable of Combined Heat and Power [CHP] generation and of taking advantage of the digestate produced. This process is demonstrated in Fig. 4, as follows:

A scheme with two digesters in series was chosen, with a coveredlagoon reactor type, insulated by concrete and other impermeable material in walls and a base, with a biogas holder at the top [27,83]. These reactors would operate with an average feeding rate of 2.4 kgVS/($m^3 \cdot d$) [29,36,42], and specifically, the fermenter would be maintained at 35 °C (mesophilic) [84,85], with an occasional stirring system of 3.5 h/day [86] and with a HRT (hydraulic residence time) of 19 d. The post-fermenter is also conditioned to operate at 35 °C (mesophilic), with no stirring, with a HRT of 11 d and decantation for generating a digestate purge. The sum of both HRT should be approximately 30 d; a number defined by numerous studies into the optimum degradation of microalgae in a mesophilic state [73,87–89].

The biogas generated in the anaerobic reactors $(5.5 \text{ kWh/m}^3 \text{ of heat} \text{power})$ [28], is usually dehydrated and desulphurized [80]. Subsequently, the biogas is stored in a biogas holder with special safety measures. A blower introduces the feed flow to the CHP system, in which the generated electricity (35%) and thermal energy (40%) [82] can be used either for internal processes or for commercialization, considering combustion losses (25%).

The final product generated in the microalgae bioenergy plant is the digestate stemming from AD. At this point there is certain industrial equipment, such as digestate separators [82], capable of separating the liquid parts (2% VS) from the solid (25% VS). A fraction of the liquid (21%) is recirculated to the beginning of the AD to be mixed with the feedstock microalgae in the pre-mixing tank. Accordingly, the total from the solid phase is sent to drying fields. Both products remain nutrient-rich, and can therefore be used as fertilizer or soil enhancer.

2.2. Economic evaluation

A project located at *Norte Grande* of Chile [55] was considered for this study, with daily operations of 12.5 h/day (07:00 to 19:30), and including the necessary work shifts and personnel required (human resource capital). The plant operates for 360 days a year, stopping only for coordinated maintenance to equipment and facilities, and for



Fig. 4. Conceptual diagram of the modelled AD. Source: Own research based on Refs. [80], [81] and [82].

15 years (the period of economic evaluation). Due to certain costs estimated at the local level, it should be noted that the exchange rate of the national currency (CLP) to the US dollar (US\$) was CLP620/US\$ as of February 2015.

2.2.1. Capital costs

Capital costs or investment will take place over the course of the first two years, depending on construction times. Amounts depend on the microalgae used, given that biomass productivity and composition of each species will require a different number of PBRs and ponds to be in operation.

For scaling equipment costs, an exponential formula was used in line with specific attributes [90]:

$$\frac{C_A}{C_B} = \left(\frac{E_A}{E_B}\right)^n.$$

 E_A : Attribute scale of known equipment. E_B : Desired scale for evaluation. CA: Cost of known investment. C_B : Desired cost for estimation. n: Exponential scaling factor (n = 0.6, except when the literature stipulates a different exponent value or a different cost function). Within engineering companies, the exponents have been seen to depend on the type of equipment, but in this study, 0.6 will be used as the most common value.

Biorefinery equipment must be installed within conditioned infrastructure for its correct and safe operation. In this area, three scales of biorefinery (2000, 5000 and 9000 m²) were assumed and constructed according to a fixed estimated per m² construction cost, using information from the Ministry of Housing and Urban Planning relating to the category of A2 construction (storehouse type) (US\$353/m²) [91,92]. Furthermore, investment also considers certain additional costs, both direct and indirect, for each one the microalgae production process stages (laboratory, preparation of medium, farming, harvesting, pre-treatment, processing, and AD). Due to the coordination and synchronization required between the unit operations, an automatic monitoring and control system is needed, which was considered as 10% of the sub-group investment in the evaluated equipment [90]. In addition, all industrial projects must consider the indirect costs of engineering, hiring and contingencies, which represented 20%, 5% and 10% of investment, respectively [90].

2.2.2. Unit and operational costs

Operations at the plant, in conjunction with its associated costs, would begin in the third year, and their scale would depend primarily on production levels. Energy and reagents are undoubtedly the main components of operational costs, for which constant values are assumed throughout the entire evaluation period. Electricity was estimated in accordance with contracts with the Chilean *Norte Grande* Interconnected System (SING) at US\$0.1/kWh [93]. Similarly, heat energy was defined using the costs of steam processes and natural gas in Chile at US\$0.04/kWh [77,94]. Prices of the reagents used in the different unit operations depend on the product in question and are calculated in accordance with local market analysis.

Acquisition of land was not considered as part of the capital costs, as a project of this type can be implemented with a land lease from National Assets. The lease was estimated at approximately US\$775/ha/year, adjustable in line with the Unidad de Fomento (Unit of Account) (UF).

Human capital is diverse and depends on the scale of the project. The recruitment of university-trained professionals is deemed necessary (i.e., researchers, civil engineers, business graduates, accountants and lawyers), in addition to individuals with technical degrees (administrative staff, technicians and chemical plant operatives), as well as non-skilled labour (grooming and security staff).

All plant equipment must be subject to regular maintenance in order to ensure its correct working. This generates annual operational expenditure associated with maintenance, equivalent to 5% of the original equipment capital costs.



Fig. 5. Comparison of total investment and operational costs by selected farming technology (MM = millions).

Finally, as is the case with other techno-economic feasibility studies, the issues of income tax and interest accrued as a result of financing were not considered [34].

2.2.3. Income

Income depends on the scenario evaluated, with a total of six possible marketable products: electricity, heat, biodiesel, microalgae fishmeal, digestate, and proteins.

Both electricity and heat energy depend on internal plant consumption. If surpluses are generated, these can be sold at the same prices mentioned regarding costs. On the other hand, biodiesel should be sold in conjunction with, or compete against, fossil diesel, the global average price of which is around US\$1/L (as of February 2015) [20]. However, the selling price of biodiesel, which makes the microalgae industry profitable, is an indicator of costs obtained through sensitivity analysis.

Other valuable microalgae products are less traditional. For the isolated proteins, a value similar to dietary supplements derived from soybean or milk was considered, of US\$3.25/kg. The price of fishmeal (for bivalves, crustaceans and fish) was estimated at US\$0.1/kg [95]. Finally, commercialization of the liquid and solid digestate stemming from AD rose by 80%, to US\$0.9/ton and US\$8/ton [96,97], respectively.

2.2.4. Discount rate and economic profitability indicator

The discount rate (dr) was not unique. Rather, it was measured across different percentages associated with industries that might show an interest in a project of this type. Specifically, four rates were defined for calculating the economic profitability of the project (net present value, NPV): social (3%); and, private with low risk (8%), medium risk (14%) and high risk (20%).

$$NPV = \sum_{t=0}^{n} \frac{(Income - Cost)_{t}}{(1 + dr)^{t}}$$

2.2.5. Energy return rate

In order not to lose the goal of producing energy from microalgae [41], the energy return on investment (EROI) can be calculated for all

Table 3	
Quantities of RWP and PBR considered in the different evaluated scales.	

Microalgae	Scale	Farmir	ıg tech		
Species	Small	Medium	Large	Unit	
Tetraselmis sp.	144.00	1035.00	1854.00	RWP	Only RWP
Isochrysis sp.	80.00	575.00	1030.00	RWP	
Tetraselmis sp.	10,136.00	278,504.00	1,519,048.00	PBR	Only PBR
Isochrysis sp.	6335.00	174,065.00	949,405.00	PBR	
Tetraselmis sp.	1016.00	27,568.00	150,304.00	PBR	Hybrid
	117.00	918.00	1665.00	RWP	
Isochrysis sp.	635.00	17,230.00	93,940.00	PBR	
	65.00	510.00	925.00	RWP	

the scenarios evaluated, using the following formula [98]:

 $EROI = \frac{Energy \text{ provided to society}}{Energy \text{ required to produce the energy provided}}$

As such, although it may be economically profitable to promote the development of the pre-treatment alternative evaluated, comparisons will always be possible from the energy perspective and its efficacy can be evaluated.

3. Results and discussion

3.1. Results presentation

The constructed model possesses a huge number of possible dimensions and scenarios. As such, it would be impossible to provide the reader with all the results without outlining a preliminary filter or a strategic approach regarding the adequate comprehension thereof.

The results help to provide an analysis of the most advisable farming technology. The alternatives evaluated consisted of the exclusive use of PBRs, RWPs, or a combination of both options, known as a hybrid system. By using PBRs, greater concentrations of biomass can be produced. Accordingly, the selection of this technology type would imply less consumption of culture medium, thereby indirectly could reduce, the costs of pumping, f/2 preparation and harvesting. The use of ponds, in contrast, provides less biomass productivity but energy consumption is less during farming and the unit cost of construction by volume should be lower.

By producing averages of all the scenarios evaluated and production scales considered in the model, the following bar charts (see Fig. 5) show the investment and operational costs according to the farming technology used (see Table 3 for some scale details). It is worthwhile comparing farming according to the scenario, since it relates to a predifferentiation stage. In other words, all scenarios share the same farming costs and then differ subsequently according to the treatment applied to post-harvest biomass.

As Fig. 5 demonstrates, the sole use of PBRs would imply capital costs almost three times greater than using other technologies, in addition to operational costs on average 37% higher. The sole use of PBRs could have been justified if its operational costs were lower than those of the RWPs, thereby having a positive impact on economic profitability. Unfortunately, this situation is technically impossible. As a result, the sole use of PBRs can be discarded from future analysis.

However, PBRs do possess an advantage not evaluated or quantified in the model: the isolation of the culture regarding the environment for reducing the risk of contamination. This characteristic, in addition to the fact that the costs in terms of the sole use of RWPs and the hybrid system are similar, allows for discrimination between the farming technologies. It also means that from now onwards only the results of the energy-economic evaluation of a hybrid system of microalgae production will be presented. This system means that PBRs will be used as

Table 4

Results of the energy-economic evaluation of the multi-scenarios of Isochrysis sp. biomass production, using a hybrid system of farming.

	MM US	5\$													
Scenario	Total capital costs		Total operational costs		Total income		NPV with dr of 14%		EROI (%)						
Scale	Small	Medium	Large	Small	Medium	Large	Small	Medium	Large	Small	Medium	Large	Small	Medium	Large
BL	3.47	36.53	165.41	0.96	9.73	44.82	0.24	6.25	34.01	-6.17	-46.32	- 186.09	0.51	0.63	0.64
DAD	3.65	43.43	196.71	0.87	7.79	33.97	0.09	2.38	12.94	-6.58	-60.56	-257.41	0.56	0.73	0.77
PE	3.67	45.99	210.63	1.07	12.10	56.89	0.78	20.35	110.71	-4.38	0.56	77.54	0.18	0.20	0.21
LE	3.77	42.71	193.03	1.08	8.56	37.19	0.24	6.16	33.50	-7.00	-46.23	-174.98	0.86	1.92	2.06
TiS	3.96	45.61	208.06	2.32	44.07	230.97	0.22	5.72	31.12	-12.92	-214.37	-1091.98	0.65	0.79	0.81
CoD	3.94	49.14	222.46	0.97	9.72	44.03	0.16	4.09	22.27	-6.96	-65.94	-280.60	0.95	1.23	1.28
CDisr	3.79	45.55	206.07	0.90	8.03	34.94	0.12	3.02	16.41	-6.73	-60.35	-253.06	0.73	0.95	1.00
CDisr + CoD	4.14	52.39	236.90	0.97	9.89	44.84	0.20	5.18	28.21	-6.96	-64.19	-268.07	1.24	1.59	1.67
PE + CoD	3.96	51.63	236.05	1.03	12.26	58.77	0.84	22.05	120.00	-4.10	3.37	92.21	0.35	0.38	0.39
LE + CoD	4.08	48.95	221.20	1.03	8.78	39.38	0.31	8.02	43.62	-6.69	-43.35	-159.56	1.08	2.39	2.55
TiS + CoD	4.18	50.00	227.89	2.25	44.01	231.65	0.28	7.43	40.41	-12.50	-209.57	-1067.50	0.85	1.03	1.06

 $MM = million (10^6)$; NPV = net present value; dr = discount rate; EROI = energy return on investment; BL = baseline; DAD = direct anaerobic digestion; PE = protein extraction; LE = lipid extraction; TiS =*insitu*transesterification; CoD = co-digestion; CDisr = cell disruption.

producers of inoculum and RWPs as the primary technology for farming and scaling.

As Table 4 demonstrates, all scenarios possess negative economic profitability (NPV < 0), except those which consider PE on a medium and large scale. This result was expected, since microalgae production is currently considered as marketable if the final product is designated for animal feed or for obtaining specific high-value metabolites, such as the proteins destined for human dietary supplements.

Regarding EROI, if this indicator exceeds the unit, the alternative evaluated can be conceived of as providing more energy to society than the amount it consumes during the production process. Conversely, and if the aforementioned does not apply, this option could be deemed unfavourable in energy terms. Table 4, outlines the EROI results for all evaluated scenarios.

Fig. 6 shows only four alternatives that present favourable EROI, none of which are profitable. Only the scenarios in which PE is considered are NPV positive. However, and counterproductively, these are the worst alternatives from the energy point of view. This figure also demonstrates that the CoD is an alternative that increases the EROI for any pre-treatment to which it is coupled, without significantly altering the NPV. Ideally, scenarios with NPV-EROI combinations would be expected, to be located over zero on the y-axis and to the right of one in the x-axis.

As Fig. 6 also shows, TiS is an extremely negative scenario in terms of economic profitability. Its value here even distorts the graph when compared to the values of the other scenarios evaluated. The dry TiS evaluated shows overly expensive operational costs that bring about an excessively negative NPV. In all analysed cases, the TiS never represented a viable or relatively attractive economic alternative. As a result, its future analysis will be considerably

reduced and it will be discarded as a feasible alternative, in terms of the described technologies and considerations regarding its current state of the art and modelling.

This study presents the NPV and EROI indicators as two tools for analysing the project from independent perspectives: economic and energy-related, respectively. However, it is possible to submit the EROI to economic evaluation by incorporating the results of a social evaluation of similar projects. It may be the case that scenarios with an EROI < 1 or with values unattractive to the energy industry, due to the displacement of fossil fuels generated, might provide social benefits greater than the projected economic returns. The EROI is reflected by the economic, environmental and social evaluation, because the nature of input and output energy transfer is not the same. For example, current electricity generation has an EROI < 1 due to the second law of thermodynamics. Nevertheless, it is currently accepted by society because the usefulness of electricity is greater than coal, diesel or natural gas. Similarly, if biofuel can be produced by consuming primary energy that is less costly to humanity (eventually, for example, solar heat, tides, wind, etc.), located in or near to deserts, this biofuel could be produced because its usefulness would be greater than the forms of energy used as an input in the process. It is clear that the cost of input energies can only be established through comprehensive research, using tools to conduct lifecycle analysis of the process, and attempting to avoid the incorporation of a simple idea about the type of energy being used.

Due to the fact that a large majority of the results produced NPV < 0, analysis will now turn to seeking critical variables which have a more substantial influence on NPV and EROI in each one of the scenarios and scales evaluated. The idea is to identify arbitrary configurations of these variables via an in-depth sensitivity analysis, which affords profitability to certain scenarios distinct to PE.





Fig. 6. NPV vs EROI for all scenarios evaluated on the basis of average production scales (MM = millions).



Fig. 7. Pie charts of the breakdown of investment by average scenarios and stages of microalgae production using hybrid farming (MM = millions).

3.2. Sensitivity analysis

In order to conduct a robust and significant sensitivity analysis, it was decided to first analyse the breakdown of costs (capital and operational). This approach helps to identify the stages of the production process with a greater average impact on NPV and, therefore, allows a definition to be devised of the variables and levels due to be measured.

As Fig. 7 shows, there are three main components of capital costs: farming, harvest, and anaerobic digestion. The particular microalgae species produced have a direct impact on farming capital costs. As such, a second analysis allows the lipids percentage of the microalgae to be seen, as well as the biomass productivity and the farming technology costs. These may represent high-impact variables in the sensitivity analysis, as they help to modify capital costs in the three main components detected.

Choosing the correct microalgae species for production is a key factor in achieving economic profitability. As Fig. 7 shows, there is a significant difference in the capital costs using Tetraselmis sp. (35% greater) with that of Isochrysis sp. The difference in the cost structure of using different microalgae is largely the result of the fact that scaling is dependent on a minimum level of generation of marketable products. For example, if the scenario relates to the production of biodiesel, changing the microalgae for another species with a different lipids percentage will change the number of farming ponds, harvesting systems and processing equipment required to obtain the annual volume of biodiesel established by that particular scale.

From the pie charts shown in Fig. 8, four main components of the operational costs can be seen: culture medium production, farming stage, harvest, and selected pre-treatment. These four stages entail intensive electricity consumption. As such, in order to modify the operational costs, the energy efficiency of the plant could be measured, as well as the energy cost, the percentage of lipids, and productivity from the farming technologies.

While the effect caused by the microalgae species being produced was analysed in the capital costs, this difference is not so clear or significant regarding the operational costs (8% greater for *Tetraselmis sp.*).

By adding the selling price of biodiesel to the variables already detected through the breakdown of capital and operational costs (lipids percentage, biomass productivity, farming technologies cost, energy efficiency of the plant, and electricity cost), modifications and measurements of income are also expected. This allows the following Table 5 to be devised, which demonstrates the variables to be measured in the sensitivity analysis, in conjunction with their respective levels of change considered technically feasible.

The aforementioned sensitivity analysis involved the generation of 10,692 combinations in order to obtain the same amount of results for both the NPV and EROI. Among all these results, only the PE and PE + CoD scenarios considered for medium and large scales of any given microalgae produced positive NPV values. Subsequently, the BL, LE y LE + CoD scenarios only achieved positive NPV under specific *Isochrysis sp.* production conditions, on the medium and large scales. The remaining scenarios (DAD, TiS, CoD, CDisr, CDisr + CoD and TiS + CoD) and the use of *Tetraselmis sp.* always produce negative economic profitability, under any possible combination of the levels and variables submitted to the sensitivity analysis.

As a result of the aforementioned, scale is a key determining factor for the NPV. On the small scale, all scenarios and combinations of





Operational cost Tetraselmis sp.

Fig. 8. Pie charts of the breakdown of operational costs by average scenarios and scales of microalgae production using hybrid farming (MM = millions).

Table 5

Variables detected with greater potential impact on the NPV and submitted to sensitivity analysis.

Variable	%Lipid ^a	Prod.CT ^b	\$CT ^c	%EffE ^d	\$EE ^e	\$BD ^f
Level	Δ%	Δ%	∆%capital cost	∆%consumption	US\$/kWh	US\$/L
Small Medium	- 50% 0%	-20% 0%		- 15% - 5%	0.05 0.1	0.8 1.1
Large	+70%	+20%	0%	0%	0.15	1.4

^a %Lipid = percentage of change to the composition of biomass lipids; the percentage change applies to the base lipids of each microalgae species.

^b Prod.CT = biomass productivity in farming technologies; the percentage change applies to the base value of g/L/day, in both RWPs and PBRs.

^c \$CT = cost of investment in farming technologies; the percentage change applies to the total value of investment in RWPs and PBRs.

^d %EffE = percentage of energy efficiency achieved; the percentage change applies to the total energy consumed in the plant.

^e \$EE = cost of electricity.

^f \$BD = selling price of biodiesel.

variables measured are negative, including the PE. From the medium scale, the NPV begins to turn positive for certain scenarios under specific combinations of the variables measured. The best results were achieved on the large scale. This makes sense, as the model considers economies of scale for both capital and operational costs.

On the other hand, the species of microalgae considered is also a relevant factor in economic profitability. The choice between *Tetraselmis sp.* and *Isochrysis sp.*, as will be demonstrated below, can significantly determine the economic attractiveness of this industry; primarily because of their biomass composition (see Fig. 9). Slight changes to the biomass composition can mean the success or failure of the business.

While this model presents a comparison of two particular species, it allows for the evaluation of any particular marine microalgae species. However, in order to analyse the production of freshwater species, and in relation to the considerations posed by this research, the modification of processes and associated costs of the culture medium preparation is recommended. Furthermore, such projects should ensure an abundant and local supply of freshwater, as well as reconsider the pumping methods used within the farming technologies.

To summarize the results of the sensitivity analysis, three contexts or scenarios will be devised regarding combinations between the variables measured (see.

Table 6). The first is the "moderate" context, in which the variables have conservative values in relation to the state of the art practices. The second is the "intermediate (lipids)" context, which consists of values similar to the base level, except for an optimistic percentage change regarding the lipid composition of the biomass (+70%). The final scenario is the "optimistic" context, which combines all the measured levels that favour economic profitability.

A frequently used indicator is the biodiesel minimum selling price that makes production economically profitable (NPV = 0). As shown by Fig. 1, an average price of US3.7/L was provided, in accordance with data collected from the literature review. The graphs in Fig. 10 are explained in the same manner, in that they represent the main results of the contexts measured in the sensitivity analysis (moderate,



Fig. 9. Biomass composition of the microalgae considered by the model.

intermediate and optimistic), regarding the biodiesel minimum selling price for the most attractive scenarios from an economic point of view (BL, LE and LE + CoD), as well as for both microalgae species.

Fig. 10 shows that a change in the lipid percentage (%Lipid) of the biomass, either through an increase (an effect of the intermediate context) or a change of microalgae, reflects an important reduction in the biodiesel minimum selling price. Specifically in the aforementioned intermediate context, the microalgae is considered to increase its lipid composition by 70%, which for *Isochrysis sp.* means achieving 40% lipids in the biomass. However, this is insufficient in approaching competitive prices in terms of fossil diesel (US\$1/L). In order to achieve this, a combination of key variables is required, defined according to the optimistic context, in which only large-scale *Isochrysis sp.* generated a price of US\$1.01/L, for both BL and LE.

It is therefore clear as to the significant effect of %Lipid on NPV in the three contexts evaluated in Fig. 10. However, it is difficult to quantify the parallel impact of the other variables measured. Therefore, the effect of all the factors measured on the NPV and EROI was evaluated by means of a statistical regression analysis which considered a fixed effects model. Fig. 11 summarizes part of the results of this analysis (only for medium scale), demonstrating the average weight of the factors or percentages associated with each of the variables measured for modifying the NPV. Fig. 11 also shows a variable called Int.Fact, which represents the sum of all the interaction effects between the factors. However, these interaction effects are usually minimal (less than 14%) in the NPV variations. Since the changes to the weight of the effects are extremely subtle between the evaluated scales, incredibly similar charts are produced. Consequently, the decision was taken to show the full results in the Appendices section of this study (NPV in Appendix B and EROI in Appendix C).

Fig. 11 confirms that %Lipid is the most relevant factor for the BL, LE, LE + CoD, PE and PE + CoD scenarios, followed by \$BD. Both variables are directly linked to the production and commercialization of the biodiesel produced, and indirectly linked to obtaining a different volume of proteins. However, there is an additional and more interesting factor which is particularly evident from this analysis. Since %Lipid has a greater impact on NPV than \$BD, this suggests that subsidies for the selling price or distribution conditions of biodiesel are less attractive options than finding a new species of microalgae with lipid hyperaccumulation or farming conditions which ensure a greater production of oils.

Alternatively, analysis of Fig. 11 allows for a more in-depth enquiry into the scenarios that failed to achieve economic profitability. As previously mentioned, the operational costs of TiS make it economically unfeasible, which is also the case for TiS + CoD. However, regarding DAD, CoD, CDisr and CDisr + CoD, it can be seen that the biomass productivity of farming technologies (Prod.CT) is the most influential factor on the NPV, followed by variables including \$TdC and %EffE. As such, in order to enhance the economic profitability of these scenarios, efforts must be focused on developing improvements to the aforementioned variables, exceeding the levels proposed in the sensitivity analysis. In these scenarios, the %Lip and \$BD factors have no meaningful influence, since they are scenarios with no biodiesel commercialization.

The effect of the electricity price (\$EE) on NPV is more uniform across all evaluated scenarios. It is possible to explain its variations throughout the scenarios that rely on technologies with intensive electricity consumption or which possess EE as a primary product for commercialization.

Regarding the effect on EROI, the situation is very different. Fewer variables are measured that impact on EROI (%Lipid, Prod.CT, %EffE and interaction between the factors), since the others are clearly economic in nature.

As Fig. 12 shows, %Lipid and %EffE are the variables with the largest influence on EROI across all scenarios evaluated. This means that these factors are mutually exclusive. When one is determinant in a given scenario, the other has little influence on the changes to the EROI.

Table 6

Definition of the contexts for comparison, following the sensitivity analysis.

Variable	%Lipid	Prod.CT	\$CT	%EffE	\$EE
Context	Δ%	Δ%	∆%capital cost	∆%consumption	US\$/kWh
Moderate Intermediate (lipids) Optimistic	0% + 70% + 70%	0% 0% +20%	0% 0% 15%	0% 0% — 15%	0.1 0.1 0.05

4. Conclusions

From the results of this study, it seems that microalgae are still not a sustainable source of bioenergy capable of competing against fossil diesel; including addressing their production by means of a biorefinery able to value all biomass fractions. Considering the use of different aforementioned treatment technologies and moderate values of the most significant or critical variables, none of the scenarios evaluated in this study were capable of producing more energy than it consumed (EROI > 1) while simultaneously achieving economic profitability (NPV > 0).

As expected, the PE is the most profitable scenario. However, it had the worst energy evaluation. This result produces a warning which must be heeded by the industry agenda, since it could be economically attractive in terms of entering the pharmaceutical market and the commercialization of proteins, which would mark a departure from the bioenergy approach. However, the interest in studying microalgae is based on a need to find sustainable energy sources. As such, future research and business in the area of microalgae depends on its ability to remain motivated with the issue of energy as its guiding principle.

The sensitivity analysis conducted in this study allows the critical variables to be detected for the entire process of bioenergy production based on microalgae, in addition to certain optimistic scenarios, through which economic profitability can be achieved. Throughout the entire process, reference has been made to the evaluation of stages prior to farming and up to the commercialization of the final products. This allows comparisons to be made of the magnitude of the investment and operational costs of the different technologies used throughout the production process. Other economic evaluation studies adopt a "door to door" methodological approach, which limits analysis of the results obtained. This model, however, proposes a "cradle to door" analysis (i.e., from the extraction of the raw material to its positioning on the market), allowing a broader and more holistic view to be generated to the advantage of the industry.

Other research contends that costs associated with the concentration and oil extraction stages represent the greatest percentage within the breakdown of biomass production costs, thereby becoming an important factor and limiting economic profitability in the process. However, from the results of this research, it can be observed and concluded that both the farming stage and the particular characteristics of the chosen species are the most influential factors in terms of economic profitability and energy use.

The costs incurred during farming are primarily explained by the excessive transport and management of water involved in the production of microalgae using RWPs. Considering that the maximum possible concentration in the ponds is 1 g/L, this means that 99.9% (in mass) is just water that requires pumping, enrichment and subsequent separation from the biomass. Figuratively, it can be said that the initial process



Fig. 10. Minimum selling price of biodiesel for scales and scenarios showing positive economic profitability, microalgae species, and contexts defined by measured variables.



NPV effect - Medium scale

Fig. 11. Chart of the weight of factors measured on the NPV for the hybrid farming of Isochrysis sp. on the medium scale.

research to approach the threshold of US\$1/L of biodiesel (the price of fossil diesel). This was possible due to the optimistic contexts or consid-

erations applied to the critical variables detected. This allows for continued hope regarding the microalgae bioenergy industry and helps to

determine future fields of study in which effort and resources can be

greatest impact on the NPV and EROI, it can be concluded that farming

technologies need to keep reducing their capital costs (reaching im-

proved economies of scale), while obtaining greater maximum concentrations and higher productivity. Simultaneously, regarding the study of

different microalgae species, the hyperaccumulation of lipids seems to

By addressing in detail the critical variables detected with the

placed in Research and Development.

unit operations work with water and not microalgae. Consequently, a change to the farmed species (an improved biomass productivity and increased lipid accumulation) has a significant impact on NPV. This is because it helps to reduce the volume of water circulating in the plant and the amount of equipment required to maintain the operation.

The AD is undoubtedly an attractive energy recovery alternative applicable to the different fractions of the microalgae biomass obtained from a biorefinery. In all scenarios evaluated, the EROI improves by coupling the CoD with other recovery alternatives. However, its direct and sole application is not profitable after the energy-economic evaluation.

The results of the multi-scenario economic evaluation of a biorefinery, despite being largely negative, allowed the authors of this



EROI effect - Medium scale

Fig. 12. Chart of the weight of factors measured on the EROI for the hybrid farming of Isochrysis sp. on the medium scale.



Fig. 13. Theoretical example of the view of the results of three energy alternatives or projects following a standardized multi-dimensional analysis.

be key to the alternatives that consider biodiesel commercialization. However, it is also necessary to keep searching for species which might have not yet been discovered and which possess characteristics attractive to bioenergy. Effort should also be made to improve the resistance of microalgae to environmental variables during farming, as well as the biomass productivity of already known species.

Throughout this study, the evaluation of bioenergy microalgae projects has been addressed from the economic (NPV) and energy (EROI) perspectives. However, this analysis could be more comprehensive and sustainable by adding new dimensions of evaluation. For example, an environmental evaluation could be incorporated to measure the carbon footprint in CO_2e emitted into the atmosphere.

A methodology is required that allows the evaluation and comparison of different energy alternatives using a homogenous standardized multi-dimensional criterion, as shown in Fig. 13. Research into the evaluation of related projects usually only shows or promotes certain characteristics favourable to that particular evaluation, concealing the overall impact of the project and making its direct comparison with other alternatives impossible. It would be interesting to be able to observe and compare projects using just one methodology that ensures an evaluation process which is clear, reproducible and sustainable.

Supplementary data to this article can be found online at http://dx. doi.org/10.1016/j.algal.2016.03.028.

Abbreviations

- PBR photobioreactors
- RWP raceway pond
- NPV net present value
- EROI energy return on investment
- MM million
- AD anaerobic digestion
- BL baseline
- DAD direct anaerobic digestion
- LE lipid extraction
- PE protein extraction
- TiS in situ transesterification
- CoD co-digestion
- CDisr cell disruption
- %Lipid lipids percentage change in biomass composition
- Prod.CT biomass productivity in farming technologies
- \$CT capital cost in farming technologies
- %EffE reached energy efficiency percentage
- \$EE electric energy cost
- \$BD biodiesel selling price

Acknowledgements

This research was partially supported by the National Commission for Scientific and Technological Research (Comisión Nacional de Investigación Científica y Tecnológica, CONICYT) of the Chilean Government, via a Master's scholarship awarded to Cristián P. Bravo-Fritz (ID No. 22130431). Additional support was provided by the Algae Fuels S.A. business consortium, funded by the Chilean Economic Development Agency (Corporación de Fomento de la Producción, CORFO), and by the Chilean Ministry of Energy (INNOVACORFO 09CTEI-6861), via its agreement with the Renewable Energies Research Group of the Chemical and Bioprocess Engineering Department, of the Pontificia Universidad Católica de Chile.

References

- EIA, EIA projects world energy consumption will increase 56% by 2040, http:// www.eia.gov/todayinenergy/detail.cfm?id=122512013 (accessed February 11, 2015).
- [2] A.P. Ballantyne, C.B. Alden, J.B. Miller, P.P. Tans, J.W.C. White, Increase in observed net carbon dioxide uptake by land and oceans during the past 50 years, Nature 488 (2012) 70–72, http://dx.doi.org/10.1038/nature11299.
- [3] NOAA, trends in atmospheric carbon dioxide, prod. trends CO₂ glob, http://www. esrl.noaa.gov/gmd/ccgg/trends/global.html2015 (accessed February 16, 2015).
- [4] National Research Council, advancing the science of climate change, 2010 489 http:// dge.stanford.edu/labs/caldeiralab/Caldeira_research/pdf/ACC_Science_2010.pdf.
- [5] T. (IPCC) Barker, Climate Change, An assessment of the intergovernmental panel on climate change, Change. 446 (2007) (2007) 12–17, http://dx.doi.org/10. 1256/004316502320517344.
- [6] R.D. Perlack, LL. Wright, A.F. Turhollow, R.L. Graham, B.J. Stokes, D.C. Erbach, Biomass as feedstock for a bioenergy and bioproducts industry : the technical feasibility of a billion-ton annual supply, Agriculture (2005) 78 DOE/GO-102. (doi: 10.2172/885984).
- [7] A. Bauen, G. Berndes, M. Junginger, F. Vuille, M. Londo, Bioenergy a sustainable and reliable energy source, Structure (2009) 1–108http://www.globalbioenergy. org/uploads/media/0912_IEA_Bioenergy_-_MAIN_REPORT_-_Bioenergy_-_a_sustainable_and_reliable_energy_source_A_review_of_status_and_prospects.pdf.
- [8] S. Ahmed, A. Jaber, M. Konukiewitz, R. Dixon, M. Eckhart, D. Hales, et al., Renewables 2011 Global Status Report, Paris, France, http://www.ren21.net/ ren21activities/globalstatusreport.aspx2011.
- [9] A.E. Farrell, R.J. Plevin, B.T. Turner, A.D. Jones, M. O'Hare, D.M. Kammen, Ethanol can contribute to energy and environmental goals, Science 311 (80-) (2006) 506–509, http://dx.doi.org/10.1126/science.1121416.
- [10] Y. Chisti, Biodiesel from microalgae, Biotechnol. Adv. 25 (2007) 294–306, http:// dx.doi.org/10.1016/j.biotechadv.2007.02.001.
- [11] R. Andersen, Algal Culturing Techniques, Elsevier Academic Press, San Diego, USA, 2005 http://books.google.com/books?hl=en&lr=&id=9NADUHyFZaEC&oi= fnd&pg=PR7&dq=Algal+Culturing+Techniques&ots=BvCr1fKOTi&sig= 2GyivBrX6QdpcD6t0fOFwi68Hdw (accessed June 4, 2014).
- [12] J. Sheehan, T. Dunahay, J. Benemann, P. Roessler, Look Back at the U.S. Department of Energy's Aquatic Species Program: Biodiesel from Algae; Close-Out Report, Golden, CO, 1998 http://dx.doi.org/10.2172/15003040.
- [13] I. Rawat, R. Ranjith Kumar, T. Mutanda, F. Bux, Biodiesel from microalgae: a critical evaluation from laboratory to large scale production, Appl. Energy 103 (2013) 444–467, http://dx.doi.org/10.1016/j.apenergy.2012.10.004.
- [14] A. Sun, R. Davis, M. Starbuck, A. Ben-Amotz, R. Pate, P.T. Pienkos, Comparative cost analysis of algal oil production for biofuels, Energy 36 (2011) 5169–5179, http:// dx.doi.org/10.1016/j.energy.2011.06.020.
- [15] NREL DOE, Microalgae feedstocks for biofuels production, Report to Congress (EISA 2007 – Section 228), 2008.
- [16] R. Davis, A. Aden, P.T. Pienkos, Techno-economic analysis of autotrophic microalgae for fuel production, Appl. Energy 88 (2011) 3524–3531, http://dx. doi.org/10.1016/j.apenergy.2011.04.018.
- [17] C. Bravo, C. Sáez, Análisis económico-energético del uso de biomasa microalgal para la producción de bioenergía en Chile [Economic and energy analysis of the use of microalgal biomass for bioenergy production in Chile], Pontificia Universidad Catolica de Chile, 2012.
- [18] D.A. Biofuels, P.T. Pienkos, Historical Overview of Algal Biofuel Technoeconomic Analyses You Are Here2008.
- [19] F. Delrue, P.-A. Setier, C. Sahut, L. Cournac, A. Roubaud, G. Peltier, et al., An economic, sustainability, and energetic model of biodiesel production from microalgae, Bioresour. Technol. 111 (2012) 191–200, http://dx.doi.org/10.1016/j.biortech. 2012.02.020.
- [20] GlobalPetrolPrices, Diesel prices, http://www.globalpetrolprices.com/2015 (accessed February 12, 2015).
- [21] J.H. Mussgnug, V. Klassen, A. Schlüter, O. Kruse, Microalgae as substrates for fermentative biogas production in a combined biorefinery concept, J. Biotechnol. 150 (2010) 51–56, http://dx.doi.org/10.1016/j.jbiotec.2010.07.030.
- [22] P. Spolaore, C. Joannis-Cassan, E. Duran, A. Isambert, Commercial applications of microalgae, J. Biosci. Bioeng. 101 (2006) 87–96, http://dx.doi.org/10.1263/jbb. 101.87.

- [23] R. Harun, M. Singh, G.M. Forde, M.K. Danquah, Bioprocess engineering of microalgae to produce a variety of consumer products, Renew. Sust. Energ. Rev. 14 (2010) 1037–1047, http://dx.doi.org/10.1016/j.rser.2009.11.004.
- [24] P. Bohutskyi, E. Bouwer, Advanced biofuels and bioproducts. Chapter 36. Biogas production from algae and cyanobacteria through anaerobic digestion, A Review, Analysis, and Research Needs, Springer New York, New York, NY, 2013 http://dx. doi.org/10.1007/978-1-4614-3348-4.
- [25] R. Harun, M. Davidson, M. Doyle, R. Gopiraj, M. Danquah, G. Forde, Technoeconomic analysis of an integrated microalgae photobioreactor, biodiesel and biogas production facility, Biomass and Bioenergy. 35 (2011) 741–747, http://dx.doi.org/10.1016/j.biombioe.2010.10.007.
- [26] M.E. Alzate, R. Muñoz, F. Rogalla, F. Fdz-Polanco, S.I. Pérez-Elvira, Biochemical methane potential of microalgae: influence of substrate to inoculum ratio, biomass concentration and pretreatment, Bioresour, Technol. 123 (2012) 488–494, http:// dx.doi.org/10.1016/j.biortech.2012.06.113.
- [27] I. Petrick, L. Dombrowski, M. Kröger, T. Beckert, T. Kuchling, S. Kureti, Algae Biorefinery – Material and Energy Use of Algae. Chapter 8. Biogas Production, http://www.dbfz.de/web/en/publications/dbfz-reports.html2013.
- [28] A.M. Buswell, H.F. Mueller, Mechanism of methane formation, Ind. Eng. Chem. 44 (1952) 550–552, http://dx.doi.org/10.1021/ie50507a033.
- [29] B. Sialve, N. Bernet, O. Bernard, Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable, Biotechnol. Adv. 27 (2009) 409–416, http://dx.doi.org/10.1016/j.biotechadv.2009.03.001.
- [30] L. Lardon, A. Hélias, B. Sialve, J.-P. Steyer, O. Bernard, Life-cycle assessment of biodiesel production from microalgae, Environ. Sci. Technol. 43 (2009) 6475–6481, http://dx.doi.org/10.1021/es900705j.
- [31] Y. Chen, J.J. Cheng, K.S. Creamer, Inhibition of anaerobic digestion process: a review, Bioresour. Technol. 99 (2008) 4044–4064, http://dx.doi.org/10.1016/j. biortech.2007.01.057.
- [32] P.H. Chen, Factors Influencing Methane Fermentation of Micro-algae, California Univ, Berkeley (USA), 1987http://www.osti.gov/scitech/biblio/6382014.
- [33] P. Bohutskyi, M.J. Betenbaugh, E.J. Bouwer, The effects of alternative pretreatment strategies on anaerobic digestion and methane production from different algal strains, Bioresour. Technol. 155 (2014) 366–372, http://dx.doi.org/10.1016/j. biortech.2013.12.095.
- [34] C. Zamalloa, E. Vulsteke, J. Albrecht, W. Verstraete, The techno-economic potential of renewable energy through the anaerobic digestion of microalgae, Bioresour. Technol. 102 (2011) 1149–1158, http://dx.doi.org/10.1016/j.biortech.2010.09. 017.
- [35] L.A. Ribeiro, P.P. Da Silva, Surveying techno-economic indicators of microalgae biofuel technologies, Renew. Sust. Energ. Rev. 25 (2013) 89–96, http://dx.doi.org/10. 1016/j.rser.2013.03.032.
- [36] P. Collet, A. Hélias, L. Lardon, M. Ras, R.-A. Goy, J.-P. Steyer, Life-cycle assessment of microalgae culture coupled to biogas production, Bioresour. Technol. 102 (2011) 207–214, http://dx.doi.org/10.1016/j.biortech.2010.06.154.
- [37] G. Pokoo-Aikins, A. Nadim, M.M. El-Halwagi, V. Mahalec, Design and analysis of biodiesel production from algae grown through carbon sequestration, Clean Techn. Environ. Policy 12 (2010) 239–254, http://dx.doi.org/10.1007/ s10098-009-0215-6.
- [38] J.G.G. Jonker, A.P.C. Faaij, Techno-economic assessment of micro-algae as feedstock for renewable bio-energy production, Appl. Energy 102 (2013) 461–475, http:// dx.doi.org/10.1016/j.apenergy.2012.07.053.
- [39] S. Nagarajan, S.K. Chou, S. Cao, C. Wu, Z. Zhou, An updated comprehensive technoeconomic analysis of algae biodiesel, Bioresour. Technol. 145 (2013) 150–156, http://dx.doi.org/10.1016/j.biortech.2012.11.108.
- [40] L.F. Razon, R.R. Tan, Net energy analysis of the production of biodiesel and biogas from the microalgae: *Haematococcus pluvialis* and *Nannochloropsis*, Appl. Energy 88 (2011) 3507–3514, http://dx.doi.org/10.1016/j.apenergy.2010.12.052.
- [41] L.B. Brentner, M.J. Eckelman, J.B. Zimmerman, Combinatorial life cycle assessment to inform process design of industrial production of algal biodiesel, Environ. Sci. Technol. 45 (2011) 7060–7067, http://dx.doi.org/10.1021/es2006995.
- [42] C.-M. Asinari Di San Marzano, A. Legros, H.P. Naveau, E.-J. Nyns, Biomethanation of the marine algae tetraselmis[†], Int. J. Sol. Energy 1 (1982) 263–272, http://dx.doi. org/10.1080/01425918208909891.
- [43] T.M. Mata, A.A. Martins, N.S. Caetano, Microalgae for biodiesel production and other applications: a review, Renew. Sustain. Energy Rev. 14 (2010) 217–232, http://dx.doi.org/10.1016/j.rser.2009.07.020.
- [44] M.J. Griffiths, S.T.L. Harrison, Lipid productivity as a key characteristic for choosing algal species for biodiesel production, J. Appl. Phycol. 21 (2009) 493–507, http:// dx.doi.org/10.1007/s10811-008-9392-7.
- [45] J.C. Frigon, F. Matteau-Lebrun, R. Hamani Abdou, P.J. McGinn, S.J.B. O'Leary, S.R. Guiot, Screening microalgae strains for their productivity in methane following anaerobic digestion, Appl. Energy 108 (2013) 100–107, http://dx.doi.org/10.1016/j. apenergy.2013.02.051.
- [46] P.V. Phatarpekar, R.A. Sreepada, C. Pednekar, C.T. Achuthankutty, A comparative study on growth performance and biochemical composition of mixed culture of *Isochrysis galbana* and *Chaetoceros calcitrans* with monocultures, Aquaculture 181 (2000) 141–155, http://dx.doi.org/10.1016/S0044– 8486(99)00227–6.
- [47] A. Richmond, Handbook of Microalgal Culture, Biotechnol. Appl. Phycol., 577, John Wiley Sons, 2004 http://scholar.google.com/scholar?hl=en&btnG= Search&q=intitle:Handbook+of+Microalgal+Culture#2 (accessed September 10, 2014).
- [48] M.A. Borowitzka, Commercial production of microalgae: ponds, tanks, tubes and fermenters, J. Biotechnol. 70 (1999) 313–321, http://dx.doi.org/10.1016/S0168– 1656(99)00083–8.

- [50] A. Richmond, Z. Cheng-Wu, Optimization of a flat plate glass reactor for mass production of *Nannochloropsis* sp. outdoors, J. Biotechnol. 85 (2001) 259–269, http:// dx.doi.org/10.1016/S0168-1656(00)00353-9.
- [51] M.P. Gentile, H.W. Blanch, Physiology and xanthophyll cycle activity of Nannochloropsis gaditana, Biotechnol. Bioeng. 75 (2001) 1–12, http://dx.doi.org/10. 1002/bit.1158.
- [52] L. Jiang, S. Luo, X. Fan, Z. Yang, R. Guo, Biomass and lipid production of marine microalgae using municipal wastewater and high concentration of CO₂, Appl. Energy 88 (2011) 3336–3341, http://dx.doi.org/10.1016/j.apenergy.2011.03.043.
- [53] C.-H. Su, L-J. Chien, J. Gomes, Y.-S. Lin, Y.-K. Yu, J.-S. Liou, et al., Factors affecting lipid accumulation by *Nannochloropsis oculata* in a two-stage cultivation process, J. Appl. Phycol. 23 (2010) 903–908, http://dx.doi.org/10.1007/s10811-010-9609-4.
- [54] C. Drapcho, N. Nhuan, T. Walker, Biofuels Engineering Process TechnologyMcGraw Hill Professional 2008 http://dx.doi.org/10.1036/0071487492.
- [55] C.P. Bravo-Fritz, C.A. Sáez-Navarrete, L.A. Herrera-Zeppelin, R. Ginocchio-Cea, Site selection for microalgae farming on an industrial scale in Chile, Algal Res. 11 (2015) 343–349, http://dx.doi.org/10.1016/j.algal.2015.07.012.
- [56] E. Molina Grima, E.-H. Belarbi, F.G. Acién Fernández, A. Robles Medina, Y. Chisti, Recovery of microalgal biomass and metabolites: process options and economics, Biotechnol. Adv. 20 (2003) 491–515 http://www.ncbi.nlm.nih.gov/pubmed/ 14550018.
- [57] C.Y. Chen, K.L. Yeh, R. Aisyah, D.J. Lee, J.S. Chang, Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review, Bioresour. Technol. 102 (2011) 71–81, http://dx.doi.org/10.1016/j.biortech.2010. 06.159.
- [58] A. Singh, P.S. Nigam, J.D. Murphy, Mechanism and challenges in commercialisation of algal biofuels, Bioresour, Technol. 102 (2011) 26–34, http://dx.doi.org/10.1016/ j.biortech.2010.06.057.
- [59] J. Mao, X. Cao, N. Chen, L. Brennan, P. Owende, Advanced biofuels and bioproducts. Chapter 24, Biofuels from Microalgae: Towards Meeting Advanced Fuel Standards, 2013 http://dx.doi.org/10.1007/978-1-4614-3348-4.
- [60] A.V. Ursu, A. Marcati, T. Sayd, V. Sante-Lhoutellier, G. Djelveh, P. Michaud, Extraction, fractionation and functional properties of proteins from the microalgae *Chlorella vulgaris*, Bioresour. Technol. 157 (2014) 134–139, http://dx.doi.org/10.1016/j. biortech.2014.01.071.
- [61] E. Barbarino, S.O. Lourenço, An evaluation of methods for extraction and quantification of protein from marine macro- and microalgae, J. Appl. Phycol. 17 (2005) 447–460, http://dx.doi.org/10.1007/s10811-005-1641-4.
- [62] J.L. Garcia-Moscoso, W. Obeid, S. Kumar, P.G. Hatcher, Flash hydrolysis of microalgae (*Scenedesmus* sp.) for protein extraction and production of biofuels intermediates, J. Supercrit. Fluids 82 (2013) 183–190, http://dx.doi.org/10.1016/j.supflu.2013.07.012.
- [63] E.A. Ehimen, Z.F. Sun, C.G. Carrington, E.J. Birch, J.J. Eaton-Rye, Anaerobic digestion of microalgae residues resulting from the biodiesel production process, Appl. Energy 88 (2011) 3454–3463, http://dx.doi.org/10.1016/j.apenergy.2010.10.020.
- [64] A. Sathish, B.R. Smith, R.C. Sims, Effect of moisture on in situ transesterification of microalgae for biodiesel production, J. Chem. Technol. Biotechnol. 89 (2014) 137–142, http://dx.doi.org/10.1002/jctb.4125.
- [65] M.J. Haas, K. Wagner, Simplifying biodiesel production: the direct or in situ transesterification of algal biomass, Eur. J. Lipid Sci. Technol. 113 (2011) 1219–1229, http://dx.doi.org/10.1002/ejlt.201100106.
- [66] E.A. Ehimen, Z.F. Sun, C.G. Carrington, Variables affecting the in situ transesterification of microalgae lipids, Fuel 89 (2010) 677–684, http://dx.doi.org/10.1016/j.fuel.2009. 10.011.
- [67] R.B. Levine, T. Pinnarat, P.E. Savage, Biodiesel production from wet algal biomass through in situ lipid hydrolysis and supercritical transesterification, Energy and Fuels. 24 (2010) 5235–5243, http://dx.doi.org/10.1021/ef1008314.
- [68] E.A. Ehimen, S. Connaughton, Z.F. Sun, G.C. Carrington, Energy recovery from lipid extracted, transesterified and glycerol codigested microalgae biomass, Glob. Chang. Biol. Bioenergy 1 (2009) 371–381, http://dx.doi.org/10.1111/j.1757– 1707.2009.01029.x.
- [69] R. Halim, M.K. Danquah, P.A. Webley, Extraction of oil from microalgae for biodiesel production: a review. Biotechnol. Adv. 30 (2012) 709–732, http://dx.doi.org/ 10.1016/j.biotechadv.2012.01.001.
- [70] H.-W. Yen, D.E. Brune, Anaerobic co-digestion of algal sludge and waste paper to produce methane, Bioresour. Technol. 98 (2007) 130–134, http://dx.doi.org/10. 1016/j.biortech.2005.11.010.
- [71] H.M. El-Mashad, Kinetics of methane production from the codigestion of switchgrass and Spirulina platensis algae, Bioresour. Technol. 132 (2013) 305–312, http://dx.doi.org/10.1016/j.biortech.2012.12.183.
- [72] M. Salerno, Y. Nurdogan, T.J. Lundquist, Biogas production from algae biomass harvested at wastewater treatment ponds, Bioenergy Eng. Conf. (2009).
- [73] R. Samson, A. Leduy, Influence of mechanical and thermochemical pretreatments on anaerobic digestion of *Spirulina maxima* algal biomass, Biotechnol. Lett. 5 (1983) 671–676, http://dx.doi.org/10.1007/bf01386360.
- [74] F. Passos, I. Ferrer, Influence of hydrothermal pretreatment on microalgal biomass anaerobic digestion and bioenergy production, Water Res. 68 (2015) 364–373, http://dx.doi.org/10.1016/j.watres.2014.10.015.
- [75] S. Schwede, Z.U. Rehman, M. Gerber, C. Theiss, R. Span, Effects of thermal pretreatment on anaerobic digestion of *Nannochloropsis salina* biomass, Bioresour. Technol. 143 (2013) 505–511, http://dx.doi.org/10.1016/j.biortech.2013.06.043.
- [76] K. de Boer, N.R. Moheimani, M.A. Borowitzka, P.A. Bahri, Extraction and conversion pathways for microalgae to biodiesel: a review focused on energy consumption, J. Appl. Phycol. 24 (2012) 1681–1698, http://dx.doi.org/10.1007/s10811-012-9835-z.

- [77] R. Schlatter, Comparación de la sustentabilidad del diseño básico de operaciones unitarias secuenciales y el diseño de planta completa para una planta de producción de biodiesel desde microalgas [Comparison of the sustainability of basic design for unit operations...], Universidad de Chile, 2014.
- [78] F. Ma, M. a Hanna, Biodiesel production: a review, Bioresour. Technol. 70 (1999) 1–15, http://dx.doi.org/10.1016/S0960-8524(99)00025-5.
- [79] J. Van Gerpen, Biodiesel processing and production, Fuel Process. Technol. 86 (2005) 1097–1107, http://dx.doi.org/10.1016/j.fuproc.2004.11.005.
- [80] P. Weiland, Biogas production: current state and perspectives, Appl. Microbiol. Biotechnol. 85 (2010) 849–860, http://dx.doi.org/10.1007/s00253-009-2246-7.
- [81] M. Pöschl, S. Ward, P. Owende, Evaluation of energy efficiency of various biogas production and utilization pathways, Appl. Energy 87 (2010) 3305–3321, http:// dx.doi.org/10.1016/j.apenergy.2010.05.011.
- [82] A. Akbulut, Techno-economic analysis of electricity and heat generation from farm-scale biogas plant: Çiçekdag case study, Energy 44 (2012) 381–390, http:// dx.doi.org/10.1016/j.energy.2012.06.017.
- [83] Biotecsur, Biodigestores para empresas agrícolas y otras industrias, (n.d.). http:// biotecsur.cl/ (accessed February 20, 2015).
- [84] B. Wu, E. Bibeau, Development of 3-D anaerobic digester heat transfer model for cold weather applications, Trans. Am. Soc. Agric. Biol. Eng. 49 (2006) 749–758http://www. prairieswine.com/pdf/3041.pdf.
- [85] Y.C. Song, S.J. Kwon, J.H. Woo, Mesophilic and thermophilic temperature co-phase anaerobic digestion compared with single-stage mesophilic- and thermophilic digestion of sewage sludge, Water Res. 38 (2004) 1653–1662, http://dx.doi.org/10. 1016/j.watres.2003.12.019.
- [86] A. Lemmer, H.J. Naegele, J. Sondermann, How efficient are agitators in biogas digesters? Determination of the efficiency of submersible motor mixers and incline agitators by measuring nutrient distribution in full-scale agricultural biogas digesters, Energies. 6 (2013) 6255–6273, http://dx.doi.org/10.3390/en6126255.
- [87] A. Gurung, S.W. Van Ginkel, W.C. Kang, N.A. Qambrani, S.E. Oh, Evaluation of marine biomass as a source of methane in batch tests: a lab-scale study, Energy 43 (2012) 396–401, http://dx.doi.org/10.1016/j.energy.2012.04.005.
- [88] H.B. Nielsen, S. Heiske, Anaerobic digestion of macroalgae: methane potentials, pre-treatment, inhibition and co-digestion, Water Sci. Technol. 64 (2011) 1723–1729, http://dx.doi.org/10.2166/wst.2011.654.
- [89] M. Ras, L. Lardon, S. Bruno, N. Bernet, J.P. Steyer, Experimental study on a coupled process of production and anaerobic digestion of *Chlorella vulgaris*, Bioresour. Technol. 102 (2011) 200–206, http://dx.doi.org/10.1016/j.biortech.2010.06.146.

- [90] R.K. Sinnott, Chemical engineering design, Coulson and Richardson's Chemical Engineering, 6, 6 2005, p. 1056http://books.google.com/books?id= DJaxUL3numgC&pgis=1.
- [91] Ministerio de Vivienda y Urbanismo (MinVU), Tablas de Costo Unitario Estadísticas – Biblioteca, http://www.minvu.cl/opensite_20101005084843. aspx#2011 (accessed February 25, 2015).
- [92] MinVU, Fija valores unitarios de construcción para aplicar en cálculos de derechos de permisos municipales, INE, Chile, http://www.ine.cl/canales/chile_estadistico/ estadisticas economicas/edificacion/metodologia/pdf/metodologia2.pdf2001.
- [93] Comisión Nacional de Energía (CNE), Precios de nudo SIC SING Aysén y Magallanes [Node prices SIC-SING-SMA-SMM], http://www.cne.cl/estadisticas/energia/ electricidad2014 (accessed February 23, 2015).
- [94] BP, BP Statistical Review of World Energy 2014 Natural Gas, http://www.bp.com/ content/dam/bp/pdf/Energy-economics/statistical-review-2014/BP-statistical-review-of-world-energy-2014-full-report.pdf2014.
- [95] B.G. Subhadra, Sustainability of algal biofuel production using integrated renewable energy park (IREP) and algal biorefinery approach, Energy Policy. 38 (2010) 5892–5901, http://dx.doi.org/10.1016/j.enpol.2010.05.043.
- [96] M. Lantz, The economic performance of combined heat and power from biogas produced from manure in Sweden — a comparison of different CHP technologies, Appl. Energy 98 (2012) 502–511, http://dx.doi.org/10.1016/j.apenergy.2012.04.015.
- [97] Waste & Resources Action Programme (WRAP), Anaerobic digestate, Banbury, Oxon, http://www.organics-recycling.org.uk/uploads/category1060/Financial_impact_assessment_for_anaerobic_digestate.pdf2009.
- [98] C.A.S. Hall, K.A. Klitgaard, Energy Return on Investment, Energy and the Wealth of Nations, 2012 309–320, http://dx.doi.org/10.1007/978–1-4419-9398-4.
- [99] D.A. Feinberg, Fuel options from microalgae with representative chemical compositions, 1984 United States, http://dx.doi.org/10.2172/6782425.
- [100] F. Weißbach, Die Bewertung von nachwachsenden Rohstoffen für die Biogasgewin-nung, Teil I : Das Gasbildungspotenzial der fermentierbaren Nährstoffe. Pflanzenbauwissenschaften 13 (2) (2009) 72–85.
- [101] VDI guideline 4630, Fachbereich Enregiewandlung und Anwendung, Vergärung organischer Stoffe-Substratcharakterisierung, Probenahme, Stoffdatenerhebung, Gärversuche, 2006.