CHEMICAL ENGINEERING TRANSACTIONS

VOL. 49, 2016

A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet

Guest Editors: Enrico Bardone, Marco Bravi, Tajalli Keshavarz

ISBN 978-88-95608-40-2; **ISSN** 2283-9216

DOI: 10.3303/CET1649096

Sustainability Evaluation of Biodiesel from *Arthrospira platensis* and *Chlorella vulgaris* under Mixotrophic Conditions and Salinity Stress

António A. Martins*[ab], Teresa M. Mata[a], Octávio Oliveira[c], Sandra Oliveira[c], Adélio M. Mendes[a], Nídia S. Caetano[ac]

[a] LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto (FEUP), R. Dr. Roberto Frias S/N, 4200-465 Porto, Portugal
[b] Department of Environmental Engineering, Faculty of Natural Sciences, Engineering and Technology (FCNET), Oporto Lusophone University, R. Dr. Augusto Rosa, 24, 4000-098 Porto, Portugal
[c] CIETI, Department of Chemical Engineering, School of Engineering (ISEP), Polytechnic Institute of Porto (IPP), R. Dr. António Bernardino de Almeida S/N, 4200-072 Porto, Portugal
tmata@fe.up.pt

This study performs a sustainability evaluation of biodiesel production from microalgae *Arthrospira platensis* (*A. platensis*) and *Chlorella vulgaris* (*C. vulgaris*) cultivated in mixotrophic conditions, with and without salinity stress, in comparison to autotrophic conditions. The life cycle steps considered for the evaluation are microalgae cultivation, biomass harvesting, lipids extraction, biodiesel production, distribution and use. Three sustainability indicators (LCEE – Life Cycle Energy Efficiency, FER – Fossil Energy Ratio and GW – Global Warming) are calculated based on laboratory experiments conducted in this study and literature data to complement inventory data, thus allowing a more truthful and accurate sustainability evaluation. Results show that in the current conditions, production of biodiesel from these microalgae is not energy efficient, since LCEE and FER values are lower than one, except for *C. Vulgaris* at mixotrophic growth, without salinity stress. GW values are always positive, meaning that carbon captured during microalgae growth does not compensate the carbon emitted in the whole process. A comparative analysis of the various process steps is also conducted, showing that the water removal and lipids recovery are critical steps for the process sustainability. One possible solution is to explore these microalgae in a biorefinery process, where the desired product is obtained along with a host of by-products, increasing the process sustainability and competitiveness.

1. Introduction

Environmental concerns and energy security are among the major issues of the twenty-first century. Within this framework, algal-based biofuels are gaining widespread attention (Ribeiro et al., 2015). In particular, lipids from microalgae are seen as a viable alternative to edible vegetable oils that are available in limited supply and have a seasonal nature due to their agricultural origin, helping to reduce the dependence on fossil fuels, with lower environmental impacts and, at the same time, contributing to the energy supply security. The interest in using microalgae for renewable energy production started during the 1970s in the wake of the first oil crisis. In the United States of America (U.S.), the National Renewable Energy Laboratory (NREL) launched a specific R&D Program dedicated to alternative renewable fuels, which lasted for about 18 years. One of its main objectives was to study the biochemistry and physiology of lipid production in oleaginous microalgae. It concluded that the use of microalgae for biodiesel production is technically feasible, but still needs considerable long term R&D activities to achieve the high productivities required (Mata el al., 2010). Currently, there are probably no more than about 30 species and genera from about 11 taxonomic classes of autotrophic microalgae that can be efficiently cultured in large scale (Friedl et al., 2012). Among them, *C. vulgaris* and *A. platensis* were the first to be cultivated in large scale since the 1960s and 1970s, respectively, for food or feed purposes due to their high protein content and excellent nutritive value (Richmond, 2004). Although

Please cite this article as: Martins A., Mata T., Oliveira O., Oliveira S., Mendes A., Caetano N., 2016, Sustainability evaluation of biodiesel from arthrospira platensis and chlorella vulgaris under mixotrophic conditions and salinity stress, Chemical Engineering Transactions, 49, 571-576 DOI: 10.3303/CET1649096

microalgae can be cultivated autotrophically in large scale production systems, the cultures in mixotrophic growth conditions normally lead to higher biomass productivities and may become more economical by using low-cost carbon sources, such as industrial by-products or even residual streams containing nutrients such as nitrogen and carbon (Mata et al., 2013b). Despite the several advantages of microalgae a proper sustainability evaluation is needed to ensure that the best options are chosen (Mata et al., 2013a). Therefore, this study aims to perform a sustainability evaluation of biodiesel production from microalgae *A. platensis* and *C. vulgaris* cultivated in mixotrophic conditions, with and without salinity stress, in comparison with autotrophic conditions.

2. Methodology for the study and process description

2.1 Framework for deriving sustainability metrics

Various strategies for evaluating sustainability of a product or process have been proposed in literature. Among them, the framework proposed and developed by Martins et al. (2007), and applied by Mata et al. (2013a, 2014a), is a flexible, objective and rigorous methodology. It is based on life cycle approach and has a sequential and iterative nature. The methodology comprises several steps. First, one needs to define the system boundary for the study. Depending on the particularities of the system and the study goals, the various stages should be considered in more or less detail, or even not accounted for, depending on the available data. Second, the most important environmental, societal and economic aspects associated to each product life cycle stage are identified and all relevant data are collected. Third, the appropriate set of suitable metrics or indicators that best describe the system is defined and prioritized, and classified in 3D, 2D or 1D (Martins et al., 2007). To identify the most adequate indicators for the sustainability evaluation one needs to study in detail the technologies involved in each life cycle stage and the associated significant environmental, societal, and economic impacts. A literature review and also interviews with experts and people involved in R&D and industrial practice are good sources of information. The prioritization is done based on system specific information, for reducing the number of metrics after their identification and selection. The number of metrics will also depend on the data available and study goals. Fourth, the metrics are computed and the sustainability of the current process or product relative to other(s) alternative(s) is compared. Interpretation is placed in a central position relative to the other steps, as for any step a critical assessment should be performed, independently or combined with others. It is also evident the iterative nature of this method, since as technologies evolve and more knowledge is acquired about the system it may be necessary to redefine the system boundaries, reevaluate or even define other indicators, eliminate indicators, change or improve calculation methods, or reassess previously made decisions. The final set of indicators can be used for decision making, so they need to be correctly defined and calculated to ensure that the most appropriate decisions are made. If the purpose of the study is to compare among process alternatives, decisions concerning the most sustainable one(s) can be made based on this evaluation, complemented with other issues if necessary, such as an economic analysis (Mata et al., 2014a).

Following this methodology one can evaluate the sustainability of microalgae biodiesel based on the computed indicators. In this study three sustainability indicators were selected: two energy based indicators, the life cycle energy efficiency (LCEE) and the fossil energy ratio (FER), and the contribution to global warming (GW). LCEE (dimensionless) is the ratio of the total energy output, consisting of the energy content of the biofuel, plus that of byproducts only if they are used to supply energy to the biofuel production system, to the amount of energy expended to obtain the biofuel. FER (dimensionless) is the ratio between the energy content of the final fuel product (or the fuel energy output) and the amount of fossil energy input (or the non-renewable energy) required for the fuel production through the supply chain. GW (kg CO_2-eq/MJ fuel) measures the potential contribution of different greenhouse gas (GHG) emissions to global warming, expressed as equivalent CO_2 emission per unit energy of fuel product. For LCEE and FER, the larger the better, while for GW the lower the better. These indicators are well described by Mata et al. (2011, 2013a), and take into account the main features of the system under study.

2.2 System boundary definition, process description and functional unit

For the sustainability evaluation all relevant life cycle stages have to be considered, from microalgae cultivation, harvesting, biomass processing (including dewatering, high pressure homogenization and three phase centrifugation for lipids extraction), microalgae oil transportation, biodiesel production, distribution and final use, as shown in Figure 1. Microalgae are cultivated in open ponds that are easier and cheaper to operate than closed photobioreactors, even though less efficient regarding biomass productivity (Mata et al., 2010). For microalgae harvesting and first dewatering, a disk stack centrifuge is used (Molina Grima et al., 2003), for which the biomass output has the total suspended solids required to perform the cells disruption successfully in a high pressure homogenizer. The mechanical disruption has the advantage of no chemicals use, this way preserving the cell components. For lipids extraction and separation, a three-phase centrifuge is used, and the biomass at the entrance is mixed with a fraction of the lipids obtained in the centrifuge to increase the overall separation efficiency, up to 98 %, following the suggestion of Benemann and Oswald (1996).

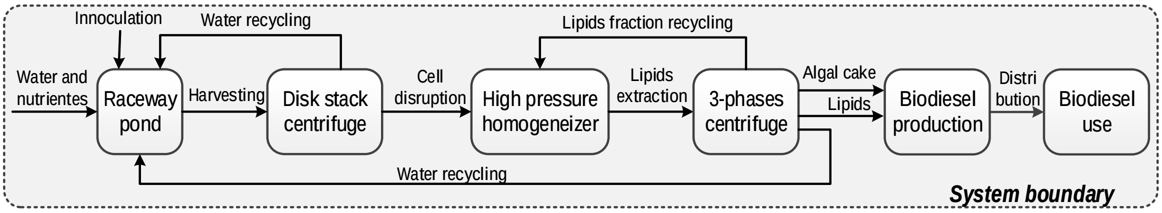


Figure 1: System boundary definition considering the life cycle stages of microalgae-based biodiesel

It is considered that the microalgae cultivation and biomass processing occurs at the microalgae producer site and the oil extracted is then transported to a dedicated external biofuel's plant at about 50 km distance from the microalgae producer. In the biofuel's plant, biodiesel is produced by transesterification of triglycerides in stoichiometric excess of methanol (Mata et al., 2012), homogenously catalyzed by a strong alkali-catalyst, in particular NaOH (González-Delgado and Kafarov, 2012; Caetano et al., 2013).
For the sustainability evaluation, the cultivation data were obtained experimentally by the authors of this study, and the data obtained includes information about the microalgae lipid content, biomass concentration, areal productivity and residence time, under mixotrophic and autotrophic conditions with and without salinity stress. The data concerning energy needs to power the process units (Molina Grima et al., 2003), fossil fuel needs for the microalgae oil transportation and respective GHG emissions (Mata et al., 2011, 2014a), were obtained from the literature and technical descriptions, assuming that energy consumption in the process is directly proportional to the quantity of microalgae biomass processed. The net GHG emissions from the process were calculated based on the energy consumption data, summing the GHG emitted in each process unit and subtracting the carbon captured during microalgae growth. The energy mix typical of Portugal was considered, corresponding to an emission of 0.094 kg of CO_2-equivalent per MJ of electricity used in the process. For estimating the carbon capture during microalgae growth it is considered that algal biomass can be described by the chemical formula $C_{106}H_{263}O_{110}N_{16}P$ (Mata et al., 2014a). Thus, as a consequence of microalgae photosynthesis, nitrate and phosphate are taken up together with carbon in the C/N/P mass proportion of about 106:16:1.
The functional unit considered for this study is 1 MJ of energy output, as the main function of biodiesel is to provide energy, allowing comparison of the sustainability of microalgae-based biodiesel with other biofuels and fossil diesel as necessary.

3. Results and discussion

3.1 Experimental study on microalgae cultivation

The cultivation step data were obtained experimentally. The measured values of the lipid content, biomass concentration, and areal productivity for a specific biomass residence time, are presented in Tables 1 and 2, for *A. platensis* and *C. vulgaris,* respectively, in two growth regimens (autotrophic and mixotrophic) and different salinity conditions. Under mixotrophy, the biomass productivity is generally higher than under autotrophy, and the maximum lipid accumulation is obtained at the highest NaCl concentration.
For *A. platensis* the lipid content varies between 7.1 and 15.4 % of biomass dry weight, and for *C. vulgaris* between 14.2 and 23.0 % of biomass dry weight. Results show that although the salinity stress induces the lipid accumulation in both microalgae, the areal productivity decreases.

3.2 Sustainability evaluation

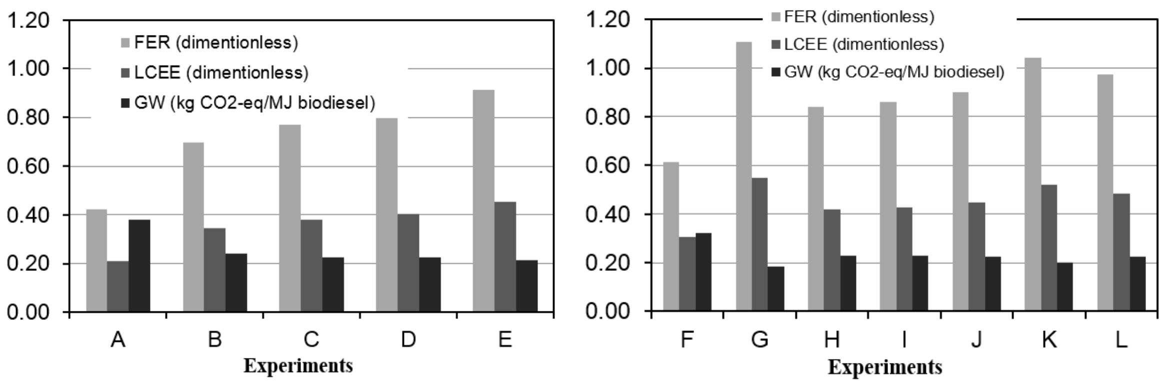
Many parameters can influence the sustainability of growing microalgae for biodiesel production, in particular: cultivation area, lipid content, biomass productivity, and efficiency of the process units. Preliminary calculations conducted by the authors of this study showed that sustainability indicators (LCEE, FER and GW) are strongly influenced by the microalgae lipid content (Mata et al., 2014a).This is because these indicators are measured in relation to the functional unit (1 MJ of energy generated) which depends on the total biomass produced and processed, assumed to be proportional to the cultivation area and energy consumption. Thus, for LCEE and FER the increase in energy consumption, due to an increase in biomass processed, is compensated by the increase in the energy obtained in the system, making both indicators basically independent of the cultivation area. A similar situation occurs for GW that is proportional to the energy consumed in the system. Figure 2 presents the values of the three sustainability indicators, calculated on the basis of the experimental results obtained for the different conditions (Tables 1 and 2).

Table 1: Lipid content, biomass concentration, areal productivity and residence time for A. platensis under mixotrophic and autotrophic conditions, with and without salinity stress

Experi-ments	Salinity stress	NaCl (M)	Glucose (g/L)	Growth regimen	Lipid content (%)	Biomass concentration (kg/m^3)	Areal productivity ($g/m^2/day$)	Biomass residence time (day)
A	No	0.017	0.00	Autotrophic	7.1	0.425	15.2	11.0
B	No	0.017	1.00	Mixotrophic	9.7	0.590	29.9	11.0
C	Yes	0.086	1.00	Mixotrophic	10.9	0.546	25.2	11.0
D	Yes	0.257	1.00	Mixotrophic	12.4	0.505	17.7	11.0
E	Yes	0.428	1.00	Mixotrophic	15.4	0.440	15.0	11.0

Table 2: Lipid content, biomass concentration, areal productivity and residence time for C. vulgaris under mixotrophic and autotrophic conditions, with and without salinity stress

Experi-ments	Salinity stress	NaCl (M)	Glucose (g/L)	Growth regimen	Lipid content (%)	Biomass concentration (kg/m^3)	Areal productivity ($g/m^2/day$)	Biomass residence time (days)
F	No	$0.43x10^{-3}$	0.00	Autotrophic	16.7	0.219	26.9	2.4
G	No	$0.43x10^{-3}$	1.00	Mixotrophic	16.6	0.492	68.2	2.2
H	Yes	$1.07x10^{-3}$	1.00	Mixotrophic	14.2	0.407	66.0	1.9
I	Yes	$2.14x10^{-3}$	1.00	Mixotrophic	15.5	0.373	63.6	1.7
J	Yes	$3.21x10^{-3}$	1.00	Mixotrophic	17.4	0.339	60.7	1.9
K	Yes	$4.28x10^{-3}$	1.00	Mixotrophic	18.5	0.386	59.4	1.9
L	Yes	$21.39x10^{-3}$	1.00	Mixotrophic	23.0	0.264	42.1	1.9



(a) **(b)**

Figure 2: Sustainability indicators LCEE, FER and GW for biodiesel production from microalgae: a) A. platensis and b) C. vulgaris.

Results show that microalgae lipid content and areal productivity significantly influence the sustainability of biodiesel, evaluated with LCEE, FER and GW indicators. For *C. vulgaris*, values of these indicators show that it is preferable to cultivate microalgae for biodiesel production in mixotrophic conditions without salinity stress, for which a lipid content of 16.6 % is obtained, followed by a moderate salinity stress inducing a lipid content of 18.5 %. For *A. platensis*, it is preferable to cultivate microalgae in mixotrophic conditions at the highest salinity stress, for which a lipid content of 15.4 % is obtained.

The values of LCEE and FER presented in Figure 2 are lower than one, except for *C. Vulgaris* at mixotrophic growth without salinity stress (experiment G) or with moderate salinity stress (experiment K). This shows that the production system is not energy efficient as it requires more energy than it generates as biofuel.

An interesting feature of the methodology used in this work for the sustainability evaluation is the possibility of identifying which life cycle stages are the most relevant, and where improvements can have bigger impact, in particular to improve the values of FER and LCEE. Figure 3 shows the relative contribution of each life cycle step to the total energy consumption as a function of microalgae lipid content (Tables 1 and 2). The life cycle steps considered include: cultivation in raceway ponds (step 1); saving from carbon capture in microalgae growth (step 2); biomass harvesting with the disc-stack centrifuge (step 3); cells disruption in the homogenizer and mixing tank (step 4); lipids separation in the 3-phase centrifuge (step 5); microalgae oil transportation (step 6); biodiesel production (step 7); and fuel use (step 8).

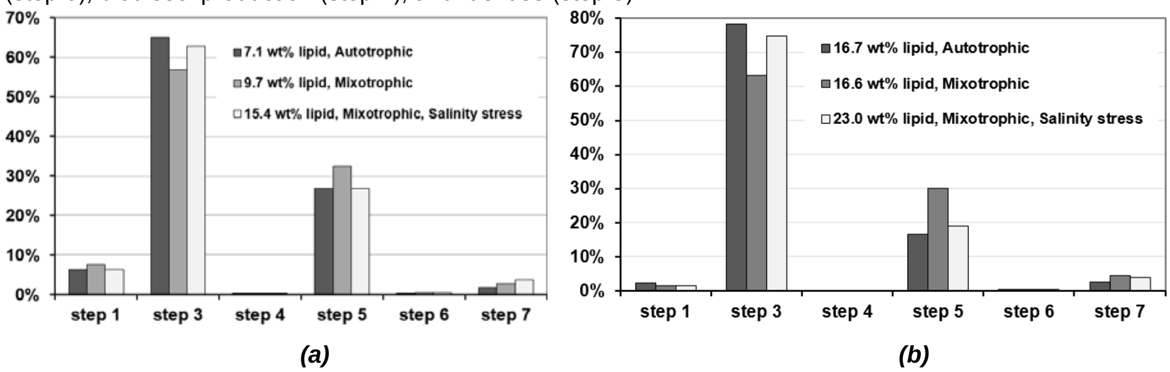


Figure 3: Relative contribution of each life cycle step to total energy consumption, considering three lipid contents under autotrophic and mixotrophic conditions with salinity stress for a) A. platensis and b) C. vulgaris.

It shows that biomass processing requires the largest energy inputs, especially for the first dewatering by disk stack centrifuge (step 3), followed by the 3-phase centrifugation for lipids, biomass and water separation (step 5). These are the dominant steps in terms of energy consumption regardless of the microalgae lipid content, leading to the conclusion that one should focus on improving the biomass processing steps, by for example, increasing the energy efficiency of the process equipment, or by using better technologies, or new production methods for biodiesel production requiring less biomass processing, among others. This is currently a very active field of research, and new developments are expected soon in this area.
Figure 4 presents the relative percentage of each life cycle step contributing to the net GHG emissions as a function of microalgae lipid content (Tables 1 and 2). These relative percentages were determined by dividing the GHG emissions of each life cycle step by the overall life cycle GHG emissions.

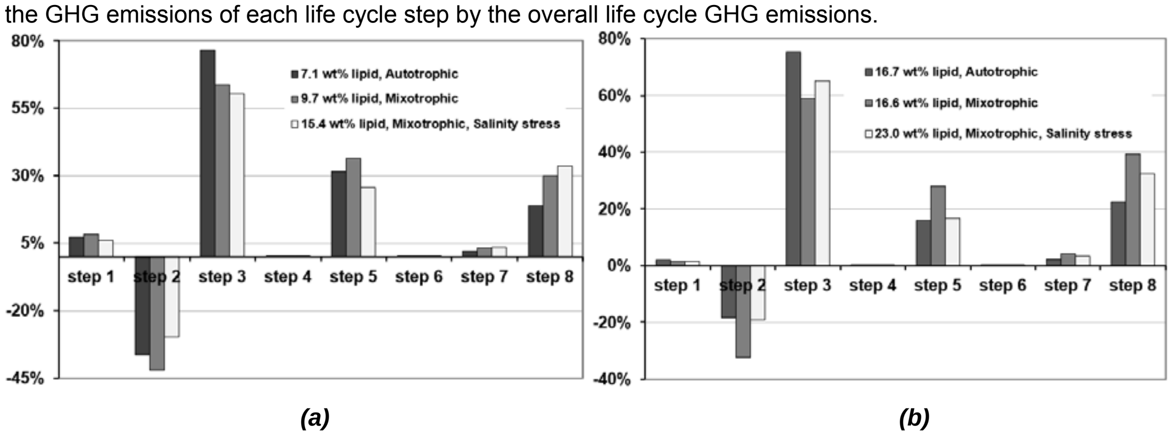


Figure 4: Relative contribution of each life cycle step to net greenhouse gas emissions of biodiesel production from microalgae, considering three lipid contents under autotrophic and mixotrophic conditions with salinity stress for a) A. platensis and b) C. vulgaris.

Results show that, when considering the overall life cycle, biomass harvesting (step 3) and lipids extraction (step 5) followed by biofuel use and combustion in diesel engines (step 8) are the most relevant steps in terms of GHG emissions. This is expected, since the biomass processing steps with higher relative energy consumption (Figure 3) are the ones with larger relative contribution to GHG emissions. The savings from carbon capture due to microalgae growth (step 2) are also relevant but this value is indicated as negative since it needs to be subtracted from the overall life cycle GHG emissions in order to determine the net GHG emissions and thus, the contribution to global warming. As expected, at higher lipid content the relative saving from carbon capture is smaller since the accumulation of lipids in microalgae is generally accompanied by a decrease in total cell and biomass productivity. The same way, the increase in the relative contribution of GHG during biomass harvesting, at higher lipid content (23 wt%), is due to the higher relative energy consumption (and thus higher emissions) to obtain the same quantity of biofuel, since although the lipid contend increases the biomass concentration and areal productivity decrease (as shown in Tables 1 and 2).

4. Conclusions

This work performed a sustainability evaluation of the biodiesel production from *A. platensis* and *C. vulgaris* in mixotrophic and autotrophic conditions, with and without salinity stress. It is concluded that, for the current process conditions, the production of biodiesel from these microalgae is not energy efficient, since the values of LCEE and FER are lower than one, except for *C. Vulgaris* at mixotrophic growth, without salinity stress. Microalgae lipid productivity is a key factor for increasing the sustainability of microalgae biodiesel but also, it is critical to have more energy efficient water removal and lipids separation. The GW values are always positive for the system under study, meaning that carbon captured during microalgae growth does not compensate the carbon emitted, mainly due to energy needs, making biodiesel not carbon neutral neither carbon negative. The use of renewable and/or carbon free energy sources in the process could significantly improve the sustainability of the life cycle of biodiesel production from microalgae.

Acknowledgments

Teresa Mata would like to thank Fundação para a Ciência e Tecnologia (FCT) for their support through provision of a research grant Ref. IF/01093/2014. This work was financially supported by: Project UID/EQU/00511/2013-LEPABE (Laboratory for Process Engineering, Environment, Biotechnology and Energy – EQU/00511) by FEDER funds through Programa Operacional Competitividade e Internacionalização – COMPETE2020 and by national funds through FCT.

References

Benemann J.R., Oswald W.J., 1996, Systems and economic analysis of microalgae ponds for conversion of CO_2 to biomass. Final Report DOE/PE/93204-T5, Department of Energy, USA, March 21.

Caetano N.S., Silva V.F.M., Melo A.C., Mata T.M., 2013, Potential of spent coffee grounds for biodiesel production and other applications, Chemical Engineering Transactions, 35, 1063 - 1068, DOI: 10.3303/CET1335177.

Friedl T., Rybalka N., Kryvenda A., 2012, Phylogeny and systematics of microalgae: An overview. In: Posten C., Walter C. (eds.) Microalgal biotechnology: potential and production, De Gruyter, Berlin, pp.11-38.

González-Delgado A.D., Kafarov V., 2012, Microalgae based biorefinery: evaluation of several routes for joint production of biodiesel, chlorophylls, phycobiliproteins, crude oil and reducing sugars, Chemical Engineering Transactions, 29, 607-612, DOI: 10.3303/CET1229102.

Martins A.A., Mata T.M., Sikdar S., Costa C.A.V., 2007, A framework for sustainability metrics. Ind. Eng. Chem. Res., 46, 2962-2973.

Mata T.M., Caetano N.S., Costa C.A.V., Sikdar S.K., Martins A.A., 2013a, Sustainability analysis of biofuels through the supply chain using indicators, Sustainable Energy Technologies and Assess., 3, 53-60.

Mata T.M., Martins A.A., Caetano N.S., 2010, Microalgae for biodiesel production and other applications, Renew. Sust. Energ. Rev., 14(1), 217-232.

Mata T.M., Martins A.A., Sikdar S., Costa C.A.V., 2011, Sustainability considerations of biodiesel based on supply chain analysis, Clean Technol. Envir. Policy, 13, 655-671.

Mata T.M., Melo A.C., Meireles S., Mendes A.M., Martins A.A., Caetano N.S., 2013b, Potential of microalgae *Scenedesmus obliquus* grown in brewery wastewater for biodiesel production, Chemical Engineering Transactions, 32, 901-906, DOI: 10.3303/CET1332151.

Mata T.M., Mendes A.M., Caetano N.S., Martins A.A., 2014a, Sustainability and economic evaluation of microalgae grown in brewery wastewater, Bioresour. Technol., 168, 151-158.

Mata T.M., Santos J., Mendes A.M., Caetano N.S., Martins A.A., 2014b, Sustainability evaluation of biodiesel produced from microalgae *Chlamydomonas* sp grown in brewery wastewater, Chemical Engineering Transactions, 37, 823-828, DOI: http://dx.doi.org/10.3303/CET1437138.

Mata T.M., Sousa I.R.B.G., Vieira S.S., Caetano N.S., 2012, Biodiesel production from corn oil via enzymatic catalysis with ethanol, Energy and Fuels, 26 (5), 3034-3041.

Molina Grima E., Belarbi E.-H., Acién Fernández F.G., Robles Medina A., Chisti Y., 2003, Recovery of microalgal biomass and metabolites: Process options and economics, Biotechnol. Adv., 20, 491-515.

Ribeiro L.A., da Silva P.P., Mata T.M., Martins A.A., 2015, Prospects of using microalgae for biofuels production: results of a Delphi study, Renewable Energy, 75, 799-804.

Richmond A., 2004, Handbook of microalgal culture: biotechnology and applied phycology, Blackwell Sci. Ltd.