

da Silva, A. Ferreira; Brazinha, C.; Costa, L.; Caetano, N. S.

## Article

# Techno-economic assessment of a Synechocystis based biorefinery through process optimization

Energy Reports

## Provided in Cooperation with:

Elsevier

*Suggested Citation:* da Silva, A. Ferreira; Brazinha, C.; Costa, L.; Caetano, N. S. (2020) : Techno-economic assessment of a Synechocystis based biorefinery through process optimization, Energy Reports, ISSN 2352-4847, Elsevier, Amsterdam, Vol. 6, Iss. 1, pp. 509-514,  
<https://doi.org/10.1016/j.egy.2019.09.016>

This Version is available at:

<https://hdl.handle.net/10419/243781>

### Standard-Nutzungsbedingungen:

Die Dokumente auf EconStor dürfen zu eigenen wissenschaftlichen Zwecken und zum Privatgebrauch gespeichert und kopiert werden.

Sie dürfen die Dokumente nicht für öffentliche oder kommerzielle Zwecke vervielfältigen, öffentlich ausstellen, öffentlich zugänglich machen, vertreiben oder anderweitig nutzen.

Sofern die Verfasser die Dokumente unter Open-Content-Lizenzen (insbesondere CC-Lizenzen) zur Verfügung gestellt haben sollten, gelten abweichend von diesen Nutzungsbedingungen die in der dort genannten Lizenz gewährten Nutzungsrechte.

### Terms of use:

*Documents in EconStor may be saved and copied for your personal and scholarly purposes.*

*You are not to copy documents for public or commercial purposes, to exhibit the documents publicly, to make them publicly available on the internet, or to distribute or otherwise use the documents in public.*

*If the documents have been made available under an Open Content Licence (especially Creative Commons Licences), you may exercise further usage rights as specified in the indicated licence.*



<https://creativecommons.org/licenses/by-nc-nd/4.0/>

6th International Conference on Energy and Environment Research, July 22–25, 2019,  
University of Aveiro, Portugal

# Techno-economic assessment of a *Synechocystis* based biorefinery through process optimization

A. Ferreira da Silva<sup>a,c,\*</sup>, C. Brazinha<sup>b</sup>, L. Costa<sup>c</sup>, N.S. Caetano<sup>a,d</sup>

<sup>a</sup> LEPABE, Faculty of Engineering, University of Porto, Portugal

<sup>b</sup> LAQV-REQUIMTE, Chemistry Department, FCT, Universidade Nova de Lisboa, Portugal

<sup>c</sup> A4F — Algae for Future, Lisbon, Portugal

<sup>d</sup> School of Engineering (ISEP), Polytechnic of Porto (P. Porto), Portugal

Received 29 July 2019; accepted 10 September 2019

Available online 1 October 2019

## Abstract

Similar to oil, biological origin feedstock can be converted into a number of products allowing to recover the maximum value from the raw biological material, within what is nowadays called a biorefinery. Among other biological materials, microalgae are a very interesting biomass due to the high economic value of certain cellular components, such as carotenoids and polyunsaturated fatty acids. In this project, a genetically modified cyanobacteria, that produces ethanol as an extracellular product, was used as the basis of this study. In order to assess the optimal configuration of the biorefinery, different scenarios were designed, each one with different sequences of unit operations, equipment and products. With the design stage completed, an economic analysis was performed to choose the 2 scenarios with the best economic performance.

© 2019 Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Peer-review under responsibility of the scientific committee of the 6th International Conference on Energy and Environment Research, ICEER, 2019.

**Keywords:** LCA; Microalgae biorefinery; Process optimization; Sustainability evaluation

## 1. Introduction

With the increase of the price of oil, the attention to find alternative, and more sustainable solutions for the production of biofuels and energy sources grew. This led to an increased interest in the study of microalgae since the fatty acids and carbohydrates contained in the cells could be used to produce biodiesel and bioethanol. In addition, the much smaller land area required to produce high amounts of biomass compared to conventional feedstocks, such as soy and sugar cane, made microalgae a very interesting and highly researched topic [1].

However, due to the high costs of harvesting and processing the biomass, the estimated cost of producing biodiesel, or bioethanol is very high [2].

\* Corresponding author at: LEPABE, Faculty of Engineering, University of Porto, Portugal.  
E-mail address: [andre.silva@algafuel.pt](mailto:andre.silva@algafuel.pt) (A. Ferreira da Silva).

Fortunately, some of the intracellular contents of microalgae, such as carotenoids, proteins and polyunsaturated fatty acids (PUFA), have a high market value [3]. This led to the development of the concept of microalgae biorefinery where, similar to a chemical refinery, not only would biodiesel and/or bioethanol be produced from/by microalgae, but also different products with high value could be obtained from the same biomass in order to compensate the previously mentioned high costs [4].

A typical microalgae biorefinery process can be described in five stages (Fig. 1):

- **Microalgae Production Stage** — Stage where the microalgae biomass is produced. This step is performed either in open reactors (such as open ponds, raceways, cascade raceways...) or in closed reactors (such as flat panel, tubular reactors...) [5];
- **Harvesting and Dewatering Stage** — This is done because usually the final concentration of biomass is around 0.1–10 g/L. The objective of this step is to increase the concentration 50 to 200-fold [6];
- **Disruption Stage** — In order to access the components found inside the microalgae, the microalgae cell wall must be ruptured. The method used, efficiency and time that it takes to achieve cell wall rupture can significantly differ, depending on the microalgae species [7];
- **Extraction Stage** — It is in this step that the different components of the microalgae are separated from each other. The most common methods are solvent extraction and supercritical extraction [8,9];
- **Conversion Stage** — Certain components can be converted into other products (polysaccharides into simpler sugars; proteins into amino acids). These conversions occur in this last step of the biorefinery [10,11].

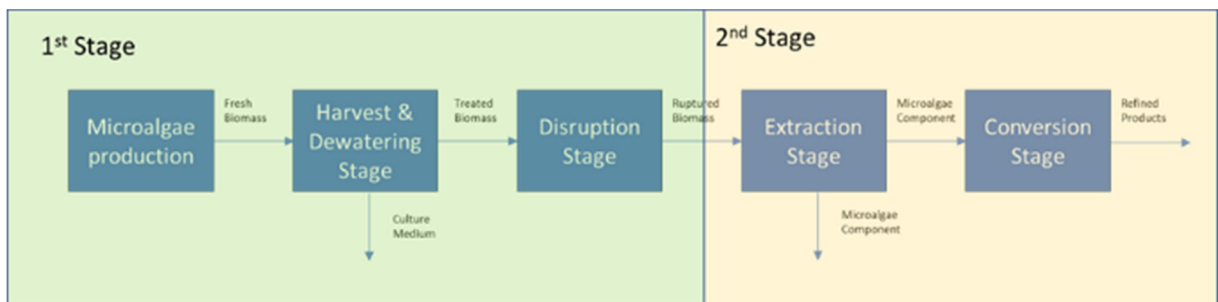


Fig. 1. Microalgae biorefinery stages.

The objective was to develop an optimal biorefinery process, using as a starting point and information source, results from a European Project, the DEMA project [12,13], where it was used a genetically modified *Synechocystis* strain that produces and excretes ethanol. In order to obtain the optimal process setup, different biorefinery scenarios were designed, each one with different equipment and final products. The economic analysis of each scenario was carried out and then, based on the economic parameters Return on Investment (*ROI*), payback time (*PBT*) and Net Present Value (*NPV*), the 2 best scenarios were selected.

## 2. Economic parameters

For the economic calculations, the annual discount rate used was 5% and the time period used was 8 years (these values were considered to be the minimum values for a rentable project [14].

The three indicators that will be calculated in order to assess the best economic performance are:

- The Return on Investment (*ROI*) — evaluates the efficiency of an investment. *ROI* directly quantifies the amount of return on a particular investment, relative to the investment's cost;
- The payback period (*PBP*) — is the length of time required to recover the initial investment. A desired payback time should be under 5 years;
- The Net present value (*NPV*) — is the difference between the present value of cash inflows and the present value of cash outflows over a certain period of time. A positive *NPV* indicates that the projected earnings generated by a project or investment exceeds the costs.

### 3. Results and discussion

Literature research and information from the DEMA project [12], found that besides ethanol, also phycocyanin, a water soluble protein, and the carotenoid zeaxanthin were interesting components [15,16]. The composition of the *Synechocystis* sp. and the most interesting components can be found in Table 1.

**Table 1.** *Synechocystis* sp. composition.

| Component            | mg/g biomass | % of total biomass |
|----------------------|--------------|--------------------|
| <b>Lipids</b> [17]   | 102.3        | 10.2%              |
| <b>Proteins</b> [18] | 675          | 67.5%              |
| Insoluble            | 314          | 31.4%              |
| Soluble              | 246          | 24.6%              |
| Phycocyanin [15]     | 115          | 11.5%              |
| <b>Zeaxanthin</b>    | 9.1          | 0.9%               |
| <b>Carbohydrates</b> | 213.6        | 21.4%              |
| <b>Total</b>         | 1000         | 100.0%             |

The biomass production stage was considered to be performed in 36 Unilayer Horizontal Tubular Photobioreactors (UHT-PBR), each with the total volume of 97 m<sup>3</sup>. With the production time of 350 days, the amount of *Synechocystis* sp. biomass produced in a year was considered as 180.5 t and the amount of ethanol produced considered was 421.7 m<sup>3</sup>.

For the Harvesting and Rupture stage, only available equipment, already used in microalgae or similar industries, was selected. The chosen equipment can be found in Table 2.

**Table 2.** Harvesting and cell rupture equipment.

| Harvesting equipment    | Efficiency | Max concentration | Ref.  | Cell disruption equipment       | Efficiency | Max concentration | Ref.  |
|-------------------------|------------|-------------------|---|---------------------------------|------------|-------------------|---|
| Membrane                | 95%        | 100 g/l           | Bhave et al. [19], Bilad et al. [20] and Drexler and Yeh [21] | Bead mill                       | 99%        | 200 g/l           | Postma et al. [22,23]                             |
| Centrifuge              | 90%        | 200 g/l           | Gerardo et al. [24] and Monte et al. [25]                     | High Pressure Homogenizer (HPH) | 96%        | 200 g/l           | Patrignani and Lanciotti [26] and Yap et al. [27] |
| Dissolved air flotation | 90%        | 60 g/l            | Al Hattab et al. [28] and Kwon et al. [29]                    | Ultrasonication                 | 100%       | 100 g/l           | A4F [14] and Kurokawa et al. [30]                 |
| Flocculation            | 85%        | 50 g/l            | Branyikova et al. [31] and Martínez [32]                      |                                 |            |                   |   |

The second part was to design the downstream section. As was mentioned before, this *Synechocystis* sp. strain produces ethanol that is excreted into the culture medium. In order to recover the excreted ethanol from the medium, two methods were selected: distillation and pervaporation. However, since distillation columns cannot achieve 99.5% of ethanol purity due to the azeotrope with water, it has to be coupled to the pervaporation membrane, so in fact two options were considered: either only pervaporation, or distillation followed by pervaporation.

In order to obtain the phycocyanin, the stream containing the ruptured biomass that enters the process goes through a diafiltration process with water, as phycocyanin is a water-soluble protein. The water stream containing the phycocyanin is then further treated using chitosan in order to obtain a purer stream of phycocyanin for higher value applications in the pharmaceutical or food industries [33,34] — Scenario 1. The remaining biomass will contain the lipids, non-water-soluble proteins and carbohydrates, and can be further treated, by hydrolysing the proteins present in the remaining biomass by enzymatic conversion producing a protein hydrolysate with bio-fertilizer potential [35] — Scenario 2. However, to remove the zeaxanthin, the biomass can previously be treated using one of two processes: One option would be to use supercritical solvent extraction with scCO<sub>2</sub> + 5% ethanol as co-solvent to

remove the zeaxanthin, and a small amount of lipids [36] — Scenario 3. The zeaxanthin can then be recovered by an extra purification step, with several saponification and separation processes, producing a metal soap and a stream of almost pure zeaxanthin — Scenario 4. The remaining biomass, without the zeaxanthin and polar lipids, can be further treated, by hydrolysing the proteins present in the remaining biomass by enzymatic conversion and producing a protein hydrolysate with bio-fertilizer potential — Scenario 5. The second extraction option would be to perform a conventional solvent extraction, using a polar solvent, ethanol, to remove zeaxanthin and the polar lipids [37] — Scenario 6. Once again, zeaxanthin can then be recovered by the extra purification step previously mentioned — Scenario 7. The remaining biomass, can again be further treated, by hydrolysing the proteins present in the remaining biomass through enzymatic conversion to produce a protein hydrolysate with bio-fertilizer potential — Scenario 8.

After the design of the different scenarios, the Capex, Opex and Revenue were calculated by combining different arrangements of the production, harvesting and rupture methods with one of the downstream processes. Using information from the DEMA project as well as some information from suppliers, the values for the capital costs and operational costs were obtained for the equipment used in the production, harvesting and rupture stage. Unlike the harvesting equipment, the rupture equipment capacity depends on the type of harvesting equipment selected, due to the final concentration. Therefore, the rupture equipment economic values are calculated for all the possible concentrations. The ultrasonication system has only one economic value since it can handle a large range of capacities (from 50 to 100 g/l). These values are shown in Table 3. Since the capacity of the downstream equipment depends on the final concentration of the ruptured biomass, the Capex values have to be calculated for all different concentrations. For each downstream scenario the Capex and Opex were calculated from 50 g/l to 200 g/l. these values can be found in Table 4.

**Table 3.** Capex and Opex for the equipment possibly used for the production, harvesting and rupture stages.

|                       |  | Capex               | Opex              |
|-----------------------|--|---------------------|-------------------|
| Production system     |  | € 13,838,941        | € 1,966,384       |
| Harvesting stage      | Membrane system                            | € 600,000           | € 139,248         |
|                       | Centrifuge system                          | € 750,000           | € 132,071         |
|                       | Flotation system                           | € 27,000            | € 91,103          |
|                       | DAF system                                 | € 60,000            | € 61,227          |
| Cell disruption stage | Ball mill (200 g/l–50 g/l)                 | € 93,253–€ 214,240  | € 14,520–€ 46,967 |
|                       | HPH (200 g/l–50 g/l)                       | € 104,583–€ 240,268 | € 17,661–€ 56,249 |
|                       | Ultrasonication                            | € 62,073            | € 38,447          |
| Ethanol recovery      | Pervaporation system                       | € 355,403           | € 172,206         |
|                       | Pervaporation system + Distillation column | € 271,207           | € 279,485         |

**Table 4.** Economic values for the *Synechocystis* ruptured biomass downstream scenarios (from 50 to 200 g/l) (for 20 kg ruptured biomass/h).

| Biomass Conc. | 50 g/l     | 200 g/l   | 50 g/l    | 200 g/l   | –            |
|---------------|------------|-----------|-----------|-----------|--------------|
| Scenario      | Capex (€ ) |           | Opex (€ ) |           | Revenue (€ ) |
| 1             | 640,719    | 299,749   | 1,319,932 | 1,240,459 | 3,672,362    |
| 2             | 661,515    | 320,545   | 1,948,289 | 1,868,815 | 8,235,125    |
| 3             | 1,625,397  | 1,284,426 | 1,805,144 | 1,697,994 | 4,146,715    |
| 4             | 1,646,193  | 1,305,223 | 2,351,329 | 2,244,180 | 8,660,819    |
| 5             | 1,070,170  | 989,282   | 1,804,126 | 1,754,910 | 3,842,191    |
| 6             | 1,090,679  | 1,009,791 | 2,340,808 | 2,291,593 | 8,080,093    |
| 7             | 1,779,309  | 1,698,421 | 1,976,664 | 1,927,448 | 4,176,821    |
| 8             | 1,799,818  | 1,718,930 | 2,513,347 | 2,464,131 | 8,414,724    |

As the downstream equipment values and the operation costs depend on both the final concentration of ruptured biomass and the harvesting efficiency, the downstream scenario economic values were calculated for different concentrations and a ruptured biomass stream of 20 kg/h (Table 4).

Afterwards, the economic values were used to calculate the economic parameters *ROI*, *PBT* and *NPV* and the 2 scenarios with the best economic performance were selected (Table 5).

**Table 5.** Data for the 2 chosen scenarios for *Synechocystis* sp. biorefinery.

| Combination | Harvesting step     | Rupture step    | Ethanol recovery step | Downstream scenario | NPV         | ROI% | PBT years |
|-------------|---------------------|-----------------|-----------------------|---------------------|-------------|------|-----------|
| 1           | Membrane filtration | Ultrasonication | Pervaporation         | 2                   | € 6,314,621 | 37   | 5         |
| 2           | Membrane filtration | Ball mill       | Pervaporation         | 2                   | € 5,839,274 | 34   | 5         |

As shown in Table 5, both conformations use a membrane to harvest the biomass. This is expected, as this is the harvesting method with the highest efficiency, which means more biomass is harvested for further treatment. In addition, the membrane is one of the harvesting systems yielding the highest maximum final concentration, which leads to smaller and cheaper downstream equipment. Another conclusion is that the conventional solvent extraction was favoured over the supercritical extraction. Concerning the ethanol extraction, the conclusion is that the pervaporation is preferred over the combination distillation plus pervaporation membrane.

#### 4. Conclusion

It is possible to conclude that the biorefinery approach has a positive impact on the production of products from microalgae since all the chosen scenarios were found to be economically viable and payed for after 5 years. The main reason behind this result is that biorefineries can produce a higher number of different high values products bringing in higher profit. However, it was shown that the scenario 1 that does not produce all the possible products possible has better economic results than the second scenario. This shows, that although biorefineries can have a positive impact on the exploitation of microalgae biomass, some purification steps are not worth it and can cost more than the profit obtained from those purifications. Moreover, all scenarios are using membrane filtration as a harvesting method, which is comprehensible since it is the method with the highest harvesting efficiency. Another conclusion is that the conventional solvent extraction was favoured over the supercritical extraction, mostly due to the high cost of the supercritical extraction process equipment, but also due to the operation costs. Similarly, the scenarios with pervaporation operation are the ones with the highest NPV as the operation costs, especially the energy costs, are lower than the combinations of distillation with pervaporation. The same analysis also demonstrated that the ethanol production is not economically viable as the revenue from the ethanol is only ca. 140,000 € and the operation costs are higher, and therefore, alternatives/improvements have to be researched.

#### Acknowledgements

Project UID/EQU/00511/2019 – Laboratory for Process Engineering, Environment, Biotechnology and Energy – LEPABE funded by national funds through FCT/MCTES (PIDDAC), Portugal; project “LEPABE-2-ECO-INNOVATION”-NORTE-01-0145-FEDER-000005, funded by Norte Portugal Regional Operational Programme (NORTE 2020), under PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF).

#### References

- [1] Hannon M, Gimpel J, Tran M, Rasala B, Mayfield S. Biofuels from algae: challenges and potential. *Biofuels* 2010;1:763–84.
- [2] Harun R, Davidson M, Doyle M, Gopiraj R, Danquah M, Forde G. Technoeconomic analysis of an integrated microalgae photobioreactor, biodiesel and biogas production facility. *Biomass Bioenergy* 2011;35:741–7. <http://dx.doi.org/10.1016/j.biombioe.2010.10.007>.
- [3] Markets and Markets, Carotenoids Market worth 1.53 Billion USD by 2021 [WWW Document]. URL <https://www.markets{and}markets.com/PressReleases/carotenoid.asp> (accessed 3.29.17), 2016.
- [4] Mata TM, Martins AA, Caetano NS. Microalgae for biodiesel production and other applications: A review. *Renew Sustain Energy Rev* 2010;14:217–32. <http://dx.doi.org/10.1016/j.rser.2009.07.020>.
- [5] Carvalho A. P., Malcata F. X., Meireles A. Microalgal reactors : A review of enclosed system designs and performances. *Biotechnol Prog* 2006;22:1490–506.
- [6] Molina Grima E, Belarbi E-H, Ación Fernández F, Robles Medina A, Chisti Y. Recovery of microalgal biomass and metabolites: process options and economics. *Biotechnol Adv* 2003;20:491–515. [http://dx.doi.org/10.1016/S0734-9750\(02\)00050-2](http://dx.doi.org/10.1016/S0734-9750(02)00050-2).
- [7] Safi C, Ursu AV, Laroche C, Zebib B, Merah O, Pontalier PY, Vaca-Garcia C. Aqueous extraction of proteins from microalgae: Effect of different cell disruption methods. *Algal Res* 2014. <http://dx.doi.org/10.1016/j.algal.2013.12.004>.
- [8] Halim R, Danquah MK, Webley PA. Extraction of oil from microalgae for biodiesel production: A review. *Biotechnol Adv* 2012;30:709–32. <http://dx.doi.org/10.1016/J.BIOTECHADV.2012.01.001>.
- [9] Kim S-K, Chojnacka K. Marine Algae Extracts: Processes, Products, and Applications, 2 Volume Set, Volume 1, 2015.



- [10] Bleakley S, Hayes M. Algal proteins: Extraction, application, and challenges concerning production. *Foods* 2017;6(33). <http://dx.doi.org/10.3390/foods6050033>.
- [11] Chen CY, Zhao XQ, Yen HW, Ho SH, Cheng CL, Lee DJ, Bai FW, Chang JS. Microalgae-based carbohydrates for biofuel production. *Biochem Eng J* 2013;78:1–10. <http://dx.doi.org/10.1016/j.bej.2013.03.006>.
- [12] Lopes TF, Cabanas C, Silva A, Fonseca D, Santos E, Guerra LT, Sheahan C, Reis A, Gírio F. Process simulation and techno-economic assessment for direct production of advanced bioethanol using a genetically modified *synechocystis* sp. *Bioresour Technol Rep* 2019;6:113–22. <http://dx.doi.org/10.1016/j.biteb.2019.02.010>.
- [13] University of Limerick, Final Report Summary - DEMA (Direct Ethanol from MicroAlgae), 2018.
- [14] A4F, 2018. Company Expertise.
- [15] Deshmukh DV, Puranik PR. Statistical evaluation of nutritional components impacting phycocyanin production *synechocystis* SP. *Braz J Microbiol* 2012;43:348–55. <http://dx.doi.org/10.1590/S1517-838220120001000041>.
- [16] Lagarde D, Beuf L, Vermaas W. Increased production of zeaxanthin and other pigments by application of genetic engineering techniques to *synechocystis* sp. strain PCC 6803. *Appl Environ Microbiol* 2000;66:64–72.
- [17] Sheng J, Vannela R, Rittmann BE. Evaluation of methods to extract and quantify lipids from *Synechocystis* PCC 6803. *Bioresour Technol* 2011;102:1697–703. <http://dx.doi.org/10.1016/J.BIORTECH.2010.08.007>.
- [18] Touloupakis E, Cicchi B, Benavides AMS, Torzillo G. Effect of high pH on growth of *Synechocystis* sp. PCC 6803 cultures and their contamination by golden algae (*Poteroiochromonas* sp.). *Appl Microbiol Biotechnol* 2016;100:1333–41. <http://dx.doi.org/10.1007/s00253-015-7024-0>.
- [19] Bhavre R, Kuritz T, Powell L, Adcock D. Membrane-based energy efficient dewatering of microalgae in biofuels production and recovery of value added co-products. *Environ Sci Technol* 2012;46:5599–606. <http://dx.doi.org/10.1021/es204107d>.
- [20] Bilad MR, Arafat HA, Vankelecom IFJ. Membrane technology in microalgae cultivation and harvesting: A review. *Biotechnol Adv* 2014;32:1283–300. <http://dx.doi.org/10.1016/j.biotechadv.2014.07.008>.
- [21] Drexler ILC, Yeh DH. Membrane applications for microalgae cultivation and harvesting: a review. *Rev Environ Sci Bio/Technol* 2014;13:487–504. <http://dx.doi.org/10.1007/s11157-014-9350-6>.
- [22] Postma PR, Suarez-Garcia E, Safi C, Olivieri G, Olivieri G, Wijffels RH, Wijffels RH. Energy efficient bead milling of microalgae: Effect of bead size on disintegration and release of proteins and carbohydrates. *Bioresour Technol* 2017;224:670–9. <http://dx.doi.org/10.1016/j.biortech.2016.11.071>.
- [23] Postma PR, Miron TL, Olivieri G, Barbosa MJ, Wijffels RH, Eppink MHM. Mild disintegration of the green microalgae *Chlorella vulgaris* using bead milling. *Bioresour Technol* 2015;184:297–304. <http://dx.doi.org/10.1016/j.biortech.2014.09.033>.
- [24] Gerardo ML, Van Den Hende S, Vervaeren H, Coward T, Skill SC. Harvesting of microalgae within a biorefinery approach: A review of the developments and case studies from pilot-plants. *Algal Res* 2015;11:248–62. <http://dx.doi.org/10.1016/j.algal.2015.06.019>.
- [25] Monte J, Sá M, Galinha CF, Costa L, Hoekstra H, Brazinha C, Crespo JG. Harvesting of *Dunaliella salina* by membrane filtration at pilot scale. *Sep Purif Technol* 2018;190:252–60. <http://dx.doi.org/10.1016/J.SEPPUR.2017.08.019>.
- [26] Patrignani F, Lanciotti R. Applications of High and Ultra High Pressure Homogenization for Food Safety 2016;7:1–13, <http://dx.doi.org/10.3389/fmicb.2016.01132>.
- [27] Yap BHI, Dumsday GJ, Scales PJ, Martin GJO. Energy evaluation of algal cell disruption by high pressure homogenisation. *Bioresour Technol* 2015;184:280–5. <http://dx.doi.org/10.1016/j.biortech.2014.11.049>.
- [28] Al Hattab M, Ghaly A, Hammoud A. Microalgae harvesting methods for industrial production of biodiesel: Critical review and comparative analysis. *J Fundam Renew Energy Appl* 2015;5. 1000154. <http://dx.doi.org/10.4172/20904541.1000154>.
- [29] Kwon H, Lu M, Lee EY, Lee J. Harvesting of microalgae using flocculation combined with dissolved air flotation. *Biotechnol Bioprocess Eng* 2014;19:143–9. <http://dx.doi.org/10.1007/s12257-013-0433-y>.
- [30] Kurokawa M, King PM, Wu X, Joyce EM, Mason TJ, Yamamoto K. Effect of sonication frequency on the disruption of algae. *Ultrason Sonochem* 2016;31:157–62. <http://dx.doi.org/10.1016/j.ultsonch.2015.12.011>.
- [31] Branyikova I, Prochazkova G, Potocar T, Jezkova Z, Branyik T. Harvesting of microalgae by flocculation. *Fermentation* 2018;4(93). <http://dx.doi.org/10.3390/fermentation4040093>.
- [32] Martínez RG. Microalgae harvesting in wastewater treatment plants : application of natural techniques for an efficient flocculation 194, 2016.
- [33] Kumar D, Dhar DW, Pabbi S, Kumar N, Walia S. Extraction and purification of C-phycocyanin from *Spirulina platensis* (CCC540). *Indian J Plant Physiol* 2014;19:184–8. <http://dx.doi.org/10.1007/s40502-014-0094-7>.
- [34] Wang WJ, Zhang XL, Xu CB, Cheng HY. Purification and concentration of c-phycocyanin from *spirulina platensis* using aqueous two-phase system. *Appl Mech Mech Eng Ii* 2012;Pts 1 2:138–9. <http://dx.doi.org/10.4028/www.scientific.net/AMM.138-139.995>, 995–1001.
- [35] Romero García JM, Acién Fernández FG, Fernández Sevilla JM. Development of a process for the production of l-amino-acids concentrates from microalgae by enzymatic hydrolysis. *Bioresour Technol* 2012;112:164–70. <http://dx.doi.org/10.1016/j.biortech.2012.02.094>.
- [36] Terme N, Boulho R, Kendel M, Kucma J-P, Gaetane W, Bourgougnon N, Bedoux G. Selective extraction of lipid classes from *Solieria chordalis* and *sargassum muticum* using supercritical carbon dioxide and conventional solid–liquid methods. *J Appl Phycol* 2017. <http://dx.doi.org/10.1007/s10811-017-1084-8>.
- [37] Koo SY, Cha KH, Song D-G, Chung D, Pan C-H. Optimization of pressurized liquid extraction of zeaxanthin from *Chlorella ellipsoidea*. *J Appl Phycol* 2012;24:725–30. <http://dx.doi.org/10.1007/s10811-011-9691-2>.