

Caetano, N. S. et al.

Article

Flocculation of *Arthrospira maxima* for improved harvesting

Energy Reports

Provided in Cooperation with:

Elsevier

Suggested Citation: Caetano, N. S. et al. (2020) : Flocculation of *Arthrospira maxima* for improved harvesting, Energy Reports, ISSN 2352-4847, Elsevier, Amsterdam, Vol. 6, Iss. 1, pp. 423-428,
<https://doi.org/10.1016/j.egyr.2019.08.083>

This Version is available at:

<https://hdl.handle.net/10419/243767>

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/>

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

Flocculation of *Arthrospira maxima* for improved harvesting

N.S. Caetano^{a,b,*}, A.A. Martins^a, M. Gorgich^{a,b}, D.M. Gutiérrez^b, T.J. Ribeiro^b, T.M. Mata^a

^a LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

^b CIETI, School of Engineering (ISEP), Polytechnic of Porto (P.Porto), R. Dr. António Bernardino de Almeida 431, 4249-015 Porto, Portugal

Received 31 July 2019; accepted 30 August 2019

Available online 12 September 2019

Abstract

The environmental impacts associated with the burning of fossil fuels coupled with growing concerns about security of energy supply, motivated the search for more sustainable forms of energy production, among which came microalgae for biofuels production. However, the commercial production of microalgae biofuels is still not competitive compared to fossil fuels, as it is necessary to solve some process bottlenecks, among which biomass harvesting, that is the focus of this work. Hence, this work intends to study the harvesting of microalga *Arthrospira maxima* through flocculation by pH variation and/or addition of CaCl₂ as flocculant. Thus, it is described the effect of pH variation (in the range 6 to 12), followed by the addition of flocculant, on the harvesting efficiency. Results show that by pH increase over 10 using NaOH, or by flocculation using CaCl₂ at a concentration of 0.2-2.0 g/L and at a 1:30 ratio (v/v) of CaCl₂/microalgae culture, it is possible to effectively harvest this microalga.

© 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: *Arthrospira maxima*; Coagulation; Flocculation; Microalgae harvesting

1. Introduction

Biofuels are a renewable energy source that can be used as an alternative to fossil fuels, with environmental advantages, such as lower CO₂ emissions and lower impacts on resources depletion and greenhouse gas emission [1]. However, first generation biofuels are produced from traditional food or feed crops, raising serious social and environmental issues [2]. Thus, the search for alternative potential feedstocks for biofuels production, has driven researchers' attention to unicellular microorganisms such as microalgae [3]. Microalgae cultivation does not need large footprint area, nor arable land, when compared to traditional food crops. Also, microalgae show high growth rate and contain higher lipid content than the traditional oil seeds [4].

* Corresponding author at: LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal.
E-mail address: nsc@isep.ipp.pt (N.S. Caetano).

<https://doi.org/10.1016/j.egy.2019.08.083>

2352-4847/© 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.

Among microalgae, this study chose *Arthrospira maxima* (*A. maxima*), a free-floating filamentous cyanobacteria characterized by cylindrical, multicellular trichomes in an open left-hand helix. These photosynthetic organisms, also considered as blue-green microalgae, occur naturally in tropical and subtropical lakes with alkaline pH and high concentrations of carbonate and bicarbonate [5]. Because *Arthrospira* is very rich in proteins (53 to 68% by dry weight) it is widely used as a food supplement for humans and animals. In addition, it contains high amounts of polyunsaturated fatty acids (PUFAs), about 1.5–2 percent of the total lipid content of 5–7% [6].

Production of microalgae biomass includes microalgae cultivation on environmental conditions that favor accumulation of target metabolites and recovery of the microalgal biomass for downstream processing [7]. The large scale harvesting of microalgal biomass from the culture medium still poses challenges, due to their reduced cells size (2–50 μm), the negative surface charge (of about $-7.5 \sim -40$ mV) and the relatively low biomass concentration (0.5–5 g L^{-1}), representing more than 30% of the total production cost of microalgae biodiesel [8].

The main factors for efficient harvesting and for making profitable the production of microalgae biofuels are the reduction of energy consumption [9] and the possibility of nutrients recycling and the reuse of the culture medium [10]. Therefore, it is required the development of cost-effective and more sustainable technologies for microalgae harvesting [11]. In this regard, flocculation can be a method with lower energy consumption when compared to filtration or centrifugation that makes it more feasible economically [12]. In addition, flocculation allows the harvesting of large amounts of biomass without disruption of the cells, thus becoming a more reliable and high-yielding process. A way to induce flocculation without the use of a flocculating agent is through pH variation in the microalgae cultures [13,14], where flocculation at high pH is normally due to precipitation of CaCO_3 , calcium phosphate and $\text{Mg}(\text{OH})_2$ [15,16]. However, depending on algal species being cultured the pH threshold for flocculation vary [17].

A wide variety of flocculants, such as organic cationic polymers or inorganic metal ions, have been exploited for algae harvesting [18–20]. However, the flocculants efficiency mainly depends on the microalgae characteristics, such as cell wall composition, cells size, culture age and growth medium composition and pH [21,22]. The large-scale harvesting using flocculants depends on the microalgae species, the amount of flocculant required and the pH at which the flocculant will have the desired efficiency [23]. For example, Bracharz et al. [24] employed auto-flocculation and biological flocculants to harvest *S. obtusiusculus* from a cultivation media containing high salt concentrations, studying flocculation induced by pH-shift and by addition of two biological flocculants, chitosan and the commercial tannin CFL-PT. These authors concluded that in comparison to biological flocculants, induction by pH shift is cheaper, but due to buffering effects of the brackish cultivation medium infeasible amounts of base are required to raise the pH-value. On the other hand, tannin seems to be superior compared to chitosan. Pandey et al. [25] performed an experimental study to evaluate the comparative efficiency of a bio-flocculant (waste egg shell), calcium carbonate and $\text{Al}_2(\text{SO}_4)_3$ for harvesting *Chlorella pyrenoidosa* and studied the influence of pH on zeta potential to explain the chemistry of flocculation process. Chen et al. [26] used ammonia as microalgae flocculant of both freshwater (*Chlorella sorokiniana*) and marine algae (*Nannochloropsis oculata*), with the advantage of the possibility to reuse the ammonia added as fertilizer in the subsequent cultures, and showing more than 99% algae removal at 12 h with this approach. Augustine et al. [23] evaluated the flocculation processes using chemo-magnetic nanoparticle (Che-MNP) and chitosan magnetic nanoparticle (CS-MNP) composites, showing that these allow efficient harvesting, being possible to reuse the flocculant (MNP) and flocculated medium without affecting the integrity of the algae cells.

Wang et al. [27] compared centrifugation of microalgae *Scenedesmus obliquus*, as a standard harvesting method, with chemical flocculation, as a cost-effective harvesting method. Results showed that lipid recovery from the centrifuged cells was 17.4%, which significantly increased by flocculation to 20.7%. Both harvesting methods presented similar thermal decomposition patterns, but the flocculated biomass showed a 15.7% higher bio-char formation than the centrifuged cells, which resulted in significant reduction in the bio-oil yield by 18.5%.

Vandamme et al. [28] studied the effect of the harvesting method on the lipid content and the lipid extraction efficiency of *Phaeodactylum tricorutum* biomass, comparing alum or alkaline flocculation, and centrifugation, showing that alkaline flocculation can be an excellent primary harvesting method for this microalga without impacting its lipid extraction efficiency. Therefore, this research aims to compare and evaluate the effectiveness of auto-flocculation and flocculation approaches applied to *Arthrospira maxima* harvesting, induced by pH variation and by the addition of CaCl_2 as flocculant.

2. Materials and methods

Arthrospira maxima SAG 84.79 from the German SAG (Sammlung von Algenkulturen Göttingen) collection was used in this work, following different cultivation scales, from 20 mL tubes to 60 L tanks. Among the several chemicals referred in literature, as potential coagulation/ flocculation agents, CaCl_2 was used and tested in this study at different concentrations.

The effect of pH variation on coagulation/flocculation of *Arthrospira maxima* was analyzed, for which the purpose of pH increase and decrease was achieved respectively by adding NaOH or HCl. Cultures with an initial pH of 10 were poured into 250 ml beakers, and the pH was adjusted to 9, 8, 7 and 6 in the culture medium. Samples were taken at 15 min intervals and absorbance was measured at 680 nm, for a maximum 4 h of settling time.

The biomass removal efficiency was calculated for each sample. Similar procedure was followed by adding NaOH to increase the culture medium pH to 11 and 12.

The effectiveness of CaCl_2 as flocculant on coagulation and flocculation was evaluated: after adding the chemical compound, the microalgae culture was stirred for 1 min at 75 rpm followed by 15 min stirring at 25 rpm, and then settling, in a jar test apparatus. Aliquots of the clarified liquid were taken for absorbance measurement (at 680 nm) at different time intervals until 4 h of settling time.

3. Results and discussion

The culture medium pH was increased from 10 to 11 and to 12 using NaOH, resulting in coagulation after 15 min. The coagulated cells remained suspended in the growth medium (as shown in Fig. 1).

Furthermore, by adding CaCl_2 as flocculant, testing concentrations from 0.2 to 2.0 g/L of 10 ml solution in 300 ml of microalgae culture, the flocculation occurred after 15 min. Similarly, the microalgae flocculated in the bottom of the recipient.

Therefore, results showed that *Arthrospira maxima* can be effectively harvested by increasing the pH over 10 with NaOH or by adding CaCl_2 as flocculant with a concentration of about 0.2–0.3 g/L or a 1:30 volume ratio of CaCl_2 /microalgae culture, respectively. These chemicals are easy to use, not expensive, and do not pose any substantial contamination risks for the recovered microalgae biomass for further processing. Other advantages include the operational simplicity and the possibility of the culture medium to be reuse and the nutrients recycled.

Literature studies on flocculation with pH variation reported similar results. For example, Sukenik and Shelef [15] reported algae auto-flocculation in outdoor autotrophic cultures of *Scenedesmus dimorphus* when culture pH was raised due to photosynthesis. Kim et al. [29] induced auto-flocculation of *Arthrospira platensis* at an optimal pH level of 9 with a flocculation efficiency of almost 90%, showing that this approach is definitely useful to harvest these microalgae in large-scale culture without any drawbacks of harvested algal biomass. Liu et al. [30] reported that metal ions, such as Mg^{2+} and Ca^{2+} , in the growth medium are hydrolyzed to form positive precipitates, which coagulate negative microalgae cells by sweeping flocculation and charge neutralization. In addition, flocculation efficiency is induced by pH increase, but it considerably decreases with increase of biomass concentrations. Vandamme et al. [31] demonstrated that *Chlorella vulgaris* flocculation can be induced by increasing medium pH to 11 and that magnesium is essential to induce flocculation. Yahi et al. [32] reported that extensive algae flocculation can be induced at pH values between 11.8 and 12 without need of adding magnesium.

Wu et al. [33] showed that by increasing the medium pH, a flocculation efficiency of up to 90% was induced for freshwater microalgae (*Chlorella vulgaris*, *Scenedesmus sp.*, *Chlorococcum sp.*) with low/medium biomass concentrations and marine microalgae (*Nannochloropsis oculata*, *Phaeodactylum tricorutum*).

Liu et al. [34] developed a flocculation method to harvest target microalgae with self-flocculating microalgae induced by decreasing pH to just below isoelectric point, showing a higher efficiency of this approach when compared to flocculation only via pH decrease. With this method the culture medium could be recycled for cultivation after neutralizing the pH and adding more nutrients.

Spilling et al. [17] studied the effect of pH increase on flocculation of the diatom *Phaeodactylum tricorutum* and of the green algae *Scenedesmus cf. obliquus*, showing that *P. tricorutum* started to flocculate at pH 10.5 and *S. cf. obliquus* at pH 11.3. Also, it was studied the combined effect of pH, turbulence, and cell density on the flocculation of *P. tricorutum*, showing that pH is the most important factor affecting flocculation. These authors also reported that since algae cultures increase the pH during photosynthesis, and *P. tricorutum* and *S. cf. obliquus* cultures increased the pH to a maximum of respectively 10.8 and 9.5, which for *P. tricorutum* is above the

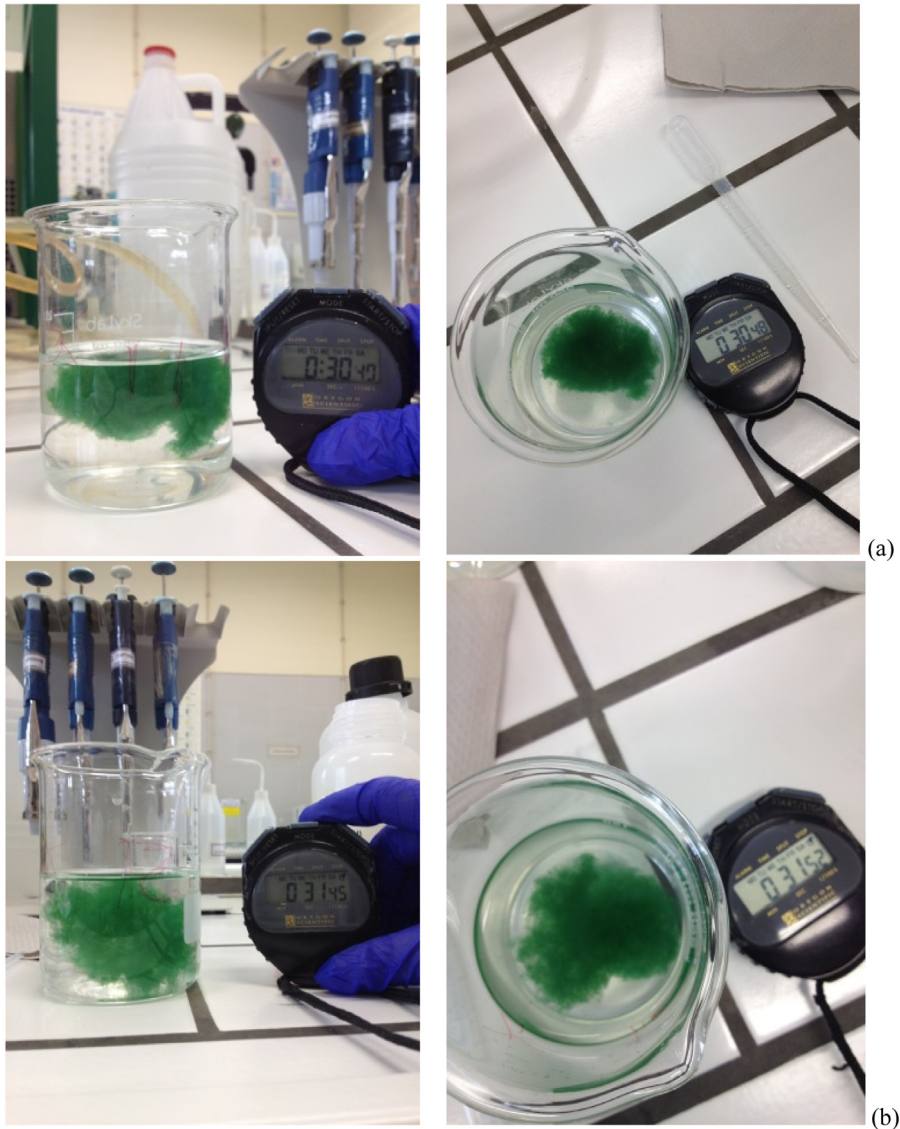


Fig. 1. Evolution of the samples after addition of NaOH, with the culture medium at (a) pH 11 and at (b) pH 12. Photos on the left are the front view and on the right the top view.

flocculation threshold, it was observed a rapid settling of this species in a matter of hours after discontinuing the CO₂ supply. Thus, the authors suggested that this could be used as a simple and low-cost initial dewatering step for *P. tricornutum*.

4. Conclusion

This study compared and evaluated the effectiveness of coagulation–flocculation methods for harvesting *Arthrospira maxima*, induced by pH variation and addition of flocculants. Results showed that, for the range of conditions tested, *Arthrospira maxima* can be effectively harvested by increasing pH over 10 with NaOH or by adding CaCl₂ as flocculant, with no need for addition of other chemicals. These chemicals are easy to use, inexpensive, and do not pose any substantial contamination risk of the recovered microalgae biomass. Therefore, this method can contribute to the economical harvesting of microalgae biomass.

Acknowledgments

Authors thank the financial support of the project IF/01093/2014/CP1249/CT0003 and research grants IF/01093/2014 and SFRH/BPD/112003/2015 funded by national funds through FCT/MCTES, and project UID/EQU/00305/2013 - Center for Innovation in Engineering and Industrial Technology — CIETI. This work was financially supported by: project UID/EQU/00511/2019 — Laboratory for Process Engineering, Environment, Biotechnology and Energy — LEPABE funded by national funds through FCT/MCTES (PIDDAC); Project POCI-01-0145-FEDER-006939 (Laboratory for Process Engineering, Environment, Biotechnology and Energy — LEPABE, UID/EQU/00511/2013) funded by FEDER through COMPETE2020-POCI and by national funds through FCT; 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] Mata TM, Sousa IRBG, Caetano NS. Transgenic corn oil for biodiesel production via enzymatic catalysis with ethanol. *Chem Eng Trans* 2012;27:19–24. <http://dx.doi.org/10.3303/CET1227004>.
- [2] Mata TM, Martins AA, Sikdar SK, Costa CAV, Caetano NS. Sustainability considerations about microalgae for biodiesel production. In: Lee J, editor. *Advanced biofuels and bioproducts*. New York: Springer; 2013, p. 745–57.
- [3] Contreras-Angulo JR, Mata TM, Cuellar-Bermudez SP, Caetano NS, Chandra R, Garcia-Perez JS, Muylaert K, Parra-Saldivar R. Symbiotic co-culture of *Scenedesmus* sp. and *Azospirillum brasilense* on N-deficient media with biomass production for biofuels. *Sustainability* 2019;11(3):707.
- [4] Rashid N, Rehman MSU, Sadiq M, Mahmood T, Han JI. Current status, issues and developments in microalgae derived biodiesel production. *Renew Sustain Energy Rev* 2014;40:760–78.
- [5] Mata TM, Martins AA, Oliveira O, Oliveira S, Mendes AM, Caetano NS. Lipid content and productivity of *Arthrospira platensis* and *Chlorella vulgaris* under mixotrophic conditions and salinity stress. *Chem Eng Trans* 2016;49:187–92.
- [6] Martins AA, Mata TM, Oliveira O, Oliveira S, Mendes AM, Caetano NS. Sustainability evaluation of biodiesel from *Arthrospira platensis* and *Chlorella vulgaris* under mixotrophic conditions and salinity stress. *Chem Eng Trans* 2016;49:571–6.
- [7] Correa DF, Beyer HL, Possingham HP, Thomas-Hall SR, Schenk PM. Biodiversity impacts of bioenergy production: Microalgae vs. first generation biofuels. *Renew Sustain Energy Rev* 2017;74:1131–46.
- [8] Mata TM, Martins AA, Caetano NS. Microalgae processing for biodiesel production. In: Luque R, Melero JA, editors. *Advances in biodiesel production: Processes and technologies*. Woodhead Publishing Series in Energy, 2012, p. 204–31. <http://dx.doi.org/10.1016/B978-0-85709-117-8.50009-5>.
- [9] Mata TM, Cameira M, Marques F, Santos E, Badenes S, Costa L, Vieira VV, Caetano NS, Martins AA. Carbon footprint of microalgae production in photobioreactor. *Energy Procedia* 2018;153:432–7. <http://dx.doi.org/10.1016/j.egypro.2018.10.039>.
- [10] Martins AA, Marques F, Cameira M, Santos E, Badenes S, Costa L, Vieira VV, Caetano NS, Mata TM. Water footprint of microalgae cultivation in photobioreactor. *Energy Procedia* 2018;153:426–31. <http://dx.doi.org/10.1016/j.egypro.2018.10.031>.
- [11] Schlesinger A, Eisenstadt D, Bar-Gil A, Carmely H, Einbinder S, Gressel J. Inexpensive non-toxic flocculation of microalgae contradicts theories; overcoming a major hurdle to bulk algal production. *Biotechnol Adv* 2012;30(5):1023–30.
- [12] Vandamme D, Foubert I, Muylaert K. Flocculation as a low-cost method for harvesting microalgae for bulk biomass production. *Trends Biotechnol* 2013;31(4):233–9.
- [13] Ayoub GM, Lee S-I, Koopman B. Seawater induced algal flocculation. *Water Res* 1986;20(10):1265–71.
- [14] Knuckey RM, Brown MR, Robert R, Frampton DMF. Production of microalgal concentrates by flocculation and their assessment as aquaculture feeds. *Aquac Eng* 2006;35(3):300–13.
- [15] Sukenik A, Shelef G. Algal autoflocculation - verification and proposed mechanism. *Biotechnol Bioeng* 1984;26:142–7.
- [16] Semerjian L, Ayoub GM. High-pH-magnesium coagulation-flocculation in wastewater treatment. *Adv Environ Res* 2003;7(2):389–403.
- [17] Spilling K, Seppälä J, Tamminen T. Inducing autoflocculation in the diatom *Phaeodactylum tricoratum* through CO₂ regulation. *J Appl Phycol* 2011;23(6):959–66.
- [18] Chen L, Sun Y, Sun W, Shah KJ, Xu Y, Zheng H. Efficient cationic flocculant MHCS-g-P(AM-DAC) synthesized by UV-induced polymerization for algae removal. *Sep Purif Technol* 2019;210:10–9.
- [19] Pugazhendhi A, Shobana S, Bakonyi P, Nemestóthy N, Xia A, Banu JR. A review on chemical mechanism of microalgae flocculation via polymers. *Biotechnol Rep* 2019;21. e00302.
- [20] Shurair M, Almomani F, Bhosale R, Khraisheh M, Qiblawey H. Harvesting of intact microalgae in single and sequential conditioning steps by chemical and biological based-flocculants: Effect on harvesting efficiency, water recovery and algal cell morphology. *Bioresour Technol* 2019;250–9.
- [21] Leite LDS, Daniel LA, Pivokonsky M, Novotna K, Branyikova I, Branyik T. Interference of model wastewater components with flocculation of *Chlorella sorokiniana* induced by calcium phosphate precipitates. *Bioresour Technol* 2019;286. 121352.
- [22] Gerde JA, Yao L, Lio J, Wen Z, Wang T. Microalgae flocculation: Impact of flocculant type, algae species and cell concentration. *Algal Res* 2014;3(1):30–5.
- [23] Augustine A, Tanwar A, Tremblay R, Kumar S. Flocculation processes optimization for reuse of culture medium without pH neutralization. *Algal Res* 2019;39. 101437.

- [24] Bracharz F, Helmdach D, Aschenbrenner I, Funck N, Wibberg D, Winkler A, Bohnen F, Kalinowski J, Mehlmer N, Brück TB. Harvest of the oleaginous microalgae *Scenedesmus obtusiusculus* by flocculation from culture based on natural water sources. *Front Bioeng Biotechnol* 2018;6(200).
- [25] Pandey A, Pathak VV, Kothari R, Black PN, Tyagi VV. Experimental studies on zeta potential of flocculants for harvesting of algae. *J Environ Manag* 2019;231:562–9.
- [26] Chen F, Liu Z, Li D, Liu C, Zheng P, Chen S. Using ammonia for algae harvesting and as nutrient in subsequent cultures. *Bioresour Technol* 2012;121:298–303.
- [27] Wang S, Yerkebulan M, Abomohra AE-F, El-Khodary S, Wang Q. Microalgae harvest influences the energy recovery: A case study on chemical flocculation of *Scenedesmus obliquus* for biodiesel and crude bio-oil production. *Bioresour Technol* 2019;286: 121371.
- [28] Vandamme D, Gheysen L, Muylaert K, Foubert I. Impact of harvesting method on total lipid content and extraction efficiency for *Phaeodactylum tricornutum*. *Sep Purif Technol* 2018;194:362–7.
- [29] Kim Y-H, Park H-J, Lee S-H, Lee J-H. Isolation and characterization of autoflocculating mutants of cyanobacterium *Arthrospira platensis*. *Korean J Chem Eng* 2013;30(2):413–6.
- [30] Liu J, Zhu Y, Tao Y, Zhang Y, Li A, Li T, Sang M, Zhang C. Freshwater microalgae harvested via flocculation induced by pH decrease. *Biotechnol Biofuels* 2013;6(1):98.
- [31] Vandamme D, Foubert I, Fraeye I, Meesschaert B, Muylaert K. Flocculation of *Chlorella vulgaris* induced by high pH: role of magnesium and calcium and practical implications. *Bioresour Technol* 2012;105:114–9.
- [32] Yahi H, Elmaleh S, Coma J. Algal flocculation-sedimentation by pH increase in a continuous reactor. *Water Sci Technol* 1994;30:259–67.
- [33] Wu Z, Zhu Y, Zhang Y, Zhang C, Huang W, Li T, Li A. Evaluation of flocculation induced by pH increase for harvesting microalgae and reuse of flocculated medium. *Bioresour Technol* 2012;110:496–502.
- [34] Liu J, Tao Y, Wu J, Zhu Y, Gao B, Tang Y, Li A, Zhang C, Zhang Y. Effective flocculation of target microalgae with self-flocculating microalgae induced by pH decrease. *Bioresour Technol* 2014;167:367–75.