Latin American Journal of Aquatic Research, 49(5): 836-842, 2021
DOI: 10.3856/vol49-issue5-fulltext-2691

Short Communication

Some implications of natural increase of pH in microalgae cultivation and harvest by autoflocculation

**Julio César Beltrán-Rocha[1], Claudio Guajardo-Barbosa[2], Humberto Rodríguez-Fuentes[1]
Gustavo Raúl Reyna-Martínez[2], Luis Osornio-Berthet[3], Magdalena García-Martínez[3]
Icela Dagmar-Barceló Quintal[3] & Ulrico Javier López-Chuken[2]**
[1]Laboratorio de Suelos, Plantas y Aguas, Facultad de Agronomía
Universidad Autónoma de Nuevo León, Nuevo León, México
[2]Centro de Investigación en Biotecnología y Nanotoxicología
Universidad Autónoma de Nuevo León, Nuevo León, México
[3]Departamento de Ciencias Básicas, Universidad Autónoma Metropolitana-Azcapotzalco
Ciudad de México, México
Corresponding author: Ulrico Javier López-Chuken (ulrico.lopezchk@uanl.edu.mx)

ABSTRACT. Microalgae hold great potential for producing purified high-value products (e.g. pigments and polyunsaturated fatty acids) and represent a source of bioavailable nutrients in aquaculture feeds; however, its production is limited by the high costs of harvesting. Recently, microalgae autoflocculation has been considered a useful solution due to the easy recovery of cell aggregates and natural increase in pH that prevents cell lysis. This work evaluates some cultivation conditions that could contribute to autoflocculation, such as natural pH increase and precipitate formation over the productivity and flocculation of 16 microalgae consortia. Results showed a biomass production of 1.4 to 4.4 dry weight (g L^{-1}) and high flocculation of 81 to ~100%, probably due to $Ca_3(PO_4)_2$ formation. Moreover, the alkaline pH could have facilitated the assimilation of CO_2 and explained the increase of inorganic carbon in the solution obtained. Likewise, results showed a marked descent in electrical conductivity (EC) in solution and high mineral content in the biomass (21.4-35.9%). Finally, this study suggests that the studied culture conditions facilitated microalgae harvest, and the resulting biomass could be a source of bioavailable dietary minerals. Therefore, some of these results might be addressed in future studies with individual microalgae species.

Keywords: algal culturing; harvesting; nutrient solution composition; pH-induced flocculation; dewatering; microalgae

While the microalgae biomass could be used as feedstock to extract high-value products (e.g. bioactive compounds such as polyunsaturated fatty acids, phyco-biliproteins, and carotenoids), the complete biomass could be directly used as food for human, aquaculture, and animal nutrition. Due to the supply of high bioavailability macro-and micronutrients (Dineshbabu et al. 2019), the use of microalgae as feed or feed supplements in aquaculture production represents an attractive alternative to replace fishmeal. Besides the nutritional value (e.g. protein, carotenoids, omega 3 fatty acid, and minerals), microalgae supplementation has shown the ability to improve the survival rates and growth in fishes (e.g. *Oreochromis niloticus*) and shrimps (e.g. *Penaeus monodon*) (Dineshbabu et al. 2019). Microalgae biomass as feed for aquaculture can be sustainably produced because it uses fewer nutrients and water than terrestrial crops. Moreover, microalgae can sequester CO_2 (greenhouse gas) more efficiently than higher plants (Rashid et al. 2014). Despite these advantages, no procedure has been developed so far for cost-effective microalgae harvesting, which is a critical step that contributes to a high cost of the total production (20-30% or even more) (Wan et al. 2015). Microalgae harvesting typically employs methods such as centrifugation, filtration, or sedimentation. Still, it

Corresponding editor: Crisantema Hernández

can become technically challenging for large volumes of operation due to the small size (2-20 μm diameter), low density (0.5-5.0 g L^{-1} dry weight; DW), and high colloidal stability of microalgae (Vandamme et al. 2013). Therefore, it is essential to develop viable strategies to harvest microalgae in large cultures. Flocculation technologies have recently been considered feasible alternatives because the formation of cell aggregates of easy recovery is induced (Wan et al. 2015). Different methods of flocculation have been developed for microalgae harvesting. For instance, chemical flocculation adding organic polymers/ polyelectrolytes (e.g. cationic starch and chitosan) shows high efficiency with minimal biomass contamination. Still, it increases the whole process cost making them unsuitable for low-value products (Vandamme et al. 2013, Rashid et al. 2014). Also, chemical flocculation using inorganic metal salts (e.g. $AlCl_3$, $Al_2(SO_4)_3$, $FeCl_3$ and $Fe_2(SO_4)_3$) and bioflocculation using bacteria, fungi, and yeast, have the disadvantage of potentially contribute to biomass contamination (Wan et al. 2015).

Autoflocculation has recently caught the attention as a viable low-cost method for harvesting microalgae. The autoflocculation process is based on the neutralization of negatively charged cells surface with positively charged salt precipitates (Ca, P, or/and Mg) at alkaline pH >8 (Vandamme et al. 2012, Nguyen et al. 2014). One of the major advantages of autoflocculation is that natural pH increases (a result of photosynthetic activity and OH^- accumulation) can be accompanied by avoiding cell lysis and loss of intracellular content (Rashid et al. 2014). Autoflocculation and pH increase can also be facilitated by culture conditions such as the low buffering capacity of the carbonate system (based upon C supply) and the use of NO_3^- as the source of N (given that 1 mole of assimilated NO_3^- produces equivalently 1 mole of OH^-) (Nguyen et al. 2014). This work evaluated the general effects of some culture conditions that ease harvest by autoflocculation during microalgae growth. Sixteen different algal consortia were cultivated under conditions that allow the natural increase of pH (low pH buffering, N-source) and theoretically formation of salt precipitates of Ca, P, and Mg already present in the nutrient solution.

Sixteen distinct native microalgae consortia (predominantly chlorophytes) (MC01-MC16) were collected from different freshwater bodies belonging to the San Juan hydrological sub-watershed in Nuevo Leon, Mexico (26°21’55”N, 98°51’15”W) were provided by the Environmental Research Lab from Autonomous University of Nuevo Leon. Details regarding for collection, propagation, and concentration of microalgae consortia are given in earlier work (Beltrán-Rocha et al. 2017). Microalgae consortia were cultivated in LC-Y nutrient solution (López-Chuken & Young 2010) containing: KNO_3 5 mM, KH_2PO_4 1 mM, $MgSO_4{\cdot}7H_2O$ 2 mM, $Ca(NO_3)_2{\cdot}4H_2O$ 6.25 mM, H_3BO_3 46 μM, $MnCl_2{\cdot}4H_2O$ 9.15 μM, $ZnSO_4{\cdot}7H_2O$ 765 nM, $CuSO_4{\cdot}5H_2O$ 320 nM, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 15 nM, $FeSO_4{\cdot}7H_2O$ and Na_2EDTA 20 μM. Likewise, the elemental concentrations of C, Ca, Mg and P in the LC-Y nutrient solution were 0.20, 6.25, 2.00 and 1.00 mM, respectively. All 16 microalgae consortia inoculums were standardized to give a DW of 0.03 (mg mL^{-1}) in a volume of 700 mL of LC-Y nutrient solution. The experiment was conducted in single batch culture (i.e. one biological replicate) for 30 days using 16 bubble column photobioreactors (1 L). Photo-bioreactors were continuously aerated from the bottom at 0.5 vvm (air volume per liquid volume per minute) to provide CO_2 and gentle agitation, using filtered (0.45 μm) atmospheric air. During the experiments, all cultures were propagated at ambient temperature (30 ± 2°C) under constant illumination (78 μM photons m^{-2} s^{-1}).

The pH in the solution was monitored daily (Hach method 8156) for 30 days to investigate the effect of pH alkalinization on microalgae flocculation and production. Electrical conductivity (EC) (Hach method 8160) and inorganic carbon (TOC-VCSH, Shimadzu, Japan) were measured at 0 and 30 days, while biomass content DW (g L^{-1}) was determined at 5, 10, 15, 20, 25, and 30 days. The % of flocculation for each microalgae culture was determined at 10, 20, and 30 days, using the following methodology: 10 mL aliquots were collected at 1/3 of the reactor height to determine both the total microalgae biomass content (from previously homogenized culture), and the microalgae suspended biomass after a settling time of 15 min. Subsequently, the aliquots were filtered and dried. The percentage of flocculation was calculated according to the equation: $(A/B) \times 100$, where A is the DW (g L^{-1}) of the suspended biomass after the settling period and B is the total biomass in DW (g L^{-1}). The microalgae biomass was then analyzed to determine the % of total mineral content (measured as ash content) using the AOAC method 942.05 (AOAC 1997).

Biomass concentration results indicated final productivity ranging from 1.42 ± 0.03 to 4.37 ± 0.10 DW (g L^{-1}) (Fig. 1). At the end of the trial, all 16 microalgae consortia showed high flocculation, ranging from 81.2 ± 0.8 to 99.5 ± 0.03% (Fig. 2). It was also observed that all cultures required only stopping agitation (i.e. aeration) from starting to settle as microalgae flocs. During the experiment, natural increases in pH varied from the initial pH (4.74) to pH values above ~8.5, highlighting consortia MC15 that reached a pH of 10.24 at the end of culture (Fig. 3), the

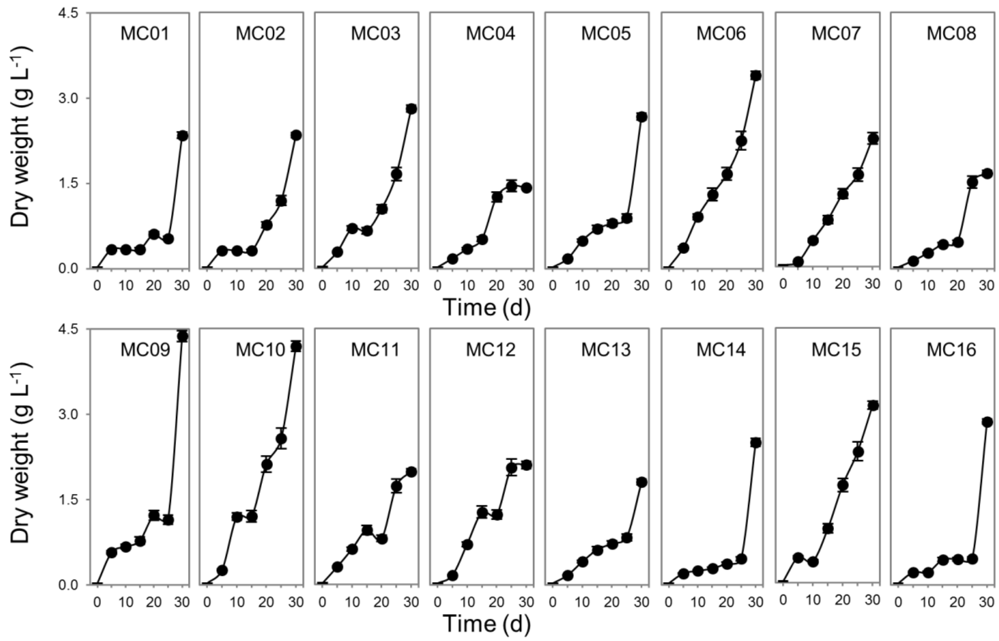

Figure 1. Microalgae biomass concentration during the culture period. Error bars indicate standard error, where n = 2.

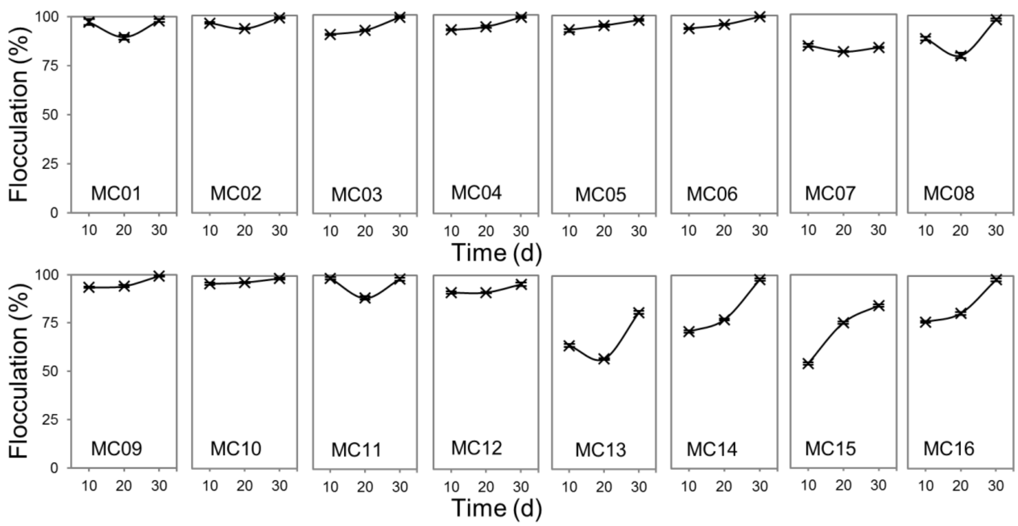

Figure 2. Flocculation of microalgae consortia at 10, 20, and 30 days of cultivation. Average ± standard error, where n = 3.

inorganic carbon concentration was analyzed to validate the close relationship of pH and atmospheric CO_2 dissociation in solution. The results of inorganic carbon indicated increases in concentration from 310 to 799% compared to the initial value (2.02 ± 0.02 mg L^{-1}) (Fig. 4). As this trial studied the potential formation of salt precipitates during the increase of pH and the microalgae capacity to recover inorganic micronutrients, our results indicated a marked EC decrease of 36.1 to 55.7% (Fig. 5) and high mineral accumulation in biomass, ranging from 21.4 ± 0.7 to 35.9 ± 0.9% (Fig. 6).

According to the settling results, all cultures resulted in high values (81 to ~100%) that fits within flocculation efficiency values (60 to 100%) reported when using chemical flocculants (e.g. $Al_2(SO_4)_3$, $FeCl_3$, cationic starch, or chitosan) after settling times of 30 or 60 min (Wan et al. 2015). Despite the high flocculation obtained, future research should address flocculation responses in monoalgal cultures, biomass properties for nutritional applications, and other considerations discussed below.

Changes in pH in the nutrient solution for microalgae flocculation can be achieved using hydroxide

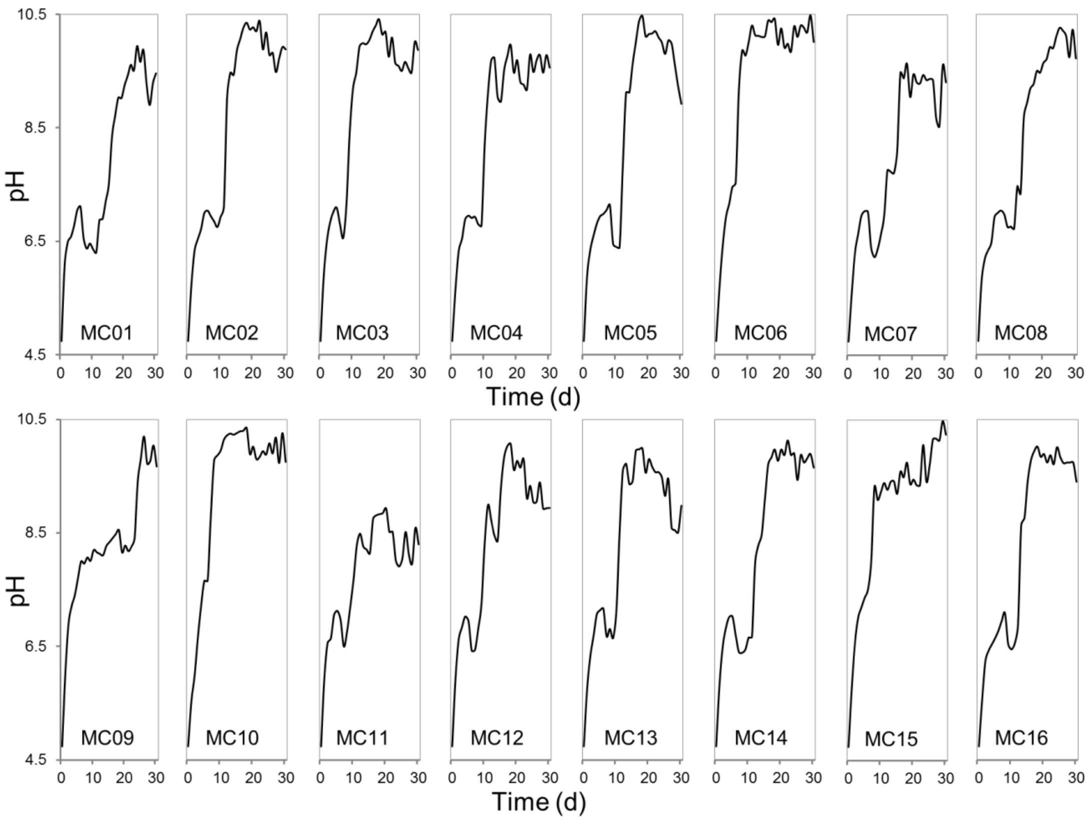

Figure 3. pH evolution during the culture period of microalgae consortia. The absence of an error bar indicates negligible standard error, where n = 3.

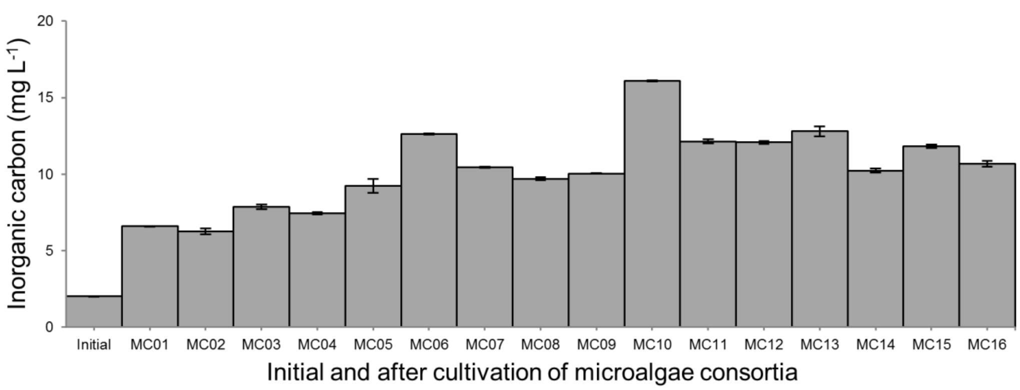

Figure 4. Initial concentration of inorganic carbon and after 30 days of microalgae consortia cultivation. Average ± standard error, where n = 3.

salts. Considering practical applications, pH adjustments in large volume operations could prove expensive and need to be carefully evaluated because the shift of pH at high values can cause cell lysis and reduce nutritional quality (Rashid et al. 2014). Conversely, a potential advantage of natural pH increase is that microalgae have biological control over pH. The photosynthetic activity could be slowed down or even stopped once that pH tolerance is reached (Spilling 2007). In this study, for all consortia, natural increases in pH reached values above ~8.5 which certainly contributed to enhancing flocculation. It is also important to note that gradual pH increases in the present experiment might be explained by processes associated with the production of OH^-. One of them is the assimilation of atmospheric CO_2 and NO_3^-, given that it provides 1 mole of OH^- under the following equation: $NO_3^- + 5.7(CO_2) + 5.4(H_2O) \rightarrow C_{5.7}H_{9.8}O_{2.3}N$

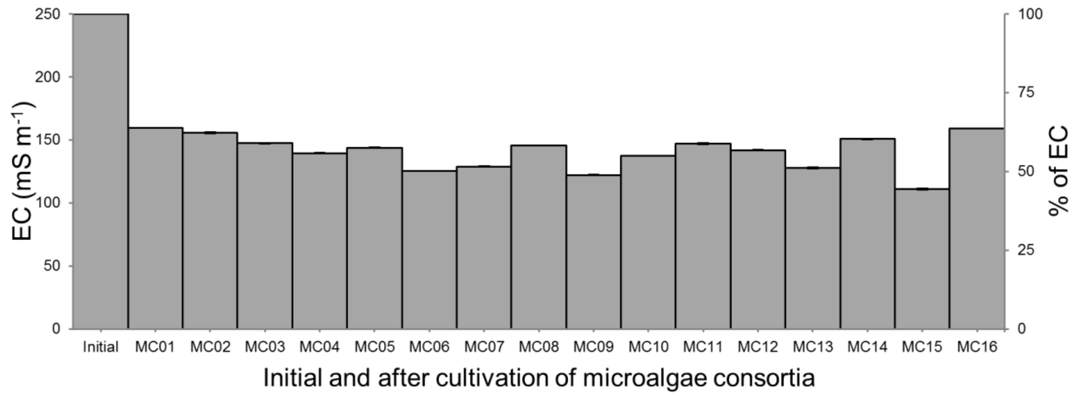

Figure 5. Initial concentration of electrical conductivity (EC) in solution and after 30 days of microalgae consortia cultivation. Average ± standard error, where n = 3.

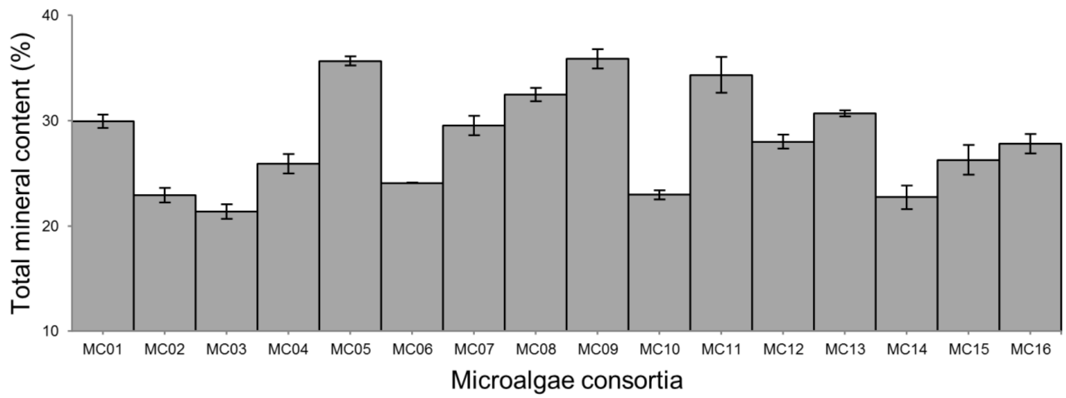

Figure 6. The total mineral content of algal biomass. Average ± standard error, where n = 3.

+ 8.25(O_2) + OH^- (Rashid et al. 2014). Also, assimilation of HCO_3^- provides CO_2 necessary for microalgae growth, at the same time that generates an accumulation of OH^-. Likewise, pH alkalinization in the present study was a factor that could have contributed to the formation of HCO_3^- that predominates at pH values ranging from 6.36 to 10.33 (Markou & Georgakakis 2011). According to this approach, biomass production in the present trial was favored by the consumption and dissolution of CO_2 (i.e. HCO_3^-) into the nutrient solution at alkaline pH. Following the formation of HCO_3^-, inorganic carbon concentration in the present experiment indicated high increases ranging from 310 to 799%. In addition, it is important to consider the decrease in microalgae growth at pH >10. It is caused by reduced bioavailability of C and P at these pH values due to limited microalgae assimilation of CO_3^{-2} forms that predominate at pH >10.33 (Markou & Georgakakis 2011), and the unavailability of phosphorus fraction that precipitates with calcium ($Ca_3(PO_4)_2$) from 8.5 to 10.5 of pH (Sukenik & Shelef 1984).

Autoflocculation has been better associated with the concentration and formation of inorganic precipitates of Ca-P and Mg in a nutrient solution rather than pH alkalinization alone (Vandamme et al. 2013). It is important to emphasize that flocculation at alkaline pH can only occur by interacting charges between cell walls and precipitates. For example, Vandamme et al. (2012) observed that precipitates of $CaCO_3$ (under pH 10.5, 11, and 12) had no contribution to flocculation of *Chlorella vulgaris* due to the absence of charge in the $CaCO_3$ crystals. On the other hand, precipitation of $Ca_3(PO_4)_2$ crystals with a positive charge under alkaline conditions (pH 8.5 to 10.5) plays an important role in autoflocculation (Sukenik & Shelef 1984, Vandamme et al. 2012), requiring concentrations of PO_4^{-3} of 0.1-0.2 mM and Ca 1.5-2.5 mM (Sukenik & Shelef 1984). Moreover, Mg at concentrations above 0.15 mM can significantly induce flocculation (Vandamme et al. 2013) since at high pH (~10.2-11.5), superficial positively charged $Mg(OH)_2$ precipitates are formed (Nguyen et al. 2014). During the trial, pH values (ranging from 8 to 10) were unsuitable for $Mg(OH)_2$ formation (except for MC15), suggesting that the main cause of flocculation in the present study was due to $Ca_3(PO_4)_2$ formation.

In this work, two indirect effects were perceived from the process of autoflocculation: 1) a marked EC decrease in solution and 2) high mineral content in the biomass. The assimilation of nutrients explains the observed EC declination in ionic forms by the microalgae and by precipitation of ionic compounds, such as NO_3^-, PO_4^{-3}, SO_4^{-2}, Na^+, K^+, Ca^{+2}, Mg^{+2}, Fe^{+2}/Fe^{+3} and trace elements that contribute to the overall EC (Cai et al. 2013). In the case of harvested biomass, an unusually high mineral concentration (21.4 to 35.9%) was found, which exceeded values of 4 to 20% generally found for freshwater and marine microalgae (Fox & Zimba 2018). It is noteworthy that microalgae biomass has been suggested as a source of highly bioavailable minerals (e.g. Ca, P, Mg, K, Na, and Fe) and biomolecules (e.g. amino acids, carotenoids, fatty acids, sterols). It could contribute to animal and human nutrition and aquaculture production (Fox & Zimba 2018, Dineshbabu et al. 2019).

In conclusion, the present cultivation conditions favored high flocculation (81 to ~100%) in all the 16 microalgae consortia studied regardless of their biomass productivity (ranging from 1.4 to 4.4 DW in g L^{-1}). The effect on rapid and efficient biomass harvest was almost certainly caused by natural pH increase and nutrient solution composition that favored the formation of $Ca_3(PO_4)_2$ as the main mechanism of flocculation. Alkaline pH in solution was favorable, allowing CO_2 assimilation and increased inorganic carbon concentration supplied by atmospheric air. Besides, two side-effects were noticed during microalgae flocculation: a pronounced decrease in EC and an elevated concentration of minerals in biomass (21.4-35.9%). As a result, the produced microalgae biomass could be a bioavailable minerals resource for feed or feed supplements in the aquaculture industry. Finally, for further research to add value, it is necessary to investigate how present culture conditions might affect pure microalgae cultures to facilitate easy harvesting.

ACKNOWLEDGMENTS

This work was supported by the Mexican National Council for Science and Technology.

REFERENCES

Association of Official Analytical Chemists (AOAC). 1997. Officials methods of analysis of AOAC International. AOAC International, Gaithersburg.

Beltrán-Rocha, J.C., Barceló-Quintal, I.D., García-Martínez, M., Osornio-Berthet, L., Saavedra-Villarreal, N., Villarreal-Chiu, J. & López-Chuken, U.J. 2017. Polishing of municipal secondary effluent using native microalgae consortia. Water Science Technology, 75: 1693-1701.

Cai, T., Park, S.Y. & Li, Y. 2013. Nutrient recovery from wastewater streams by microalgae: status and prospects. Renewable and Sustainable Energy Reviews, 19: 360-369.

Dineshbabu, G., Goswami, G., Kumar, R., Sinha, A. & Das, D. 2019. Microalgae-nutritious, sustainable aqua-and animal feed source. Journal of Functional Foods, 62: 103545.

Fox, J.M. & Zimba, P.V. 2018. Minerals and trace elements in microalgae. In: Levine, I.A. & Fleurence, J. (Eds.). Microalgae in health and disease prevention. Academic Press, London, pp. 177-193.

López-Chuken, U.J. &. Young, S.D. 2010. Modelling sulphate-enhanced cadmium uptake by *Zea mays* from nutrient solution under conditions of constant free Cd^{+2} ion activity. Journal of Environmental Sciences, 22: 1080-1085.

Markou, G. & Georgakakis, D. 2011. Cultivation of filamentous cyanobacteria (blue-green algae) in agro-industrial wastes and wastewaters: a review. Applied Energy, 88: 3389-3401.

Nguyen, T.D.P., Frappart, M., Jaouen, P., Pruvost, J. & Bourseau, P. 2014. Harvesting *Chlorella vulgaris* by natural increase in pH: effect of medium composition. Environmental Technology, 35: 1378-1388.

Rashid, N., Ur-Rehman, M.S., Sadiq, M., Mahmood, T. & Han, J.I. 2014. Current status, issues and developments in microalgae derived biodiesel production. Renewable and Sustainable Energy Reviews, 40: 760-778.

Spilling, K. 2007. Dense sub-ice bloom of dinoflagellates in the Baltic Sea, potentially limited by high pH. Journal of Plankton Research, 29: 895-901.

Sukenik, A. & Shelef, G. 1984. Algal autoflocculation-verification and proposed mechanism. Biotechnology and Bioengineering, 26: 142-147.

Vandamme, D., Foubert, I., Fraeye, I., Meesschaert, B. & Muylaert, K. 2012. Flocculation of *Chlorella vulgaris* induced by high pH: role of magnesium and calcium and practical implications. Bioresource Technology, 105: 114-119.

Vandamme, D., Foubert, I. & Muylaert, K. 2013. Flocculation as a low-cost method for harvesting microalgae for bulk biomass production. Trends in Biotechnology, 31: 233-239.

Wan, C., Alam, M.A., Zhao, X.Q., Zhang, X.Y., Guo, S.L., Ho, S.H., et al. 2015. Current progress and future prospect of microalgal biomass harvest using various flocculation technologies. Bioresource Technology, 184: 251-257.

Received: December 30, 2020; Accepted: August 16, 2021