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Original Article

Use of aluminium sulphate as flocculant for spirulina (*Arthrospira platensis*) with notes on the use of antioxidants for preservation under refrigerated conditions

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Abstract
One of the bottlenecks in microalgae harvesting is the lack of an efficient method for separating the microalgae from its culture medium. Moreover, the lack of viable and simple preservation techniques for microalgae starters hinders the immediate recovery of cultures after experiencing collapse. Hence, the present study was conducted to evaluate the use of aluminium sulphate as a flocculant for harvesting spirulina (*Arthrospira platensis*) and compare it with other flocculation techniques (electrolytic flocculation and autoflocculation). Moreover, the use of antioxidants to lengthen the storage of viable spirulina cells in refrigerated conditions was explored. The results of the study showed that the optimum dosage of aluminium sulphate for flocculation of spirulina is 200 ppm with 94.82 ± 0.59% efficiency in 15 – 45 minutes post-administration. Moreover, the combination of ascorbic acid and alpha-tocopherol at 0.01% v/v resulted in the highest viable cells at 57.76 ± 2.48% until the 3-week refrigeration period. This may help in maintaining viable starters. However, further investigations are needed to ascertain residuals of aluminium in harvested biomass and explore low-cost options for its reduction or removal and optimize the use of antioxidants in spirulina preservation in refrigerated conditions.

Keywords: blue-green algae; flocculation; microalgae; simple preservation

1 | INTRODUCTION

Spirulina (*Arthrospira platensis*) is a microscopic, planktonic, blue-green alga belonging to the class Cyanophyta. The cellular structure of this alga is spiral shape and similar to that of a simple prokaryote. They form massive populations in tropical and subtropical alkaline bodies of water with an extremely high pH. Spirulina achieved considerable popularity in the health sector, food industry, and aquaculture due to the increased awareness of its therapeutic benefits (Belay *et al.* 1997). It contains antioxidants, phytonutrients, probiotics, nutraceuticals, and essential fatty acids (Soni *et al.* 2017). Hence, the United Nations declared it as the best food for the future while UNESCO described it as the most ideal food for tomorrow (Pulz and Gross 2004). Also, it has been successfully used by astronauts as a dietary supplement on space missions

by the National Aeronautics and Space Administration and the European Space Agency (Jung *et al.* 2019; Ciani *et al.* 2021; Ramírez-Rodrigues *et al.* 2021; Soni *et al.* 2021). With this, there was a burst of activity relating to the production of spirulina biomass as specialised industries for producing health food, food additives, animal feed, biofertilizers, and assorted natural products have emerged (Vonshak 1997). This increasing demand can only be met if a reliable, predictable, and continuous supply is made available. Hence, the development of culture technology for the propagation of spirulina. As early as the 1970s, mass production technology of spirulina is already available utilising a semi-natural facility (Voshak 1997; Soni *et al.* 2017). Two decades later, the mass production technology of spirulina has been established with total annual production reaching as high as 1000 tons per year (Vonshak 1997; Richmond 2004). Currently, spirulina can be found in health food stores and is sold mainly as a dietary supplement in the form of health drinks or tablets. In the Philippines, large-scale production of spirulina remains limited even with the progress in the development of biomass production technology. Research on the optimisation of growth under local climatic conditions, post-harvest processing, and utilisation of spirulina in the country is limited (Tiburcio *et al.* 2007).

The global average production of spirulina is 73030 tons in the last 13 years (FAO 2020). As one of the most cultivated microalgae worldwide, the spirulina market achieved USD 348 million in 2018 and is expected to reach USD 779 million by 2026 (Silva *et al.* 2020). Moreover, spirulina is a premium source of the natural blue pigment phycocyanin. The market demand for naturally-derived phycocyanin pigment has continued to increase through the years with the global market size valued at USD 155.3 million in 2020 and it is expected to reach USD 409.8 million by 2030 (Thevarajah *et al.* 2022).

To efficiently harvest spirulina and use it for varied applications, efficient methods for separating and harvesting its biomass from the culture media must be employed. Harvesting spirulina can be done through different techniques with its pros and cons. Commonly, centrifugation is used but its main drawback of being energy-intensive led to the adaptation of a two-step harvesting strategy that involves pre-concentration (either by sedimentation or floatation) and centrifugation to reduce costs (Pienkos and Darzins 2009; Dassey and Theegala 2013). Also, floatation has been reported as a good harvesting technique with approximately 80% efficiency due to the presence of gas vacuoles in spirulina (Kim *et al.* 2005). Furthermore, recent developments in membrane filtration technology touted it as a promising option as it reduced energy consumption and reduced water requirements (Ismail *et al.* 2021). A more convenient process in harvesting spirulina is through flocculation as it allows the treatment of large quantities of culture. Electrolytic flocculation with the use of metal anodes / cathodes is a promising approach but its major drawback is the requirement of high energy input. On the other hand, chemical flocculation using inorganic flocculants is the most cost-effective flocculation method (Vasistha *et al.* 2021). Aluminium sulphate, commonly known as alum, has been used as a flocculant for algae and as a preferred cationic coagulant in water and wastewater treatment (Apostol *et al.* 2011) due to its ability to obtain strong flocs and a minimum amount of residual aluminium in the water (Govoreanu *et al.* 2001; Gabelich *et al.* 2006; Lin *et al.* 2008; Mihai and Dajiba 2008; Liu and Chin 2009). Previous studies in several algal species reported successful use of aluminium sulphate as an inorganic flocculant at doses ranging from 100 ppm to 1200 ppm but with pH manipulation and rotational speeds to facilitate coagulation and flocculation (Papazi *et al.* 2010; Gani *et al.* 2017; Zhu *et al.* 2020; Mohseni and Moosavi Zenooz 2022).

Another challenge in the culture of spirulina is the occurrence of contamination and inclement weather conditions which ultimately lead to die-off in outdoor cultures. This is undesirable as this interferes with the program of production which may ultimately affect profit. Thus, a readily viable starter is necessary to immediately jumpstart culture after experiencing a die-off. Both short-term and long-term storage using cost-effective methods are desirable. Recently, the production of microalgal pastes has been successful but its use has remained limited to resuspension as larval food but not as a viable starter for cultures (de la Peña and Franco 2020). The present study investigated the efficiency of different flocculation techniques (autoflocculation, chemical flocculation, and electrolytic flocculation) in harvesting spirulina (*A. platensis*) to determine the most viable technique for biomass production under local conditions. Moreover, the use of antioxidants to lengthen the storage of viable spirulina cells in refrigerated conditions was explored.

2 | METHODOLOGY

2.1 Spirulina production

The test organism (*A. platensis*) was grown in the laboratory using a commercial NPK complex fertilizer formulation under constant illumination with cool white light-emitting diodes (specification: T5 15W 6400K) and aeration (airflow rate: 90 Lh^{-1}) at ambient temperatures (24-26°C). Spirulina was harvested after three days and was ensured to be homogenous to be used for the flocculation experiments. All flocculation experiments had spirulina cultures with an optical density of 0.800 at 680 nm.

2.2 Flocculation techniques

Electrolytic flocculation was done using 10 L spirulina cultures. Two aluminium electrode plates were placed parallel and vertically in the vessel with an electrode gap of 11 cm and both plates had an area of 38.7 cm^2 and a thick-

ness of 0.5 mm. The anode and cathode were connected to the DC power supply in the constant current mode and both submerged at a depth of 30 cm. The time course for microalgae concentration was monitored by a UV–VIS (Hach DR 1900, USA) spectrophotometer with optical density values measured at 680 nm. Furthermore, to determine the microalgae recovery efficiency, a 10 ml sample within 15 cm depth was collected every five minutes during the electrolytic flocculation process.

Autoflocculation is done by allowing the spirulina to naturally flocculate. Spirulina was placed in 8 L capacity glass jars and allowed to autoflocculate. Moreover, chemical flocculation was done using aluminium sulphate ($Al_2(SO_4)_3$) at various concentrations as treatments. Aliquots of algal cultures (200 ml) were placed in 250 ml Erlenmeyer flasks. The test flocculant was then added accordingly to reach final concentrations of 50, 100, 150 and 200 ppm. Upon addition of flocculants, flasks were stirred for 1 minute and left to settle. All flocculation experiments were carried out in triplicates following a completely randomised experimental design.

2.3 Determination of flocculation efficiency

Flocculation efficiency was determined by measuring the optical density of the cell cultures before flocculation and the residual optical density of the supernatant liquid at different time intervals: every 5 minutes for electrolytic flocculation and every 15 minutes for chemical and auto flocculation. Optical density (OD) was read using a spectrophotometer at 680 nm. To determine the flocculating efficiency, the following formula by Ferriols and Aguilar (2012) was used: Flocculating Efficiency = (Initial OD – Residual OD) / Initial OD × 100.

2.4 Microscopic examination of spirulina cells and flocs

To determine whether the various flocculation methods used had any effect on the integrity of the cell walls of *A. platensis*, samples of settled material were pipetted out and observed using a compound microscope under 10X magnification and photomicrographs were taken for documentation.

2.5 Use of antioxidants for preservation

A completely randomised design experiment with different preservatives as treatments in triplicates, namely: control- distilled water, treatment 1 (ascorbic acid at 0.1% volume per volume [v/v]); treatment 2 (butylated hydroxytoluene at 0.01% v/v); treatment 3 (α-tocopherol at 0.1% v/v) and treatment 4 (a combination of ascorbic acid and α-tocopherol at 0.1% v/v).

Spirulina cells were concentrated using a centrifuge (Benchtop low-speed Centrifuge, TD-5Z, China) at 5000 rpm for 5 minutes to a volume of 15 ml and put in a test tube with the preservative and covered with paraffin film. All the test tubes were put in a rack and stored in a refrigerator with a temperature of 5.00 ± 0.30°C.

Monitoring was done once a week until the cells exhibit signs of deformation and / or disintegration. Cell viability was monitored by obtaining a 1 ml sample from each replicate and diluted in 100 ml distilled water for observation under the light microscope at 100X using a Sedgewick rafter counting chamber. Cell viability is computed using the formula: Cell viability (%) = (No. of viable filaments / Total No. of filaments) × 100

2.6 Data analyses

The collected data were subjected to descriptive analyses followed by testing of significant differences of means among treatments. Once requirements for parametric testing were satisfied, a one-way analysis of variance was conducted and a post-hoc Tukey's test followed. All statistical tests were done using Paleontological Statistics (PAST) software v. 4.0 at a 95% confidence level. Values are expressed as mean ± standard error of the means.

3 | RESULTS AND DISCUSSION

3.1 Flocculation of spirulina

Changes in the optical density of spirulina subjected to the different flocculation methods are shown in Figure 1. Among the flocculation methods, electrolytic flocculation had the fastest settling times displaying stable optical density after 20 minutes. This was followed by the chemical flocculation method using aluminium sulphate. Among the concentrations tested, 150 and 200 ppm had the fastest settling times displaying a marked decrease in optical density after 15 minutes up to 45 minutes of the settling period. In addition, the autoflocculation method reached stable optical density after 105 minutes of the settling period.

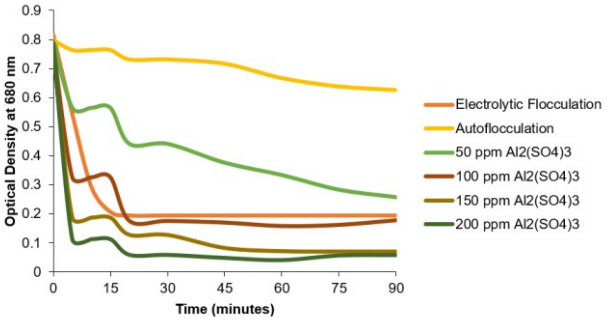

FIGURE 1 Changes in optical density of spirulina (*Arthrospira platensis*) in different methods of flocculation: A) electrolytic flocculation, B) autoflocculation and C) chemical flocculation using aluminium sulphate ($Al_2(SO_4)_3$). Values are means ± standard error of the means.

On the other hand, Figure 2 shows the different flocculation efficiencies of different flocculation methods. The highest flocculation efficiency was attained in 200 ppm $Al_2(SO_4)_3$ at 94.82 ± 0.59% followed by 150 ppm

$Al_2(SO_4)_3$, 100 ppm $Al_2(SO_4)_3$, electrolytic flocculation and 50 ppm $Al_2(SO_4)_3$ at 90.62 ± 2.46%, 79.78 ± 0.77%, 76.22 ± 2.42% and 58.10 ± 1.50% respectively. The lowest flocculation efficiency was attained in the autoflocculation method at 32.29 ± 4.10%. These differences were found to be significant (One way ANOVA, $p < 0.05$) with the 200 ppm $Al_2(SO_4)_3$ concentration significantly higher than the other methods.

Furthermore, the microscopic examination of the spirulina filaments flocculated using various methods showed that cells were in good physical condition as shown in Figure 3. No signs of cell structural damage or plasmolysis were observed under all treatments. The formation of flocs was observed in spirulina flocculated using 200 ppm $Al_2(SO_4)_3$ and electrolytic flocculation (Figure 3D and 3E) while those treated with lower concentrations of aluminium sulphate and autoflocculated, although indicating reductions in optical densities, displayed no prominent aggregations of spirulina filaments (Figure 3A–C and 3F). Compared to untreated cells, flocculated cells were observed to have not exhibited distinct changes in size and shape.

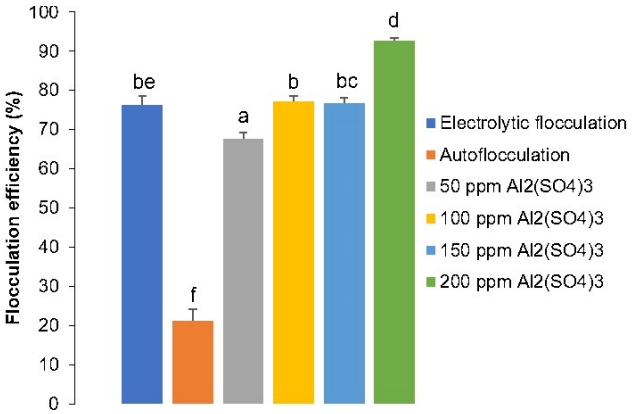

FIGURE 2 Flocculating efficiency (%) of various flocculation methods. Values are means ± standard error of the means. Different letters on top of bars indicate a significant difference (One-way ANOVA, $p < 0.05$).

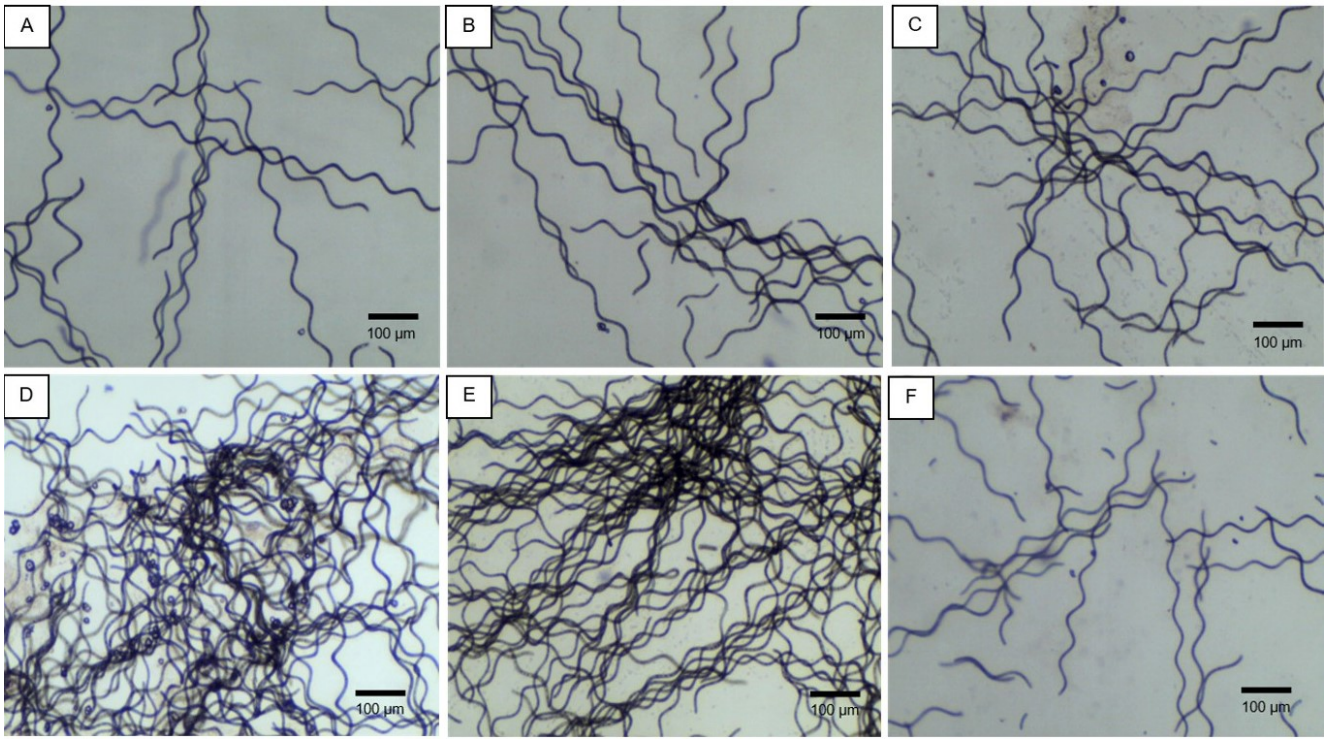

FIGURE 3 Microscopic examination of flocculated spirulina (*Arthrospira platensis*) filaments using A) 50 ppm $Al_2(SO_4)_3$, B) 100 ppm $Al_2(SO_4)_3$, C) 150 ppm $Al_2(SO_4)_3$, D) 200 ppm $Al_2(SO_4)_3$, E) electrolytic flocculation and F) autoflocculation showing no signs of plasmolysis.

The use of 200 ppm aluminium sulphate dosage in spirulina can result in high efficiencies at 15 – 45 minutes after administration. The use of aluminium sulphate as a flocculant has been tested in several species of algae. In *Botryococcus* sp., the use of 100 ppm aluminium sulphate resulted in up to 95% biomass harvest (Gani *et al.* 2017). In other reports, flocculation was successful only at higher doses and longer incubation times. Flocculation was best observed in *Chlorella minutissima* at 750 ppm in 2 h resulting in 80% efficiency (Papazi *et al.* 2010) while in *Chlorella vulagris*. Mohseni and Moosavi Zenooz (2021) reported an optimum dose at 500 ppm at pH 8.0 resulting

in 90% efficiency but Zhu *et al.* (2020) attained optimum flocculation at 2500 ppm with optimal rotation speeds for coagulation and flocculation were 150 and 25 rpm respectively.

Furthermore, the use of electrolytic flocculation for spirulina may not be a promising option due to the high cost of electrodes and high-power requirement for operation (Fuad *et al.* 2018) while food-grade aluminium sulphate, as used in this study, is readily available at 0.74 USD kg^{-1} and may not require energy inputs as coagulation-flocculation was observed even without rotation. However, further evaluation is necessary to ascertain whether levels of aluminium residues in the harvested algal biomass are at recommended levels for animal and human consumption and possible low-cost options to remove or reduce it.

3.2 Antioxidants as spirulina preservatives

The concentrated spirulina stored in refrigerated conditions lasted only for four weeks. Cell viability varied throughout the storage period depending on the preservative used. There was no observed cellular deformations / disintegration after centrifugation as shown in Figure 4. However, after 1 week of storage, cell viability is highest in treatment 1 at 81.78 ± 7.90% followed by treatment 3 at 80.51 ± 5.88%, treatment 4 at 49.21 ± 3.16% and control at 47.34 ± 13.27% while the lowest cell viability was recorded in treatment 2 at 36.90 ± 5.52%. Only treatments 1 and 2 were significantly different during this period.

In the 2nd week of storage, cell viability further declined. The highest cell viability was recorded in treatment 1 at 81.65 ± 2.45% followed by treatment 3 at 80.51 ± 5.88%, treatment 4 at 77.04 ± 0.30%, treatment 2 at 58.59 ± 6.05% while the lowest cell viability was recorded in the control at 52.01 ± 6.24%. It can be observed that the cells in treatments 2 and 4 grew with the increase in viable cells. The control is significantly different from treatments 1, 3 and 4. In the 3rd week of storage, all but treatments 2 and 4 have viable cells. In this period, significantly higher cell viability is observed in treatment 4 at 57.76 ± 2.48% compared to 13.02 ± 1.38% in treatment 2. The photomicrographs of the cells in treatments 2 and 4 are shown in Figure 5. However, in the 4th week, all stored cells in different preservatives have no viable cells.

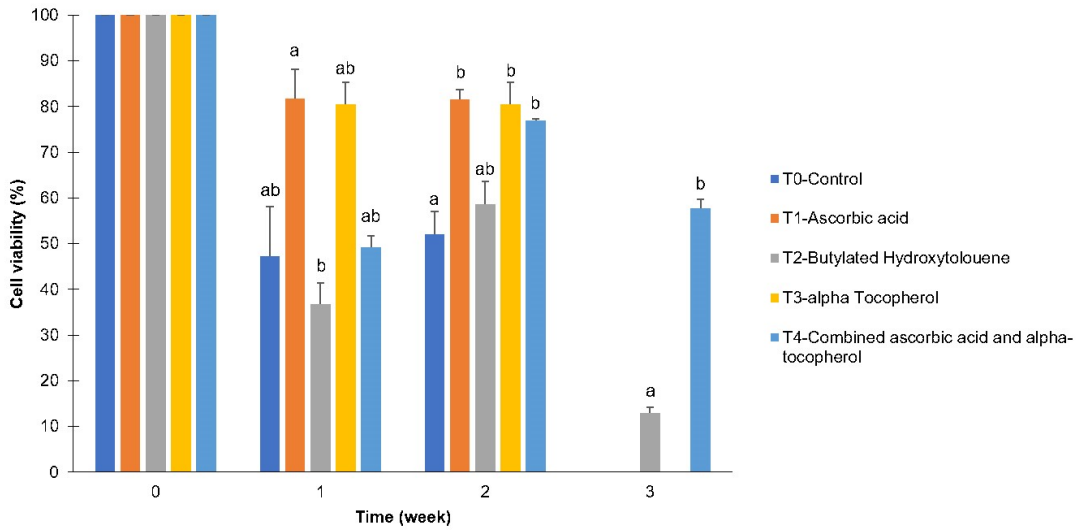

FIGURE 4 Cell viability of spirulina stored using various preservatives in refrigerated conditions. Values are mean ± standard error of the means. Bars with different letter notations indicate a significant difference (One-way ANOVA, $p < 0.05$).

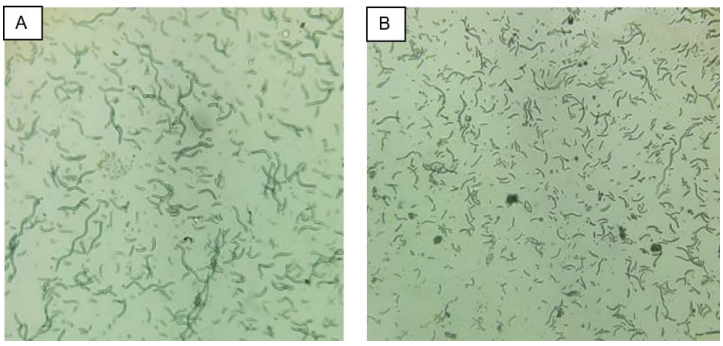

FIGURE 5 Photomicrographs of spirulina cells in treatment 4- a combination of ascorbic acid and alpha-tocopherol at (A) and treatment 2- butylated hydroxytoluene (B) after 3 weeks in refrigerated storage conditions showing the majority of cells are deformed and/or disintegrated.

The results of this experiment indicated that the use of combined ascorbic acid and alpha-tocopherol at 0.01% v/v may help preserve spirulina cells under refrigerated conditions for up to 3 weeks. Further investigation is necessary on the optimisation of this method and / or the use of other preservatives.

4 | CONCLUSIONS

The present study demonstrated that the optimum dosage of 200 ppm aluminium sulphate can be used as an inorganic chemical flocculant for spirulina (*A. platensis*) without pH adjustments and rotational inputs to facilitate coagulation and flocculation. Compared to previous studies the dosage is lower but results in faster flocculation in 15 to 45 minutes post-administration. Moreover, the combination of ascorbic acid and alpha-tocopherol at 0.01% v/v may help preserve spirulina cells under refrigerated conditions for up to 3 weeks. This may help in maintaining viable starters. However, further investigations are needed to ascertain residuals of aluminium in harvested biomass and explore low-cost options for its reduction or removal and optimize the use of antioxidants in spirulina preservation in refrigerated conditions.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

AUTHORS' CONTRIBUTION

All authors equally participated in the design and conduct of the experiment, data analysis and preparation of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on a reasonable request from the corresponding author.

REFERENCES

Apostol G, Kouachi R, Constantinescu I (2011) Optimization of coagulation-flocculation process with aluminum sulfate based on response surface methodology. UPB Buletin Stiintific Series B: Chemistry and Materials Science 73(2): 77–84.

Belay A, Kato T, Ota Y (1997) Spirulina (Arthrospira): potential application as an animal feed supplement. Journal of Applied Phycology 8: 303–311.

Ciani M, Lippolis A, Fava F, Rodolfi L, Niccolai A, Tredici MR (2021) Microbes: food for the future. Foods 10(5): 971.

Dassey AJ, Theegala CS (2013) Harvesting economics and strategies using centrifugation for cost effective separation of microalgae cells for biodiesel applications. Bioresource Technology 128: 241–245.

de la Peña MR, Franco AV (2020) Production of microalgal paste in the Philippines (pp. 67–74). In: Martinez-Goss MR, Rivera WL, Torreta NK (Eds) Methods in microalgal studies. Los Baños, Laguna, Philippines: Philippine Science Letters; University of the Philippines Los Baños.

FAO (2020) The state of world fisheries and aquaculture 2020. Sustainability in action. Rome, Italy. 244 pp.

Ferriols VMEN, Aguilar RO (2012) Efficiency of various flocculants in harvesting the green microalgae *Tetrahelmis tetrahele* (Chlorodendrophyceae: Chlorodendraceae). AACL Bioflux 5(4): 265–273.

Fuad N, Omar R, Kamarudin S, Harun R, Idris A, WAKG WA (2018) Mass harvesting of marine microalgae using different techniques. Food and Bioproducts Processing 112: 169–184.

Gabelich CJ, Ishida KP, Gerringer FW, Evangelista R, Kalyan M, Suffet IH (2006) Control of residual aluminum from conventional treatment to improve reverse osmosis performance. Desalination 190: 147–160.

Gani P, Sunar NM, Matias-Peralta H, Mohamed RMSR, Latiff AAA, Parjo UK (2017) Extraction of hydrocarbons from freshwater green microalgae (*Botryococcus* sp.) biomass after phycoremediation of domestic wastewater. International Journal of Phytoremediation 19(7): 679–685.

Govoreanu R, Apostol G, Costache C, Codescu A, Stănescu R, Constantinescu I (2001) Determination of optimal coagulant dosing rate using empirical models (pp. 207–212). In: Proceedings of 12th Romanian International Conference on Chemistry and Chemical Engineering, Bucharest, Romania.

Ismail I, Kurnia KA, Samsuri S, Bilad MR, Marbelia L, ... Susilawati S (2021) Energy efficient harvesting of *Spirulina* sp. from the growth medium using a tilted panel membrane filtration. Bioresource Technology Reports 15: 100697.

Jung F, Krüger-Genge A, Waldeck P, Küpper JH (2019) *Spirulina platensis*, a super food? Journal of Cellular Biotechnology 5: 43–54.

Kim SG, Choi A, Ahn CY, Park CS, Park YH, Oh HM (2005) Harvesting of *Spirulina platensis* by cellular flotation and growth stage determination. Letters in Applied Microbiology 40(3): 190–194.

Lin JL, Huang C, Pan JR, Wang D (2008) Effect of Al(III)

speciation on coagulation of highly turbid water. Chemosphere 72: 189–196.

Liu TK, Chin CJM (2009) Improved coagulation performance using preformed polymeric iron chloride (PICl). Colloids and Surfaces A: Physicochemical and Engineering Aspects 339(1–3): 192–198.

Mihai M, Dabija G (2008) Cationic polyelectrolytes – anionic surfactant complexes used in the coagulation processes. UPB Scientific Bulletin Series B 70(4): 29–36.

Mohseni F, Moosavi Zenooz A (2022) Flocculation of Chlorella vulgaris with alum and pH adjustment. Biotechnology and Applied Biochemistry 69(3): 1112–1120.

Papazi A, Makridis P, Divanach P (2010) Harvesting Chlorella minutissima using cell coagulants. Journal of Applied Phycology 22: 349–355.

Pienkos P, Darzins A (2009) The promise and challenges of microalgal-derived biofuels. Biofuels, Bioproducts and Biorefining 3: 431–440.

Pulz MO, Gross W (2004) Valuable products from biotechnology of microalgae. Applied Microbiology Biotechnology 6: 635–648.

Ramírez-Rodrigues MM, Estrada-Beristain C, Metri-Ojeda J, Pérez-Alva A, Baigts-Allende DK (2021) *Spirulina platensis* protein as sustainable ingredient for nutritional food products development. Sustainability 13(12): 6849.

Richmond A (2004) Handbook of microalgal culture: biotechnology and applied phycology. Blackwell Science Ltd, Oxford, UK.

Silva SC, Ferreira IC, Dias MM, Barreiro MF (2020) Microalgae-derived pigments: a 10-year bibliometric review and industry and market trend analysis. Molecules 25(15): 3406.

Soni RA, Sudhakar K, Rana RS (2017) Spirulina - from growth to nutritional product: a review. Trends in Food Science and Technology 69: 157–171.

Soni RA, Sudhakar K, Rana RS, Baredar P (2021) Food supplements formulated with *Spirulina*. In: Mandotra SK, Upadhyay AK, Ahluwalia AS (Eds) Algae. Springer, Singapore.

Thevarajah B, Nishshanka GKSH, Premaratne M, Nimarshana PHV, Nagarajan D, ... Ariyadasa TU (2022) Large-scale production of spirulina-based proteins and c-phycocyanin: a biorefinery approach. Biochemical Engineering Journal 185: 108541.

Tiburcio PC, Galvez FCF, Cruz LJ, Gavino VC (2007) Determination of shelf life of *Spirulina platensis* (MI2) grown in the Philippines. Journal of Applied Phycology 19: 727–731.

Vasistha S, Khanra A, Clifford M, Rai MP (2021) Current advances in microalgae harvesting and lipid extraction processes for improved biodiesel production: a review. Renewable and Sustainable Energy Reviews 137: 110498.

Vonshak A (1997) *Spirulina Platensis* Arthrospira: physiology, cell biology and biotechnology, 1st edition. CRC Press, Florida, USA.

Zhu L, Hu T, Li S, Nugroho YK, Li B, ... Hiltunen E (2020) Effects of operating parameters on algae *Chlorella vulgaris* biomass harvesting and lipid extraction using metal sulfates as flocculants. Biomass and Bioenergy 132: 105433.

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