Harvesting *Porphyridium purpureum* using polyacrylamide polymers and alkaline bases and their impact on biomass quality

Revision Submitted to

Science of the Total Environmental

September 2020

Hang P. Vu[a], Luong N. Nguyen[a*], Minh T. Vu[a], Leen Labeeuw[b], Benjamin Emmerton[b] Audrey S. Commault[b], Peter J. Ralph[b], T.M.I. Mahlia[c] and Long D. Nghiem[a,d]

[a] Center for Technology in Water and Wastewater, School of Civil and Environmental Engineering, University of Technology Sydney, Sydney, NSW 2007, Australia

[b] University of Technology Sydney, Climate Change Cluster, Faculty of Science, Sydney, NSW 2007, Australia

[c] School of Information, Systems and Modelling, Faculty of Engineering and Information Technology, University of Technology Sydney, NSW, 2007, Australia

[d] Nguyen Tat Thanh University, NTT Institute of Hi-Technology, Ho Chi Minh City, Vietnam

[*]Corresponding author:

Luong N. Nguyen: Centre for Technology in Water and Wastewater, School of Civil and Environmental Engineering, University of Technology Sydney, NSW 2007, Australia

Phone: (+61) 468863865 E-mail: luongngoc.nguyen@uts.edu.au

Abstract

This study aims to examine the flocculation efficiency of *Porphyridium purpureum* (i.e. a red marine microalga with high content of pigments and fatty acids) grown in seawater medium using polyacrylamide polymers and alkaline flocculation. Polymers Flopam^{TM} and FO3801 achieved the highest flocculation efficiency of over 99% at the optimal dose of 21 mg per g of dry biomass through charge neutralisation and bridging mechanism. The addition of sodium hydroxide, potassium hydroxide, and sodium carbonate also achieved flocculation efficiency of 98 and 91%, respectively, but high doses were required (i.e. > 500 mg per g of dry biomass). Calcium hydroxide was not as effective and could only achieve 75% flocculation efficiency. Precipitation of magnesium hydroxide was identified as the major cause of hydroxide-induced flocculation. On the other hand, sodium carbonate addition induced flocculation via both magnesium and calcium carbonate co-precipitation. The large mass of precipitates caused a sweeping effect and enmeshed the microalgal cells to trigger sedimentation. Cell membrane integrity analysis of flocculated *P. purpureum* indicated that polyacrylamide polymers led to significant compromised cells (i.e. 96%), compared to the alkaline bases (70-96% compromised cells). These results appear to be the first to demonstrate the high efficiency of polyacrylamide polymer and alkaline flocculation of *P. purpureum* but at the expense of the biomass quality.

Keywords: *Porphyridium purpureum*; Flopam; Alkaline flocculation; Cell membrane integrity; Algae harvesting.

1. Introduction

Microalgae have emerged as a promising platform to produce renewable feedstock for biorefinery applications (Kumar et al., 2020a), remove nutrient from wastewater (Hom-Diaz et al., 2017; Nguyen et al., 2020; Tolboom et al., 2019), and sequester carbon dioxide from flue gas (Cheng et al., 2019; Yadav et al., 2019). *Porphyridium purpureum* is a red marine microalga notable for its high content of valuable biochemicals such as red pigments (e.g. phycoerythrin), phycobiliproteins, polyunsaturated fatty acids, and exopolysaccharides (Di Lena et al., 2020; Gaignard et al., 2019; Kavitha et al., 2016). This species is particularly high in phycoerythrin, a water-soluble bioactive compound with anti-inflammatory, immunosuppressive, and antioxidant properties (Sosa-Hernández et al., 2019). The cultivation of *P. purpureum* is well understood and can be easily performed in seawater medium, thus eliminating the need for arable lands. However, microalgal biomass harvesting at large-scale remains a challenge to the overall economic viability of *P. purpureum* cultivation. *P. purpureum* cells are about 12 µm in diameter and have almost the same density as water. At stationary phase, a *P. purpureum* culture has a biomass content of 0.5 to 2 g/L, therefore intense dewatering is needed to harvest the biomass (Aizdaicher et al., 2014; Oh et al., 2009; Singh and Patidar, 2018).

Microalgal harvesting is an important step in the supply chain of algal biotechnology. It accounts for up to 30% of the total processing cost (Singh and Patidar, 2018). Common harvesting methods include: centrifugation, membrane filtration, flocculation, and flotation

(Kumar et al., 2020b; Singh and Patidar, 2018). Centrifugation can recover high (>90%) microalgal biomass concentration, but significant energy consumption is a drawback (Singh and Patidar, 2018). Membrane filtration is an emerging technology that still needs to overcome the issue of membrane fouling and high maintenance cost (Singh and Patidar, 2018). Among these methods, flocculation has proven to be an energy efficient, environmental-friendly and effective approach to harvest a wide range of microalgae (Fasaei et al., 2018; Nguyen et al., 2019). Nonetheless, the selection of harvesting methods is dependent on: i) the type of microalga and its characteristics (e.g. size and growth medium), and ii) the desired compounds to be extracted from the algal biomass.

Monitoring cell membrane integrity during harvesting is important as valuable intracellular compounds (e.g. pigments and fatty acids) can be lost if the cell membrane is damaged during the harvesting process. Due to the absence of a rigid cell wall, *P. purpureum* is likely to be susceptible to cell membrane damage (Heaney-Kieras and Chapman, 1976; Kendir Çakmak and Ugurlu, 2020). This particular species is encapsulated within a layer of gelatinous polysaccharide matrix (i.e. extracellular polymeric substances (EPS)) (Geresh et al., 2002; Heaney-Kieras and Chapman, 1976). This EPS layer contains proteins, sulfate, xylose, galactose, glucose, and glucuronic acids (Kendir Çakmak and Ugurlu, 2020). During growth in aerated cultures and harvesting process, it is expected that the EPS will partially dissolve into the medium (Heaney-Kieras and Chapman, 1976). Harvesting methods may introduce hydraulic forces (e.g. differential pressure on two sides of membrane filtration or radial centrifugal forces exerted on biomass during centrifugation) and chemical bonding or bridging (i.e. flocculation) to the cells that can potentially damage the cell membrane. Compromised (i.e. damaged) cell membrane could lead to intracellular leakage. The effects of harvesting methods on *P. purpureum* cell membrane are still largely unknown.

P. purpureum biomass is cultivated in seawater culture medium that contains a large amount of alkaline earth metal ions such as Mg^{2+} and Ca^{2+}. At high pH >9 and under atmospheric conditions, these ions can precipitate as magnesium hydroxide and calcium carbonate (Besson and Guiraud, 2013; Mayers et al., 2020; Vandamme et al., 2015). The large mass of precipitates, while settling down due to gravitational force, entangles with the algal cells by a sweeping effect. The sedimentation of the algal cells is thus facilitated. Alkaline flocculation has been reported for seawater and freshwater microalgal cultures (e.g. *Phaeodactylum tricornutum*, *Chlorella vulgaris, Scenedesmus* sp., and *Nannochloropsis oculata*) (Vandamme et al., 2015; Wu et al., 2012) and a *Dunaliella salina* hypersaline culture (Besson and Guiraud, 2013). However, it has not been studied on a *P. purpureum* culture.

Recent studies have also demonstrated the effectiveness of cationic polyacrylamide polymers as flocculants for microalgae harvesting from freshwater and seawater cultures (Nguyen et al., 2019; Vu et al., 2020). These previous studies have not examined the effectiveness of polyacrylamide polymer for *P. purpureum* harvesting. Due to the specific composition and structure of the cell membrane of *P. purpureum*, it is necessary to elucidate the effect of flocculation on cell integrity to assess the practicality of this harvesting method for *P. purpureum*.

This study aims to investigate the harvesting performance of *Porphyridium purpureum* in seawater medium using: (a) polyacrylamide polymers Flopam^{TM} and FO3801, and (b) alkaline flocculation at high pH through the addition of common bases (i.e. sodium hydroxide, potassium hydroxide, calcium hydroxide and sodium carbonate). Flocculation experiments are further conducted in saltwater medium lacking calcium or magnesium to determine the influence of these cations on the harvesting efficiency of *P. purpureum.* Cell membrane integrity analysis was performed to examine the impact of polyacrylamide polymers and the alkaline bases on the quality of the microalgal cells after flocculation. The new understanding of the floc harvesting of *P. purpureum* in this study will contribute to the process optimisation of biorefinery for a wider range of microalgal species.

2. Materials and methods

2.1. Microalgae strains and growth conditions

The marine red microalgae *Porphyridium purpureum* was obtained from the Australian National Algae Collection at CSIRO Microalgae Research (Hobart, Tasmania, Australia). It was maintained in marine f/2 media (Guillard, 1975) using 0.22 µm filtered autoclaved seawater collected from Sydney Harbour (salinity of 33-35 g/L). The chemical composition of the seawater medium was analysed using Agilent Microwave Plasma-Atomic Emission Spectrometry (Section 2.4). Stock cultures were maintained at the Climate Change Cluster (C3, University of Technology Sydney).

The *P. purpureum* culture for flocculation experiments was scaled-up from a 1 L Schott's bottle to a 350 L bag following the procedure described in previous studies (Labeeuw et al., 2021; Nguyen et al., 2019). The bag bioreactor was bubbled with air through air lines on either side of the bioreactor and maintained at 23 °C and 400 μmol photons/m^2/s light in a 16:8 hour light:dark cycle. The seawater medium for the large-scale bioreactor was first sterilized by the addition of 100 mL of 12% sodium hypochlorite, followed by 100 mL of 2 M sodium thiosulphate. Filter sterilized stock components of f/2 media for marine water were then added. The pH of the algal culture was checked twice a day and maintained below 9.3 by carbon dioxide sparging. This cultivation protocol was developed at the Climate Change Cluster facility (University of Technology Sydney, Australia). Microalgal suspension at mid-stationary growth phase was used for flocculation experiments as it was previously determined to be the best growth phase for harvesting other species (Section 2.2 & 2.3) (Labeeuw et al., 2021).

2.2. *P. purpureum* flocculation

2.2.1. Experimental set up

The flocculation experiments were conducted using a 4G Platypus Jar Tester (Australia Scientific, Kotara NSW Australia). Samples of 500 mL *P. purpureum* suspension were added to 2 L beakers. The jar test was carried out following the procedure from Vu et al. (2020). The microalgal suspension was rapidly mixed at 200 rpm for one minute followed by slow mixing at 50 rpm for 15 min. The flocculated microalgal biomass was allowed to settle for one hour. To measure the flocculation efficiency, 15 mL of the supernatant was pipetted from the suspension at between one- and two-third from the bottom. The optimal flocculant dose was determined by a dose-response relationship protocol (Section 2.4). All experiments were conducted in three technical replicates using one biological replicate of the microalga.

2.2.2. Flocculants and chemicals preparation

Two cationic polyacrylamide flocculants (FO3801 and Flopam™) with high-charge (>80% charge), high-molecular weight (>15 Megadalton) (SNF Pty Ltd; Corio, VIC, Australia) were used in the first set of flocculation experiments. A stock solution of each polymer (2 g/L) was prepared in accordance to Vu et al. (2020) and used within one day of preparation. FO3801 and Flopam™ were dosed at a concentration of 5 to 20 mg/L microalgal suspension (i.e. 7 to 36 mg polymer/g dry biomass), followed by the jar test. The flocculation efficiency was determined using optical density measurement as described in section 2.4.

Solutions of 0.1 M sodium hydroxide, potassium hydroxide, calcium hydroxide and sodium carbonate were prepared for alkaline flocculation experiments. These chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). The pH of the *P. purpureum* suspensions was adjusted to 9.5, 10 and 10.5 using the alkaline solutions, followed by the jar tests. The volume of 0.1 M stock solution required to raise the pH to the desired level was recorded for each alkali. The flocculation efficiency was calculated as described in section 2.4.

2.3. Effect of cations on *P. purpureum* flocculation

The mechanisms governing the flocculation of marine *P. purpureum* in seawater culture through pH adjustment using 0.1 M sodium hydroxide and sodium carbonate were investigated. These bases represent widely available and effective approaches to increase the pH of the solution i.e. sodium hydroxide releases hydroxide ions while sodium carbonate removes hydrogen atoms from the suspension. Since magnesium and calcium cations are dominant elements in seawater medium, their relative importance to the alkaline flocculation of *P. purpureum* using two different bases was evaluated. The cation Na^+, Mg^{2+}, Ca^{2+}, and K^+ concentrations in the seawater medium were 10.55, 1.36, 0.44, and 0.46 g/L, respectively.

P. purpureum suspensions of 35 mL volume were centrifuged at 4000*g* for 10 min to separate the biomass from the initial medium. The resultant biomass was rinsed gently with Milli-Q water to remove residual medium and resuspended in a new medium of 35 mL containing 38 g/L NaCl to maintain the equivalent Na^+ level (10.55 g/L) as in the initial medium. Likewise, $MgSO_4$ was added to the new medium (i.e. contain only NaCl) to maintain Mg^{2+} concentration of 1.36 g per litre of algal suspension. This experiment was to investigate the role of magnesium in alkaline flocculation. In another new NaCl medium containing algal biomass, calcium chloride was dosed at 0.44 g Ca^{2+} per litre of algal suspension to study the role of calcium in alkaline flocculation. These concentrations of Mg^{2+} and Ca^{2+} corresponds to their concentration in the initial microalgal seawater medium. The alkaline flocculation at pH 10.5 using sodium hydroxide and sodium carbonate were carried out as described in section 2.2. The initial microalgal suspension was used as the control. The description of the samples is provided in Table 1.

Table 1: Samples of 35 mL (incl. 2 technical replicates) for studying the influence of calcium and magnesium in *P. purpureum* alkaline flocculation

Assay	Sample name	Description	Dosage (g/g dry biomass)
1	Control	Initial algal suspension without chemical addition	Nil
2	1 (NaOH)	Algal suspension subjected to NaOH induced flocculation	0.57 (NaOH)
3	2 (Na_2CO_3)	Algal suspension subjected Na_2CO_3 induced flocculation	4.5 (Na_2CO_3)
4	Mg Control	Suspended algal biomass in a $MgSO_4$ + NaCl medium	9.6 ($MgSO_4$) 38.3 (NaCl)
5	Mg (NaOH)	Suspended algal biomass in $MgSO_4$ + NaCl medium subjected to NaOH flocculation	
6	Mg (Na_2CO_3)	Suspended algal biomass in $MgSO_4$ + NaCl medium subjected to Na_2CO_3 flocculation	
7	Ca Control	Suspended algal biomass in a $CaCl_2$ + NaCl medium	1.7 ($CaCl_2$) 38.3 (NaOH)
8	Ca (NaOH)	Suspended algal biomass in $CaCl_2$ + NaCl medium subjected to NaOH flocculation	
9	Ca (Na_2CO_3)	Suspended algal biomass in $CaCl_2$ + NaCl medium subjected to Na_2CO_3 flocculation	

2.4. Analytical methods

The optical density of the microalgae medium before and after flocculation was measured by spectrophotometer (UV 6000 Shimadzu) at wavelength of 730 nm.

The flocculation efficiency was calculated based on the change in the optical density of the suspension before and after flocculation occurs, as shown in the following equation.

$$\text{Flocculation efficiency (\%)} = \left(\frac{OD_{i} - OD_{f}}{OD_{i}}\right) \times 100$$

Where OD_i and OD_f is the optical density of the culture before and after flocculant addition.

The *P. purpureum* biomass concentration was determined gravimetrically. A 150 mL sample of microalgae suspension was filtered through a 1.1 μm pre-weighed glass fiber filter paper. The weight of the final filter paper after 12 h drying at 60 °C was used to calculate the dry microalgal biomass.

The solution pH was measured using a pH/conductivity meter (Orion 4-Star Plus Thermo Scientific; Waltham, MA, USA).

Statistical analysis of flocculation efficiency and biomass quality measurements was performed in Microsoft Excel using Student's unpaired *t*-Test, with a two-tailed distribution.

The chemical analysis (Mg, Ca, K, Na) was conducted using Microwave Plasma Atomic Emission Spectrometry (MP-AES, Agilent). The sample was diluted 1000 times (i.e. 50 µL stock into 49.95 mL Milli-Q water) before the analysis.

Cell membrane integrity of the flocculated *P. purpureum* biomass under conditions described in Section 2.2 was determined in an endpoint assay using Celltox Green kit (Promega; Madison, WI, USA) and CytExpert v2.4 (flow cytometer, Becton, Dickinson and Company). This assay measures the loss of cell membrane integrity using a non-toxic dye that can enter a damaged cell membrane to bind to the DNA. Intact algal cells have a lower amount of fluorescence as the dye cannot enter the cells. Damaged and intact cells are then counted by flow cytometry.

3. Results and Discussion

3.1. *P. purpureum* characteristics and floc formation

The biomass concentration of the *P. purpureum* suspension used in this study was determined gravimetrically to be 0.7 g/L. The initial optical density (730 nm) was 0.601. The algal suspension had a pH of 8.9.

Differences in the morphology of the flocs from polyacrylamide polymers and alkaline flocculation at optimal doses were visually observed (Fig. 1). Polyacrylamide polymers (Flopam^{TM} and FO3801) flocculated the microalgal cells into a large clump. The clump settled to the bottom of the beaker and the clear supernatant was observed (Fig. 1b). It is relatively easy to collect the floc formed by polyacrylamide polymer from the solution through a strainer. On the other hand, alkaline chemicals (i.e. sodium hydroxide, calcium hydroxide, and calcium carbonate) induced a foamy and powdery layer of flocculated biomass at the bottom of the beakers (Fig. 1b). This layer can be easily disintegrated, making algal biomass recovery difficult. The appearance of the supernatant also varied among different types of alkaline flocculation. Dosing *P. purpureum* suspension with sodium hydroxide achieved a much clearer supernatant than when using calcium hydroxide and sodium carbonate (Fig. 1b). The supernatant from calcium hydroxide flocculation still contained an amount of *P. purpureum* microalgal cells as suggested by its red colour and low flocculation efficiency (75%) at the optimal pH of 10.5 (Fig. 1b). Sodium carbonate induced flocculation caused the supernatant to become cloudy due to the precipitation of calcium carbonate (Fig. 1b).

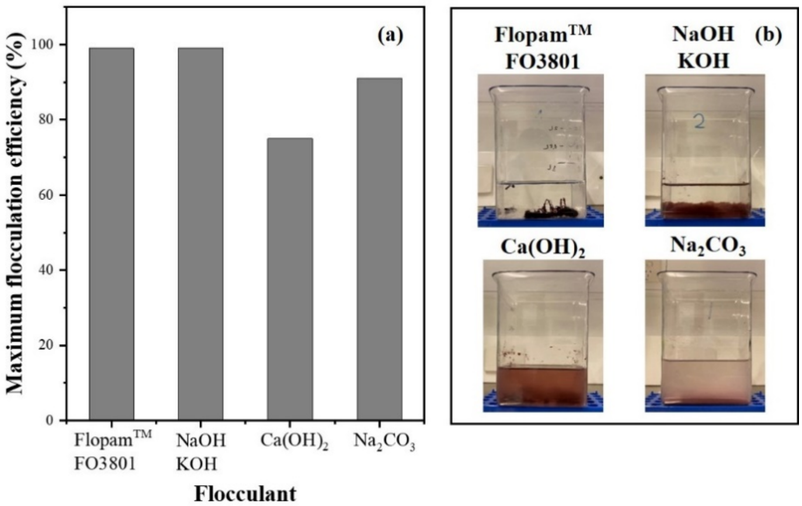

Figure 1: (a) The maximum *P. purpureum* flocculation efficiency achieved by dosing polyacrylamide polymers (21 mg/g dry biomass), NaOH (571 mg/g dry biomass), $Ca(OH)_2$ (875 mg/g dry biomass), and Na_2CO_3 (4,542 mg/g dry biomass) and (b) their corresponding floc formations observed visually. Values represent mean (*n = 2,* technical replicates).

3.2. *P. purpureum* flocculation using polyacrylamide polymers

Both polyacrylamide polymers in this study show high *P. purpureum* flocculation efficiency (Fig. 2). Flocculation efficiency of 80 and 97% was observed for low doses of 7 and 14 mg polymer per g dry biomass, respectively. Flocculation efficiency was over 99% for both FO3801 and Flopam^{TM} at the optimal dose of 21 mg polymer per g dry biomass. The further increase in polymer doses did not improve the flocculation efficiency of the *P. purpureum* suspension (Fig. 2). Overdosing polymers may cause a counteractive effect (i.e. reduced flocculation efficiency and increased polymer residual in the medium) on the flocculation efficiency (Nguyen et al., 2019; Vu et al., 2020).

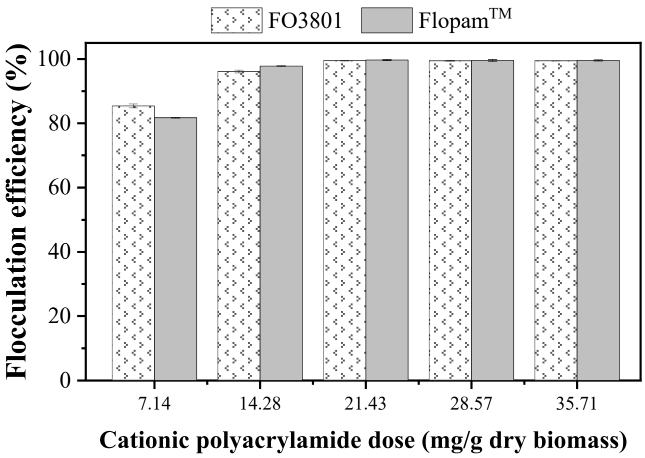

Figure 2: Flocculation efficiency of *P. purpureum* using cationic polyacrylamide polymers Flopam™ and FO3801. Value and error bars represent mean and standard deviation (*n = 3 technical replicates*).

Polyacrylamide polymers neutralise the negatively charged microalgal cells due to their highly charged and cationic characteristic. When algal cells lose their negative surface charge, electrostatic repulsion force between the cells diminish and flocculation can occur (Nguyen et al., 2019). These polyacrylamide polymers are also high molecular weight polymers (MW = 15 MDa) to facilitate entanglement and bridging with the algal cells to form large and stable flocs (Fig. 1b). The excellent performance of FO3801 and Flopam™ for other microalgae species has been reported in the literature (Nguyen et al., 2019; Vu et al., 2020). The optimal doses to achieve 90-99% flocculation efficiency for *Chlorella vulgaris* and *Phaeodactylum tricornutum* were 18.9 and 13.7 mg polymer/g dry biomass, respectively (Nguyen et al., 2019). In this study, similar doses (14-21 mg polymer/g dry biomass) were required to obtain 97-99% flocculation efficiency for *P. purpureum.* This demonstrated that marine *P. purpureum* can be effectively harvested from seawater culture using polyacrylamide polymers at a low dose.

3.3. Alkaline flocculation of *P. purpureum*

The alkaline flocculation of marine *P. purpureum* was pH-dependant. The flocculation efficiency was low (i.e. 11%) at pH 9 for all samples using sodium hydroxide, potassium hydroxide, calcium hydroxide and sodium carbonate (Fig. 3). This is because the initial pH of the algal suspension was pH 8.9. When the pH was increased to 9.5, 10, and 10.5 using sodium hydroxide or potassium hydroxide, a significant improvement in the harvesting performance was observed (Fig. 3). The flocculation efficiency was 51.5, 65.3, and 97.5% for pH 9.5, 10, and 10.5, respectively. Thus, the optimal pH to obtain the highest efficiency for sodium hydroxide and potassium hydroxide induced flocculation was pH >10.5. Calcium hydroxide was not as effective at inducing microalgal agglomeration. The flocculation efficiency was 20 and 50% when the pH was raised to 9.5 and 10, respectively, using calcium hydroxide. The maximum flocculation efficiency obtained was 75% at pH 10.5. In terms of sodium carbonate, the alkaline flocculation remained low (11%) at pH 9 and 9.5. It started to significantly increase when more sodium carbonate was added to reach pH >10. The highest flocculation efficiency of 91% was recorded at pH 10.5.

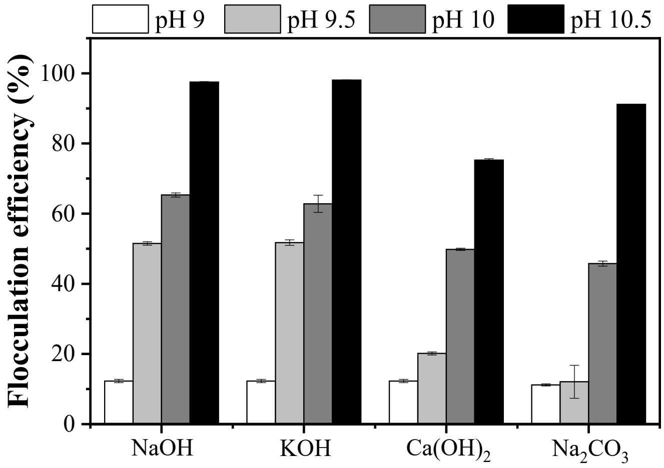

Figure 3: *P. purpureum* alkaline flocculation efficiency using: sodium hydroxide (NaOH), potassium hydroxide (KOH), calcium hydroxide ($Ca(OH)_2$), and sodium carbonate (Na_2CO_3). Value and error bars represent mean and standard deviation (*n = 3* technical replicates).

The dose of alkaline bases necessary to increase the pH and induce effective microalgal flocculation is an important factor in considering large-scale applications. Sodium hydroxide and potassium hydroxide required the lowest doses (i.e. 571 and 800 mg chemical/g dry biomass, respectively) to achieve the highest flocculation efficiency of 98% at pH 10.5 (Table 2). Sodium carbonate showed effective performance (i.e. 91% flocculation efficiency) at pH 10.5 but required a very high dose of 4,542 mg chemical/g of algal suspension.

Table 2: Concentration of bases required to adjust the pH to desired values.

pH	Alkaline	Concentration (mg chemical/g dry algal biomass)
9.5	NaOH	194
	KOH	271
	Na_2CO_3	861
10	NaOH	308
	KOH	431
	Na_2CO_3	2482
10.5	NaOH	571
	KOH	800
	Na_2CO_3	4542

The bases (i.e. sodium hydroxide, potassium hydroxide, and calcium hydroxide) studied in this paper were chosen because of their accessibility and common use as pH adjusting agents (Vandamme et al., 2012). However, they are hazardous chemicals that can impact the quality of the harvested microalgae biomass (i.e. loss of lipid and fatty acid contents), thus limiting its industrial applications (Borges et al., 2016). Sodium carbonate was investigated in this study as a widely available, and less hazardous alternative to sodium hydroxide. Since sodium carbonate also provides an inorganic carbon source for algal growth (Duan et al., 2020), there is the potential of recycling the supernatant as culture media.

3.4. The role of cations in *P. purpureum* flocculation

The results showed a 26% of Mg^{2+} and 50% of Ca^{2+} reduction in the medium after sodium hydroxide or sodium carbonate flocculation occurred at pH 10.5 (Fig. 4). The flocculation efficiency was >99% and 78% using sodium hydroxide and sodium carbonate, respectively. This observation indicates that magnesium and calcium salt precipitation at high pH can lead to good alkaline flocculation of *P. purpureum* in a seawater medium. The large mass of metal precipitate rapidly forming and settling induced the sweeping flocculation of the microalgae cells (Besson and Guiraud, 2013; Vandamme et al., 2012). When the sweeping phenomena occurred, microalgal cells were enmeshed in the precipitate and settled. This explains the layer of powdery flocculated *P. purpureum* observed on the bottom of the beaker after pH adjustment (Fig. 1b). In the medium containing the same concentration of Na^2, $Mg^{2+,}$ and Ca^{2+} but without algal biomass, the precipitation and sedimentation of magnesium and calcium salts were also observed.

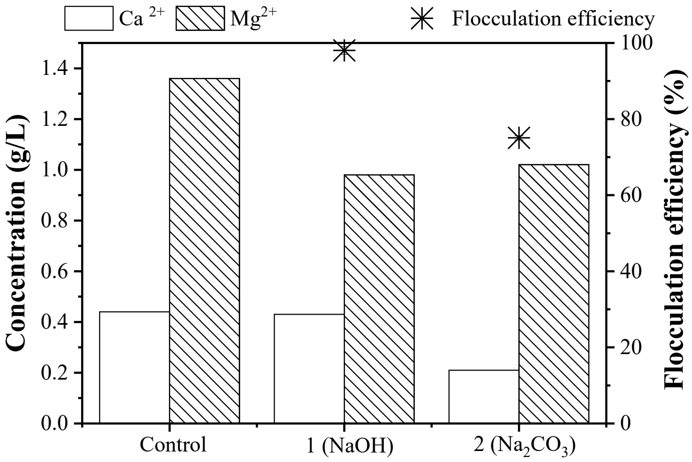

Figure 4: The change in magnesium and calcium concentration in the microalgal solution (supernatant) due to alkaline flocculation. Values represent mean (*n = 2,* technical replicates).

The role of magnesium and calcium precipitation in microalgal flocculation depends on the alkaline base (Fig. 5). In the new medium containing Na^+ (10.55 g/L) and Mg^{2+} (1.36 g/L), sodium hydroxide was able to achieve effective flocculation (i.e. 94%) (Fig. 5a). A 47% reduction in magnesium concentration in the medium was recorded. On the other hand, flocculation did not occur when sodium hydroxide was added to the medium containing only calcium (Fig. 5b). The reduction in calcium concentration in the medium was also minimal (i.e. 5%). This indicates that the main cause for flocculation of *P. purpureum* by sodium hydroxide is the precipitation of magnesium hydroxide. Meanwhile, sodium carbonate caused magnesium carbonate precipitation in medium containing only magnesium, thus a 39% of Mg^{+} reduction (Fig. 5a). However, magnesium carbonate is a white solid that can affect the light absorbance measurement. This caused the flocculation efficiency induced by sodium carbonate to be significantly lower (i.e. 60%) than that of sodium hydroxide (i.e. 94%). In the medium containing Na^{+} (10.55 g/L) and Ca^{2+} (0.44 g/L), flocculation efficiency of 75% was observed for sodium carbonate. This is due to the 51% reduction in calcium concentration, which had precipitated out of the medium in the form of calcium carbonate (Fig. 5b). Carbonate precipitates caused the supernatant to be cloudy, as observed in Fig. 1b. Thus, both magnesium carbonate and calcium carbonate are involved in sodium carbonate induced flocculation of marine *P. purpureum.*

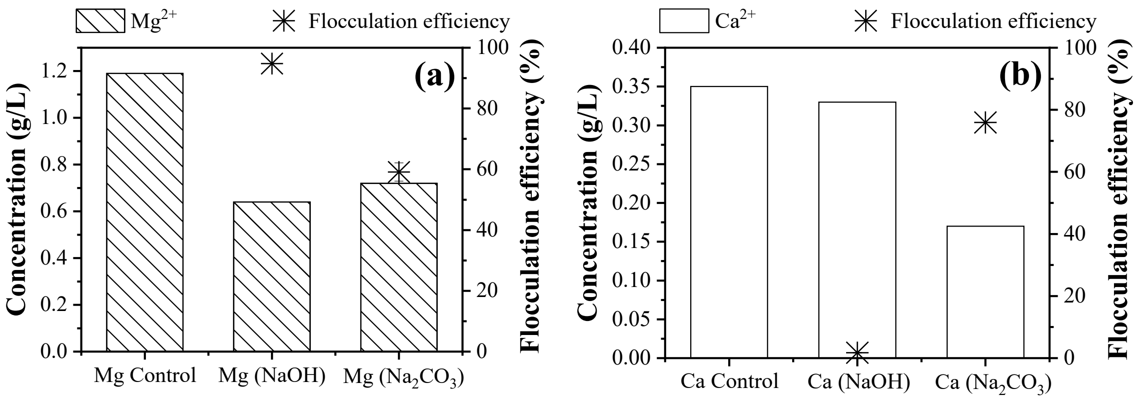

Figure 5: Alkaline flocculation efficiency of *P. purpureum* with respect to (a) medium containing sodium chloride and magnesium sulphate only, and (b) medium containing sodium chloride and calcium hydroxide only. Values represent mean (*n = 2,* technical replicates).

3.5. Biomass quality after flocculation

Polyacrylamide polymers and alkaline agents could potentially damage the cell wall of *P. purpureum*. The proportion of compromised cells was 96% and 68-96% for polyacrylamide polymer and alkaline bases, respectively (Fig. 6). This suggests that polyacrylamide polymers induced the highest degree of damage to *P. purpureum* cell membrane despite being the most efficient flocculants (> 99% flocculation efficiency). Sodium hydroxide and sodium carbonate caused high cell membrane damage at 68-70% but has the least impact on *P. purpureum* among the chemicals tested. A comparable observation was reported in a *Euglena gracilis* harvesting study where the sodium hydroxide induced flocculation at pH >10 caused the microalgal cells to be completely ruptured (Wu et al., 2020). On the other hand, Sales et al. (2019) revealed that *Nannochloropsis oculata* cells, a green marine microalga, were intact after being subjected to a three-step harvesting process: (1) pH-induced flocculation using sodium hydroxide, (2) Flopam FO4800 (1 mg/L), and (3) 6000*g* centrifugation. Harvested *N. oculata* showed 99-100% cell viability (i.e. 0-1% damaged cells compared to the fresh microalgal culture). Sales et al. (2019)'s results suggest that polymer and alkaline flocculation has a negligible effect on the microalgal cell membrane, which disagrees with our results. This is attributed to the different morphologies of *P. purpureum* and *N. oculata* (e.g. *N. oculata* has a thick cell wall resistant to shock while *P. purpureum* has no rigid cell wall) and the chemical doses used. The effect of polyacrylamide polymers and alkaline bases on cell membrane integrity is, therefore, dependant on the microalgal species, its cell wall characteristics, and operational parameters.

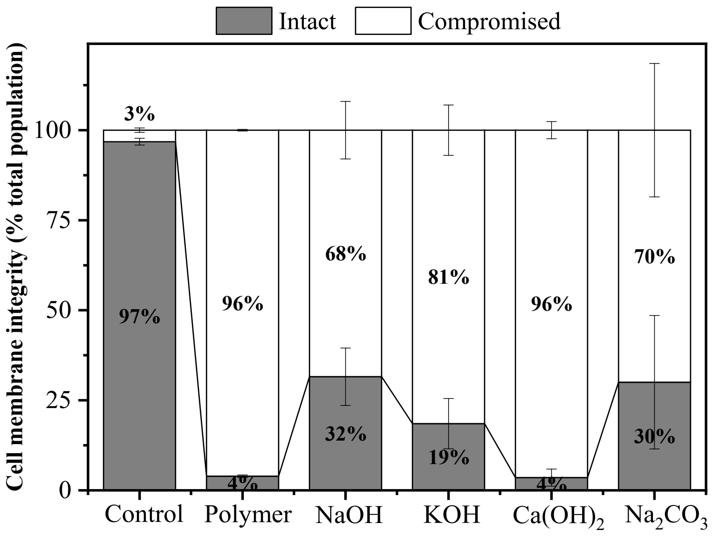

Figure 6: *P. purpureum* cell membrane integrity after flocculation (intact vs compromised). Value and error bars represent mean and standard deviation (*n* = *3*, technical replicates).

Polyacrylamide polymers (e.g. Flopam™ and FO3801) have high molecular weight and positive surface charge, thus strongly attracting the negatively charged EPS cell wall of *P. purpureum*. When the EPS is attached to the tails and loops of the polyacrylamide polymer, the structural arrangement of EPS on the microalgal cell wall is likely to be modified. This could lead to severe damages on the cell walls (e.g. the EPS gel no longer covers and protects the cells and the intracellular matters can leak through the cell wall). Meanwhile, after alkaline addition, extremely high pH (10-11) causes the EPS proteins to lose their natural shapes and the gelatinous EPS layer to be solubilised. These disruptions are facilitated through chemical degradation and ionisation of the hydroxyl group (OH -> O^-) (Wingender et al., 1999). The subsequent swelling and solubilisation of the EPS cell wall could cause the microalgal cell to lose its viability as it can no longer maintain an appropriate turgor pressure (i.e. the hydrostatic pressure within the cell against the cell wall) and disrupts the cell membrane (Neyens et al., 2004). The cell cytoplasm and nucleus are exposed to the environment, thus releasing intracellular components (e.g. organic matter and pigments) into the medium (Du et al., 2020).

Cell rupturing is an important step to extract valuable products (e.g. carbohydrates, lipids, and proteins) from microalgal biomass for biorefinery applications (Günerken et al., 2015). However, it should take place after biomass harvesting to minimise the processing volume (i.e. economic viability). In this study, polyacrylamide polymers induced *P. purpureum* floc formation that is dense and easy to remove (section 3.1). Their impact on the cell membrane was, however, the most severe with only 4% of the population remaining entirely intact after flocculation. Alkaline flocculation not only caused significant microalgal cell damage, its flocs contained a high concentration of salt precipitates and were difficult to collect from the medium (i.e. the settled biomass was highly disintegrated) (section 3.1). The composition of the biomass before and after flocculation, together with the extent of intracellular compound leakage will be a topic of interest for future study to further investigate the impact of these flocculants on biomass quality and potential end-products.

4. Conclusions

Polyacrylamide polymers effectively neutralise charge and bridge algal cells to flocculate over 99% of red marine *P. purpureum* at a low dose (i.e. 21 mg/g dry biomass). The floc formation was dense and easy to remove from the supernatant. However, cell membrane integrity results showed that polymers compromised the membrane of 96% of algal cells (i.e. the most negative impact on *P. purpureum* cells among the chemicals). Alkaline flocculation achieved up to 98% flocculation efficiency, but the alkaline doses were high at around 500 to 4,500 mg/g dry biomass. These high doses of alkali are not cost-effective and practical. In addition, the algal flocs induced by alkaline flocculation were powdery and disintegrated, making it harder for subsequent algal biomass recovery compared to polyacrylamide polymer flocs. Around 70% of microalgal cells were compromised after sodium hydroxide or carbonate addition, lower than that caused by polymers (96%), potassium hydroxide (81%), and calcium hydroxide (96%).

5. Acknowledgments

The authors acknowledged the scholarship support to Hang P. Vu by the University of Technology Sydney.

6. Declarations

The authors declare that there is no conflict of interest regarding the publication of this article.

References

Aizdaicher NA, Stonik IV, Boroda AV. The development of Porphyridium purpureum (Bory de Saint-Vincent) Drew et Ross, 1965 (Rhodophyta) from Amursky Bay, Sea of Japan, in a laboratory culture. Russian Journal of Marine Biology 2014; 40: 279-285.

Besson A, Guiraud P. High-pH-induced flocculation–flotation of the hypersaline microalga Dunaliella salina. Bioresource Technology 2013; 147: 464-470.

Borges L, Caldas S, Montes D'Oca MG, Abreu PC. Effect of harvesting processes on the lipid yield and fatty acid profile of the marine microalga Nannochloropsis oculata. Aquaculture Reports 2016; 4: 164-168.

Cheng D, Li X, Yuan Y, Yang C, Tang T, Zhao Q, et al. Adaptive evolution and carbon dioxide fixation of Chlorella sp. in simulated flue gas. Science of The Total Environment 2019; 650: 2931-2938.

Di Lena G, Casini I, Lucarini M, Sanchez del Pulgar J, Aguzzi A, Caproni R, et al. Chemical characterization and nutritional evaluation of microalgal biomass from large-scale production: a comparative study of five species. European Food Research and Technology 2020; 246: 323-332.

Du X, Tao Y, Liu Y, Li H. Stimulating methane production from microalgae by alkaline pretreatment and co-digestion with sludge. Environmental Technology 2020; 41: 1546-1553.

Duan Y, Guo X, Yang J, Zhang M, Li Y. Nutrients recycle and the growth of Scenedesmus obliquus in synthetic wastewater under different sodium carbonate concentrations. Royal Society open science 2020; 7: 191214.

Fasaei F, Bitter JH, Slegers PM, van Boxtel AJB. Techno-economic evaluation of microalgae harvesting and dewatering systems. Algal Research 2018; 31: 347-362.

Gaignard C, Gargouch N, Dubessay P, Delattre C, Pierre G, Laroche C, et al. New horizons in culture and valorization of red microalgae. Biotechnology Advances 2019; 37: 193-222.

Geresh S, Adin I, Yarmolinsky E, Karpasas M. Characterization of the extracellular polysaccharide of Porphyridium sp.: molecular weight determination and rheological properties. Carbohydrate Polymers 2002; 50: 183-189.

Günerken E, D'Hondt E, Eppink MHM, Garcia-Gonzalez L, Elst K, Wijffels RH. Cell disruption for microalgae biorefineries. Biotechnology Advances 2015; 33: 243-260.

Heaney-Kieras J, Chapman DJ. Structural studies on the extracellular polysaccharide of the red alga, Porhyridium. Carbohydrate Research 1976; 52: 169-77.

Hom-Diaz A, Jaén-Gil A, Bello-Laserna I, Rodríguez-Mozaz S, Vicent T, Barceló D, et al. Performance of a microalgal photobioreactor treating toilet wastewater: Pharmaceutically active compound removal and biomass harvesting. Science of The Total Environment 2017; 592: 1-11.

Kavitha MD, Kathiresan S, Bhattacharya S, Sarada R. Culture media optimization of Porphyridium purpureum: production potential of biomass, total lipids, arachidonic and eicosapentaenoic acid. Journal of Food Science and Technology 2016; 53: 2270-2278.

Kendir Çakmak E, Ugurlu A. Enhanced biogas production of red microalgae via enzymatic pretreatment and preliminary economic assessment. Algal Research 2020; 50: 101979.

Kumar M, Sun Y, Rathour R, Pandey A, Thakur IS, Tsang DCW. Algae as potential feedstock for the production of biofuels and value-added products: Opportunities and challenges. Science of The Total Environment 2020a; 716: 137116.

Kumar R, Ghosh AK, Pal P. Synergy of biofuel production with waste remediation along with value-added co-products recovery through microalgae cultivation: A review of membrane-integrated green approach. Science of The Total Environment 2020b; 698: 134169.

Labeeuw L, Commault AS, Kuzhiumparambil U, Emmerton B, Nguyen LN, Nghiem LD, et al. A comprehensive analysis of an effective flocculation method for high quality microalgal biomass harvesting. Science of The Total Environment 2021; 752: 141708.

Mayers JJ, Landels AR, Allen MJ, Albers E. An energy and resource efficient alkaline flocculation and sedimentation process for harvesting of Chromochloris zofingiensis biomass. Bioresource Technology Reports 2020; 9: 100358.

Neyens E, Baeyens J, Dewil R, De heyder B. Advanced sludge treatment affects extracellular polymeric substances to improve activated sludge dewatering. Journal of Hazardous Materials 2004; 106: 83-92.

Nguyen LN, Labeeuw L, Commault AS, Emmerton B, Ralph PJ, Johir MAH, et al. Validation of a cationic polyacrylamide flocculant for the harvesting fresh and seawater microalgal biomass. Environmental Technology & Innovation 2019; 16: 100466.

Nguyen LN, Truong MV, Nguyen AQ, Johir MAH, Commault AS, Ralph PJ, et al. A sequential membrane bioreactor followed by a membrane microalgal reactor for nutrient removal and algal biomass production. Environmental Science: Water Research & Technology 2020; 6: 189-196.

Oh SH, Han JG, Kim Y, Ha JH, Kim SS, Jeong MH, et al. Lipid production in Porphyridium cruentum grown under different culture conditions. Journal of Bioscience and Bioengineering 2009; 108: 429-434.

Sales R, Derner RB, Tsuzuki MY. Effects of different harvesting and processing methods on Nannochloropsis oculata concentrates and their application on rotifer Brachionus sp. cultures. Journal of Applied Phycology 2019; 31: 3607-3615.

Singh G, Patidar SK. Microalgae harvesting techniques: A review. Journal of Environmental Management 2018; 217: 499-508.

Sosa-Hernández JE, Rodas-Zuluaga LI, Castillo-Zacarías C, Rostro-Alanís M, de la Cruz R, Carrillo-Nieves D, et al. Light Intensity and Nitrogen Concentration Impact on the

Biomass and Phycoerythrin Production by Porphyridium purpureum. Marine Drugs 2019; 17: 460.

Tolboom SN, Carrillo-Nieves D, de Jesús Rostro-Alanis M, de la Cruz Quiroz R, Barceló D, Iqbal HMN, et al. Algal-based removal strategies for hazardous contaminants from the environment – A review. Science of The Total Environment 2019; 665: 358-366.

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. Bioresource Technology 2012; 105: 114-119.

Vandamme D, Pohl PI, Beuckels A, Foubert I, Brady PV, Hewson JC, et al. Alkaline flocculation of Phaeodactylum tricornutum induced by brucite and calcite. Bioresource Technology 2015; 196: 656-661.

Vu HP, Nguyen LN, Lesage G, Nghiem LD. Synergistic effect of dual flocculation between inorganic salts and chitosan on harvesting microalgae Chlorella vulgaris. Environmental Technology & Innovation 2020; 17: 100622.

Wingender J, Jaeger K-E, Flemming H-C. Interaction Between Extracellular Polysaccharides and Enzymes. In: Wingender J, Neu TR, Flemming H-C, editors. Microbial Extracellular Polymeric Substances: Characterization, Structure and Function. Springer Berlin Heidelberg, Berlin, Heidelberg, 1999, pp. 231-251.

Wu M, Li J, Qin H, Lei A, Zhu H, Hu Z, et al. Pre-concentration of microalga Euglena gracilis by alkalescent pH treatment and flocculation mechanism of Ca3(PO4)2, Mg3(PO4)2, and derivatives. Biotechnology for Biofuels 2020; 13: 98.

Wu Z, Zhu Y, Huang W, Zhang C, Li T, Zhang Y, et al. Evaluation of flocculation induced by pH increase for harvesting microalgae and reuse of flocculated medium. Bioresource Technology 2012; 110: 496-502.

Yadav G, Dash SK, Sen R. A biorefinery for valorization of industrial waste-water and flue gas by microalgae for waste mitigation, carbon-dioxide sequestration and algal biomass production. Science of The Total Environment 2019; 688: 129-135.