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Marine microalgae flocculation and focused beam reflectance measurement

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ABSTRACT

The production of biodiesel from fast growing and lipid containing marine microalgal species is sustainably and economically more promising than fresh water microalgae. However, like fresh water microalgae, the process is limited by numerous factors such as the generally dilute nature of microalgal cultures, and the small size of microalgae cells, except for multi-cellular species such as Spirulina. Current dewatering technologies are sufficiently able to separate microalgae from the culture media; however the economics of the process makes these technologies ineffective as they are all too expensive when compared with the low cost of biodiesel from other sources. Centrifugation, which is one of the current technologies, is highly energy intensive especially in a large-scale setting. Filtration techniques such as tangential flow filtration has the potential to be a low cost dewatering technique, however there is a cost issue associated with the replacement of clogged membranes. Flocculation is another commonly used dewatering technique that has the advantage of using less energy under optimum conditions. Thus process development for marine microalgae flocculation could be an essential step to revolutionize biodiesel production from microalgae.

In this work, mixed cultures of marine microalgal species were obtained from semi-continuous laboratory reactors and flocculation was investigated using polyelectrolyte (polymer) flocculants. Cationic, anionic and non-ionic polyelectrolyte flocculants were tested using the standard jar stirrer test at varying pH and temperature. All three flocculant types displayed suitability for microalgae flocculation with the cationic polymer obtaining the highest flocculation efficiency of 89.9% at an optimum concentration of 4 mg/L. Focused beam reflectance measurements (FBRM) showed real time changes in microalgal flocs size during the flocculation process. This data is essential to understand the kinetics of microalgal flocs formation, to ensure the stability of the floc formation process, and to monitor and evaluate the performance of the flocculation process.

1. Introduction

With increasing concerns regarding sustainability and the environment, it has become a common practice to reduce carbon dioxide emissions resulting from human activity and thus global warming. This is due to the evidence suggesting that the increase in anthropological carbon dioxide and global warming is linked. Biodiesel is thought of as a practical alternative transport fuel that may in the future, play a significant part in the reduction of transportation related CO_2 emissions. The biodegradable, renewable and non-toxic properties of biodiesel make it a very promising alternative fuel. The production and use of biodiesel contribute virtually no net carbon dioxide and sulphur into the atmosphere and emit fewer gaseous pollutants than petroleum diesel [1,2]. The use of oil seed crops as the feedstock for biodiesel production has been questioned on sustainability and global equity grounds, and therefore alternative sources of triglyceride oil which do not consume agricultural resources such as land and water are of considerable interest. For these reasons substantial research is being carried out on the use of microalgae as a lipid source for biodiesel production [3,4].

Microalgae have advantages over traditional biodiesel feedstocks; these include high growth rate (able to double their biomass within a period of 24 h) [3,5], high lipid content, and the ability to grow on arid regions of land whilst making use of water that is not suitable for conventional agriculture [4,6]. Biodiesel from microalgae may offer carbon neutrality in the ideal scenario, as other operations such as culture mixing and down stream unit operations can results in carbon dioxide emissions. There also exists the possibility of recycling downstream carbon dioxide emissions for microalgae cultivation. Amongst all these advantages, the bioprocess engineering of microalgae is limited by the dewatering of extremely dilute cultures of small-sized microalgal cells, and this is one of the major challenges obstructing the emergence of algae-based fuels. Dewatering of microalgal cultures requires high costs and energy due to its dilute nature and this highly impacts on the economics of bioprocess engineering.

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Production of biodiesel from marine microalgal species is seen as a strategy to improve the economics and sustainability of the algae-to-biodiesel process [3,4]. Whilst this strategy offers the platform to cultivate the microalgae in the most saline water or sewage streams, it is still limited the by high cost of dewatering which has been a major bottleneck to microalgae bioprocess engineering. Several dewatering techniques, including centrifugation, gravity sedimentation, filtration and screening, flotation and flocculation have been used for microalgae dewatering [7–12]. However, each has its disadvantages that affect the overall economics of the process. Centrifugation requires high energy input and high initial capital cost. Filtration and screening require regular replacement of filters, screens and membranes. Gravity sedimentation is a slow process and electroflotation requires the replacement of worn electrodes that have been consumed and a high cost of electricity. Flocculation is a low energy process, but can be expensive if the flocculant is costly and the dosage is high. However, most microalgal systems rely on cheap flocculants such as ferric chloride, aluminium sulphate, chitosan and various polymeric flocculants [13]. This makes flocculation a potentially viable option for microalgae dewatering.

This work contributes to improving the dewatering of marine microalgal cultures by developing an optimum flocculation process with real time monitoring of the flocculation performance using a FBRM analysis. Flocculation is the process where a solute particle in a solution forms an aggregate called a floc. Flocculation is the result of collisions between solute particles and the adherence to each other in a suspension [14,15]. Most microalgal cells have a size range between 5 and 50 μm [12] and form stable suspensions with negatively charged cellular surfaces. The stability of these microalgal suspensions is dependant on the forces that interact between the cells themselves and between the cells and water. Hence they are considered as hydrophilic bio-colloids [12] which aid in the understanding of the mechanisms of flocculation; namely charge neutralisation and polymer bridging. Flocculation of microalgae results from charge neutralization due to the reduction in the electrostatic force of repulsion between charged microalgal cells in suspension and intra-particle bridging. Flocculants that have a high charge density are therefore more effective [16]. Flocculation performance is seen to be dependent on the molecular weight of the polymer flocculant, with a higher molecular weight polymer giving better flocculation performance [16,17]. Other factors that influence flocculation performance include the charge density, dosage, pH, salinity and the characteristics of the microalgae. Previous studies have found success with freshwater microalgae flocculation using cationic polymers and inorganic flocculants such as aluminium sulphate [10,12]. There have been only a few studies on the flocculation of marine microalgae. Sukenik et al. [11] found that polymeric flocculants were ineffective in flocculating marine microalgae (with salinity up to 36 g/L). Further studies by Bilanovic and Shelef (1988) showed that reducing the salinity improved flocculation for all cationic polymers [18].

Focused beam reflectance measurement (FBRM) is a tool that allows in situ measurements of the distribution of particle size over a wide range of solid concentrations. FBRM operates by scanning through a highly focused laser beam at a fixed speed across particles in suspension and measures the time duration of the backscattered light from each particle or floc, which is multiplied by the velocity of the scanning laser [19,20]. The size and number of particles is given in terms of the chord length. The principle of FBRM has been described previously by Blanco et al. [19] and Heath et al. [20]. Although FBRM is a widely acknowledged technique for monitoring flocculation processes [19], little amount of work has been reported on its use for microalgal flocculation. In this study, the use of FBRM analysis is used to obtain real time data on the kinetics of floc formation during microalgal flocculation. With this, it is possible to set flocculation parameters, such as pH, conductivity and temperature, to enhance or optimize the stability of the floc formation processes, and hence the performance of the flocculation process.

2. Experimental

2.1. Microalgae culture description

Laboratory scale semi-continuous marine microalgal culturing reactors with a maximum volume of 100 L were used to produce the algae used in this study. The predominant species of microalgae was *Chlorococcum* sp. The marine algae solutions were stored at 4 °C after collection and were tested within 168 h. The average dry mass microalgae concentration was 0.6 g/L. All experiments were performed with cells from a single harvest (at the stationary phase) to ensure the same growth phase and storage conditions in order to nullify any variations that could result from differences in these conditions. The influence of microalgae cultivation growth phase on dewatering performance has been reported by Danquah et al. [21].

2.2. Polymer flocculants

The flocculants investigated were cationic, anionic and non-ionic polyelectrolytes. Cationic flocculants 71305, 71301, 71303 and anionic flocculant 82240 were obtained from Nalco (Australia). Anionic flocculants Magnafloc 155, Magnafloc 156 and non-ionic flocculant Magnafloc 351 were obtained from Ciba (Australia). The concentration of the stock flocculant solutions used was 0.5 wt% (Table 1).

2.3. Flocculation jar test

The flocculation experiments were carried out in 1 L batch jar tests. A desired dose of the flocculant was added into the microalgae solution. The mixture was agitated at 200 rpm for 10 s for a fast mix followed by a slow mix for 10 min at 50 rpm. It was then left to settle for 30 min. A sample of the solution was pipetted from a fixed height in the jar corresponding to a volume of 0.8 L. The absorbance of this sample was measured using a UV-VIS-2450 spectrophotometer (Shimadzu, Australia). The zeta potential of the sample was measured using a Zetasizer Nano (Malvern, Australia).

The effects of pH and temperature were also investigated by adjusting the pH with hydrochloric acid and the temperature prior to the addition of the flocculant. The microalgal cells were maintained at the desired temperature for 30 min before testing. The cell viability after increasing the temperature was measured with a PAM-210 Chlorophyll Fluorometer (WALZ, Germany). This instrument induces the chlorophyll in the microalgae to emit photons by the exposure of blue light into the microalgae solution. The higher wavelength of red light is emitted due to the fluorescence and then measured. The instrument gives the output measurement as a yield, with inactive chlorophyll (dead algae) giving a low yield.

Table 1
Charge density and molecular weight properties of polymer flocculants.

Polymer flocculant	Charge density	Molecular weight
71303	Low/medium cationic	Medium
71305	Low cationic	Medium/high
71301	Medium cationic	Medium/high
82230	Low/medium anionic	Medium/high
Magnafloc 156	Medium anionic	High
Magnafloc 155	Low/medium anionic	High
Magnafloc 351	Non-ionic	High

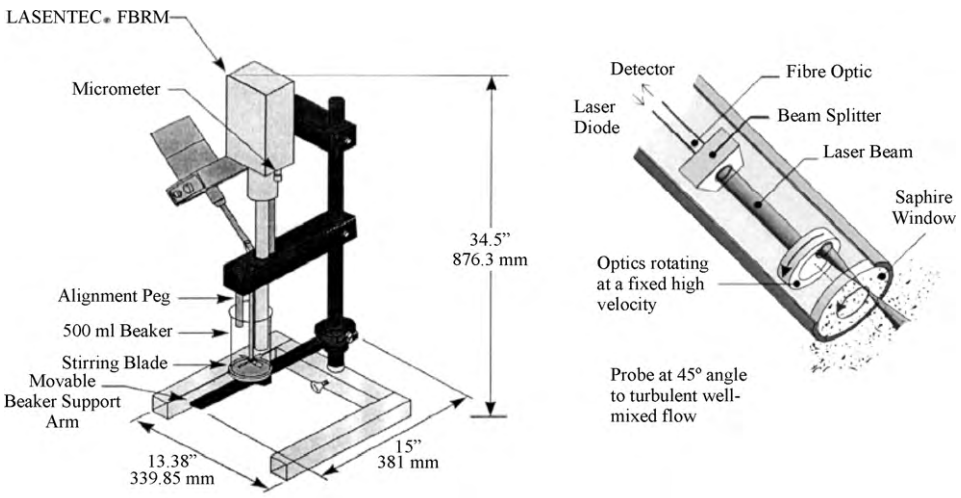

Fig. 1. Schematic diagram of FBRM setup [20].

2.4. Focused beam reflectance measurement

Two types of measurements were performed using the FBRM. The flocculation process was monitored in situ with a focused beam reflectance measurement (FBRM) (Mettler Toledo, Australia). The FBRM was also used to determine the change in chord size distributions before and after flocculation. This involved obtaining the distributions for algae before flocculation, performing the flocculation experiment with the jar stirrer, decanting the supernatant after the settling period and obtaining the distribution of the supernatant. The speed of the mixer was set at 400 rpm. The FBRM system was set under constant mixing to limit the amount of microalgae settling that can occur. Fig. 1 presents a schematic diagram of the FBRM setup.

3. Results and discussion

3.1. Flocculation measured by jar test

The efficiency of the flocculation process is determined by the probability of collision of the polymer and microalgal particles, as well as the capability of these particles to stick together once brought together by collision [15]. The results from the jar tests show that all the flocculants investigated can flocculate the microalgal cells to some extent and hence improve the separation rate of the microalgal cells from the culture. The optimum dose for each flocculant is presented in Table 2. The cationic polymer flocculants 71303 and 71305 were found to be the most effective, with the highest microalgae recovery and the lowest optimum dose (3–4 mg/L). This compares well with those obtained in freshwater microalgae flocculation studies, with the optimum dose ranging from 2.5 mg/L for a cationic polyamide [12] to 10 mg/L for a cationic polyacrylamide Zetag 51 [9]. Acrylamide, the monomer of polyacrylamide is known to be carcinogenic [22]. The non-ionic polymer shows to be the worst flocculant overall, with the highest optimum dose and the second lowest algae recovery. The recovery of microalgae refers to the relative mass of flocculated microalgae compared to the total mass (Table 2).

As an example, Fig. 2 shows the percentage recovery of microalgae for varying doses of flocculant 71303 and 71305. It can be clearly seen that an optimum dose is achieved at 4 mg/L for flocculant 71303 and 3 mg/L for flocculant 71305. The flocculation mechanism for cationic polymers is already well known to be a combination of charge neutralisation and polymer bridging [23,24].

Earlier studies on freshwater microalgae have shown minimal success using anionic and non-ionic polymer flocculants [12,23]. In the present study, both anionic and non-ionic polymer flocculants were found to flocculate the marine microalgae, however the degree of flocculation was somewhat less than the best cationic

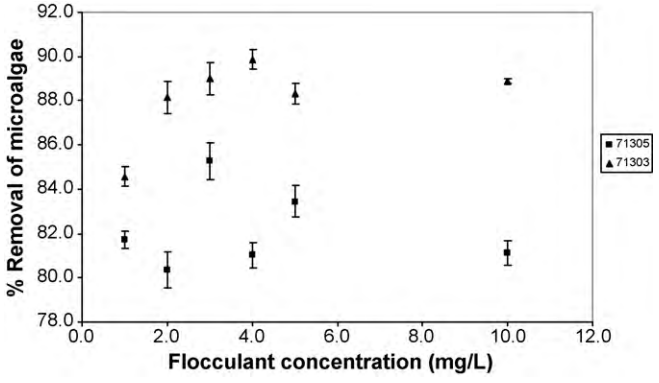

Fig. 2. Recovery of microalgae at varying flocculant dosage for cationic polymer 71303 and 71305.

Table 2
Flocculant optimum dose and percentage removal based on an average microalgae concentration of 0.6 g/L.

Flocculant	Charge	Optimum dose (mg flocculant/L algae)	mg flocculant/g algae	mg flocculant/g flocculated algae	% Removal
71303	+	4.0	6.6	12.2	89.9 ± 0.2
71305	+	3.0	4.9	9.7	85.3 ± 0.5
82230	–	5.0	8.2	16.2	84.5 ± 0.3
Magnafloc 156	–	3.0	4.9	12.5	84.5 ± 0.4
Magnafloc 155	–	2.0	3.3	7.0	83.9 ± 0.6
Magnafloc 351	No charge	10.0	16.5	43.7	79.9 ± 0.6
71301	+	3.0	4.9	9.7	78.0 ± 0.3

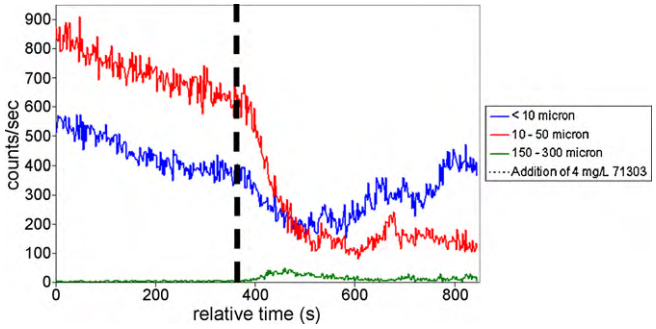

Fig. 3. FBRM analysis – counts per second versus time for flocculation using cationic polymer 71303 at 4 mg/L for three broad ranges of particle chord size.

polymer. The mechanism of anionic and non-ionic polymer attachment to the microalgae surface is governed by chemical forces rather than electrostatic forces, as well as interaction with cations formed on the microalgal colloid surface [12]. Anionic and non-ionic polymer flocculation can also occur by adsorption of the polymer onto the microalgal surface through hydrogen bonding [25].

In an industrial perspective, the top five polymers can be seen as successful. Although 71303 may be the best flocculant, the next four flocculants have similar recovery efficiencies. Flocculants such as 71305 and Magnafloc 155 have a lower efficiency than 71303 but also use a lower amount of polymer per litre of microalgae. Depending on the cost of the flocculant, it may be more advantageous to choose a cheaper flocculant with lower efficiency over an expensive flocculant with slightly higher efficiency. However, it can also be seen in Fig. 2 that a lower than optimum dose of 71303 can still achieve a better recovery efficiency than the optimum dose of the other flocculants.

3.2. Flocculation measured by FBRM

The process of microalgal flocculation can be observed more clearly using in situ focus beam reflectance measurements (FBRM). From Figs. 3 and 4, it can be seen that immediately after administration of the flocculant, the particle counts in the chord size ranges of less than 10 μm and 10–50 μm significantly drop while those in the chord size range of 150–300 μm increase. This corresponds to the agglomeration of the smaller size particles into larger ones; hence the results show a decrease in the count of smaller particles and a simultaneous increase in the larger particles. The fluctuations of counts in the size ranges of <10 μm and 10–50 μm seen for the flocculated microalgae after the addition of flocculant (Fig. 3) can be explained as the breakage and re-agglomeration of microalgal flocs due to the shearing forces of the FBRM mixer blades. This

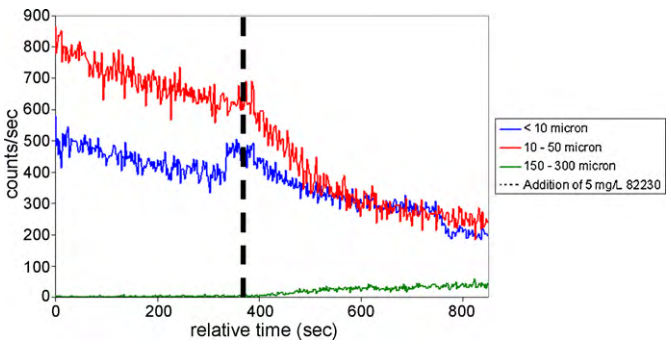

Fig. 4. FBRM analysis – counts per second versus time for flocculation using anionic polymer 82230 at 5 mg/L for three broad ranges of particle chord size.

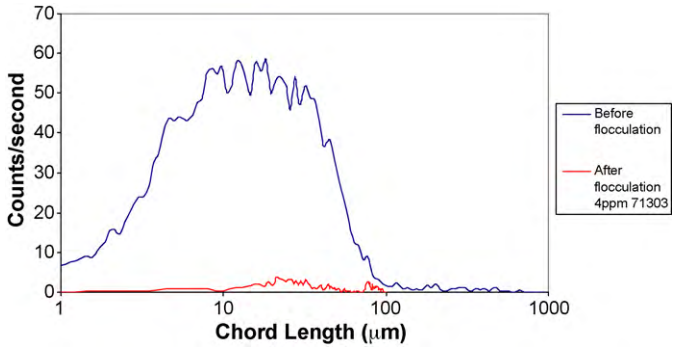

Fig. 5. FBRM analysis – before and after flocculation using cationic polymer 71303 at 4 mg/L.

fluctuation of the microalgae particle count of this size range is not as evident for microalgal flocculation using anionic flocculants (Fig. 4) because the floc size is much smaller, and therefore the agglomerates are more able to resist shearing forces. The initial pre-flocculation decrease in the chord length (up to 360 s) is as a result of large microalgae agglomerates unable to stay in suspension even in the presence of 400 rpm mixing, and this could be as a result of the physiological characteristics of the microalgal culture at the harvested stationary phase and/or the long culture storage time.

In the second type of FBRM test the particle count before flocculation is compared with the count for the supernatant liquid after flocculation in the jar test. These results also give a measure of the flocculation efficiency for a specific flocculant and dose. As seen in Fig. 5 for cationic polymer 71303 at the optimum dose of 4 mg/L, the counts per second significantly decrease after flocculation. By contrast, decreasing the dose to 2 mg/L (half the optimum dose) results in higher counts per second after flocculation (Fig. 6), thus indicating a greater number of unflocculated cells and a lower flocculation efficiency.

The result from Fig. 7 further verifies the ability of anionic polymers to flocculate marine microalgae. A similar trend to the results of the jar tests can be seen from FBRM results for the microalgae flocculation process using different types of flocculants. At the optimum dose, the cationic polymer 71303 gives the lowest particle counts followed by the anionic flocculant 82230.

3.3. Effect of pH on microalgae flocculation

The original microalgae media had a pH of approximately 8. The effect of pH on the flocculation of microalgae was determined by performing the flocculation process at pH 4, 6 and 8, respectively. Flocculation was not carried out at high alkaline pH because at pH

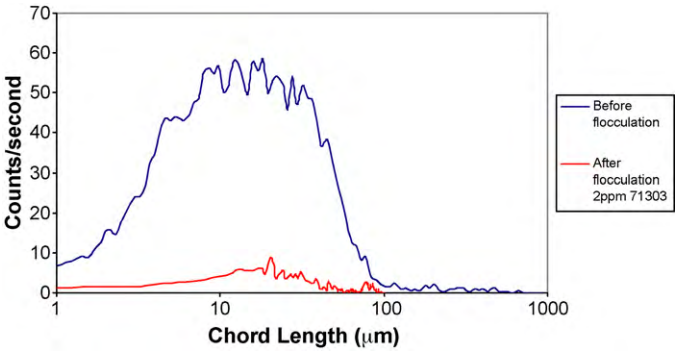

Fig. 6. FBRM analysis – before and after flocculation using cationic polymer 71303 at 2 mg/L.

Table 3
Percent removal and zeta potential of unflocculated algae at varying pH, done at the optimum dose for each flocculant.

Flocculant	pH 4		pH 6		pH 8	
	% Removal	Zeta potential (mV)	% Removal	Zeta potential (mV)	% Removal	Zeta potential (mV)
71303	86.5	−4.9 ± 1.6	89.8	−6.8 ± 0.6	89.9	−7.04 ± 0.2
82230	56.3	−4.6 ± 0.7	77.5	−7.3 ± 1.5	84.5	−7.64 ± 0.9
Magnafloc 156	54.5	−7.8 ± 0.8	74.3	−12.6 ± 0.4	84.5	−13.08 ± 0.7

values greater than 10.5 the recovery of the marine microalgae can be achieved very efficiently without the use of polyelectrolyte flocculants. At this pH Mg^{2+} and Ca^{2+} ions can be made to precipitate and as these cations surround the negatively charged microalgal cells, the precipitate will also sweep the algae from the suspension [26,27]. Although this is an effective means of removing the algae from solution, in a process where the algae requires further treatment to extract lipids, this is not an attractive option, because the algae must be still be separated from the precipitate.

Table 3 presents the results of microalgae recovery by flocculation at different pH values using the most successful cationic and anionic polymer. The results show that microalgal recovery increases with pH, unlike freshwater microalgae which showed the opposite effect in previous studies [12]. Reducing the pH to acidic conditions brings the freshwater microalgae closer to its isoelectric point and at this point the microalgal cells are more likely to flocculate. This effect is more significant for the anionic flocculants. For marine microalgal species, a higher pH will also affect the precipitation of salts, which has a significant effect on microalgal recovery. The cationic flocculants show a minimal change in microalgae recovery efficiency over the three pH values. This is because the charge neutralisation mechanism for cationic polymers persists with changing pH. However, for the anionic polymers, decreasing the pH brings an increase in the amount of H^+ ions in solution, which binds to the negatively charged polymer, thus giving it a lower efficacy. The pH variation could also affect the optimum pH range required for successful polymer activity.

The zeta potential was also measured for each flocculation test. It was found that with decreasing pH, the zeta potential of the un-flocculated microalgal solution became more positive (the charge becoming closer to the isoelectric point). This is expected because microalgae surface charge becomes more positive as the pH decreases.

3.4. Effect of temperature on microalgae cultivation

Temperature was seen to have a noticeable effect on microalgae recovery by flocculation. Temperature effect on freshwater microalgae flocculation has been previously reported by Al-Layla and Middlebrooks [28]. In their study, alum was used as the flocculant. The results showed a decrease in flocculation efficiency with

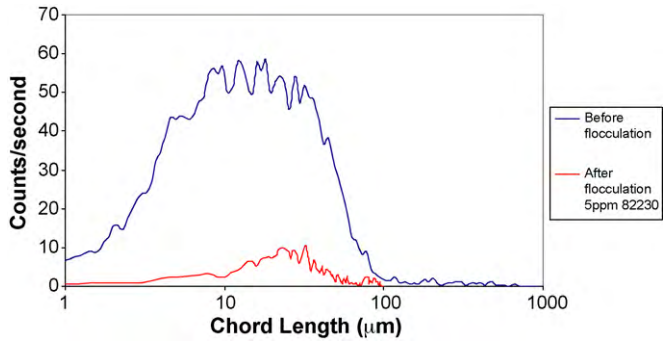

Fig. 7. FBRM analysis – before and after flocculation using 5 mg/L anionic polymer 82230.

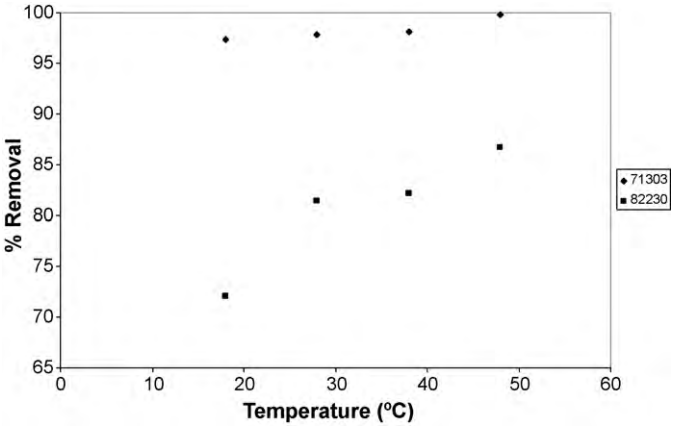

Fig. 8. Recovery of microalgae at varying temperatures. Experiments were performed using the optimum dose for each flocculant.

increasing temperature, resulting from the minimal solubility of aluminium hydroxide at lower temperatures. An opposite scenario was observed in this study, resulting from the differences in flocculant properties in the marine context.

It was found that an increase in the percentage of microalgal recovery was obtained with increasing temperature (Fig. 8). This can be explained by the theory of collision. With increasing temperature, there is a greater probability that the polymer and microalgae cells will collide due to the increasing mobility of the cellular particles at higher temperature. Increasing the number of collisions increases the number of possible interactions that can occur, which in turn improves the flocculation rates. The increasing mobility resulting from temperature increase relates to the molecular mobility of the flocculant molecules which results in an increase in flocculant–algae interactions per time, hence productivity and recover. The settling rates are also improved with increasing temperature due to a greater density difference. However, a third factor is the viability of the microalgae.

The WALZ PAM-210 chlorophyll fluorometer measures the dimensionless yield of active chlorophyll in the microalgae. This yield reduced with increasing temperature up to 48 °C. Healthy, viable microalgal cells can achieve yields of around 0.6–0.7, however at 48 °C, the microalgae had a yield of only 0.03, indicating that nearly all the microalgae had been killed. It is not clear why the dead microalgae should be easier to separate, possible explanations may include a change in the cell charge and/or the excretion of polymeric substances, and this is a subject of on-going research.

4. Conclusion

In early studies of marine microalgae flocculation only cationic polyelectrolytes were shown to be effective. Batch flocculation tests of cationic, non-ionic and anionic polymers were all found to be successful with microalgae recovery of 89.9%, 84.5% and 79.9% for cationic polymer 71303, anionic polymer 82230 and non-ionic polymer Magnafloc 351, respectively. In situ FBRM was used successfully to evaluate the microalgae flocculation process by measuring particle counts and the chord size distribution during

flocculation and also the distribution of unflocculated cells in the supernatant after flocculation. Both the pH and the temperature were also shown to have an effect on marine microalgae flocculation. Both offer potential to improve flocculation performance in conjunction with polymer flocculants.

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