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The transition away from chemical flocculants: commercially viable harvesting of *Phaeodactylum tricornutum*

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Abstract

Harvesting can contribute up to 15% of the total production cost for microalgal biomanufacturing. Using flocculants is potentially a cost-effective approach but there has been considerable debate on the efficacy, cost, toxicity to the cell and environment, and the effect on the biomass further downstream. In this study, a range of biobased flocculants (chitosan from crab shells, *Moringa oleifera* seed extract, pectin from bananas, tannic acid-based derivatives from *Acacia* tree bark and egg shell powder) were compared with traditional chemical flocculants (aluminium sulphate, iron chloride and sodium hydroxide) for harvesting the diatom *Phaeodactylum tricornutum*. It was concluded that Tanac's tannin based Tanfloc 8025 (SL range) was the most promising, requiring a low concentration (10.4 kg ton^{-1} biomass), and low cost ($27.04 ton^{-1} microalgal biomass). The flocculant was effective over a wide pH (7.5-10.0) and temperature (15-28°C) range and harvesting (>85% efficiency) occurred in 10 mins, which resulted in a biomass concentration factor of ≥5.69.

Keywords: microalgae; harvesting; biobased; bioflocculant, waste

1. Introduction

The microalga *Phaeodactylum tricornutum* is a model diatom that is known to accumulate a spectrum of marketable natural and engineered products (Butler et al., 2020). It is a commercially viable species for large-scale cultivation, and in outdoor mass culture systems, it has been shown to dominate and outcompete other microalgal species with a tolerance for high pH and an ability to grow under low light (Piiparinen et al., 2018; Remmers et al., 2017). *P. tricornutum* is typically cultivated in suspended culture, requiring cell harvest and extraction stages, which represent a target for increasing the cost competitiveness for the development of commercially viable manufacturing processes.

Harvesting alone can contribute up to 15% of the total microalgal production cost (Fasaei et al., 2018). Typically, less than 0.5 g L^{-1} dry weight (DW) (0.05% solids) is obtained in open raceway ponds, with a higher order of cell density achieved in photobioreactors, and industrial fermenters (Pahl et al., 2013). For commercial viability, the harvesting technique must meet specific criteria: increase the solid content to 10-25% total dry matter, be time efficient (requiring less than 1 h), be of high efficacy (>80%), be cost effective, require a low energy input, leave behind little or no toxic residues, and not affect the quality of the biomass downstream (’t Lam et al., 2018). Centrifugation (including conventional cream separators, disc stack-, bucket, and super-centrifuges) is the current option, industrially. However, centrifugation is energy intensive (1 KWh m^{-3}) and can account for 20-25% of the cultivation costs (’t Lam et al., 2018; Molina Grima et al.,

2003). Using flocculation can result in an energy demand reduction from 13.8 to 1.34 MJ kg^{-1} when compared to centrifugation (Hesse et al., 2017).

A wide variety of harvesting methods have been suggested for *P. tricornutum*. However, the most well researched area has focussed on flocculation with this method being scalable. Flocculants are well characterised and are effective through different modes; charge neutralisation (inorganic flocculants), sweeping, polymeric bridging (organic flocculants) and electrostatic patch mechanisms (Vandamme et al., 2010).

The concentrations of chemical flocculants required for flocculating marine microalgae are often five to ten-fold higher than for freshwater microalgae (Sukenik et al., 1988; Uduman et al., 2010). Metal salts (ionic) such as aluminium and iron chloride are the most commonly used flocculants for harvesting microalgae and are already applied commercially in breweries, drinking water supplies, mining, and wastewater treatment (Vandamme et al., 2013). However, these metals have been found to remain in microalgal biomass after lipid and carotenoid extraction and the residues can be toxic (Rwehumbiza et al., 2012; Singh and Patidar, 2018). Consequently, an additional step is required further downstream to remove the flocculant from the biomass, incurring additional expense.

To date, many flocculants have been tested (cationic, anionic, and non-ionic) for harvesting microalgae but there has been an inconsistency with the setup design, flocculant concentration used, the initial biomass density, optimal pH, and the sedimentation time for comparing commercially viable flocculants. In addition, few studies have addressed the impact on final product quality. The efficiency of a flocculant depends on several factors;

including the species, strain, culture conditions, ionic environment, stirring rate, concentration of flocculant, pH, and temperature. Flocculant costs in excess of \$0.10 kg^{-1} DW algae constrain commercial implementation and targets of \$0.04 kg^{-1} are required (Rogers et al., 2014). In transitioning towards a sustainable circular economy there is a desire to use biobased flocculants. Biobased flocculation offers considerable potential as an environmentally friendly and potentially cost-effective approach. Chitosan (10 mg L^{-1}) has been observed to be the optimal flocculant tested to date (in terms of efficacy and time) for *P. tricornutum* (biomass density 0.2-0.5 g L^{-1}) resulting in a 100% harvesting efficiency after 15 mins at pH 8 (Lubián, 1989). However, the cost of harvesting (\$0.32 kg^{-1} DW) is prohibitive for commercial harvesting applications. In this investigation, the use of sustainable biobased flocculants were explored which have shown promise in the literature: chitosan from crab shells (Şirin et al., 2012), eggshell powder (Choi, 2015), *Moringa oleifera* seed powder (Abdul Hamid et al., 2016), pectin from bananas (Kakoi et al., 2016), and two commercial *Acacia* tree bark tannin derived products from Tanac (Brazil); Tanfloc 6025 (SG range) and 8025 (SL range) (Roselet et al., 2015). Our focus was to identify the most effective natural or biobased harvesting solution that was commercially viable and environmentally sustainable.

2. Materials and methods

2.1. *Phaeodactylum tricornutum* culture and growth conditions

P. tricornutum CCAP 1055/1, obtained from the Culture Collection of Algae and Protozoa (CCAP) was cultivated in modified f/2 medium (Guillard, 1975) (elevated nitrate and phosphate: 4.41 mM nitrate and 0.17 mM phosphate) with 33 g L^{-1} of artificial seawater (Instant Ocean, Aquarium Systems, US), in 5 L Duran bottles, in fed-batch mode to ensure nitrogen and phosphorus replete conditions. A continuous irradiance of ca. 240 µmol photons m^{-2} s^{-1} was used using 45 W red:blue (70:30) 225 LED (Excelvan) with the cultivation at 21 °C (± 1 °C). The culture was aerated at 3 lpm (0.7 vvm) through a 0.22 µm air filter. The pH of the culture was observed to range from 9.16 to 10.15 over the cultivation period.

2.2. Inorganic and biobased flocculants

Unless mentioned otherwise, all chemicals were purchased from Sigma Aldrich, UK. The inorganic chemical flocculants tested as a baseline were iron chloride (Fisher Scientific, UK) and anhydrous aluminium sulphate (stock solution of 10 g L^{-1}). Low molecular weight chitosan (deacylated chitin) was dissolved in 1% acetic acid solution under constant magnetic stirring to form a 10 g L^{-1} stock (pH approximately 4). In order to form a homogeneous clear solution, the mixture was stirred overnight. Three sources of pectin were used, two commercial sources, CU 201 (high methyl-esterified 70%) and 701 (low methyl-esterified 36%) (Herbstreith and Fox, Sweden) heated to 100ºC and dissolved

in deionised water, and a pectin extract from banana peels (locally sourced). The banana peels were dried at 60°C for 24 h and then coarsely ground and stored at room temperature prior to extraction of pectin. The pectin was extracted according to Emaga et al. (2008) with minor modification. The pectin was extracted with sulphuric acid (pH 2, 90ºC for 1 h), the residue freeze dried using a CoolSafe freeze dryer (Labogene, Denmark) and then a stock solution equivalent of 10 g L^{-1} was prepared in deionised water. Waste eggshells were prepared after boiling, according to Kothari et al. (2017) with minor modification. The fine powder formed was dissolved in 10 mL of 0.1 mol L^{-1} of hydrochloric acid solution and the mixture was stirred for 30 mins to obtain a stock solution of 10 g L^{-1}. *Moringa oleifera* (Moringa) seeds were purchased from Herbalii, UK. The seeds were unshelled and mechanically ground as only the seed powder results in flocculation. The powder was sieved with a 600 µm mesh for homogeneity (Abdul Hamid et al., 2014) and used directly for the test. Tanfloc 6025 and 8025, two commercial quaternary ammonium polymers based on tannins extracted from the black wattle tree (*Acacia mearnsii*), were obtained from Tanac (Brazil) in a concentrated solution of 25% (w/w) and were diluted 100-fold with deionised water. These polymers were kindly provided by a distributor of the manufacturer, Lansdown Chemicals (OQEMA, UK).

2.3. Harvesting experimental setup

Before beginning the flocculation trials, the concentration of the microalgae suspension was always adjusted to 5 x 10^6 cells mL^{-1} (~0.07 g L^{-1} DW) [determined using an Advanced

Neubauer haemocytometer (BS-748, Hawksley) using a microscope (BX51, Olympus)]. Flocculation efficiency was evaluated in 500 mL glass columns (42 cm height with a 25 cm working height, 5.5 cm diameter, height/diameter ratio = 4.55) arranged as shown in Fig. 1. The cylinders were supported on a magnetic stirrer (RT10 Power, IKA, UK). To each glass cylinder, 500 mL of the culture ($5x10^6$ cells mL^{-1}) at pH 9.16-10.15 was aliquoted, different concentrations of flocculants were added as detailed below, and stirred at 1000 rpm for 10 mins for uniform flocculant dispersal. Preliminary experimentation showed that there was no effect of centrifugation and re-suspension in the fresh medium compared with the cultivated biomass (data not shown). The pH was then adjusted to pH 7.5 and pH 9.0 with 1 M of HCl or 100% CO_2 and the culture was mixed for 15 mins at 250 rpm to allow floc formation. The pH was monitored using a pH meter (FE20 FiveEasy, Mettler Toledo). After mixing, the microalgae suspension settling time was monitored every 10 mins over a 60 min time period, with a 1 mL aliquot drawn 5 cm from the top layer of the suspension in the glass column, and the optical density at 750 nm measured by spectrophotometry (SPECTROstar Nano, BMG Labtech, Germany). The harvesting efficiency was calculated from the difference in optical density (OD at 750 nm) between the *P. tricornutum* suspension at the start of the harvesting experiment (t_0) and that after a defined settling time (t_t), a commonly utilised method (Şirin et al., 2012; Vandamme et al., 2018):

$$\text{Harvesting efficiency}\,(\%) = \frac{OD_{750_{t_0}} - OD_{750_{t_t}}}{OD_{750_{t_0}}} * 100$$

2.4. Flocculation and sedimentation experiments

Sodium hydroxide, calcium oxide, and calcium hydroxide (Sigma Aldrich, UK) were tested for alkaline flocculation. To standardise the dosage of base, the pH of the culture medium was adjusted to pH 6.5 with 1 M HCl and a 1 M stock of the base was used to increase the pH to 10.5. The effect of autoflocculation was explored without the addition of any flocculant to the starting culture (pH 9.16 to 10.15) to determine if high pH alone was responsible for flocculation.

The zeta potential was measured using a phase amplitude light-scattering (PALS) zeta potential analyser (BrookHaven ZetaPALS, UK). The electrode was initially equilibrated to the new medium for 10 mins. Microalgae cultures with sodium hydroxide were centrifuged at 6000 rpm for 5 mins to obtain the mother liquor, and a 50-fold dilution was conducted on the original culture in this mother liquor as recommended by the manufacturer. The Smoluchowski model was applied as the solution was non-aqueous. Measurements were conducted using an electric field of 2.5 V/cm at a frequency of 2.0 Hz. The reported values represent the average of five successive runs of 20 cycles each, using the ZetaPALS. Three replicates of each treatment were used in the analysis.

For the flocculant screening trials, aluminium sulphate and iron chloride (chemical flocculants) and Moringa seeds, eggshell, chitosan, Tanfloc's 8025 and 6025 (bio-based flocculants) were evaluated at 100, 50 and 20 mg L^{-1}. Tests were conducted without the addition of a flocculant to determine if 100% CO_2 could replace 1 M HCl for lowering the pH. As CO_2 did not induce flocculation, the pH value was reduced to pH 7.5 and 9.0 using

100% CO_2 as a more environmental and cost-effective approach. All the tests were performed in triplicates with biomass at different time points of cultivation except the control (nine biological replicates were examined as the pH was highly variable from 9.16 to 10.5).

2.5. Effect of flocculant dosage, flocculation time, flocculation temperature, and biomass concentration on flocculation efficiency

Iron chloride was compared to Tanfloc 6025 and 8025 at 5 mg L^{-1} and the harvesting efficiency was recorded after 10 and 20 mins. To evaluate the performance and behavior of Tanfloc 6025 and Tanfloc 8025 the concentration of the selected bio-flocculant was tested at 100, 50, 20 and 5 mg L^{-1} over a more rigorous pH range (pH 7.5, 8.0, 8.5, 9.0, 9.5, and 10.0) and a 2D response surface interaction was conducted (Hesse et al., 2017).

The effect of temperature during the harvesting process was studied as this will be applicable when operating in an industrial location. Three different temperatures were tested 15 ºC [optimal for eicosapentaenoic acid (EPA) content], 21ºC (optimal for biomass and EPA productivity), and 28 ºC (upper thermal tolerance for *P. tricornutum* CCAP 1055/1). Those temperatures were chosen between the ranges at which microalgae physiology is not affected and all cells remained viable. Temperature was fixed in the incubator and the tests were conducted after thermal equilibrium was achieved between the culture and the environment.

Different initial cell concentrations were tested by diluting with Instant Ocean Sea Salts (33 g L^{-1}) or concentrating the sample by centrifugation (Sorvall, Thermo Scientific) as required. The concentrations tested were 1, 2, 5, 10, 20 and 40 (x 10^6) cells mL^{-1}. Dry weights were determined using a speedvac centrifugal freeze dryer (ScanVac Coolsafe 110-4).

The concentration factor (CF) is a parameter which provides information about the residual water content of the particulate phase. The CF is the ratio of final product concentration to the initial concentration (Şirin et al., 2012). The height of the examined microalgal culture ($h_{culture}$) and the final height of the concentrated algae ($h_{conc.\ algae}$) was used to determine the CF. The CF was calculated after 10 mins as in Şirin et al. (2012).

2.6. Effect of flocculants on fatty acid methyl ester (FAME) composition using a wet processing method

For the effect on fatty acid methyl ester (FAME) composition, aliquots of 5 mL of algal culture were harvested by centrifugation at 4000 rpm for 10 mins, the pellets washed with 0.01 M phosphate buffered saline (PBS) and transferred to 2 mL Eppendorf safe-lock tubes, centrifuged at 13,000 rpm for 5 mins and stored at -20°C for analysis. Flocculants which resulted in >80% harvesting efficiency at their lowest respective dose [aluminium sulphate, iron chloride, Tanfloc 6025 and Tanfloc 8025 at 20 mg L^{-1} and sodium hydroxide at 264 mg L^{-1} (6.6 mM)] were compared to the control (no flocculant addition). For comparative dry weight calculations, the samples were then freeze dried using a CoolSafe

freeze dryer (Labogene, Denmark). These pellets were weighted using a 5 d.p. balance for subsequent data analysis (Sartorius, UK). A modified version of Kapoore (2014) was used for FAME analysis using direct transesterification on the wet biomass with methanolic-HCl (7%) replacing BF_3 as the acid catalyst.

2.7. FTIR analysis of Tanfloc 6025 and 8025

Fourier transformation infrared spectroscopy (FTIR) of both Tanfloc 6025 and 8025 flocculants was conducted using an IR Prestige-21 Fourier Transformation Infrared Spectrophotometer (Shimadzu, UK). Samples were dried on a diamond Attenuated Total Reflectance (ATR) apparatus (Pike Technologies, USA) separately attached to the FTIR. A blank spectrum was run as a background using the ATR without the samples and the baseline shift of the spectra was corrected using the IR solution software provided with the Shimadzu FTIR instrument. Two replicates for each sample were analysed. At least 64 scans, with resolution of 4 cm^{-1} using the Happ-Genzel apodization function were collected for all samples. Spectral processing was carried out using the IR solution software, including correction for carbon dioxide, atmospheric water vapour, and baseline correction. A second derivative of the spectra was conducted, and the spectra were overlaid to identify the differences and putatively identify the chemical origins of the regions showing difference.

2.8. Statistical analysis

Each treatment was performed in triplicate except for the control culture without pH control (n=9), and the mean and standard error of the dependent variable (harvesting efficiency) are presented in the results. Statistical analysis of the experimental data was conducted using SPSS statistical software (SPSS Statistics 26, IBM). The data was tested for normality using a Kolmogorov-Smirnov test and if these data were normally distributed ($P>0.05$) they were subsequently tested for equal variance using Levene's test. If variances were considered equal, then a one-way/two-way ANOVA or MANOVA and a *post-hoc* Tukey's test was utilised to understand where the differences were. If samples were not normally distributed ($P<0.05$) then a Kruskall-Wallis and *post-hoc* Dunn's non-parametric comparison was undertaken to understand the changes. The statistical difference of each set of experiments was studied with the analysis of the P value. In addition, this value was used to select parameters that were significant.

3. Results and Discussion

3.1. Autoflocculation and alkaline flocculation

The concept of autoflocculation was first devised by Golueke and Oswald (1970) and was understood to occur at high pH due to CO_2 depletion. In later years autoflocculation was determined to be induced by chemical coprecipitation with magnesium and calcium salts in the culture medium (Shelef et al., 1984). Autoflocculation

can also be induced by increased extrapolymeric substance (EPS) production which can neutralise cell surface charge (increasing illumination/temperature/dissolved oxygen/CO_2 or through nutrient deficiency or darkness) and also through increased cell density (ageing of microalgal culture) (González-López et al., 2013; Şirin et al., 2012).

Alkaline flocculation (sometimes incorrectly termed autoflocculation) is another method to induce flocculation. Flocculation for *P. tricornutum* CCAP 1055/1 commences at pH 10 and reaches 90% harvesting efficiency at pH 10.5 when magnesium and calcium are present in the medium, but in the absence of these elements no flocculation occurs (Vandamme et al., 2015b). The harvesting efficiency remains effective up to pH 12 (Vandamme et al., 2015b) but a decrease in photochemical efficiency is observed and the culture becomes lighter brown in colour (Spilling et al., 2011). It has been revealed that 3 mM sodium hydroxide at pH 10.6 resulted in 91% harvesting efficiency in *P. tricornutum* CCAP 1055/1 (Vandamme et al., 2018). However, in an earlier report with the same strain, 4 mM of sodium hydroxide (pH 10.3) was reported to be necessary to maximise harvesting efficiency (71%). However, this resulted in a white sludge with a magnesium content of 5% and a mineral content greater than 10%, requiring hydrochloric acid addition to remove the magnesium from the biomass (Vandamme et al., 2015a), as this is above the recommended level (<10%) for food and feed applications.

In the current investigation, the objective was to determine if autoflocculation was possible without the addition of chemicals. We altered environmental factors, including flocculation in the dark, high temperature (up to 45°C) and high light (250-1000 μmol

photons/m^2/s), all of which resulted in poor harvesting efficiencies after 1 h (<13%) (data not shown). By manipulating pH it was observed that the culture pH alone (even in the alkaline region), at 9.16-10.15, at different time points during the fed-batch experiments, did not result in 'auto' flocculation even after 1 h (Fig. 2a). However, when the culture pH was reduced to pH 6.5, and sodium hydroxide added to adjust the pH back to the alkaline range, a critical concentration of sodium hydroxide (>5.4 mM-6.6 mM) was required for flocculation (Fig. 2b), suggesting that pH in itself (pH 9.5 – 10) is not sufficient for flocculation. It was further validated through zeta potential analysis that at higher concentrations of sodium hydroxide (>5.4 mM), the zeta potential increased, indicating charge neutralisation of the cells (Fig. 2b). As observed by Vandamme et al. (2015a), the formation of a white precipitate was observed when flocculation occurred (6.6 mM) (Fig. 2b). As autoflocculation could not be induced without chemical addition, and a white sludge was formed, requiring the addition of hydrochloric/nitric acid for deflocculation, a series of biobased flocculants were explored for more natural flocculation methods.

3.2. Bio-based flocculation

As autoflocculation was not observed to be effective without the addition of sodium hydroxide, and to avoid chemical addition, a series of non-toxic biobased flocculants (pectin, Moringa seeds, eggshell powder, chitosan, Tanfloc 6025 and Tanfloc 8025) were examined to evaluate their flocculation ability and their performance compared with that of

the conventional chemical flocculants (aluminium sulphate and iron chloride) at two pH conditions (7.5 and 9.0).

Typically, pH 7.0 to 8.5 has been used for flocculation studies for *P. tricornutum* (’t Lam et al., 2014; Schlesinger et al., 2012). As sodium hydroxide was revealed to induce flocculation at 5.4-6.6 mM and given the cultures were already at a high pH (pH 9.16-10.15) the culture pH had to be reduced to accommodate the addition of sufficient sodium hydroxide. This was achieved with 1 M HCl (up to 1.5 mM required to attain pH 7.5) or by sparging the culture medium with 100% CO_2. Either approach for pH reduction did not affect the harvesting efficiency compared to the control (Fig. 3a) and CO_2 offers an environmentally favourable method with reduced cost.

To date, various chemical flocculants (hydroxides, aluminium sulphate and ammonium chloride), bioflocculants (chitosan, poly-gamma-glutamic acid and cationic starch), and synthetic polymers (e.g. Zetag) have been investigated for harvesting *P. tricornutum* (’t Lam et al., 2014; Schlesinger et al., 2012; Şirin et al., 2012; Vandamme et al., 2010; Zheng et al., 2012). The minimum concentration of flocculant found to result in >90% harvesting efficiency for *P. tricornutum* was 10 mg L^{-1} chitosan at pH 8 (100% harvesting efficiency) (Lubián, 1989). For chemical flocculation, the lowest concentration of flocculant tested for harvesting *P. tricornutum* was 25.7 mg L^{-1} aluminium sulphate which resulted in 94% harvesting efficiency at pH 7 (Vandamme et al., 2018). The concentration of chemical flocculant in the literature has varied considerably up to a maximum of 270 mg L^{-1} using aluminium sulphate (Şirin et al., 2012). In this study, an

intermediate value of 100 mg L^{-1} was selected to observe if there were any differences at pH 7.5 and 9.0 and this was reduced further (50 and 20 mg L^{-1}) to observe the combined impact of flocculant dosage and pH on the harvesting efficiency of *P. tricornutum*. To date, no data exists on harvesting efficiency using the chemical flocculant iron chloride for harvesting *P. tricornutum*.

Aluminium sulphate was found to be the most effective flocculant at pH 7.5 (>92% harvesting efficiency) at all three concentrations tested, but in all cases the flocculation efficacy decreased at pH 9.0 (30-88% harvesting efficiency) (Fig. 3b-d). In alkaline conditions, monomeric hydroxoaluminium anions dominate the solution and reduce the absorption capacity of microalgal cells and it has been reported that sweeping and enmeshment is responsible for sedimentation (Barros et al., 2015).

Iron chloride was found to operate effectively at pH 7.5 with >90% harvesting efficiency. Iron chloride was equally effective at pH 7.5 and 9.0 at 100 mg L^{-1} (90-94% harvesting efficiency), but at 50 and 20 mg L^{-1}, the harvesting efficiency was found to decrease at the higher pH (Fig. 3c-d). There was no statistically significant difference between iron chloride and aluminium sulphate at pH 7.5 at the tested concentrations. However, the difference in performance between the two chemical flocculants became apparent at pH 9.0 at reduced concentrations (50 and 20 mg L^{-1}), where iron chloride had a significantly greater harvesting efficiency than aluminium sulphate (ANOVA and *post hoc* Tukey's test, $F = 85.73$, $P < 0.01$, $df = 1$) and was revealed to be suitable as a non-toxic chemical replacement.

In terms of the bio-based flocculants, pectin has not been tested for harvesting microalgae, Moringa seeds and eggshell powder have not been tested on marine microalgae, and tannin based flocculants have only been tested for freshwater microalgae and *Nannochloropsis oculata* in seawater (Roselet et al., 2015). Preliminary experimentation with pectin resulted in no significant difference in harvesting efficiency compared to the control without flocculant addition over both pH ranges (data not shown). Moringa seeds and eggshell powder were ineffective at pH 7.5 and 9.0 (<22% harvesting efficiency) (Fig. 3b). The high ionic strength of the medium due to the salinity may have caused coiling of the flocculants (Roselet et al., 2015). Moringa seed derivatives and eggshell powder are both natural polymers. Moringa seeds contain an active biocoagulating agent (dimeric protein) with a peptide of 6-20 kDa (Abdul Hamid et al., 2014). The presence of positively charged amino acids causes charge neutralisation through adsorption but it also acts through interparticle bridging (Sapana et al., 2012). For Moringa seeds it is the tertiary protein extract powder (29% protein by weight) and the primary seed powder which are responsible for flocculation (Abdul Hamid et al., 2016; Kandasamy and Shaleh, 2018) due to their cationic charges (Sapana et al., 2012). Eggshell has a high cationic charge density and is known to act by sweeping and charge neutralisation due to the presence of divalent cations of calcium and magnesium (Choi, 2015). Eggshells are about 95 % calcium carbonate and the remainder is 5 % calcium phosphate (Kothari et al., 2017). As these flocculants were not effective for *P. tricornutum* in this study they were not used for further experimentation at the lower flocculant dosages.

Chitosan was more effective at pH 9.0 at 100 and 50 mg L^{-1} (Fig. 3b; c). A reduction in chitosan concentration from 100 to 20 mg L^{-1} at all pH values tested resulted in a decrease in harvesting efficiency (Fig. 3a-c). Chitosan is proposed to act through a sweeping mechanism induced by the precipitation of the chitosan polymers (Blockx et al., 2018). Other studies have reported that chitosan acts through a combination of charge neutralisation (cationic polymer) and by bridging (Şirin et al., 2012).

Tanfloc 6025 was found to work more effectively at pH 9.0 at all the concentrations tested. The highest harvesting efficiency was attained at pH 9.0 at 20 mg L^{-1} (82%), but this was not statistically significant compared with 100 and 50 mg L^{-1}. It could be observed that Tanfloc 8025 resulted in a harvesting efficiency >93% at all concentrations tested (100, 50, and 20 mg L^{-1}), at both pH values (pH 7.5 and pH 9.0), and was determined to be the most viable bio-based flocculant screened in this study. Roselet et al. (2016a) reported that the performance of Tanfloc is reduced in saline environments (30‰ salinity) compared to brackish water (10‰ salinity) when applied at 1-10 mg L^{-1}. However, in the current study in a saline medium (33‰ salinity) Tanfloc 8025 was effective, but this could be because the medium we used differed in ionic composition and higher concentrations of flocculant were used (20-100 mg L^{-1}). It was interesting to note than Tanfloc 6025 was not as effective as 8025. The effectiveness of the Tanfloc based products has been attributed to their branched phenolic structure compared with other polysaccharides such as starch (Roselet et al., 2017). It has been found that Tanfloc POP (>80 mg L^{-1}) resulted in a harvesting efficiency of >80% with a pH range of 7 to 10 (Cassini et al., 2017). Similar results (>90% harvesting

efficiency) were obtained using a modified tannin coagulant (Q-TN) at 5 mg L^{-1} for *Microcystis aeruginosa* cultivated in BG-11 medium at pH 7 to 8 but at pH 9 the efficacy decreased (Wang et al., 2013). It has been reported that as the Tanfloc concentration increases (69 to 100 mg L^{-1}), the excess cationic charges may destabilise the system and reduce the harvesting efficiency (Roselet et al., 2017). However, in the current study Tanfloc performed well at 20-100 mg L^{-1}. It is interesting that differences were observed between the two Tanfloc products in the current study for harvesting *P. tricornutum*, as Roselet et al. (2015) reported that there was no significant difference in the performance of four tannic acid-based products (SG 1500, POP, SG and SL) for harvesting *Nannochloropsis oculata* at ≥ 15 mg L^{-1}. It is possible that there is a difference in charge density which accounts for the better performance of Tanfloc 8025.

It was concluded that Tanfloc 8025 is an effective biobased flocculant for *P. tricornutum* requiring no additional chemicals, such as sodium hydroxide. At pH 9.0 it was the most effective flocculant and at pH 7.5 the harvesting efficiency was similar to aluminium sulphate and iron chloride. In addition, for downstream processing of wet biomass no change in cell morphology, photosynthetic activity (data not shown) or fatty acid composition was observed (Fig. 4) compared with the control (no flocculant addition).

3.3. Effect of lowering Tanfloc dosage and flocculation time

In the current study, the ability of Tanfloc 8025 and Tanfloc 6025 to flocculate *P. tricornutum* at a reduced concentration of 5 mg L^{-1} and in a shorter time period (10 mins)

compared with iron chloride was investigated. At pH 7.5 and 9.0, Tanfloc 8025 performed the best and Tanfloc 6025 outperformed iron chloride at pH 9.0. At 20 mg L^{-1}, with Tanfloc 8025, the harvesting efficiency at pH 7.5 was 94% and at pH 9.0 it reached almost 97% (Fig. 5a). When the concentration of Tanfloc 8025 was reduced to 5 mg L^{-1} the harvesting efficiency was observed to decrease to 83-86%. However, reducing the sedimentation time to 10 mins did not result in a further significant decrease (P>0.05). At 5 mg L^{-1}, Tanfloc 8025 performed better at both pH values than Tanfloc 6025 (55-61% harvesting efficiency). Roselet et al. (2015) also found at reduced concentrations (1.66-5 mg L^{-1}) of Tanfloc that the SL range (Tanfloc 8025) was a more effective flocculant for harvesting *N. oculata* and *C. vulgaris*. Here, it was found that Tanfloc 8025 performed equally well at pH 7.5 and 9 with >82% harvesting efficiency and there was no statistically significant difference (P>0.05).

To confirm the ability of Tanfloc 8025 to operate effectively over a wide pH range a more detailed pH range (7.5 to 10.0 at 0.5 increments) was investigated (Fig. 5b). There was no statistically significant difference in the harvesting efficiency over the pH range investigated and an average harvesting efficiency of 81.18% ± 3.55 S.E. was attained (P>0.05). From the 2D response surface graphs it could be observed that Tanfloc 6025 generally performed better at a higher pH (pH 10.0) and concentration (100 mg L^{-1}) (Fig. 5c) but Tanfloc 8025 was found to perform well at all pH values and concentrations (>76% harvesting efficiency) (Fig. 5b; c). At a higher pH of 9.0, Roselet et al. (2017) found that an increased dosage (70 mg L^{-1}) was required for both *Chlorella vulgaris* and

Nannochloropsis oculata for a harvesting efficiency >90%. The reduced effectiveness at higher pH has been attributable to the denaturation of the molecule at an alkaline pH (Sánchez-Martín et al., 2009) and could be related to differences in the protonation of the amine group, the macromolecular chain and the structure of the flocs (Selesu et al., 2016). At pH 8.0 and 9.0 Tanfloc had little cationic charge and the mechanism of action was most likely enmeshment (sweep coagulation) in Tanfloc precipitates (Graham et al., 2008).

The minimum concentration of Tanfloc SL (8025) required is reported in the literature to range from 1.66 mg L^{-1} to 5 mg L^{-1} which resulted in 94-97% harvesting efficiency for *Nannochloropsis oculata* and 90-100% harvesting efficiency for *Chlorella vulgaris* at pH 8.0 after 30 mins (Roselet et al., 2015). In the current study, reducing the concentration of flocculant to 5 mg L^{-1} resulted in a decrease in harvesting efficiency compared with 20 mg L^{-1} for *P. tricornutum*, but still a harvesting efficiency of 81% on average could be obtained over a wide range of pH values and in only 10 mins. It is notable that this is the lowest concentration of flocculant tested in the literature for *P. tricornutum*. One possible reason the lower dosage of Tanfloc 8025 at 5 mg L^{-1} did not result in ≥94% harvesting efficiency could have been because algal organic matter (AOM) was present. High doses of Tanfloc (60 mg L^{-1}) can be necessary to achieve a harvesting efficiency (>90%) in the presence of AOM, and the organic matter is known to interact with cationic polymers (Henderson et al., 2007; Roselet et al., 2017). Alternatively, even though the stirring speed used in this study may have been sufficient to disperse the

flocculant evenly amongst the cells, the floc formation time may need to be extended for optimal floc formation and altering the mixing conditions would be useful to investigate.

3.4. Surface functional groups of Tanfloc

Tanfloc 8025 was observed to outperform Tanfloc 6025 at the pH range investigated (pH 7.5-10.0). An FTIR analysis was undertaken to determine why this may have been the case. The second derivative was applied to the raw FTIR spectra, recorded between 850 and 3800 wavenumbers, to bring out minute differences of the *Acacia* tree extracts of Tanfloc 6025 and Tanfloc 8025 (Fig. 6). Notable differences in the spectral regions include at 1020-1030 cm^{-1} (C-O stretching of ether group), 1280-1290 cm^{-1} (C-O stretching, O-H deformations), 1320-1330 cm^{-1}, 1340-1360 cm^{-1} (C-O stretching of pyrogallic moieties), 1720-1730 cm^{-1} (C=O stretching from formaldehyde activation), and several peaks in the 3000-3800 cm^{-1} region (combined effect of O-H stretching and N-H groups) (Fig. 6). These suggest that Tanfloc 8025 is perhaps more activated with formaldehyde and quaternary amine derivatisation (tannin modification by Mannich reaction) (Arbenz and Avérous, 2015), and hence it is better crosslinked and charged at all pH values to be effective in flocculation. Tanfloc is a moderate-to-high molecular weight C16 polymer (~570,000 g/mol), at 1.7 kDa, with approximately 1000-2000 repeating units with a proposed monomer molecular weight of 399 g mol^{-1} and an elemental ratio of N:O:C:H of 1:4.2:7.2:12 (Graham et al., 2008; Barrado-Moreno et al., 2016). The cationic nature of the polymer is thought to be a single tertiary amine group (produced by the

Mannich reaction) per monomer resulting in a charge density of 3.1 mequiv g^{-1} at pH 4 and 0.2 mequiv g^{-1} at pH 9 (Graham et al., 2008). Elemental analysis should be conducted on Tanfloc to provide an indication to the degree of substitution from the precursor tannic acid which may aid in understanding the high flocculation ability.

3.5. Effect of temperature and biomass concentration on flocculation with Tanfloc 8025

From a commercial perspective it was important to determine how Tanfloc 8025 would perform at different temperatures and with increasing biomass concentrations which would be more appropriate for industrial facilities. Most species of microalgae can grow at 10 to 35°C (Barrado-Moreno et al., 2016), but the maximum temperature that *P. tricornutum* CCAP 1055/1 can be cultivated at is 28°C (data not shown). It is important in high temperature environments that the biomass is harvested as quickly as possible because the biomass can deteriorate (Gerardo et al., 2015).

Most flocculant screening studies have been conducted at room temperature or under temperature control (20-25°C) (Roselet et al., 2015). To the best of the authors' knowledge only one study has investigated the effect of a flocculant (chitosan) over a range of temperatures (5-20°C) for *P. tricornutum* at pH 8 with no reduction in efficacy observed (Lavoie and de la Noüe, 1983). To the authors' knowledge it is apparent that no study has investigated Tanfloc over a range of temperatures. From the current study, at all temperatures and pH values tested the harvesting efficiency was greater than 82% (Fig. 7a)

and there appeared to be no significant effect of pH and temperature (P>0.05). Therefore, Tanfloc 8025 offers potential in colder and warmer climates.

For *P. tricornutum*, limited studies have been conducted on determining the concentration factor of the flocculant and how it varies with changes in biomass concentration. Furthermore, in calculating the concentration factor few individuals have normalised the data to the harvesting efficiency which has often resulted in overestimates. In the current study, the harvesting efficiency increased up to a maximum (92%) at a biomass density of 0.26-0.48 g L^{-1} (92% harvesting efficiency) and a further increase to 1.01 g L^{-1} resulted in a decrease in harvesting efficiency (75%) (Fig. 7b). From 0.07 g L^{-1} to 1.01 g L^{-1} there was a decrease in concentration factor from 23.45 to 5.69 (Fig. 7b). It was clear the biomass became less compacted.

Most studies investigating flocculation of *P. tricornutum* have used low biomass densities (<0.5 g L^{-1}) and if the data in the current study is compared, a concentration factor of 12.82 is comparable with sodium hydroxide and chitosan in the literature. Tanfloc 8025 applied to higher biomass densities resulted in a lower concentration factor and further experimentation is required to improve this. Using properly designed settling vessels with an inclined bottom at an angle would help improve flocculation and is likely to result in higher compaction. Furthermore, optimising the mixing time would likely improve the sedimentation rate and compaction.

3.6. Cost analysis of Tanfloc 8025 compared with other commercially viable flocculants

A cost analysis based on the dosage and efficiency can be found in Table 1 for commercially viable flocculants for harvesting *P. tricornutum*. In the current study, with a starting biomass of 0.48 g L^{-1}, 92% of the culture could be harvested in 10 mins with a concentration factor of 12, requiring only 5 mg L^{-1} of Tanfloc 8025. Tanfloc 8025 was more economically favourable than chitosan, aluminium sulphate and sodium hydroxide (Table 1) but calcium hydroxide has been proposed as a more economically viable alternative. However, calcium hydroxide for harvesting *P. tricornutum* has not been proven and this process would still require a de-flocculation step (addition of hydrochloric or nitric acid) to remove the minerals in the biomass which can cost \$40 ton^{-1} (Vandamme et al., 2015a) and is environmentally unfavourable. Consequently, Tanfloc 8025 would be more suitable. The flocculant cost per ton of biomass in the current study (\$20.80-27.04) compared favourably to the cost reported for harvesting *Nannochloropsis oculata* with 1.66 to 5 mg L^{-1} Tanfloc 8025 at \$38 ton^{-1} (Roselet et al., 2016).

In addition, the concentration required (10.4 μg g^{-1} biomass) was vastly lower than the quantities of aluminium and iron required for flocculating *P. tricornutum* (200-270 mg g^{-1} biomass) (Şirin et al., 2012). As Tanfloc is a natural biopolymer it offers not just an ecological alternative, but it is economically viable and has great potential to replace chemical flocculants. For future studies, the mixing time and optimal harvesting apparatus should be considered as these are critical factors in floc formation and could maximise the performance of this effective biobased flocculant.

Conclusion

In transitioning towards a biobased and circular economy the replacement of chemical flocculants is essential. Tanfloc 8025 requires a low concentration of flocculant (10.4 kg per ton biomass), is cost effective ($27.04 per ton microalgal biomass), effective over a wide pH (7.5-10.0) and temperature range (15-28°C) with harvesting occurring in as little as 10 mins, resulting in a concentration factor of ≥5.69. Further experimentation needs to be conducted at laboratory scale observing the effect of AOM on the behaviour of this flocculant. Further work is required at pilot scale ensuring this method upscales before implementation at a commercial level.

E-supplementary data of this work can be found in online version of the paper

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Author contributions

TOB designed the experiments, conceived and wrote the paper with guidance from SV. TOB, KAC, MMD and OC conducted all experiments. TOB and KAC composed the

Figures. JM and TOB conducted the FTIR analysis. TOB revised and submitted the manuscript. All authors declare that they have read and agree with the manuscript.

Conflicts of interest

The authors declare no conflict of interest. The funding sponsors had no role in the writing of the manuscript and the decision to publish the results. The company Tanac supplied the Tanfloc products but had no role in the work, including writing of the manuscript and the decision to publish.

References

't Lam, G.P., Vermuë, M.H., Eppink, M.H.M., Wijffels, R.H., Van Den Berg, C., 2018. Multi-Product Microalgae Biorefineries: From Concept Towards Reality. Trends Biotechnol. 36, 216–227.

't Lam, G.P., Vermuë, M.H., Olivieri, G., van den Broek, L.A.M., Barbosa, M.J., Eppink, M.H.M., Wijffels, R.H., Kleinegris, D.M.M., 2014. Cationic polymers for successful flocculation of marine microalgae. Bioresour. Technol. 169, 804–807.

Abdul Hamid, S.H., Lananan, F., Din, W.N.S., Lam, S.S., Khatoon, H., Endut, A., Jusoh, A., 2014. Harvesting microalgae, *Chlorella* sp. by bio-flocculation of *Moringa oleifera* seed derivatives from aquaculture wastewater phytoremediation. Int. Biodeterior. Biodegrad. 95, 270–275.

Abdul Hamid, S.H., Lananan, F., Khatoon, H., Jusoh, A., Endut, A., 2016. A study of coagulating protein of *Moringa oleifera* in microalgae bio-flocculation. Int. Biodeterior. Biodegrad. 113, 310–317.

Arbenz, A., Avérous, L., 2015. Chemical modification of tannins to elaborate aromatic biobased macromolecular architectures. Green Chem. 17, 2626–2646.

Barrado-Moreno, M.M., Beltrán-Heredia, J., Martín-Gallardo, J., 2016. Microalgal removal with natural coagulants. Phycologia 55, 688–695.

Barros, A.I., Gonçalves, A.L., Simões, M., Pires, J.C.M., 2015. Harvesting techniques applied to microalgae: A review. Renew. Sustain. Energy Rev. 41, 1489–1500.

Blockx, J., Verfaillie, A., Thielemans, W., Muylaert, K., 2018. Unravelling the Mechanism of Chitosan-Driven Flocculation of

Microalgae in Seawater as a Function of pH. ACS Sustain. Chem. Eng. acssuschemeng.7b04802.

Butler, T., Kapoore, R.V., Vaidyanathan, S., 2020. *Phaeodactylum tricornutum*: A Diatom Cell Factory. Trends Biotechnol. 1–17.

Cassini, S.T., Francisco, S.A., Antunes, P.W.P., Oss, R.N., Keller, R., 2017. Harvesting microalgal biomass grown in anaerobic sewage treatment effluent by the coagulation-flocculation method: Effect of pH. Brazilian Arch. Biol. Technol. 60, 1–12.

Choi, H.J., 2015. Effect of eggshells for the harvesting of microalgae species. Biotechnol. Biotechnol. Equip. 29, 666–672.

Fasaei, F., Bitter, J.H., Slegers, P.M., van Boxtel, A.J.B., 2018. Techno-economic evaluation of microalgae harvesting and dewatering systems. Algal Res. 31, 347–362.

Gerardo, M.L., Van Den Hende, S., Vervaeren, H., Coward, T., Skill, S.C., 2015. Harvesting of microalgae within a biorefinery approach: A review of the developments and case studies from pilot-plants. ALGAL 11, 248–262.

Golueke, C., Oswald, W., 1970. Surface Properties and Ion Exchange in Algae Removal. J. Water Pollut. Control Fed. 42.

González-López, C. V., Cerón-García, M.C., Fernández-Sevilla, J.M., González-Céspedes, A.M., Camacho-Rodríguez, J., Molina-Grima, E., 2013. Medium recycling for *Nannochloropsis gaditana* cultures for aquaculture. Bioresour. Technol. 129, 430–438.

Graham, N., Gang, F., Fowler, G., Watts, M., 2008. Characterisation and coagulation performance of a tannin-based cationic polymer: A preliminary assessment. Colloids Surfaces A Physicochem. Eng. Asp. 327, 9–16.

Guillard, R.R.L., 1975. Culture of Phytoplankton for Feeding Marine Invertebrates. In: Culture of Marine Invertebrate Animals. Springer US, Boston, MA, pp. 29–60.

Happi Emaga, T., Ronkart, S.N., Robert, C., Wathelet, B., Paquot, M., 2008. Characterisation of pectins extracted from banana peels (Musa AAA) under different conditions using an experimental design. Food Chem. 108, 463–471.

Henderson, R.K., Baker, A., Parsons, S.A., Jefferson, B., 2007. Characterisation of algogenic organic matter extracted from cyanobacteria, green algae and diatoms. Water Res. 42, 3435–3445.

Hesse, M.C.S., Santos, B., Selesu, N.F.H., Corrêa, D.O., Mariano, A.B., Vargas, J.V.C., Vieira, R.B., 2017. Optimization of flocculation with tannin-based flocculant in the water reuse and lipidic production for the cultivation of *Acutodesmus obliquus*. Sep. Sci. Technol. 52, 936–942.

Kakoi, B., Kaluli, J.W., Ndiba, P., Thiong'o, G., 2016. Banana pith as a natural coagulant for polluted river water. Ecol. Eng. 95, 699–705.

Kandasamy, G., Shaleh, S.R.M., 2018. Flotation removal of the microalga Nannochloropsis sp. using Moringa protein–oil emulsion: A novel green approach. Bioresour. Technol. 247, 327–331.

Kapoore, R.V., 2014. Mass spectrometry based hyphenated techniques for microalgal and mammalian metabolomics.

Kothari, R., Pathak, V. V., Pandey, A., Ahmad, S., Srivastava, C., Tyagi, V. V., 2017. A novel method to harvest *Chlorella* sp. via low cost bioflocculant: Influence of temperature with kinetic and thermodynamic functions. Bioresour. Technol. 225, 84–89.

Lavoie, A., de la Noüe, J., 1983. Harvesting Microalgae With Chitosan. J. World Maric. 694, 685–694.

Lubián, L.M., 1989. Concentrating cultured marine microalgae with chitosan. Aquac. Eng. 8, 257–265.

Molina Grima, E., Belarbi, E.-H., Acién Fernández, F.., Robles Medina, A., Chisti, Y., 2003. Recovery of microalgal biomass and metabolites: process options and economics. Biotechnol. Adv. 20, 491–515.

Pahl, S.L., Lee, A.K., Kalaitzidis, T., Ashman, P.J., Sathe, S., Lewis, D.M., 2013. Harvesting, thickening and dewatering microalgae biomass. In: Algae for Biofuels and Energy.

Piiparinen, J., Barth, D., Eriksen, N.T., Teir, S., Spilling, K., Wiebe, M.G., 2018. Microalgal CO2 capture at extreme pH values. Algal Res. 32, 321–328.

Remmers, I.M., Wijffels, R.H., Barbosa, M.J., Lamers, P.P., 2017. Can We Approach Theoretical Lipid Yields in Microalgae? Trends Biotechnol.

Rogers, J.N., Rosenberg, J.N., Guzman, B.J., Oh, V.H., Mimbela, L.E., Ghassemi, A., Betenbaugh, M.J., Oyler, G.A., Donohue, M.D., 2014. A critical analysis of paddlewheel-driven raceway ponds for algal biofuel production at commercial scales. Algal Res. 4, 76–88.

Roselet, F., Burkert, J., Abreu, P.C., 2016. Flocculation of *Nannochloropsis oculata* using a tannin-based polymer: Bench scale optimization and pilot scale reproducibility. Biomass and Bioenergy 87, 55–60.

Roselet, F., Vandamme, D., Roselet, M., Muylaert, K., Abreu, P.C., 2015. Screening of commercial natural and synthetic cationic polymers for flocculation of freshwater and marine microalgae and effects of molecular weight and charge density. Algal Res. 10, 183–188.

Roselet, F., Vandamme, D., Roselet, M., Muylaert, K., Abreu, P.C., 2017. Effects of pH, Salinity, Biomass Concentration, and Algal Organic Matter on Flocculant Efficiency of Synthetic Versus Natural Polymers for Harvesting Microalgae Biomass. Bioenergy Res. 10, 427–437.

Rwehumbiza, V.M., Harrison, R., Thomsen, L., 2012. Alum-induced flocculation of preconcentrated *Nannochloropsis salina*: Residual aluminium in the biomass, FAMEs and its effects on microalgae growth upon media recycling. Chem. Eng. J. 200–202, 168–175.

Sánchez-Martín, J., González-Velasco, M., Beltrán-Heredia, J., 2009. *Acacia mearnsii* de Wild tannin-based flocculant in surface water treatment. J. Wood Chem. Technol. 29, 119–135.

Sapana, M.M., Sonal, C.G., D, R.P., 2012. Use of *Moringa Oleifera* (Drumstick) seed as Natural Absorbent and an Antimicrobial agent for Ground water Treatment. Res. J. Recent Sci. Res.J.Recent Sci 1, 31–40.

Schlesinger, A., Eisenstadt, D., Bar-Gil, A., Carmely, H., Einbinder, S., Gressel, J., 2012. Inexpensive non-toxic flocculation of microalgae contradicts theories; overcoming a major hurdle to bulk algal production. Biotechnol. Adv. 30, 1023–1030.

Selesu, N.F.H., de Oliveira, T. V., Corrêa, D.O., Miyawaki, B., Mariano, A.B., Vargas, J.V.C., Vieira, R.B., 2016. Maximum microalgae biomass harvesting via flocculation in large scale photobioreactor cultivation. Can. J. Chem. Eng. 94, 304–309.

Shelef, G., Sukenik, A., Green, M., 1984. Microalgae Harvesting and Processing: A Literature Review.

Singh, G., Patidar, S.K., 2018. Microalgae harvesting techniques: A review. J. Environ. Manage. 217, 499–508.

Şirin, S., Trobajo, R., Ibanez, C., Salvadó, J., 2012. Harvesting the microalgae *Phaeodactylum tricornutum* with polyaluminum chloride, aluminium sulphate, chitosan and alkalinity-induced flocculation. J. Appl. Phycol. 24, 1067–1080.

Spilling, K., Seppälä, J., Tamminen, T., 2011. Inducing autoflocculation in the diatom *Phaeodactylum tricornutum* through CO2 regulation. J. Appl. Microbiol. 23, 959–966.

Sukenik, A., Bilanovic, D., Shelef, G., 1988. Flocculation of microalgae in brackish and sea waters. Biomass 15, 187–199.

Uduman, N., Qi, Y., Danquah, M.K., Forde, G.M., Hoadley, A., 2010. Dewatering of microalgal cultures: A major bottleneck to algae-based fuels. J. Renew. Sustain. Energy 2, 12701–23104.

Vandamme, D., Beuckels, A., Markou, G., Foubert, I., Muylaert, K., 2015a. Reversible Flocculation of Microalgae using Magnesium Hydroxide. Bioenergy Res. 8, 716–725.

Vandamme, D., Foubert, I., Meesschaert, B., Muylaert, K., 2010. Flocculation of microalgae using cationic starch. J. Appl. Phycol. 22, 525–530.

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

Vandamme, D., Gheysen, L., Muylaert, K., Foubert, I., 2018. Impact of harvesting method on total lipid content and extraction efficiency for Phaeodactylum tricornutum. Sep. Purif. Technol. 194, 362–367.

Vandamme, D., Pohl, P.I., Beuckels, A., Foubert, I., Brady, P. V., Hewson, J.C., Muylaert, K., 2015b. Alkaline flocculation of *Phaeodactylum tricornutum* induced by brucite and calcite. Bioresour. Technol. 196, 656–661.

Wang, L., Liang, W., Yu, J., Liang, Z., Ruan, L., Zhang, Y., 2013. Flocculation of *Microcystis aeruginosa* using modified larch tannin. Environ. Sci. Technol. 47, 5771–5777.

Zheng, H., Gao, Z., Yin, J., Tang, X., Ji, X., Huang, H., 2012. Harvesting of microalgae by flocculation with poly (Î³-glutamic acid).

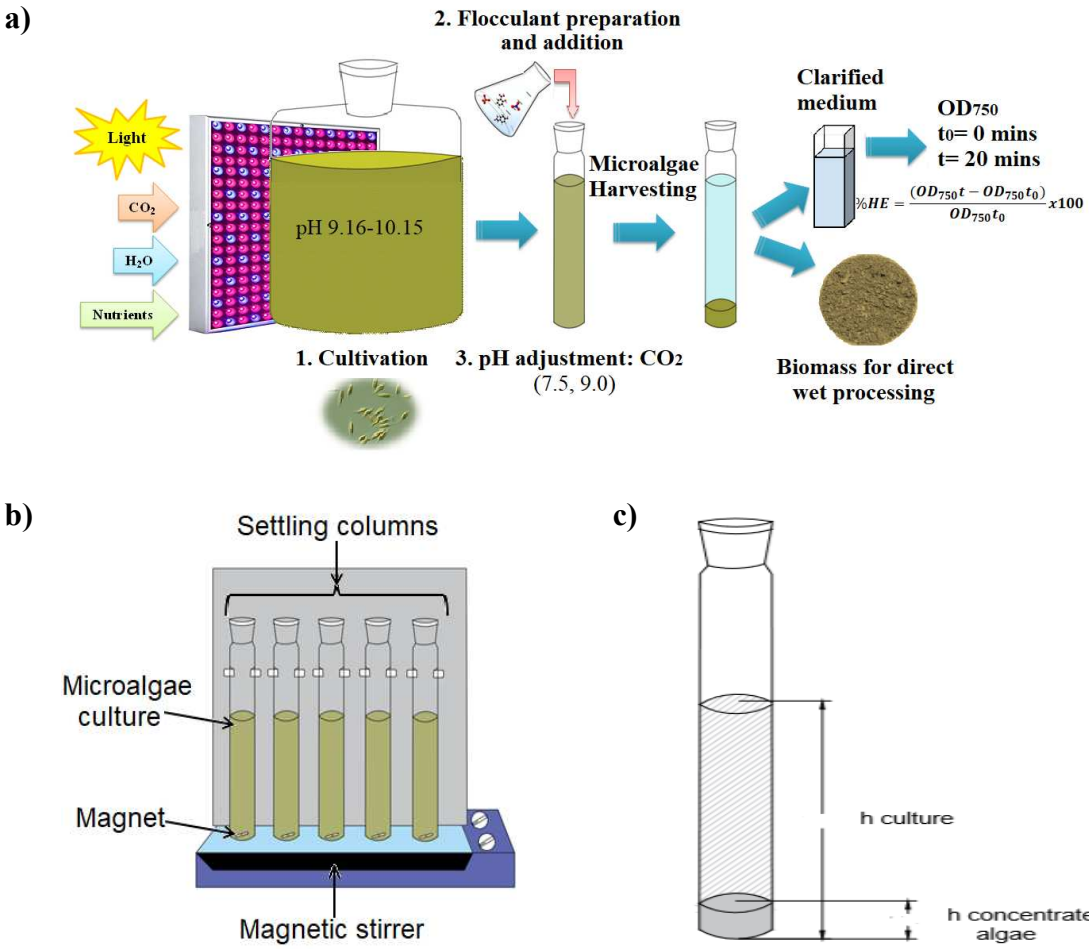

Fig. 1. Harvesting experimental setup: a) the experimental workflow, b) Settling column arrangement with 500 ml algal culture and c) concentration factor determination. The cells were cultivated under red:blue light in 5 L Duran bottles, 500 ml was diluted to the required cell density (5 x 10^6 cells mL^{-1}), the flocculant added, pH adjusted and the samples were withdrawn 5 cm from below the liquid miniscus at ~20 cm height from the bottom of the column.

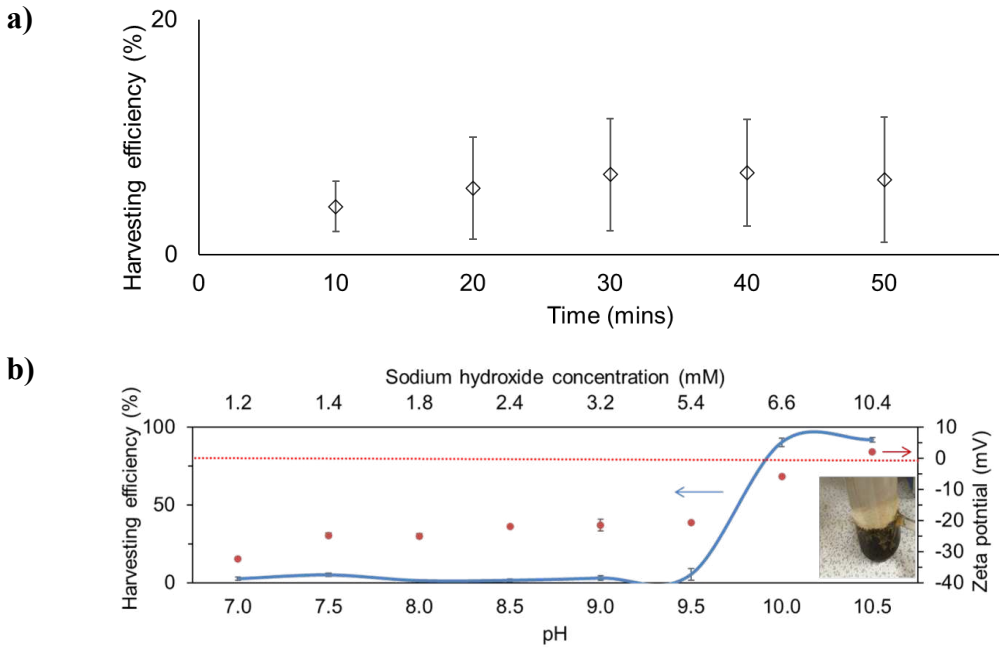

Fig. 2 - Autoflocculation and alkaline flocculation: a) autoflocculation with culture obtained directly without pH adjustment (pH of the culture ranged from 9.16 to 10.15) (n=9) from 10 mins to 1 h and b) the effect of sodium hydroxide concentration and pH (starting pH 6.5) on harvesting efficiency and zeta potential after 20 mins (n = 3)

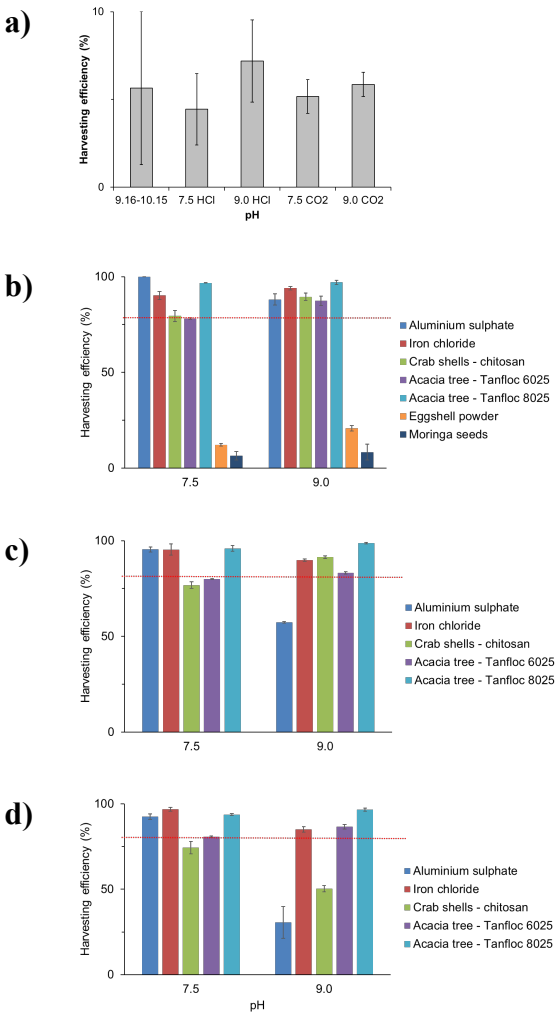

Fig. 3. Chemical and bio-based flocculation: a) Effect of adding hydrochloric acid compared with 100% CO_2 for pH reduction with harvesting efficiency reported after 20 mins, b) Performance of biobased flocculants (chitosan, calcium carbonate from eggshells, Moringa seeds and Tanfloc products from *Acacia* tree bark) compared with conventional chemical based flocculants (aluminium sulphate and iron chloride) at 100 mg L^{-1} after 20 mins of flocculation (n= 3 replicates). Effects of lowering the flocculant dosage for the most effective flocculants are shown in c) 50 mg L^{-1} and d) 20 mg L^{-1}. Red dotted line shows 80% harvesting efficiency.

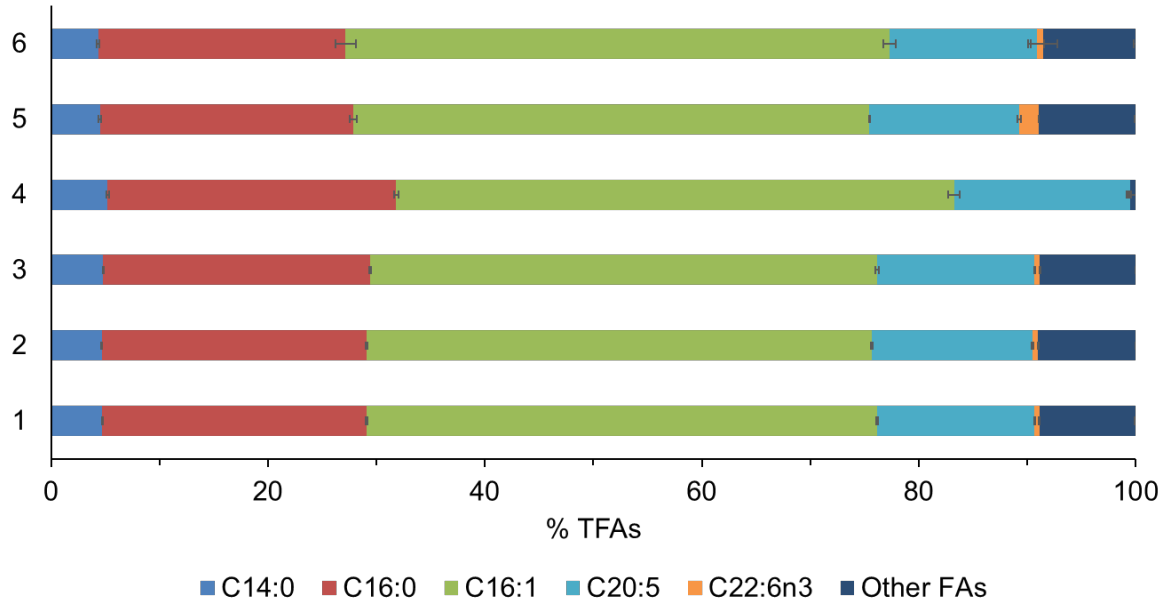

Fig. 4. Fatty acid profile *of Phaeodactylum tricornutum* for each treatment (% of total fatty acids). (1) Control - no flocculant addition; (2) aluminium sulphate; (3) iron chloride; (4) sodium hydroxide; (5) Tanfloc 6015 and (6) Tanfloc 8025. Each flocculant (aluminium sulphate, iron chloride, Tanfloc 6025 and Tanfloc 8025) was applied at 20 mg L^{-1} and sodium hydroxide was added at 264 mg L^{-1} (6.6 mM) compared to the control (no flocculant addition)

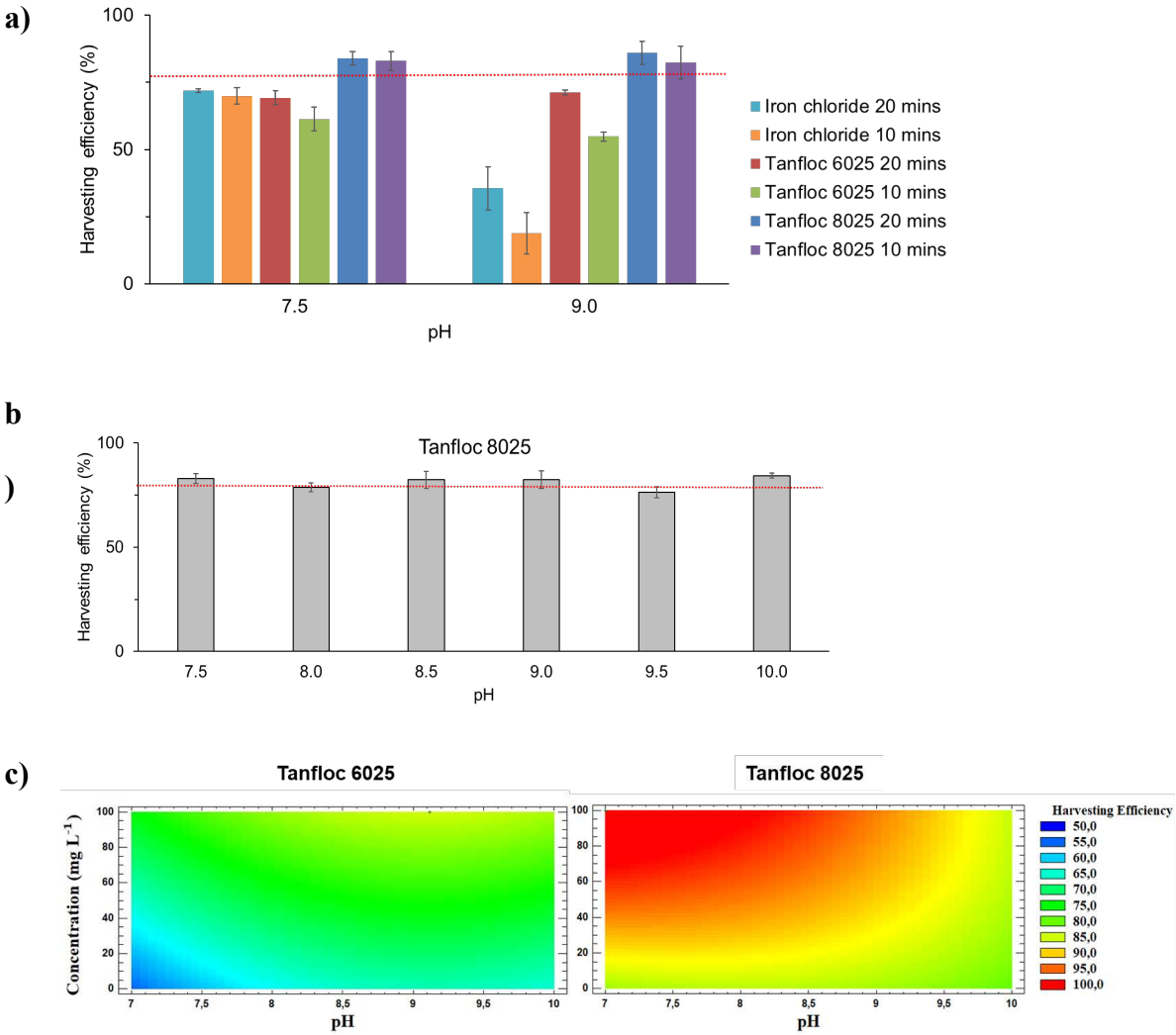

Fig. 5. Performance of reduced dosages of Tanfloc: a) effect of reducing the flocculant dosage of iron chloride and Tanfloc 6025 and 8025 to 5 mg L^{-1} at pH 7.5 and 9.0 and the effect of sedimentation after 10 mins compared with 20 mins, b) performance of Tanfloc 8025 over a wider pH range and c) response surface methodology 2D plot of harvesting efficiency represented by colour after 10 mins for flocculant concentration (100, 50, 20 and 5 mg L^{-1}) and pH (7.5, 8, 8.5, 9, 9.5, and 10) for Tanfloc 6025 compared with Tanfloc 8025. Red dotted line indicates 80% harvesting efficiency

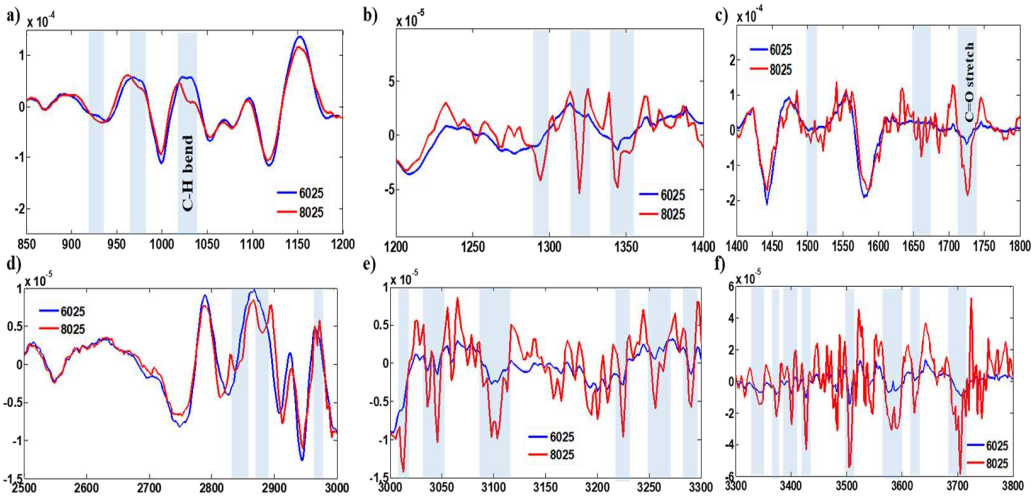

Fig. 6. Second derivative FTIR spectra of Tanfloc 6025 and 8025 from 850 – 3800 wavenumbers with a-f showing the differences in the spectra

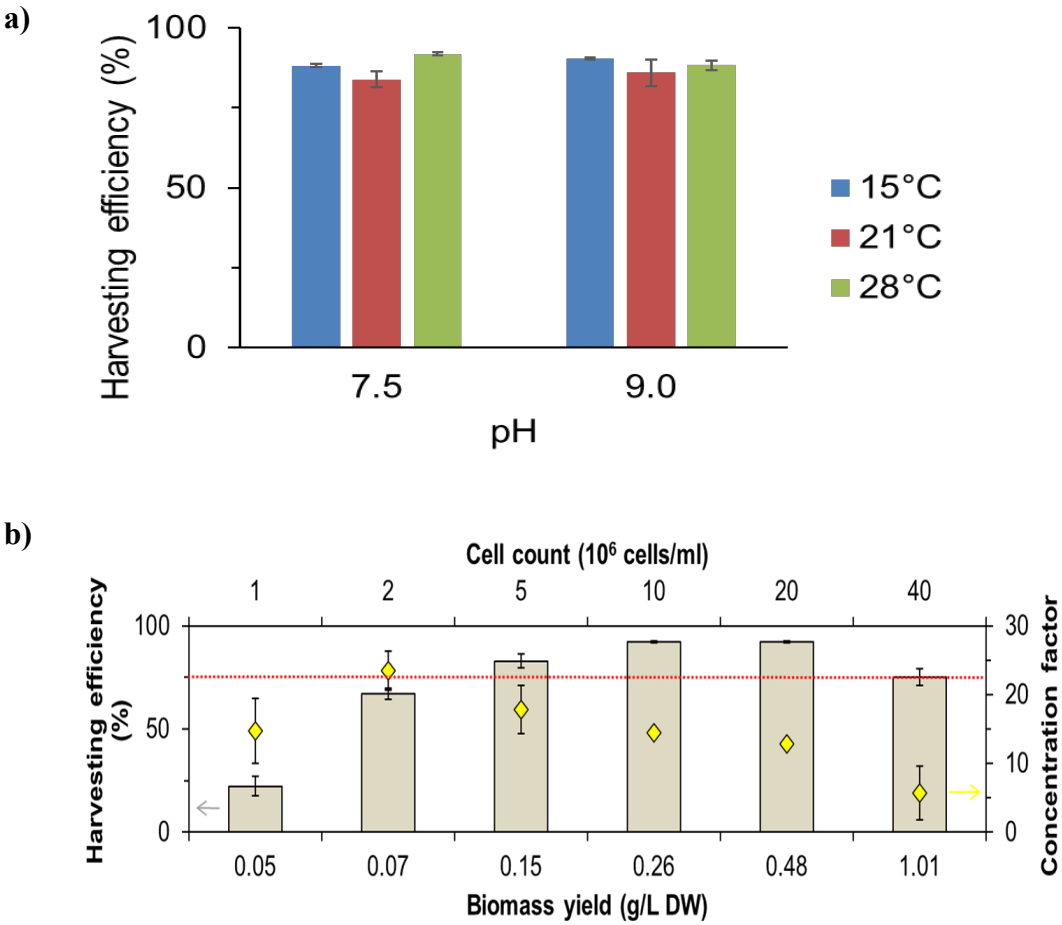

Figure 7. Performance of Tanfloc 8025 at different temperatures and biomass densities after *10* mins harvesting: a) Effect of temperature on harvesting efficiency of Tanfloc 8025 applied at 5 mg L^{-1}. b) Effect of biomass concentration (0.05-1.01 g/L DW) on the flocculation potential and concentration factor (CF) of Tanfloc 8025 (5 mg L^{-1}). Red dotted line shows 80% harvesting efficiency.

Table 1 - Cost analysis of harvesting *P. tricornutum* with chemical (aluminium sulphate, sodium hydroxide, and calcium hydroxide) and bio-based flocculants (chitosan and Tanfloc 8025). All costs are in US$.

	Chitosan [a]	Aluminium sulphate [a]	Sodium hydroxide [b]	Calcium hydroxide [b]	Tanfloc 8025
Harvesting efficiency (%)	91.8	82.6	73	-	92.16
Concentration factor	8.9	7.2	-	-	12.82
Flocculant dosage (mg L^{-1})	20	30	60	-	5
Biomass concentration (g L^{-1})	0.105	0.105	0.5	0.5	0.48
Flocculant dosage (ton ton^{-1} biomass)	0.18	0.27	0.12	-	0.0104
Flocculant cost ($ ton^{-1})	2000 to 100,000	976 to 2073	350	150	2000[c] to 2600[d]
Flocculant cost ($ ton^{-1} biomass harvested)	360 to 18,000	263.52 to 559.71	42	17	20.80 to 27.04

Data for the efficacy and cost analysis of chitosan and aluminium sulphate was obtained from (Şirin et al., 2012) [a] and for sodium hydroxide and calcium hydroxide from Vandamme et al. (2015a)[b]. It has already been shown that a de-flocculation step would be required for sodium hydroxide and calcium hydroxide to remove the magnesium from the biomass which would cost \$40 ton^{-1} (Vandamme et al., 2015a). The flocculant cost for Tanfloc 8025 was obtained from Roselet et al. (2015) [c] and Selesu et al. (2016b) [d].