Microalgae harvesting using flocculation and dissolved air flotation: selecting the right vessel for lab-scale experiments

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ABSTRACT: Flocculation combined with dissolved air flotation (DAF) is a promising technology for harvesting microalgae; therefore, optimisation of flocculant–DAF operating conditions are frequently explored in laboratory experiments. DAF systems have jars of differing volumes, height to diameter ratios, shapes and materials used to manufacture the jars; thus, the harvesting efficiency (η) may differ between these jars. The aim was to systematically compare η between different types of benchtop DAF jars. Evaluation of 30 different types of DAF jars revealed that η was not influenced by the volume of the jars, but was impacted by the height to diameter ratio, with optimal η at a ratio ranging between 1.6 to 2.05. There was no difference in η between cylindrical and cuboid jars, but jars made of hydrophobic (polypropylene) plastic resulted in a lower η. Overall, these results are useful to guide the design of lab-scale DAF microalgae harvesting experiments.

Keywords: Biofuels; DAF sizing; Microbubbles; Polymers; Water treatment.

1. Introduction

Microalgae are an attractive and novel source of biomass for production of food, feed or biofuels because they combine a high productivity with a biomass composition that is low in fibre and high in protein and carbohydrates or lipids [1-3]. They are also a promising technology to recover valuable nutrients such as N and P from wastewater [4]. An important challenge in large-scale production of microalgae is the harvesting of the biomass [5]. The standing crop biomass concentration in microalgal cultures is relatively low (1 – 5 $g \cdot L^{-1}$) due to self-shading of the microalgal cells, therefore large volumes of water need to be processed at an acceptable cost to harvest microalgae. To conserve energy, this is best done in a two-stage process in which the bulk of the water is removed during a pre-concentration step that generates a sludge with dry matter content of ~50 $g \cdot L^{-1}$ and a second step in which all extracellular water is removed using centrifugation to generate a biomass paste with a dry matter content of 20 % [6]. Flocculation is an interesting approach to be used during the pre-concentration step as it generates large aggregates of cells that can then be easily separated from the liquid using simple gravity settling (sedimentation) [5, 7]. As the density of the microalgal flocs is close to that of water, sedimentation is a slow process (1.25 - 2.5 $m \cdot h^{-1}$) and often results in a loose sludge with a high water content (< 2 % solids) [8-10]. An alternative approach to separate microalgal flocs from the liquid is to use dissolved air flotation (DAF). In DAF, air-water mixture is pressurised in a saturator (pressure tank), released through a nozzle and introduced to the bottom of flotation jars containing a microalgal suspension that has been previously mixed with a flocculant [3, 11, 12]. The small air bubbles attach to the flocs and concentrate the flocs in a float layer that can be skimmed off the surface. DAF is much faster (hydraulic rates of 10 - 25 $m \cdot h^{-1}$) and can generate a sludge with a higher dry matter content (2 - 7 %) compared to gravity sedimentation [8, 9].

This results in a harvesting system with a smaller footprint and in lower volumes of sludge that need to be dewatered using centrifugation. Hence, DAF is increasingly being explored as a promising technology for harvesting microalgae [13].

The efficiency of DAF for microalgae harvesting depends on the interaction between microalgal flocs and air bubbles. This interaction can be quite variable, depending on the type of flocculant used, the microalgae species, culture conditions or the chemistry of the water [14]. Additionally, several theoretical and pilot-scale studies have established that the removal of particles via DAF is greatly dependent on optimising the design of the separation tank as it can influence bubble residence times and bubble-floc interactions. For instance, varying the H : D ratios of the separation tank can impact the residence time of microbubbles and thus, the microbubble-floc interactions [15]. When the H : D ratio is low, the residence time of bubbles decreases as the bubbles do not have sufficient time to interact with flocs before they reach the liquid surface, thereby decreasing the DAF harvesting efficiency [16, 17]. For instance, Lundh, Jönsson [17] noted increasing microbubble residence times from 28 - 61 s as the H : W ratio increased from 2.1 to 4.6 in a pilot-DAF study. In another study, Yang, del Pozo [18] noted that when the H : W ratio was > 4.5 in a pilot-DAF plant, greater bubble coalescence occurred due to the turbulent flow of bubbles in the vertical direction, which decreased the bubble residence time. Hence, it is clear that the design of the separation tank could impact DAF separation outcomes.

Until now the design of the separation vessel has been evaluated only in experimental and theoretical pilot-scale DAF studies and not in lab-scale studies that use benchtop DAF systems. A review of the literature revealed that the DAF jars used in commercially available

and in-house made benchtop DAF testers are of different shapes, sizes and materials (**Table 1**). The volume of the DAF jars used in benchtop lab-scale DAF systems varied between 0.5 – 2 L and shapes of the DAF jars were cuboidal or cylindrical. The use of different jars and/or variable volumes have also resulted in a height to width ratio (H : W for cuboidal jars) or height to diameter ratio (H : D for cylindrical jars) of 1.4 – 2.1 of the liquid volume in the jar (**Table 1**). Similar to the pilot-scale systems, the variations in the design of the benchtop DAF jars could influence the DAF harvesting efficiency. Additionally, the material from which the jars are made may also influence flotation outcomes. A review of the benchtop DAF systems used also revealed that the DAF jars were made of differing materials including plexiglass, polycarbonate, glass and plastic (with the type of plastic not specified) (**Table 1**). Air bubbles are relatively hydrophobic [15] and may therefore interact with the wall of the jar. This may be especially consequential when the jars are made from hydrophobic polymers and/or when jars have a small volume and hence, a high surface to volume ratio. Overall, there has been no methodical study on the influence of dimensions and other properties of DAF jars on DAF performance. A systematic investigation is essential to allow comparison of results of different studies using different types of DAF jars.

(Table 1)

The use of DAF in combination with flocculation is a relatively complex process involving the formation of flocs after addition of a flocculant followed by the harvesting of the flocs from the liquid by flotation. This degree of complexity may be reflected in a high degree of variability between replicate tests. Because flotation experiments require quite large volumes of culture, it may be necessary to use different batches to evaluate all possible combinations

of flocculant concentration, flocculation time, microalgae concentration or DAF recycle ratio (ratio of pressurised water to feed volume). It is therefore also important to have an understanding of the degree of variability between DAF experiments carried out on different batches of culture of the same species. For instance, the surface properties of microalgal cells are known to change depending on the growth stage of a culture [30] and thus, different results may be obtained when the growth stage differs slightly between different batches of cultures of the same microalgae species.

Overall, the twin novelties of a systematic evaluation of the benchtop DAF jars and estimation of the variability in harvesting efficiencies across multiple microalgal batches form the basis of this study. Hence, the aim was to examine several DAF jars with differing attributes – volume, H : W or H : D ratios and materials - to identify the most appropriate jar to conduct lab-scale DAF testing. Additionally, the coefficient of variation was estimated between replicate DAF experiments using the same batch of microalgae and between DAF experiments carried out on different batches of the same microalgae species. All experiments were carried out using the freshwater microalgae *Chlorella vulgaris* as a model species and the model flocculant used was cationic poly(2-(dimethylamino) ethyl methacrylate) or pDMAEMA. *Chlorella vulgaris* is a commercially important species of freshwater microalgae [31] that is frequently used as a model species in microalgae harvesting studies [32]. PDMAEMA was synthesised in-house as a sustainable alternative to the toxic polyacrylamide-based flocculants, which have been frequently used in microalgae flocculation – sedimentation/ DAF experiments [33].

2. Materials and methods

2.1 Chemicals

The following chemicals were purchased from Sigma-Aldrich and used as received: 2-(dimethylamino)ethyl methacrylate (DMAEMA monomer) (98%, contains 700-1000 ppm hydroquinone as inhibitor), 2-cyano-2-propyl benzodithioate (reversible addition − fragmentation chain transfer [RAFT] agent) (>97%), 1,1′-azobis(cyclohexanecarbonitrile) (radical initiator) (98%), dioxane (99.5%), n-heptane (99%), chloroform with 0.6% ethanol stabiliser (reagent grade), tetrahydrofuran stabilised with 250 ppm BHT (>99.9%), and triethylamine (>99.5%).

2.2 Microalgal cultivation

The freshwater microalgae *Chlorella vulgaris* (211-11b SAG, Germany) was used in all experiments. The microalgae was cultivated in 30 L plexiglass bubble column photobioreactors (length – 100 cm; diameter – 20 cm). The photobioreactors were illuminated from two sides with daylight fluorescent tubes that each produced a photosynthetic photon flux of 100 $\mu mol \cdot m^{-2} s^{-1}$ at the surface of the reactor (24 h light cycle). The algal suspension was mixed and oxygen was purged by bubbling the reactor with 0.2 μm-filtered air (5 $L \cdot min^{-1}$). The pH was maintained at 8.0-8.5 by supplying pure carbon dioxide (2 - 3%) using a pH-controller system. The culture medium consisted of Wright's cryptophyte medium. The biomass concentration was monitored by measuring optical density

at 750 nm (OD_{750}) using a Genesys 10S UV-Vis spectrophotometer (Thermo Scientific, UK). Flocculation – DAF experiments were carried out with late-exponential growth phase cultures with a biomass concentration of ~0.5 $g \cdot L^{-1}$ (corresponding to an OD_{750} of ~0.70, ~ 5 $\times 10^8$ $cells \cdot mL^{-1}$), which is a typical biomass concentration obtained in extensive raceway pond cultivation systems. Optical density was calibrated against dry weight, which was determined gravitationally after filtering a known volume of culture on pre-weighed GF/F glass fibre filters (Whatman, UK). A total of 22 batches of *Chlorella vulgaris* were cultured over a total period of 11 weeks in 30 L bubble column photobioreactors to provide sufficient volume of algal suspension to be used in the experiments. The optical density varied from 0.646 to 0.732 between these different batches (0.696 ± 0.02; n = 187). Replicate experiments were always done using the same batch. Several experimental conditions were repeated with different batches to determine the experimental variability in harvesting efficiency between different batches of the same microalgae species cultured under identical conditions.

2.3 Polymer synthesis and characterisation

The DMAEMA monomer (3.0 g, 19.1 mmol, 1000 eq.), RAFT agent (4.22 mg, 0.019 mmol, 1.0 eq.), and the radical initiator (0.470 mg, 0.00191 mmol, 0.1 eq.) were dissolved in 3.0 ml of dioxane in a glass vial equipped with a stirring bar. The glass vial was sealed with a septum and degassed by bubbling the reaction solution with argon for 15 minutes. Subsequently, the glass vial was placed in a pre-heated oil bath and stirred for 48 h at 90 °C. The reaction mixture was allowed to cool down to room temperature and was diluted with chloroform. The obtained slightly viscous solution was then twice precipitated in cold heptane and dried on a rotary evaporator under reduced pressure. Further drying in a vacuum

oven at 60 °C for 24 h yielded a sticky solid with a pinkish hue as the product. A single batch of pDMAEMA was used in the entire study.

The molecular weight of the obtained polymers was determined using gel permeation chromatography (GPC). GPC measurements were conducted on an Agilent 1260 infinity system operating in THF + 1% triethylamine and equipped with an autosampler, a refractive index detector, and a variable wavelength detector. The setup was complemented with a light scattering detector (miniDAWN from Wyatt Technologies) and a viscometer (ViscoStar from Wyatt Technologies). The system was equipped with a PLgel 5 mm guard column (50 × 7.5 mm) and two separation columns from Agilent (PLgel mixed-C) which were kept at 35 °C in a column oven. Samples were prepared by dissolving a known amount of the polymer in HPLC grade THF + 1% triethylamine. All samples were filtered through 0.2 μm PTFE filters before analysis. Measurements were conducted at a flow rate of 1 mL min^{-1} and 100 μL of sample solution were injected. The values for Mn, Mw, and polydispersity index (PDI) were determined from the RI detector signal and the light scattering detector signal. The dn/dc values of purified samples were determined by the 100% mass recovery method, measuring solutions with known polymer concentrations. Data analysis was conducted using Astra 8.1 software from Wyatt Technologies.

The pDMAEMA flocculant synthesised in this study had a degree of polymerisation (number of monomer units in each polymer molecule) of 50, and the polymer molecular weight was observed to be 9.5 kDa as determined by GPC. The dispersity, which measures the degree of variability (broadness) in the molecular weights of the different polymer molecules was 1.02. A dispersity of 1 indicates that the polymer molecules are fully monodisperse and have no

variability. This suggested that the pDMAEMA molecules in this study were predominantly monodisperse with nearly identical molecular weights.

2.4 Flocculation–dissolved air flotation experiments

2.4.1 Flocculation-dissolved air flotation protocols

A Platypus DAF Tester (Aquagenics Pty Ltd, Australia) was used in all experiments. Conditions for flocculation followed by DAF were initially optimised in the standard 2 L DAF jars that are supplied with the Platypus DAF Tester. These jars are made of polycarbonate, are cuboid in shape and have a height of 19.7 cm and width of 10.5 cm (H : W ratio 1.9). The algal suspensions were first flocculated with pDMAEMA. The optimal polymer dose was determined in exploratory experiments (see Section S1) and was kept constant in all DAF trials in this study (25 $mg \cdot L^{-1}$). Prior to addition of the polymer the pH of the algal culture was adjusted to pH 6 by addition of 1M HCl or NaOH in order to ensure protonation of the amine groups on the pDMAEMA. After addition of the polymer, the algal suspension was stirred at 200 rpm for 2 min, followed by slow stirring at 30 rpm for 20 min to promote floc growth [34]. Mixing was done using the 7.6 × 2.5 cm flat paddle impellers of the Platypus jar tester. DAF was conducted immediately after the slow stirring phase.

The saturator of the Platypus DAF Tester was used to generate the microbubbles. The saturator was filled with demineralised water that was pressurised to 450 kPa with air using an oil-free compressor (OS20P, Abac, Belgium). Depressurised water containing the

microbubbles was introduced at the bottom of the flotation jar through polyethylene tubing (5 mm diameter) connected to the outlet of the saturator. The volume of depressurised water introduced (referred to in DAF process technology as the recycle ratio) was 10 % of the influent volume. After introduction of the depressurised water, a 10 min waiting time for the bubble blanket to rise and create a froth-float layer on the surface was provided. After 10 min, a 5 mL sample was collected from the middle of the clarified zone. The harvesting efficiency (η) was estimated from the initial OD_i and final OD_f optical density (750 nm) of the liquid according to equation (1):

$$\eta = \frac{OD_i - OD_f}{OD_i} \times 100 \qquad \text{(Equation 1)}$$

2.4.2 Evaluating different jars for dissolved air flotation testing

DAF performance was compared between different types of DAF jars that varied in volume, height, and width, and that were made from different materials. The concentration of the polymeric flocculant (25 $mg \cdot L^{-1}$), the mixing time and intensity (200 rpm for 2 min followed by 30 rpm for 20 min), the percentage of pressured water added (recycle ratio – 10 %) as well as the flotation time (10 min) were maintained constant over all experiments. The 2 L Platypus DAF jar (height 19.7 cm, width 10.5 cm) was used as a benchmark and compared with other recipients in a series of experiments. When jars other than the 2 L Platypus DAF jar were used, flocculation was first conducted in the 2 L Platypus DAF jar under conditions described in Section 2.4.1. The flocculated suspension was subsequently gently transferred into the test jar before depressurised water containing bubbles was introduced to the bottom of the jar. Exploratory experiments had indicated that the transfer of the flocculated algal

suspension between jars prior to the DAF had no significant influence on the harvesting efficiency (see SI Section S1). The flocculation – DAF performance in all tests was evaluated in triplicate using a single batch of *Chlorella vulgaris*.

- Test-1 – Varying shape of DAF jars: The variation in the harvesting efficiency due to changes in the shape of the DAF jar – cuboidal (Platypus DAF jar) and cylindrical (Duran 2 L Schott bottle) was first evaluated. The volume of liquid in the jars was kept constant at 2 L. The cylindrical jar had similar dimensions (height – 18.9 cm; diameter – 11.6 cm) to the Platypus jar (height – 19.7 cm; width – 10.5 cm).
- Test 2 – Varying fill level of DAF jar: The changes to the harvesting efficiency when decreasing the fill level of the standard Platypus DAF jars was then examined. The volume of algal suspension introduced into the jar varied between 2 to 0.5 L, resulting in a decrease in fill level from 19.7 to 0.56 cm and a reduction in the H : W ratios from 1.9 to 0.5.
- Test-3 – Varying liquid volumes at a constant H : D ratio of the DAF jars: In test-2, the reduction in fill level did not allow the evaluation of whether changes in harvesting efficiency were due to changes in volume or changes in H : D ratio. Therefore, in one series of follow-up experiments, jars with decreasing volume but a H : W ratio fixed at 1.2 – 1.3 were used. Like the standard Platypus jar tester, these jars were cuboidal in shape and were made of polycarbonate.
- Test-4 – Varying H : D ratios of the DAF jars at constant liquid volumes: In a series of follow-up experiments, jars with a decreasing H : D ratio (1.9 to 0.4) but a fixed volume (2 L) were examined. For these experiments cylindrical glass jars were used. Finally, an additional series of experiments were carried out in which the H : D was increased relative to the H : D ratio of the standard Platypus DAF jars. These experiments were carried out

in cylindrical glass jars that were filled to different levels, resulting in H : D ratios varying from 2.06 to 6.09.

To confirm the influence of volume and H : D ratio on harvesting efficiency in benchtop DAF experiments, additional experiments were carried out in 16 additional types of jars with varying volume and H : D ratio.

- Test-5 – Varying DAF jar material: To evaluate the influence of jar material properties on the harvesting efficiency, jars with comparable H : D ratio and identical volumes (0.4 L) but made of different materials were used: glass, stainless steel, polycarbonate, and polypropylene.

An overview of all DAF jars used in this study and their properties is provided (see supplementary material).

2.4.3 Biological variability in DAF harvesting efficiencies

Replicate experiments were conducted using different batches of *Chlorella vulgaris* to evaluate if there was statistically significant biological variability in harvesting efficiencies when using multiple batches of algae. The flocculation – DAF harvesting performance was tested in the standard Platypus DAF jars for 5 independent batches of *Chlorella vulgaris* cultured at intervals of at least one week over a period of 4 months. For each batch the harvesting efficiency was tested in triplicate.

2.4.4 Statistical analysis

Differences in the harvesting efficiency between different types of jars (fill level, volume, H : D ratio, materials) and between different batches of microalgal culture were evaluated using one-way analysis of variance (ANOVA) with Tukey's post-hoc test.

A total of 187 DAF experiments were carried out in 30 types of jars with H : D ratio varying between 0.46 and 6.09 and volume ranging from 0.05-2 L. These included replicate experiments using the same batch of microalgae as well as using different batches of microalgae. The influence of volume and H : D (H : W) ratio on the harvesting efficiency across all experiments was evaluated using second order polynomial regression. The H : D (or H : W) ratio and volume were log-transformed as a Shapiro test and had indicated that data did not conform to normal distribution. To evaluate whether H : D (or H : W) and volume independently explained variation in removal efficiency, a step-wise forward linear regression was carried out. To quantify the experimental variability in harvesting efficiency, the coefficient of variation (standard deviation / mean) was calculated between replicate experiments using the same batch of microalgae. All statistical analyses were carried out in R (version 4.2.0).

3. Results and Discussion

3.1 Evaluating optimal polymer concentrations for dissolved air flotation experiments

The harvesting efficiency of *Chlorella vulgaris* after flocculation by pDMAEMA and subsequent concentration using DAF increased from < 25 % at a pDMAEMA concentration of 15 $mg \cdot L^{-1}$ to 80.2 ± 1.9 % at 20 $mg \cdot L^{-1}$ (see supplementary material). A further increase in the polymer concentration resulted in an increase in the DAF harvesting efficiency up to 88 %, with no further increase in DAF harvesting efficiencies observed at higher doses of polymer (see supplementary material). Therefore, a pDMAEMA dose of 25 $mg \cdot L^{-1}$ was chosen for all the subsequent experiments conducted in this study. The maximum DAF harvesting efficiency achieved for *Chlorella vulgaris* when using pDMAEMA in the current study was comparable to the 85 – 90 % obtained for *Chlorella sorokiniana* with the polyacrylamide-based Zetag [35] and ~ 90 % achieved for *Chlorella vulgaris* with a cationic polyacrylamide [36]. This shows that pDMAEMA used in this study is an effective flocculant for harvesting microalgae via DAF.

3.2 The influence of the shape of the dissolved air flotation jar on flotation efficiency

When cuboidal (standard jar) and cylindrical DAF jars were used to evaluate if the shape of the DAF jars had an influence on the harvesting outcomes (test-1), no significant differences

in the harvesting efficiencies were observed between the DAF jars (ANOVA $F[1,4] = 0.18$, $p = 0.689$) (**Fig.1**). This indicated that any variation in DAF separation efficiencies observed in this study were independent of whether the shape of the DAF jar used was cuboidal or cylindrical.

(Figure 1)

3.3 Varying the volume and height : diameter or width ratios of the dissolved air flotation jars

The initial optimisation of the dose of pDMAEMA was done in the standard Platypus DAF jars filled to the maximum fill level (2 L). When the volume of liquid was decreased from 2 L to 0.5 L (test-2) a significant reduction in the harvesting efficiency was observed (ANOVA $F[4, 10] = 29.6$, $p < 0.001$) (**Fig.2**). While the decrease in the fill level from 2 to 1.5 L had no effect, fill levels of 1.25 L and lower resulted in significantly lower harvesting efficiencies compared to the 2 L level in the Platypus DAF jar (**Fig.2**). The harvesting efficiency declined from 83 ± 5 % at the 2 L level to 54.5 ± 7.2 % when the jar was filled with only 0.5 L.

(Figure 2)

Varying the fill levels in the DAF jar results in differing H : D (or H : W) ratios as well as different volumes of the liquid suspension. However, it was unclear whether the algal

harvesting efficiency was mostly influenced by a change in volume or a change in H : D ratio. To isolate and examine these effects, additional experiments were carried out where the volume was reduced but H : D ratio was constant (test-3) and where the volume was kept constant but H : D ratio was reduced (test-4). When the H : D ratio of the liquid volume was reduced while keeping the volume constant at 2 L (test-4), a significant decrease in the algal harvesting efficiency was observed (ANOVA $F[4,10] = 212$, $p < 0.001$) (**Fig.3**). While a decrease in H : D ratio from 1.9 in the standard jar of the DAF tester to 1.6 had no significant effect, further decreases to 1.3 or lower resulted in a significantly lower algal harvesting efficiency (**Fig.3**). On the contrary, when the volume of the algal suspension was reduced while maintaining a H : D ratio constant between 1.3 - 1.4 (test-3), no significant differences in the algal harvesting efficiency were observed, even though the volume was lowered by almost 20 times from 2 L to 125 mL (ANOVA $F[4,100] = 0.75$, $p = 0.582$) (**Fig.4**). This demonstrates that the volume of the DAF jar can be decreased without impacting the harvesting efficiency as long as the H : D ratio of the jar is close to its optimum. Interestingly, while the harvesting efficiency did not significantly differ between the DAF jars evaluated in this test, it was on average ~ 66 %, which was lower than the ~ 83 ± 5 % achieved using the standard Platypus DAF jar. This can be ascribed to the fact that the H : D ratio of the jars with varying volume (1.3) was lower than H : D ratio of the Platypus DAF jar (1.9).

(Figure 3, 4)

In the experiments carried out so far, the highest algal harvesting efficiency was observed for the jar with the highest H : D ratio (**Figs.1-2**). To test whether an even higher harvesting efficiency may be achieved by further increasing the H : D ratio, several jars with variable

volumes but increasing H : D ratio were examined. Interestingly, increasing the H : D ratio from 2.06 to 6.09 did not result in an increase, but rather a decrease in the harvesting efficiency (ANOVA $F[6,14] = 8.9$, $p < 0.001$) (**Fig.5**). While an increase in H : D ratio from 2 to 2.5 had no significant effect, a further increase to 3 or higher resulted in a significantly lower harvesting efficiency (**Fig.5**).

(Figure 5)

The experimental results presented above indicate that the harvesting efficiency of flocculation-DAF is related in a unimodal way to H : D ratio of the jars used while volume has no significant effect. Multiple regression was used to explore the dependence of harvesting efficiency on H : D ratio and volume. Results of a total of 16 flocculation-DAF experiments were used for this analysis. The harvesting efficiency was related in a unimodal way to the H : D ratio and increased following a saturating curve with the volume. The H : D ratio explained more variation in the harvesting efficiency ($R^2 = 0.33$) compared to the volume ($R^2 = 0.18$) (**Table 2**). As the volume and H : D ratio were negatively correlated (Pearson correlation coefficient 0.32), the response of harvesting efficiency to volume may be partly explained by its response to H : D ratio. Indeed, when the residuals of the regression of the harvesting efficiency against the H : D ratio were regressed against the volume, the volume explained very little of the residual variation in the removal efficiency ($R^2 = 0.08$) (**Table 2**). These further reiterate that lab-scale DAF harvesting efficiencies would not get impacted by the volumes of the liquid as long as the H : D of the liquid volume was within the optimal range of 1.6 - 2.06.

The importance of H : D ratio in determining the harvesting efficiency can be attributed to the behaviour of the bubble blanket during the flotation process. When the H : D ratio decreases below its optimum (< 1.6), there is limited coalescence of bubbles due to a greater number and movement of microbubbles in the horizontal plane [37, 38]. However, the distance to the surface of the liquid decreases which reduces the microbubble residence times and reduces opportunities for bubble-floc collisions. Overall, it is suggested that low microbubble residence times outside the optimal H : D ratio range inhibits microbubble-floc collisions and mixing, consequently impacting the DAF harvesting efficiency [15, 38]. As the height of the jar increases while the diameter remains constant (at H : D ratio > 2.5), the ratio of microbubble size to cross-sectional area of the jar increases, resulting in a greater number and movement of microbubbles in the vertical rather than horizontal plane [18, 39]. Owing to the turbulence created during bubble injection, a velocity gradient exists between the different bubbles which enhances bubble coalescence [18, 37]. As the coalesced bubbles rise faster than DAF microbubbles, the bubble residence time and bubble-floc collisions decrease, thereby impacting flotation efficiency.

Interestingly, the optimal H : D ratio range observed in this study was lower than the: (a) 2.75-4.1 range observed by Lundh, Jönsson [17] for pilot-scale DAF, and (b) 2.5-3.5 range suggested for designing pilot- and full-scale DAF jars [15]. One hypothesis behind the discrepancy is that unlike the lab-scale batch DAF process, there is a constant feed inflow into the pilot- and full-scale DAF systems as they are continuous processes. Therefore, the height of the flotation tanks in pilot- and full-scale systems are increased to combat the constant feed inflow and prolong the microbubble residence times in the contact zones of

pilot- and full-scale DAF systems. This results in elevated H : D ratios for these systems. The lab-scale batch DAF experiments are carried out in batch rather than continuous mode and have no such requirements, and hence, DAF separation efficiencies can be optimised at lower H : D ratios.

3.4 Influence of materials of the dissolved air flotation jar on harvesting efficiency

As microbubbles are hydrophobic, the use of hydrophobic material can influence DAF outcomes. In this study, no differences were observed between polycarbonate, glass and steel jars but a significantly lower removal efficiency was observed for the polypropylene jar (ANOVA $F[3,8] = 61$, $p < 0.001$) (**Fig.5**). Visually, a large proportion of the microbubbles when introduced into the jar preferentially interacted with the walls of the polypropylene DAF jar than attach to the flocs. This was unsurprising given that polypropylene presents a hydrophobic surface [40, 41], thereby increasing the entropic driving force for bubble attachment to the wall [42]. Hence, it is suggested that hydrophobic materials are to be avoided when selecting DAF jars.

(Figure 6, Table 2)

3.5 Degree of variability across microalgal batches during dissolved air flotation testing

To understand the degree of variability in DAF harvesting efficiencies across replicate experiments using the same batch of microalgae, the coefficient of variation was calculated for a total of 39 experiments. The coefficient of variation was on average 7.6 % and varied between < 1 % and 20 %. The coefficient of variation was negatively correlated with the harvesting efficiency (Pearson correlation coefficient $r = -0.47$, $p = 0.003$), indicating that the variability between replicate experiments was lowest when the harvesting efficiency was high. To evaluate whether the harvesting efficiency was different when different batches of microalgae were used, a comparison of the results of flocculation – DAF test carried out in the 2 L Platypus DAF jar with five different microalgal culture batches cultured over a period of four months was undertaken. Analysis of the data revealed that the average harvesting efficiencies for the five batches varied between 78-88% but did not differ significantly between the batches (ANOVA $F[4,10] = 1.9$, $p = 0.172$). This implied that there was no variability in the harvesting efficiencies across batches as long as the culture conditions in every batch remained identical.

3.6 Recommendations for selecting appropriate dissolved air flotation jars

The use of benchtop DAF systems has been gaining traction in algal harvesting studies. This systematic study demonstrates that the properties of jars used in benchtop DAF experiments can influence the algal harvesting efficiency. In this study, the H : D (or H : W) ratio and the material used to manufacture the DAF jar were found to be the critical determinants of algal harvesting efficiency via DAF, while there was no impact of shape of the jar or volume of the liquid used (**Table 2**). Maximum DAF harvesting efficiencies of 76 – 88 % were observed for DAF jars with differing volumes, all of which had an H : D (or H : W) ratio between 1.60 –

2.05 (**Fig.4**); the harvesting efficiencies decreased sharply to < 60 % on either side of this range irrespective of the liquid volumes (**Fig.4**), indicative of a narrow window for the most appropriate H : D (or H : W) ratio. When DAF jars made of different materials were trialled, the harvesting efficiencies declined only when using polypropylene, suggesting that DAF jars made of hydrophobic materials should be avoided (**Table 2**). Overall, the recommendation is to use DAF jars that can hold liquid volumes between 0.125 – 2 L as long as the H : D ratio of the liquid volume ranges between 1.60 – 2.06 and the DAF jar is not made of hydrophobic materials. While the DAF jars used in commercially available and in-house made benchtop DAF testers largely fall into this category, there were also some DAF jars which had H : D < 1.60 and were made of unspecified material which may not be suitable for DAF testing according to the current study (**Table 1**). This study also suggests that DAF jars that are smaller than those supplied by manufacturers can be used for algal harvesting experiments, enabling a greater number of experiments to be conducted using a given volume of algal culture.

This study was performed using the freshwater microalgae species *Chlorella vulgaris* and the polymeric flocculant pDMAEMA. While overall harvesting efficiencies may be very different when using different microalgae species and flocculants, the analysis of the results from this study indicate that the effect of DAF jar properties on harvesting efficiency will be similar in different settings. No statistically significant differences in harvesting efficiency were observed between replicate DAF experiments using different batches of exponential growth phase *Chlorella vulgaris* cultures. In non-exponential growth phase cultures and/or cultures experiencing stress conditions, the cell properties may change quickly over time and even minor variations in the timing of harvesting experiments may result in differences in

harvesting efficiency. Thus, when using different culture batches, the recommendation is to compare identical flocculation and DAF experimental conditions between different batches of culture to control differences in cell properties between culture batches.

It is to be noted that in addition to the DAF jar properties and microalgal batches, other factors such as flocculant and its solvent properties, flocculation and DAF operating conditions including flocculation time, flotation time, recycle ratio can influence the microalgal harvesting efficiency via DAF [43]. Hence, it is recommended that these factors be evaluated in detail in the future to get a holistic understanding of how harvesting efficiencies can be tailored via DAF.

4. Conclusions

A systematic comparison of different benchtop DAF jars to harvest *Chlorella vulgaris* via DAF revealed significant differences in harvesting efficiency. Optimal H : D ratios of 1.60 – 2.05 of the liquid volume in the DAF jar and the use of non-hydrophobic materials to manufacture DAF jars is recommended to optimise lab-scale DAF. No statistically significant differences were observed between cuboid or cylindrical shaped jars, and volumes of liquid between 0.125–2 L. Multiple batches of microalgae could be used provided that the culture conditions throughout the growth period remain the same across the multiple batches.

E-supplementary data for this work can be found in e-version of this paper online.

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Table 1. Commonly used bench scale dissolved air flotation jar testers in several studies.

Model	Jar shape	Height:Width ratio	Volume (L)	Material	References
DBT6, EC Engineering DAF Tester, UK and Canada	Cuboid	2.1	1	Plexiglass (polymethyl methacrylate)	[14, 19, 20]
Platypus DAF Tester, Aquagenics, Australia	Cuboid	1.8 – 2.05	2	Polycarbonate	[21, 22]
Multiplace Orchidis™ FTH3 Flottatest, France	Cylindrical	1.4 – 2.1	0.6 – 1	Glass	[23, 24]
TA6 DAF Tester, Hengling, China	Cylindrical	N/A	0.4	Glass	[25]
In-house built DAF testers	Cuboid, cylindrical	1.4 – 1.8	0.5 – 2	Glass, plastic, unspecified	[26-29]

Table 2
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Table 2. Output of second order polynomial ($y = a + bx + cx^2$) of the harvesting efficiency (Eff) modeled against the independent parameters height to diameter ratio (HD) and volume (Vol). The independent variables were log-transformed to approach normality. The model R^2, F degrees of freedom, F statistic, p-value and estimates for parameters a, b and c are given.

Model	R^2 adj	df	F	p	a	b	c
$Eff = a + b \cdot logHD + c \cdot logHD^2$	0.33	2, 183	46	< 0.001	70***	-4.3	-77***
$Eff = a + b \cdot logVol + c \cdot logVol^2$	0.18	2,183	22	< 0.001	70***	52***	-28**

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Table S1. List of dissolved air flotation jars and their design parameters used in the current study.

DAF Jar Details				**Height**	**Width or Diameter**	**H:D ratio**	**Liquid Volume**
Jar used	Manufacturer	Shape	Material	cm	cm	$cm \cdot cm^{-1}$	L
Platypus DAF jar	Aquagenics, Australia	Cuboid	Polycarbonate	19.70	10.50	1.90	2.00
2 L Duran bottle	Schott, Germany	Cylinder	Glass	18.90	11.60	1.63	2.00
Platypus DAF jar	Aquagenics, Australia	Cuboid	Polycarbonate	5.60	10.50	0.53	0.50
Platypus DAF jar	Aquagenics, Australia	Cuboid	Polycarbonate	10.80	10.50	1.02	1.00
Platypus DAF jar	Aquagenics, Australia	Cuboid	Polycarbonate	12.70	10.50	1.21	1.25
Platypus DAF jar	Aquagenics, Australia	Cuboid	Polycarbonate	15.20	10.50	1.44	1.50
Platypus DAF jar	Aquagenics, Australia	Cuboid	Polycarbonate	19.70	10.50	1.90	2.00
Nalgene 0.125 L	Thermo Fisher, Germany	Cuboid	Polycarbonate	6.60	5.20	1.27	0.125
Nalgene 0.25 L	Thermo Fisher, Germany	Cuboid	Polycarbonate	8.10	5.90	1.37	0.22
Nalgene 0.65 L	Thermo Fisher, Germany	Cuboid	Polycarbonate	10.10	7.60	1.33	0.45
Nalgene 1 L	Thermo Fisher, Germany	Cuboid	Polycarbonate	12.10	9.20	1.32	0.73
Nalgene 2 L	Thermo Fisher, Germany	Cylinder	Polycarbonate	13.70	9.90	1.38	0.96
3 L Duran bottle	Schott, Germany	Cylinder	Glass	8.40	18.20	0.46	2.00
3 L Beaker	AARK, China	Cylinder	Glass	13.10	14.60	0.90	2.00
2 L Beaker	AARK, China	Cylinder	Glass	16.50	13.00	1.27	2.00
2 L Duran bottle	Schott, Germany	Cylinder	Glass	18.90	11.60	1.63	2.00
Platypus DAF jar	Aquagenics, Australia	Cuboid	Polycarbonate	19.70	10.50	1.90	2.00
Measuring cylinder	AARK, China	Cylinder	Glass	13.80	6.70	2.06	0.50
Measuring cylinder	AARK, China	Cylinder	Glass	17.00	6.70	2.54	0.60
Measuring cylinder	AARK, China	Cylinder	Glass	12.00	4.00	3.00	0.15
Measuring cylinder	AARK, China	Cylinder	Glass	22.70	6.70	3.39	0.80
Measuring cylinder	AARK, China	Cylinder	Glass	28.30	6.70	4.22	1.00
Measuring cylinder	AARK, China	Cylinder	Glass	12.80	2.60	4.92	0.07
Measuring cylinder	AARK, China	Cylinder	Glass	13.40	2.20	6.09	0.05
Storage bottle	Corning, Cole-Palmer, UK	Cylinder	Polycarbonate	12.80	6.20	2.06	0.40
Measuring cylinder	AARK, China	Cylinder	Glass	12.00	6.50	1.84	0.40
Steel vessel	Not available	Cylinder	Steel	13.00	7.30	1.78	0.40
Centrifuge tube	Thermo Fisher, Germany	Cylinder	Polypropylene	14.00	6.50	2.15	0.40

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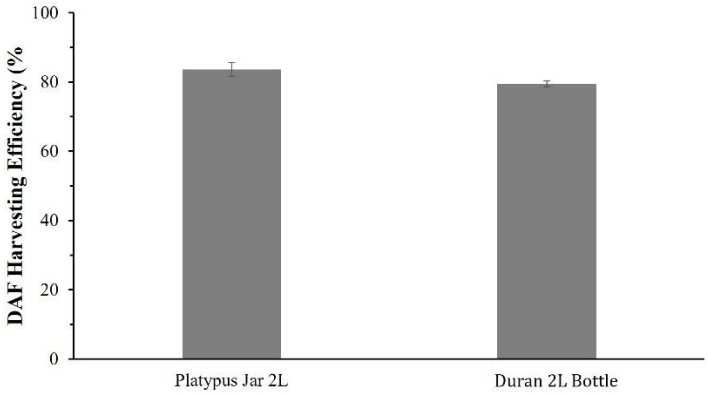

Fig.1. Comparisons of separation efficiencies when conducting dissolved air flotation (DAF) in the 2 L Platypus DAF jar and a 2 L Schott Duran bottle. Cuboidal Platypus DAF jars (height 197 mm, width 105 mm) made of polycarbonate and a cylindrical Schott Duran bottle (height 189 mm, width 116 mm) made of glass were used. Poly(dimethylaminoethyl methacrylate) was used as the flocculant.

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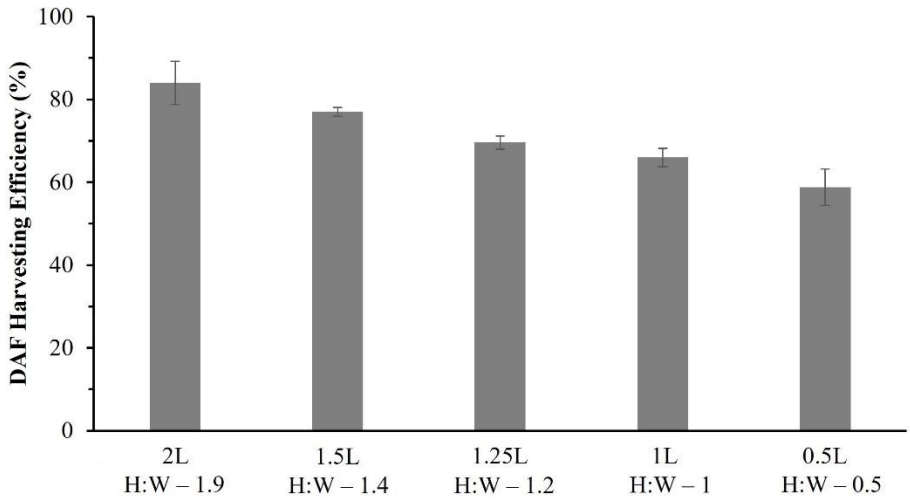

Fig.2. Dissolved air flotation performance when using Platypus dissolved air flotation jar filled to different heights (5.60-19.70 cm) and volumes (0.50-2.00 L) of *Chlorella vulgaris* culture suspension. Error bars represent standard deviations obtained for experimental variability.

Click here to access/download;Figure;Figure 3.docx

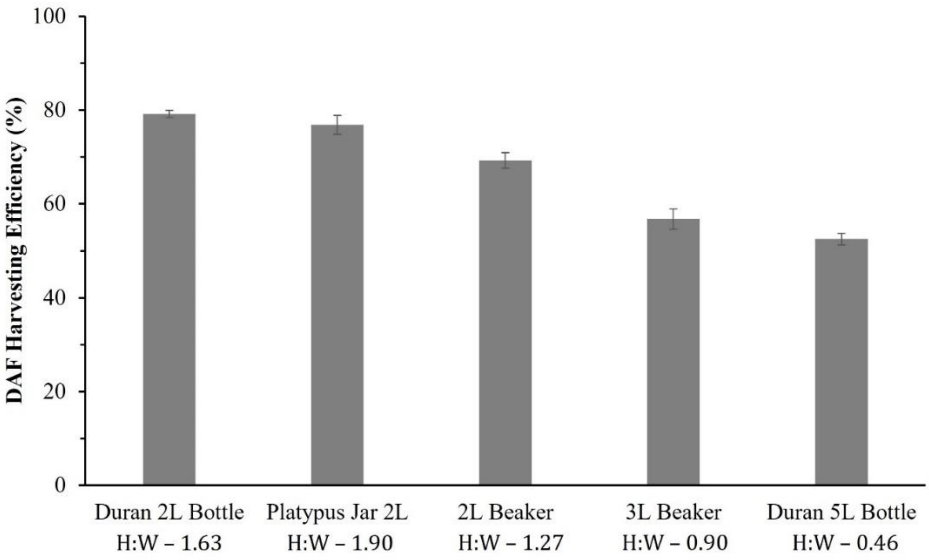

Fig.3. Dissolved air flotation performance when using different DAF jars that had fixed volumes of the flocculated *Chlorella vulgaris* suspension (2 L) but differing H : W ratio from 0.46 – 1.90. Error bars represent standard deviations obtained for experimental variability. The volume of the DAF jars listed in the figure represent the total volume of the DAF jars (2 – 5 L) and not the volume of the flocculated *Chlorella vulgaris* suspension (2 L) used.

Click here to access/download;Figure;Figure 4.docx

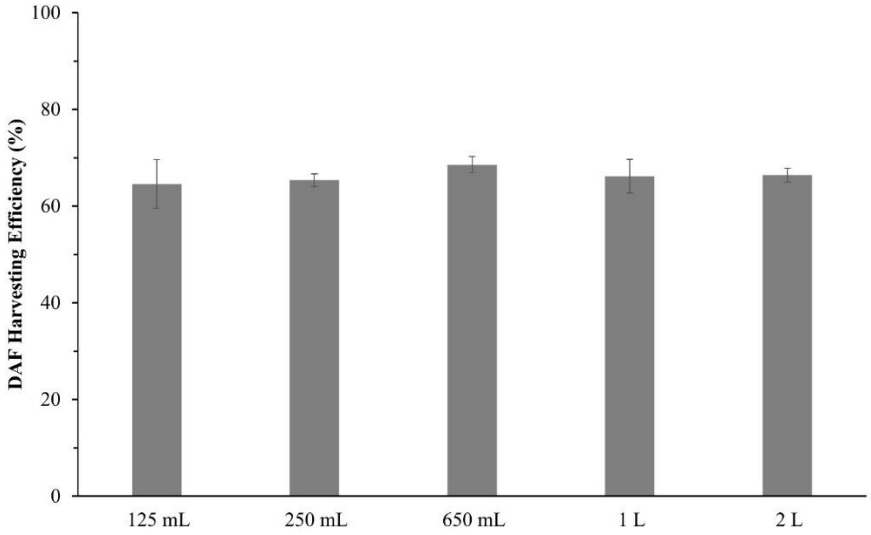

Fig.4. Dissolved air flotation performance when using different Nalgene DAF jars that had similar H : W ratio (~1.2 – 1.3) but differing volumes (125 mL – 2L) of the flocculated *Chlorella vulgaris* suspension. Error bars represent standard deviations obtained for experimental variability.

Click here to access/download;Figure;Figure 5.docx

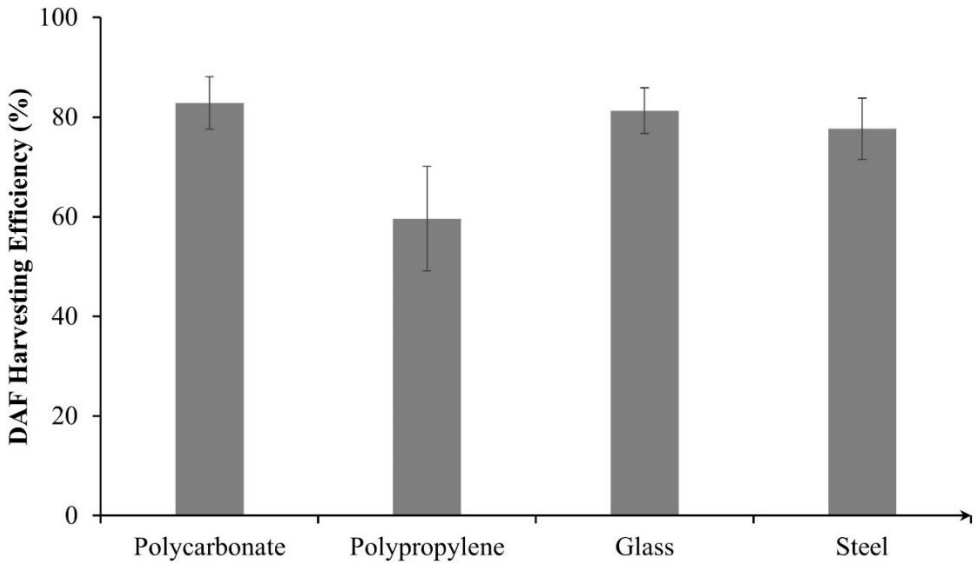

Fig.5. Algal harvesting efficiency when using dissolved air flotation jars made of polycarbonate, polypropylene, glass and steel. The jars were cylindrical and had H : D ratios ranging from 1.78-2.15. See Table S2 for details.

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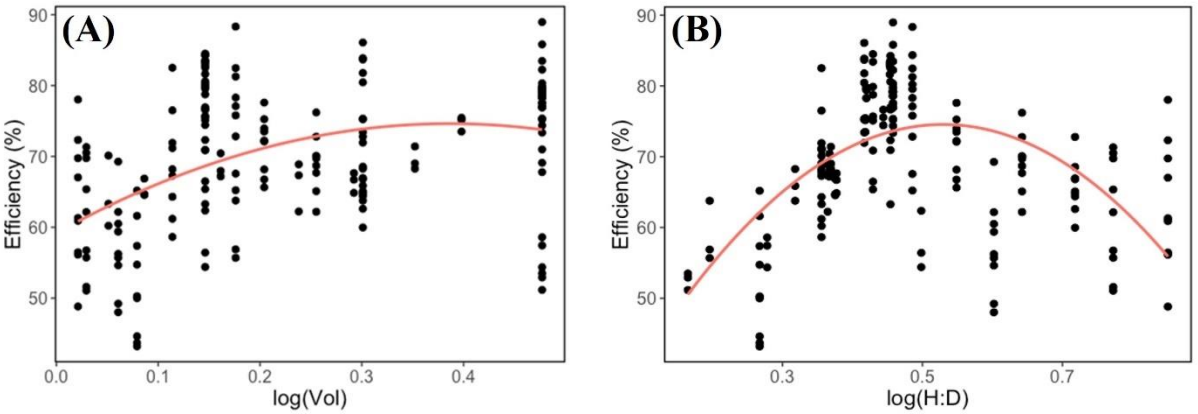

Fig.6. Algal harvesting efficiency versus log-transformed (a) volumes, and (b) H : D ratios when using several dissolved air flotation jars. 187 tests were undertaken 30 different types of jars with H : D ratio varying between 0.46 and 6.00 and volume ranging from 0.05-2 L.

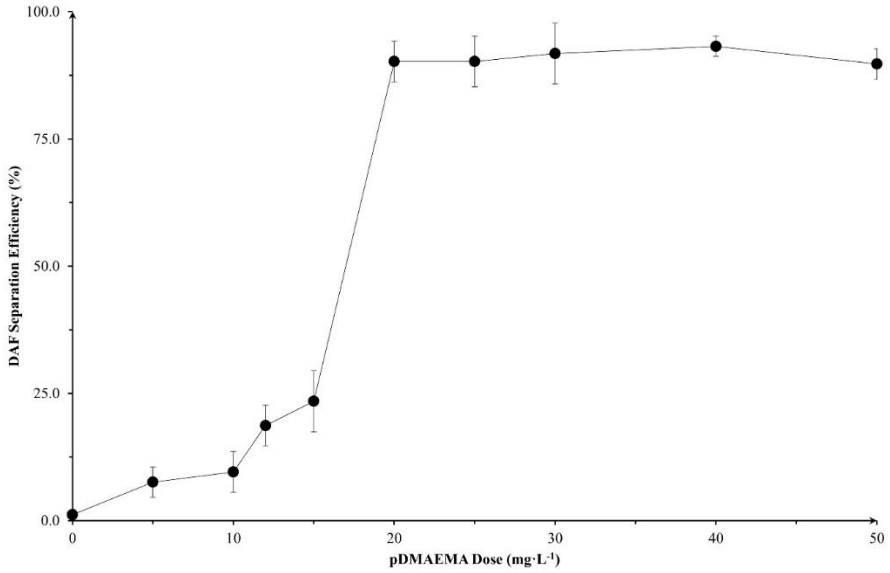

Fig S1. Dose response curve for *Chlorella vulgaris* separation via dissolved air flotation using poly(dimethylaminoethyl methacrylate) as the flocculant.

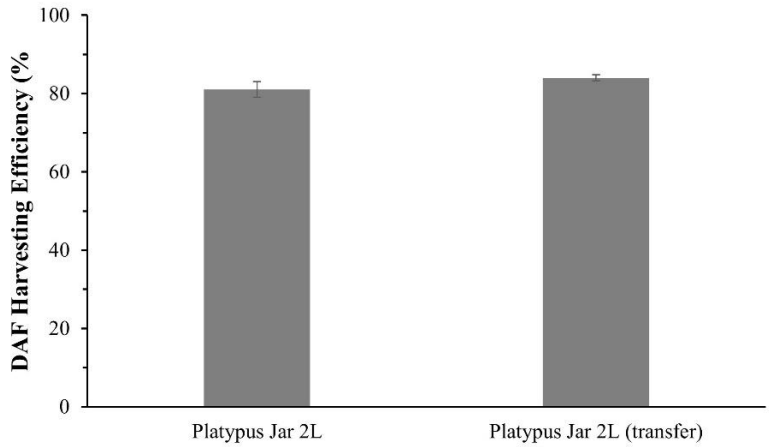

Fig S2. No statistically significant changes (ANOVA F[1,4] = 2.27, p = 0.206) were observed in separation efficiencies when conducting dissolved air flotation (DAF) (a) in the same jar in which flocculation was conducted, and (b) after transferring the flocculated suspension from one DAF jar to another. Cuboidal Platypus DAF jars (height 197 mm, width 105 mm) made of polycarbonate were used. Two liters of *Chlorella vulgaris* culture was separated. Poly(dimethylaminoethyl methacrylate) was used as the flocculant