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Harvesting optimization and Omega-3 recovery improvement from *Schizochytrium* DT3 using surfactant-aided dispersed air flotation: Response surface methodology

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ABSTRACT

Microalgae's potential to produce high value bioactives is contingent on the cost-effective harvesting of algal biomass. The use of CTAB (cetyl trimethylammonium bromide) assisted dispersed air flotation as a harvesting technique for *Schizochytrium* biomass, was optimized as a cost-effective means for recovery (*R*) and concentration factor (*CF*) using Response Surface Methodology. To the best of our knowledge, this is the first study to employ surfactant assisted dispersed air flotation (SDAF) in the recovery of a heterotrophic thraustochytrid strain. A Box-Behnken design of experiment investigating the operating parameters of CTAB amount, air flow rate and volume on *Schizochytrium* was employed. Initially, both responses were analyzed individually and then used to simultaneously maximize both variables. The optimized conditions of CTAB (500 mg/L), air flow of 2 L/min and volume of 600 mL resulted in a *R* of 91 % and *CF* of 19 times. Although a secondary step is necessary for further concentration, this technique utilizes 70 times less energy as compared to conventional centrifugation techniques which are used to recover *Schizochytrium* sp. This is significant as this technique can be easily adapted to existing bioreactors, as they are already equipped with gas spargers. In addition, the presence of surfactant carried through demonstrated an improvement in the recovery of long chain poly unsaturated fatty acid (PUFA) by 6 %, particularly in DHA and DPA which was not observed in washed biomass.

1. Introduction

Currently, the majority of long-chain polyunsaturated fatty acids (LC-PUFA) are derived from fish sources which are both unsustainable and non-renewable, only meeting 30 % of the global demand for omega-3 supplementation [1]. Thraustochytrids (such as *Schizochytrium*, and *Aurantiochytrium* sp.) are heterotrophic marine organisms classified as oleaginous microbes, with commercial potential for the production of DHA which makes up 35 to 55 % of their total fatty acids (FA) [2,3]. DHA production from these microorganisms involves both upstream and downstream processes. Upstream includes strain development, fermentation media tweaking to induce biomass and lipid production, which have been extensively studied [4–6]. Downstream processes on the other hand, which include harvesting of the biomass, has had very little attention in heterotrophic fermentation processes, despite this being one of the major bottlenecks noted in photosynthetic microalgae research [7–11]. Typically, single cell oils are produced in small-scale and the costs for processing and extraction are not considered in smaller settings [12]. However, for large-scale production, economical downstream processing techniques are essential for improving the feasibility of single cell oil production for the extraction of dietary lipids, and even more so for biofuels [12].

Biomass harvesting techniques are energy-intensive, and can account for 20–60 % of the total costs of production [13–15]. These greater costs are attributed to conventional centrifugation techniques and costly membrane replacement for filtration [16]. However, dewatering of thraustochytrids are primarily performed using energy intensive centrifugation techniques [7], with reported recovery efficiencies of up to 80–90 % [7,17]. Centrifugation techniques are not economically feasible, with greater energy consumption reported with decreases in particle size, increases in rotational speed, and increases in centrifugal bowl radius [18], which have hampered microalgae biotechnology [17]. The use of centrifugation for harvesting *Aurantiochytrium* sp. was explored by Kim et al. [19], which reported a loss in biomass as a result

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of cell rapture bringing the efficiency down to 87 %. The use of other techniques or the pre-concentration using alternate methods could significantly decrease the production costs as was discussed in a recent review [17]. It is essential to investigate alternate cost-effective harvesting techniques suitable for large scale operation in order to improve the overall economics of this biotechnology.

Other harvesting techniques such as filtration, gravity sedimentation, flocculation and flotation can also be employed [10]. Sedimentation is effective for specific strains, however, settling times can be lengthy with low biomass concentrations attained [7]. The use of flocculants to achieve greater settling rates, is suitable for a wide range of species, but the chemicals used are difficult to separate from the biomass and can be harmful to the environment [10]. Filtration techniques are prone to membrane clogging, with high costs for replacement [10]. The study performed by Kim et al. [19] also evaluated other harvesting techniques on *Aurantiochytrium* sp., which included coagulation, electrocoagulation-flotation, and membrane filtration with reported efficiencies as high as 99, 88 and 99.9 %, respectively. However, despite the high efficiency achieved in coagulation, the water content in the harvested biomass was much greater than that achieved with membrane filtration, but the efficiency of this technique was noted to be highly influenced by the degree of membrane fouling. Flocculation was also employed on *Auranticohyltrium* using chitosan by Zamri et al. [7], with reported efficiencies of >95 %, but no mention of the degree of dewatering was made. However, flotation techniques have been shown efficient in the harvesting of microalgae biomass, with low operational costs and space requirements, and suitable for large scale operation [20,21].

Dispersed air flotation processes only utilize a fraction of the energy requirements for operation, as compared to dissolved air flotation [10] and centrifugation [22]. The efficiency of the technique as well as other methods such as filtration and flocculation has been further improved with the aid of surfactants such as cetyl trimethylammonium bromide (CTAB) [16,20,23,24]. CTAB is an organic bromide salt with surface active and topical antiseptic properties, used in medicine as an apoptosis-promoting anticancer agent [25], for nanoparticle synthesis [26] and is considered a food-grade compound [27]. In dispersed air flotation, it is thought that effective recovery is achieved through the hydrophobic tail ends of CTAB which embed into the air bubbles creating an overall positive charge that electrostatically attracts the negatively charged biomass cells, and float to the surface [20,28,29]. Furthermore, harvesting using surfactant aided dispersed air flotation of photoautotrophic microalgae biomass, has also shown improvements in lipid and polyunsaturated fatty acid recoveries [20,30]. In our earlier study, CTAB was used to assist in the recovery of *Chlorella saccharophila* biomass using dispersed air flotation, and the presence of CTAB was found to improve the polyunsaturated fatty acid (PUFA) recovery, as compared to that harvested by centrifugation [30]. The study of Coward et al. [20] also employed CTAB to effectively harvest *Chlorella* sp. and noted improvements in lipid recovery, as compared to that collected using centrifugation technique.

Although the use of surfactant aided dispersed air flotation has been shown effective in the recovery of photoautotrophic microalgae biomass [20,30–32], to the best of our knowledge, it has not yet been investigated on heterotrophic thraustochytrids. Response surface methodology (RSM) is a powerful and efficient mathematical approach widely applied in biotechnology for the optimization of fermentation processes. It can give information about the interaction between variables, provide the information necessary for design, and process optimization, simultaneously [33,34]. In this study we examined the effectiveness of CTAB aided dispersed air flotation in the recovery and concentration of *Schizochytrium* DT3 biomass, as a low cost means for biomass harvest, and to assist in the improvement of PUFA recovery. Response surface methodology was used to determine and predict the optimal conditions (surfactant amount, air flow rate and working volume) required for achieving high levels of cell recovery and concentration. Experimental validation of the predicted results was performed.

2. Materials and methods

2.1. Cultivation of Schizochytrium species

Cultivation of *Schizochytrium* DT3 strain, isolated as previously described in Gupta et al. [35] and maintained in glycerol 80 % (*v*/v) solution kept at −80 °C, was revived on yeast (2 g/L), peptone (2 g/L), glucose (5 g/L) agar plate. After 5 days of growth the cells were transferred into 500 mL Erlenmeyer flasks containing 200 mL of sterilized fermentation medium. Briefly, this consisted of yeast (4 g/L), peptone (0.4 g/L), monosodium glutamate (20 g/L), glucose (80 g/L) made up with 50/50 artificial seawater and Mili Q water. The culture was left to grow for 5 days in an incubator shaker at 180 rpm and 30 °C. At the end of the incubation period, the optical density (T70, pg instruments, Wibtoft, United Kingdom) of the culture was measured ($\lambda = 660$ nm) and adjusted to a concentration of 5.9 g/L using Mili Q water prior to use. Only cultures that could be diluted down to the standard cell density were used. All experiments were conducted within a 6-week period. All chemicals were purchased from Sigma-Aldrich (Macquarie Park, NSW, Australia) and ThermoFisher Scientific (Scoresby, VIC, Australia).

2.2. Determination of biomass concentration

At the end of the cultivation period and after adjusting the biomass concentration, a 10 mL sample was transferred into a pre-weighed 15 mL falcon tube. Next, it was centrifuged (5810 R, Eppendorf, Macquarie Park, NSW, Australia) at 3500 rpm for 5 min and the supernatant was decanted. The remaining biomass was washed with 10 mL of Mili Q and centrifuged 3500 rpm for 5 mins, and the decanted biomass was frozen at −80 °C and freeze dried (Beta 2–8 LSCbasic, Christ, Sciteck, Lane Cove, NSW, Australia) for the initial dry cell weight determination, Eq. (1). The harvested biomass slurry from dispersed air flotation, was further centrifuged at 3500 rpm for 5 min and frozen at −80 °C for freeze drying. The collected biomass amounts were used to determine the recovery efficiency.

$$\text{Biomass concentration (g/L)} = \frac{Dried\ cell\ weight\ (g)}{Volume\ sampled\ (L)} \quad (1)$$

2.3. Lipid extraction and FAME analysis

Lipid extraction and FAME conversion was performed using a method adapted from Gupta et al. [36]. Briefly, 10 mg of freeze-dried biomass, was weighed into a 1.5 mL microcentrifuge tube to which 600 μL of chloroform: methanol (2:1) was added. Next, the samples were vortexed for 2 min and centrifuged (5420, Eppendorf, Macquarie Park, NSW, Australia) for 10 min at 10,000 rpm. The supernatant was collected and the extraction process was repeated twice more. The collated extracts were filtered using 0.22 μm filter membranes into a pre-weighed 4 mL glass vial and left to dry at 50 °C on a heating block (DBH30, Ratek Instruments, Boronia, VIC, Australia). The dried lipid weight was recorded for lipid yield quantification.

The extract obtained was methylated and preserved by the addition of 500 μL of toluene, 200 μL of butylated hydroxytoluene, and 500 μL of 10 % acetyl chloride (prepared in methanol). The reaction was allowed to take place at 50 °C overnight. Next, the liquid samples were transferred into a 10 mL test tube with the addition of 1 mL of sodium chloride solution (5 % *w/v* in Mili-Q (MQ) water), and 1 mL of hexane. The organic layer containing the fatty acid methyl esters was pipetted into another 10 mL test tube, to which 1 mL of potassium bicarbonate (2 % w/v in MQ water) was added for washing. The organic layer was again pipetted off into a third 10 mL test tube, where a small aliquot of sodium sulfate was added to dry the mixture. Finally, the hexane layer was transferred into a 2 mL glass vial for gas chromatography (GC)

analysis. Samples were analyzed using GC equipped with a barrier discharge ionization detector (GC-2030, Shimadzu Scientific Instruments (Oceania)). The chromatograms were integrated using LabSolutions software (Version 5.92 Shimadzu Scientific Instruments (Oceania)). The FA were identified by comparison with fatty acid methyl ester (FAME) standard (Supelco 37 component FAME Mix, CRM47885, Merck). Quantification of individual FA was reported as a percentage of total FAME.

2.4. Dispersed air flotation unit and components

The recovery of *Schizochytrium* DT3 biomass was performed using an in-house built dispersed air flotation unit (Fig. 1), similar to that previously described [21]. The unit consists of three main components: air supply, diffuser and main vessel.

2.4.1. Air supply

The laboratories central air supply, which was fed by compressed air, was connected to the inlet of the air flow meter which was used to control the air flow rate (0–5 L/min). The outlet end of the flow meter was connected to a one eight-inch PVC elbow fitting, which was attached to the bottom of the chamber and connected to the diffuser.

2.4.2. Diffuser

A cylindrical porous gas diffusing stone (TS-HA006, Aquarium Shop, QLD, Australia), made from quartz sand, was anchored to the interior of the PVC elbow fitting. It was mounted to the center of the culture chamber. This was used to provide the microbubbles for flotation. The diffuser consisted of an internal diameter of 22.2 mm.

2.4.3. Main vessel

The cultivated biomass for each flotation experiment, was loaded into the main cylindrical vessel, which was made of Plexiglas. It consisted of an inner diameter of 145 mm, outer diameter of 153 mm and a height of 250 mm. The diffuser was placed at the center of the bottom of the vessel. The top of the chamber was fitted with a 795 mm high column, with inner and outer diameters of 58 and 63.5 mm, respectively, to allow foam to travel upwards during experimentation and avoid spilling over.

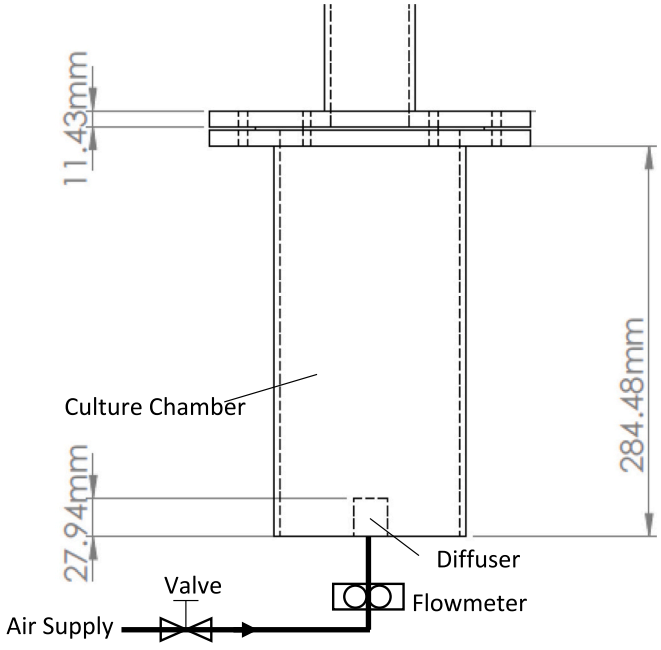

Fig. 1. Dispersed air flotation harvesting unit and culture chamber dimensions.

2.5. Experimental procedure

A working volume of 150 mL of *Schizochytrium* DT3 culture was used for each run. The experimental run volume (600–1300 mL) was adjusted with Mili Q water and stirred for 5 mins. A cationic surfactant, cetyl trimethylammonium bromide (CTAB), selected based on other studies performed that reported effective recoveries, suitable charge interaction, and for its consideration as a food-grade agent [20,21,24,27,37], was added to the biomass and further mixed for 10 mins to ensure complete mixing of the CTAB in solution. Once complete, the suspension was added to the culture chamber (main vessel), Fig. 1, of the dispersed air flotation unit. The top column was sealed in place and the required air flow was adjusted to the run parameters. Each run was terminated after 30 mins, and the biomass collected at the top of the chamber was skimmed off and collected in a pre-weighed 50 mL falcon tube for recovery and cell concentration factor determination.

2.6. Experimental design and data analysis

2.6.1. Design of experiments - Box-Behnken

A response surface, Box-Behnken design of experiment (Minitab® version 21.4.1Software, Minitab LLC., State College, PA, USA) consisting of 15 runs (3 center point replicates) was used in this study. The optimal harvesting conditions for maximum recovery of *Schizochytrium* DT3 using surfactant assisted dispersed air flotation were predicted using response surface methodology. The variables and levels selected were based on previous literature findings which reported on photoautotrophic microalgae harvesting [20,21] and initial preliminary experimental work. The three variables investigated were CTAB amount, air flow rate and culture volume, Table 1. In the preliminary experimental runs, it was identified that the dense nature of fermented *Schizochytrium* DT3 cell biomass, required a certain amount of CTAB to achieve foaming. As such, the surfactant amount and final volume were investigated separately to determine the ideal concentration of CTAB surfactant necessary. The CTAB amounts of 50 to 300 mg were selected to include the critical micelle concentration (CMC) range of 335–364 mg/L [38], which correspond to a concentration that varies from 25 to 500 mg/L with the working volume. A working volume of 600–2000 mL was selected as the minimum volume required for the apparatus and 2000 mL as the maximum volume that allows for sufficient headspace for collection. The air flow rate of 0.5 L/min was selected as the minimum flow required to achieve foaming with the minimum CTAB amounts and biomass with the apparatus, and the maximum air flow of 2 L/min was based on providing an acceptable performance at the highest CTAB

Table 1
Box-Behnken design matrix for harvesting *Schizochytrium* DT3 with recovery (*R*) and concentration factor (*CF*) achieved from experimental investigation.

Exp. no.	Air flow rate (L/min)	CTAB (mg)	Volume (mL)	*R* (%)	*CF*
1	2	175	2000	2.1	0.1
2	1.25	300	600	40.0	14.4
3	0.5	175	2000	0.2	0.2
4	1.25	50	2000	17.1	5.1
5[a]	1.25	175	1300	6.7	2.0
6	2	300	1300	50.1	10.0
7	2	50	1300	14.1	4.5
8	1.25	300	2000	36.5	7.3
9	1.25	50	600	13.3	4.0
10	0.5	175	600	0.7	0.5
11	2	175	600	13.9	5.2
12	0.5	50	1300	15.4	3.9
13[a]	1.25	175	1300	4.5	1.4
14	0.5	300	1300	13.6	4.1
15[a]	1.25	175	1300	11.0	3.3

Exp. No.: Experiment number; CTAB: cetyl trimethylammonium bromide; *R*: recovery; *CF*: concentration factor.
[a] Center point experimental runs (replicates).

amounts without flowing above the experimental area for recovery.

2.6.2. Data analysis

Recovery (*R*) and enrichment ratio or concentration factor (*CF*) were the response variables used to assess the effectiveness of the surfactant-aided dispersed air flotation in harvesting *Schizochytrium* DT3 biomass. *R* represents the percentage of cells recovered in the fomate compared to the initial culture, and was calculated using Eq. (2) for all experiments. Further validation of the *R* in the optimized experimental trails was also performed using UV spectroscopy measured at a wavelength of 660 nm, Eq. (3). While *CF* expresses the degree to which the cells are concentrated by comparing the cell concentration in the fomate with that of the initial sample, Eq. (4). The Box-Behnken design matrix and the experimental results for *R* and *CF* are tabulated in Table 1.

$$R = \frac{Cell\ weight\ (g)_{recovered}}{Cell\ concentration\ (g/L)_{initial}\ x\ Volume_{initial}} \times 100 \tag{2}$$

$$R = 100 - \frac{OD_{Initial\ culture}(\lambda\ 660\ nm)}{OD_{Residual\ media}\ (\lambda\ 660\ nm)} \tag{3}$$

$$CF = \frac{Cell\ concentration\ (g/L)_{fomate}}{Cell\ concentration\ (g/L)_{initial}} \tag{4}$$

Initially, the results for each individual response variable were assessed separately and fitted to a second-degree polynomial Eq. (5), using Minitab, in order to determine the variable levels for maximizing *R* and *CF*.

$$y = \beta_o + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j \tag{5}$$

where y is the response, β_o, β_i, β_{ii}, and β_{ij} represent the regression coefficients for the intercept, linearity of the input factor X_i, the quadratic interaction X_i, and the interaction of X_iX_j, respectively. The terms X_i and X_j represent the interaction coefficients between the factors [22]. This equation is used to determine the relationship between the response variables and the independent parameters in Table 1.

Once a fitted model was determined separately for each response, both responses were simultaneously optimized using the desirability function [39] to predict the maximum values in Minitab. The optimal parameters identified were experimentally validated by comparing the predicted and experimental responses at the optimal conditions.

2.6.3. Energy consumption

The energy consumption for operating the dispersed air floatation unit was calculated using Eq. (6). Where, the air compressors power consumption (watts) was theoretically based on small to large industrial air compressor units, capable of generating air flow rates of up to 6.3 m^3/min and 42.3 m^3/min, respectively, consuming 42.6 to 271.9 kW [40]. This suggests that the small and large systems consume approximately 0.11 kWh of electrical power to generate 1 m^3 of air. This value was used to determine the energy consumption for generating the optimized air flow rate found in this study.

$$E = \frac{W \times H}{S_t} \tag{6}$$

where *E* is the total energy consumed (kWh/m^3), *W* is the power consumption in (W), *H* is the run time (hours) and S_t is the sample volume (m^3).

3. Results and discussion

3.1. Surfactant aided dispersed air flotation (SDAF) harvesting optimization using RSM

In this study, the *R* and *CF* attained from harvesting *Schizochytrium* DT3 using surfactant aided dispersed air flotation (SDAF) following the Box-Behnken design, ranged from 0.2 to 50.1 % and 0.1 to 14.4, respectively, Table 1. The three center point replicate runs performed using the same operational conditions, experiment numbers 5, 13 and 15, resulted in varying recovery efficiencies (4.5–11 %) with a standard deviation of 3.3 %. This variation may be attributed to the manual collection process of skimming the floating biomass. Although this may seem high in the context of the lower recovery values obtained for this combination of parameters, the reverse can be said when compared with the higher recoveries achieved of up to 50 %. Future studies, however, should consider an automated skimmer or inserting a side slit opening at the top collection point to allow for a more controlled collection process.

The full quadratic model was used to assess the analysis of the variance (ANOVA) for each response variable separately, using an α of 0.05 level of significance. Next, a backward stepwise elimination was performed to selectively remove the coefficients with a greater *p*-value of α = 0.1 (unless required for hierarchical purposes), in order to identify the most significant terms that could best explain the effects of *Schizochytrium* DT3 *R* and *CF* using SDAF.

3.1.1. Recovery model using RSM

The ANOVA of the full quadratic model on the *R* response variable was highly significant with a *p*-value = 0.004 with R^2, *adjusted* R^2 and *predicted* R^2 of 96 %, 90 % and 52 %, respectively, however, some of the variables were not significant. The ANOVA of the reduced model (Eq. (7)) which best explain the results for the recovery of *Schizochytrium* DT3 using SDAF, Table 2, indicate that the model is highly significant (*p*-value <0.001). The significant parameters (*p*-value of <0.003) identified for the *R* of *Schizochytrium* DT3 were surfactant addition, flow rate, the interactions between them, and the square term. Additionally, the non-significant lack-of fit for the model (*p*-value = 0.347) suggests it is a good model predictor [41]. This indicates that flow rate, surfactant amount and their interactions best explain the *R* of *Schizochytrium* DT3 using SDAF, and can be used to predict the *R* using Eq. (7).

$$R = 1.22 - 9.29FR - 0.48CTAB + 0.0012CTAB^2 + 0.0101FR{\cdot}CTAB \tag{7}$$

where *R* is the recovery response, *FR* is the flow rate (L/min), and CTAB is the surfactant amount (mg).

The reduced model correlation coefficient (R^2), *adjusted* R^2, and *predicted* R^2 were 93 %, 90 % and 80 %, respectively. This suggests that only 7 % of the variability cannot be explained by this model, which may be attributed to the higher interaction orders for which the model does not account for. The *adjusted* R^2 value of 90 % reflects the variation fraction that is explained by factor changes, and since this is in excess of 50 %, it suggests that the most important variables are known and identified during testing as noted by Allen [42,43]. The contour plot which illustrates the relationship of the significant variables on the *R*, Fig. 2, show that increasing the flow rate and the CTAB amount were

Table 2
ANOVA reduced model results for the recovery (*R*) of *Schizochytrium* DT3 biomass harvested using dispersed air flotation using a Box-Behnken design of experiments.

Source	DF	SS	MS	F-Value	*p*-Value
Model	4	2889.21	722.30	33.63	<0.001
Linear	2	1121.49	560.75	26.11	<0.001
Flow rate	1	315.88	315.88	14.71	0.003
Surfactant	1	805.61	805.61	37.51	<0.001
CTAB * CTAB	1	1409.37	1409.37	65.61	<0.001
Flow rate * CTAB	1	358.34	358.34	16.68	0.002
Error	10	214.80	21.48		
Lack-of-Fit	8	193.12	24.14	2.23	0.347
Pure Error	2	21.67	10.84		
Total	14	3104.01			

Df: Degree of freedom; SS: Sum of squares; MS: Mean square; CTAB: cetyl trimethylammonium bromide.

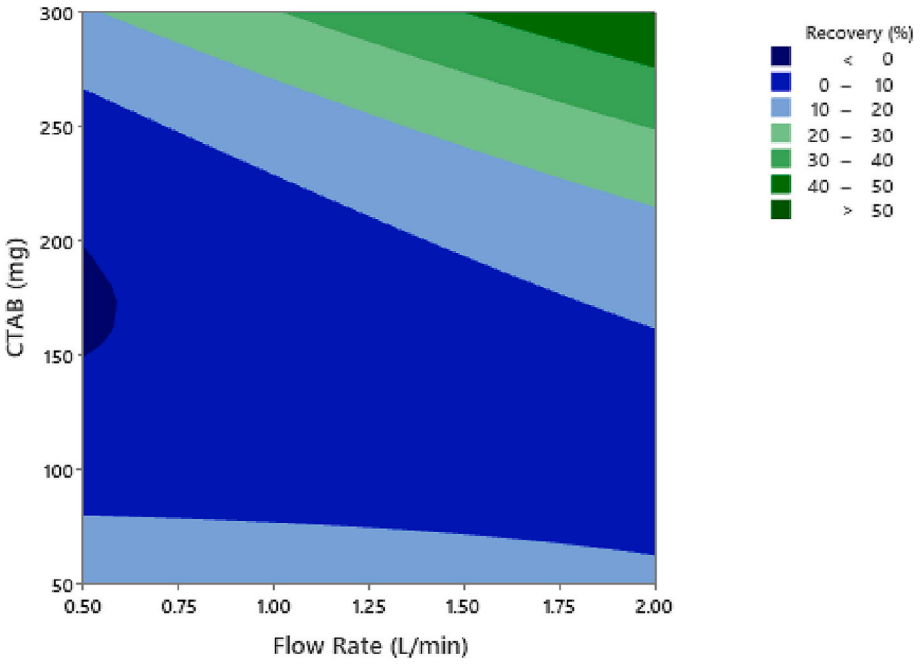

Fig. 2. Contour plot illustrating the relationship between flow rate (L/min) and surfactant CTAB (mg) on the recovery of *Schizochytrium* DT3 biomass using surfactant aided dispersed air flotation.

correlated with greater *R*. The *R* response is a measure of the number of cells which have been recovered, Eq. (2). The mechanism for SDAF is thought to occur through the hydrophobic tail ends of the CTAB which embed into the air bubbles creating an overall positive charge that electrostatically attracts the negatively charged biomass cells, and float to the surface [20,28,29]. As such, both the surfactant amount and flow rate were found significant as they directly impact the number of cells recovered at the surface. However, the volume was not significant in explaining this response term, as it does not account for the degree of dewatering, which is captured in the concentration factor term.

3.1.2. Concentration factor

The effect of the various variables on the *CF* response term was also investigated. The ANOVA of the full quadratic model resulted in a *p*-value of 0.005 which suggest the model is highly significant, with an R^2, *adjusted* R^2 and *predicted* R^2 of 96 %, 88 and 49 %, respectively. However, some of the linear, square and interaction terms were insignificant with *p*-values greater than $\alpha = 0.05$. A backward stepwise elimination ($\alpha = 0.1$) was performed to identify the most significant terms and interaction terms. The reduced model with fewer square and interaction terms, Table 3, was highly significant as suggested by the low *p*-value of 0.002 and the non-significant lack-of fit of 0.205, with an R^2, *adjusted* R^2 and *predicted* R^2 value of 85 %, 77 % and 47 %, respectively. The reduced model for predicting the *CF* is depicted in Eq. (8). As noted above, the adjusted R^2 of >50 % suggests the significant variables have been identified [42,43].

$$CF = 3.08 + 1.86FR - 0.0597CTAB + 0.0021V + 0.0003CTAB{\cdot}CTAB - 0.00002CTAB{\cdot}V \quad (8)$$

where *CF* is the concentration factor, *FR* is the flow rate (L/min), CTAB is the surfactant amount (mg), and *V* is the final volume (mL).

The relationship between the variables and the *CF* response term are graphically depicted in Fig. 3. These contour plots show that greater *CF* was attained using higher flow rates, greater surfactant additions using less volume. *CF* is a term which takes into consideration the degree of dewatering (Eq. (3)), in which case surfactant, volume and air flow rate were significant ($\alpha = 0.1$) in explaining this response variable.

Table 3
Reduced model ANOVA results for the concentration factor (*CF*) of *Schizochytrium* DT3 biomass harvested using dispersed air flotation using a Box-Behnken design of experiments.

Source	DF	Adj SS	Adj MS	F-Value	*p*-Value
Model	5	179.173	35.8346	10.41	0.002
Linear	3	74.291	24.7636	7.19	0.009
Flow rate	1	15.652	15.6520	4.55	0.062
CATB	1	42.136	42.1362	12.24	0.007
Volume	1	16.503	16.5025	4.79	0.056
CTAB * CTAB	1	87.908	87.9079	25.54	0.001
CTAB * Volume	1	16.974	16.9744	4.93	0.054
Error	9	30.982	3.4425		
Lack-of-Fit	7	29.015	4.1450	4.21	0.205
Pure Error	2	1.967	0.9837		
Total	14	210.155			

Df: Degree of freedom; SS: Sum of squares; MS: Mean square; CTAB: cetyl trimethylammonium bromide (surfactant).

3.1.3. Effect of air flow rate on R and CF

The effects of increasing the air flow rate (up to 2 L/min) resulted in greater *R* and *CF*, Figs. 2 and 3. Higher air flow rates reduce the foam residence time [44], and produce more bubbles that provide more area for cells to bind [45]. In a porous ceramic medium, lower air flow travels through the bigger pores and higher flow activates the smaller pores which allow for an increased number of bubbles that bind and carry the cells to the surface [37,46]. It was also reported by Wu et al. [47] that ceramic stones produced bubbles that were smaller than those generated by other mediums such as metal. In this study, the higher air flow rate of 2 L/min is still considered low enough to avoid disturbances which have been noted to influence recoveries above values of 4 L/min [48].

In terms of *R*, greater flow rates (2 L/min) accelerate the foaming processes and allow for increased bubble availability for biomass to be carried to the top of the chamber, which may be attributed to the higher *R* attained here (> 50 %). *CF* is typically found to increase as a function of decreased flow rate as lower air flow rates allow for more residence time for dewatering [16,21,49]. However, this was largely investigated on dilute suspensions, whereas fermented *Schizochytrium* DT3 biomass is

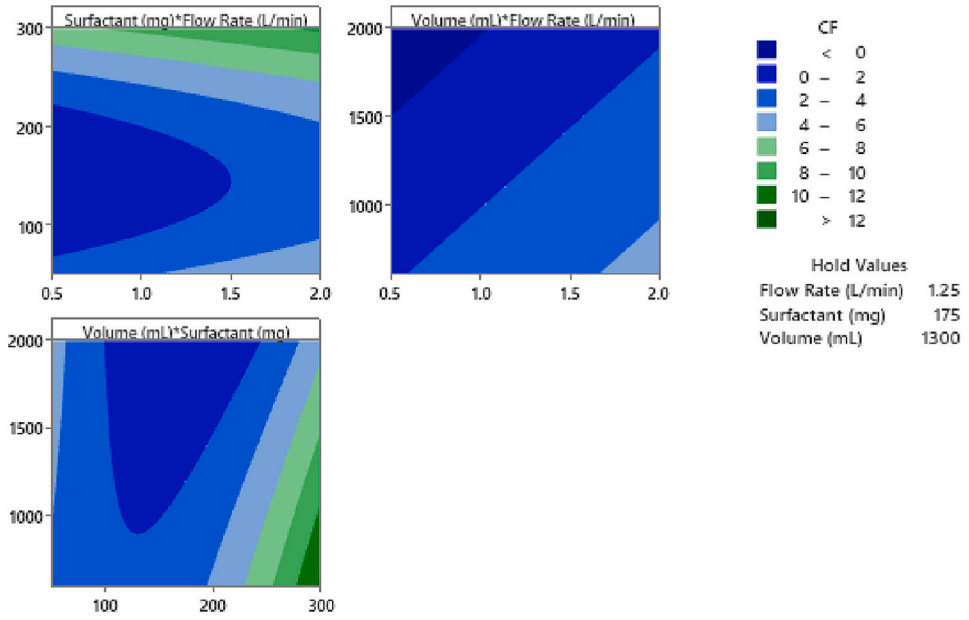

Fig. 3. Contour plot illustrating the relationship between flow rate (L/min), surfactant CTAB amount (mg), and final volume (mL) on the concentration factor of *Schizochytrium* DT3 biomass using surfactant aided dispersed air flotation.

much more concentrated requiring greater air flow rates to generate sufficient bubbles and foam to effectively carry the biomass to the top of the chamber. It was also observed that greater flow rates generated foam more rapidly allowing for longer dewatering period at the top of the chamber until the run has ended, which would provide more time for dewatering. In an earlier study investigating the flotation of *C. saccharophila*, it was noted that improved concentration factors were achieved with increasing column height which allowed for longer dewatering [21]. This was also in agreement with other studies [22,37].

3.1.4. Effect of surfactant addition

In this study, the effect of CTAB surfactant addition on both response variables, *R* and *CF*, demonstrated improvements with increasing CTAB addition, Figs. 2 and 3. The CTAB concentration range studied varied from 25 to 500 mg/L, where the upper range covers the CMC concentration of 335–364 mg/L [38]. The highest concentration of 500 mg/L was determined most effective in concentrating *Schizochytrium* DT3 biomass up to 14 times. Despite it being well reported that increased surfactant concentrations result in wetter foams which would lower the *CF* [21,49,50], the higher concentrations noted here for effective *R* (>50 %) and *CF* (>12) may be attributed to the more dense biomass of 5.9 g/L. More cells would require more CTAB surfactant to bind to the cell surface [51]. The effective recovery of microalgae cells in SDAF is thought to be a result of the electrostatic and hydrophobic van der Waals interactions, between the positively charged head group and long alkyl chain of CTAB with the negatively charged algal surface. Where both interactions lead to the CTAB binding which assists in the flotation [16,20,28,29]. The study of Taghavijeloudar et al. [23], compared the effect of short (C12) and long (C16) alkyl chain agents in the flocculation recovery efficiency of *C. sorokiniana* and noted that CTAB was more effective and required less dosage to achieve 60 % as compared with the shorter alkyl chain agent DTAB (C12). Furthermore, a concentration of 500 mg/L of CTAB is greater than the CMC, which results in micelles formation that could carry the biomass to the surface by flotation [52]. In this regard, more surfactant maybe required, to effectively form sufficient binding to carry more *Schizochytrium* DT3 biomass to the surface. Similarly, Al-Humairi et al. [49] noted that a CTAB concentration of 30 mg/L did not sufficiently produce enough foam in the presence of microalgae cells as compared to that without. They found a concentration of 45 mg/L resulted in higher *CF*. Although lower concentrations of CTAB were observed to result in dryer foams, the cell *R* was low thus lowering the overall *CF*. The greater *R* achieved with higher surfactant dosage maybe attributed to increased effective binding, as a result of micelle formation with the greater biomass concentrations, resulting in greater recoveries. This observation is consistent with other findings [16,21,31,44,49,53].

It should be noted, that the use of higher concentrations of cationic surfactants, poses environmental hazardous in wastewater discharge [54,55]. As such, surfactant concentrations in residual water should be investigated and recycled to limit further additions.

3.1.5. Effect of volume

Lastly, the effect of volume was not significant on the *R* response, Table 2, however, it was significant in predicting the *CF* (also referred to as enrichment ratio), Eq. (8). As noted above, *R* is a measure of the cells recovered as compared to the initial suspension which would be less influenced by the volume in the suspension. However, *CF* which takes into consideration the amount of volume carried with the foam, would be influenced by the amount of working volume, which in this case dictates the final CTAB surfactant concentration. Lower surfactant concentrations (25 mg/L) are achieved with higher volumes used in the experimental runs (2000 mL) and higher concentrations (500 mg/L) using lower volumes (600 mL) and greater CTAB surfactant amounts (300 mg). The *CF* attained in the experimental runs ranged from 0.1 to 14.4. It was noted by Al-Humairi et al. [49] that a CTAB increase from 30 to 35 mg/L and the dilution of the *C. vulgaris* biomass reduced the surface tension, which allows for more foam. The surfactant concentration needs to be high enough to facilitate foaming as increasing the CTAB concentration reduces the surface tension [49], however, more cells would also require more CTAB to compensate. They noted that an increase in CTAB concentration from 30 to 45 mg/L resulted in improved *CF* as the lower concentration may not have been sufficient to form foam [49]. In this study, it was observed that less surfactant in the highly dense biomass resulted in less foam. The use of greater CTAB concentrations (500 mg/L) in this study, resulted in lower *CF* (0.1 to 14.4) as compared to that of Al-Humairi et al. [49] which ranged from 30 to 145 times using CTAB concentrations of 30 to 45 mg/L. These variations may be attributed to the difference in biomass concentrations used in this study of 5.9 g/L as compared to the 1.3 g/L used by Al-Humairi et al. [49]. However, the study of Taghavijeloudar et al. [16] demonstrated

concentration factor improvements of 2.4 folds with increasing CTAB dosage from 50 to 1500 mg/L in filtration assisted harvesting of *Chalmydomonas sp.*

3.2. Optimization of Schizochytrium DT3 harvest using SDAF

After determining suitable models for predicting the *R* (Eq. (7)) and *CF* (Eq. (8)), the desirability function *(D)* in Minitab was used to predict the conditions that would result in maximizing both response variables, *R* and *CF,* simultaneously. In this study, the *CF* is of key importance as we are interested in dewatering the biomass, to recover the majority of the cells from liquid suspension in as minimal volume as possible. Accordingly, an importance value of 3 was assigned to the *CF* with a weighted value of 1. As for the *R* both importance and weighted value were kept at 1, to signify that the *CF* is more highly desired. This is necessary, as having a high *R* with lower *CF* may not suggest effective dewatering ability as the collected volume needs to be less than the original suspension to signify effective dewatering.

The optimized predicted parameters for achieving maximum *R* and *CF*, with greater importance assigned to the *CF*, were using a flow rate of 2 L/min, surfactant CTAB addition of 300 mg in a volume of 600 mL, signifying a surfactant concentration of 500 mg/L. The maximum expected values for *CF* and *R* were 13.8 ± 3.6 and 50.7 % ± 8.3 %, respectively. Four experimental runs were carried out to validate the predicted optimum values, where the average experimental *CF* was 19.1 ± 1.3 with a *R* of 91.4 % ± 3.6 %. It was observed that the remaining media was almost near clear at the end of the run as compared to that before starting, Fig. 4. The average experimental value for the *R* obtained was nearly double the predicated value. However, the standard deviation in the optimized conditions for recovery remained the same as those achieved in the Box-Behnken experimental replicate center points, but in this case the standard deviation is drastically smaller as compared to the higher recovery value obtained in the optimized conditions. As for the *CF*, the experimental value attained was within two standard deviations of the predicted. The experimental value obtained for *R* was much higher than that anticipated which may be a result of the lower ranges investigated, and the lower number of experimental runs carried out. These results were also validated by optical density measurement carried out at a λ of 660 nm on the initial biomass (diluted 10×) before starting the SDAF run, and compared with the optical density of the residual de-celled suspension media remaining after flotation, (diluted 10×), Fig. 4. The results of these readings showed a *R* of 95.6 % ± 1.7 % calculated using Eq. (3). The 4 % variation maybe attributed to the cells lost during skimming of the biomass for collection. Nonetheless the identified parameters proved to be effective in achieving a 91 % recovery for *Schizochytrium* DT3 biomass in 7.5 mL slurry, signifying a concentration factor of 19 times.

Literature on SDAF has employed this technique on dilute microalgae biomass. However, to the best of our knowledge, no studies have investigated surfactant aided dispersed air flotation for harvesting of the heterotrophic thraustochytrid biomass, although it has been noted effective, scalable and low in operational cost for the recovery of dilute microalgae cells [22,56,57]. In fact, very little effort has been placed on the harvesting of thraustochytrid using other techniques aside from centrifugation and flocculation [7,19,58], which grow achieving high biomass concentrations. In this study, the thraustochytrid *Schizochytrium* DT3 achieved a biomass concentration of 5.9 g/L in only 5 days of growth. Nevertheless, much of literature has noted different operating parameters were necessary to achieve either a high *R* or *CF*, where one is obtained at the expense of the other [22,56,57]. However, using the desirability function the optimized conditions were simultaneously achieved which resulted in high *R* of 91 % and *CF* of 19 times, which are high values for both responses.

The *CF* achieved in the experimental run using the optimized parameters corresponds to a *Schizochytrium* DT3 concentration of 164 g/L. The optimized SDAF conditions reduced the working volume by 19 times to that which is 7.5 mL (*R* of 91 %). Although the working volume has been significantly reduced, conversion operations require concentrations of 300–400 g/L [22], thus a secondary step will be required to achieve further concentrations.

These results are comparable with other harvesting techniques which have been employed on the thraustochytrid *Aurantiochytrium* sp. Centrifugation was evaluated by Kim et al. [19] in the harvesting of *Aurantiochytrium* sp., where a recovery efficiency of 87 % was reported as a result of loss due to cell rapture. They also compared this with other techniques which included coagulation, electro-coagulation-flotation, and membrane filtration with achieved recovery efficiencies as high as 99, 88 and 99 %, respectively. However, despite the higher efficiency achieved in coagulation, the water content in the harvested biomass was >90 % which was greater than that achieved with membrane filtration of 80 %, but the efficiency of filtration was found to be highly influenced by the degree of membrane fouling. The concentration factor achieved of 6 times using filtration is less than that achieved in this study. The flocculation efficiency using chitosan was also employed on *Auranticohyltrium* sp. by Zamri et al. [7], with reported efficiencies of >95 %, but no mention of the degree of dewatering was made.

3.3. Energy consumption and comparison

Despite the need for a secondary concentrating technique to achieve greater concentrations of 300–400 g/L [20], SDAF energy consumption of 0.013 kWh/m^3 calculated based on the optimized conditions achieved

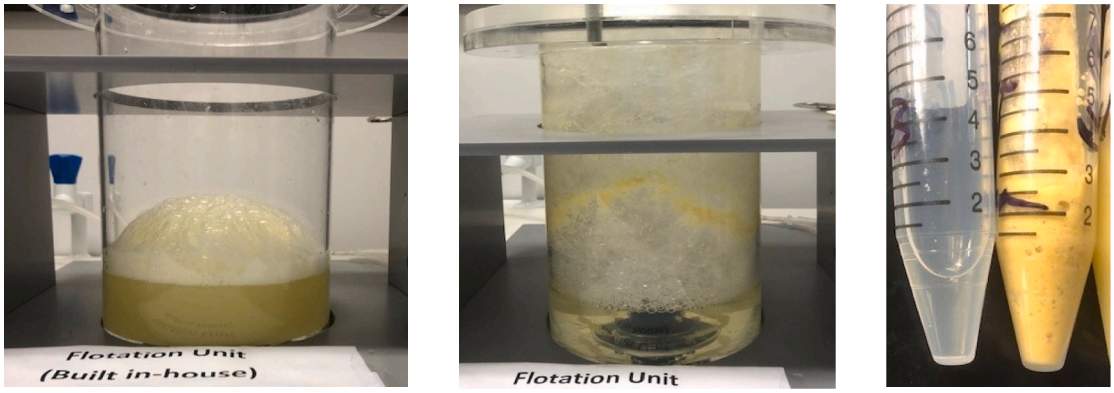

Fig. 4. Surfactant aided dispersed air flotation recovery of *Schizochytrium* DT3 biomass using optimized run parameters (a) at the start of the run, (b) end of the experimental run, (c) residual media at the end of the run, and (d) harvested *Schizochytrium* DT3 biomass slurry.

Table 4
Energy consumption comparison of surfactant aided dispersed air flotation with commonly used harvesting techniques.

Harvesting technique	Algae	Energy consumption (kWh/m^3)	Concentration factor	Reference
Centrifugation				
Disc-stack	*Scenedesmus, Coelastrum proboscideum*	1	120	[60]
Nozzle discharge		0.9	20–150	
Decanter bowl		8	11	
Hydrocyclone		0.3	4	
Filtration				
Chamber filtration	*C. proboscideum*	0.88	245	[60]
Vacuum filtration	*C. proboscideum*	5.9	180 s	
Tangential flow filtration	*Tetraselmis suecica/ Chlorococum* sp.	0.38–0.51	23–48	[61]
Flotation				
Dissolved air flotation	*Chlorella, Scenesdesmus*	7.6	NR	[62]
Dispersed air flotation		0.003		
Foam flotation	*Chlorella* sp.	0.015	250	[22]
Foam flotation	*Schizochytrium* DT3s	0.013[a]	19	This study

[a] Theoretical value estimated based on small to large air compressors capable of supplying up to 6.3 to 42.3 m^3/min of air, utilizing 0.11 kWh of energy per m^3 of air.

in this study, Table 4, are much less than those using conventional centrifugation techniques (0.3–8 kWh/m^3) [59,60]. Where the lower energy consuming centrifugation technique resulted in a poor concentration factor of only 4 times. However, good concentration factors were achieved using centrifugation techniques that utilize a minimum of 1 kWh/m^3 [60], which corresponds to >70 times the energy required to operate SDAF. Tangential flow filtration showed good concentrations factors, with lower energy requirements as compared to centrifugation, but this technique suffers from high membrane cost, membrane fouling, and the energy consumption is >25 times that of SDAF [61].

The energy consumption reported in this study for SDAF of 0.013 kWh/m^3 is comparable with other reports for dispersed air flotation which ranged from 0.003 to 0.015 kWh/m^3, Table 4 [19,22,62]. An initial pre-concentration of 19 times utilizing this technique which consumes 70 times less energy than that of centrifugation, would improve the economics of this biotechnology dramatically. However, more studies need to be dedicated to investigate the use of SDAF for the recovery of thraustochytrid as it could significantly reduce large scale production costs. The use of surfactants has also proven beneficial in preserving long chain fatty acid as discussed below.

3.4. Impact of surfactant aided dispersed air flotation on FAME profile

The FAME analysis for each of the SDAF runs was carried out on the initial biomass recovered using centrifugation (prior to surfactant exposure), and on that harvested using SDAF for comparison. This was performed on both the optimized SDAF harvesting conditions (Table 5) and the experimental runs performed for the RSM optimization (Supplementary S1).

Results showed greater total lipid (% DCW) in the biomass harvested using SDAF, as compared to the initial biomass concentrated by centrifugation prior to surfactant exposure. The lipid yields attained from the initial centrifuged biomass ranged from 19.2 to 24.0 % (DCW)

Table 5
FAME analysis of optimized surfactant aided dispersed air flotation recovery of *Schizochytrium* DT3 biomass.

Run number	OPTIM 1		OPTIM 2		OPTIM 3[a]		OPTIM 4[a]	
FAME	I	C	I	C	I	C	I	C
C12:0	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1
C14:0	6.4	6.6	6.3	6.5	7.1	7.1	7.1	7.4
C16:0	31.7	30.3	32.2	30.7	29.4	29.2	30.9	29.8
C18:0	1.8	1.9	1.9	2.0	2.0	2.0	2.1	2.0
C20:3ω6	0.5	0.6	0.6	0.6	0.6	0.6	0.6	0.6
C20:4ω6	1.0	1.0	0.8	1.2	1.0	1.1	1.2	1.2
C20:5 EPA	1.2	1.2	1.3	1.3	1.1	1.1	1.1	1.2
C22:5ω6 DPA	12.4	13.4	11.8	13.3	12.6	12.7	12.2	12.6
C22:6 DHA	19.7	20.7	19.6	19.8	19.6	19.4	18.2	18.9
Lipid %	21.5	42.2[b]	19.6	50.0[b]	24.0	26.6	23.8	29.9
SFA	65.2	63.1	65.9	63.8	65.0	64.9	66.7	65.4
MUFA	0.0	0.1	0.0	0.1	0.1	0.1	0.0	0.1
PUFA	34.8	36.9	34.1	36.1	34.9	35.0	33.3	34.5
R (%)		87.0		91.8		95.8		92.1
CF		17.4		18.4		20.5		19.7

FAME: fatty acid methyl ester; I: initial biomass; C: collected biomass; EPA: eicosapentaenoic acid; docosapentaenoic acid; DHA: docosahexaenoic acid; OPTIM: optimized run.

[a] Harvested biomass washed three times prior to freeze drying for FAME analysis.

[b] Higher lipid values in unwashed biomass are attributed to the presence of surfactant.

in the experimental DOE runs, while those recovered using SDAF ranged from 21.1 to 54.9 % (DCW), Supplementary S1. This variation maybe attributed to the presence of surfactant in the biomass, which was validated by performing 3 additional washes on the harvested biomass in the optimized run conditions, OPTIM 3 and OPTIM 4, Table 5. The optimized run conditions resulted in an initial biomass lipid yield that varied from 19.6 to 23.8 %, while that of the collected ranged from 42.2 to 50 % and 26.6 to 29.9 % in the unwashed and washed biomass, respectively. This confirms that the presence of surfactant in the biomass interferes with the lipid yields. Therefore, the biomass should be washed well before lipid yield quantification. However, the presence of surfactant in the biomass would not limit its application as CTAB is a food-grade agent and has been incorporated in the downstream processing of food based applications [27,63].

The FAME profile from the *Schizochytrium* DT3 strain, consisted mainly of palmitic acid (C16:0), docosapentaenoic acid (C22:5ω6), docosahexaenoic acid (C22:6ω3) as the major FAME which made up the majority of the lipids (Table 4). The highest fraction (% of TFA) was attributed to C16:0 in both surfactant exposed and unexposed samples, followed by DHA > DPA. In all optimized runs the PUFA content in the surfactant exposed biomass was higher as compared to the initial biomass harvested using centrifugation. Interestingly, however, the biomass recovered by SDAF resulted in PUFA improvements of 5.8 to 6 % as compared to 0.1 to 3.5 % observed in washed biomass, Table 4. This was also observed in the experimental runs performed for the response surface methodology (Supplementary S1), where higher *CF* were achieved, suggesting the presence of surfactant has an influence on PUFA. However, the degree of improvements in PUFA observed in these runs was much higher (up to 60 %) as compared to those observed in the optimized run conditions. This variation maybe attributed to the lower recoveries which suggest less biomass is there for binding indicating more surfactant available to interact with the cells present. This may also be attributed to the different operational parameters where some runs have more surfactant and less/more volume. In an earlier study performed, using the microalgae *C. saccharophila*, a similar phenomenon was also observed in that the presence of surfactant improved the recovery of total PUFA from 12 to 39 % in the presence of surfactant used in dispersed air flotation harvested biomass [30].

Other studies have also reported improvements in product yields as a

result of chemical treatment. Surfactant treatment was used by Lai et al. [64] to treat *Scenedesmus* biomass which resulted in a 16 fold improvement in FAME as compared to undisturbed biomass. The study of Ulloa et al. [65] also noted an improvement in the antioxidant extraction with the application of Triton 114 surfactant as compared to that disrupted using ultrasound processes.

It is thought that the mechanism for cell disruption is based on two interactions, electrostatic and hydrophobic, whereby the electrostatic interaction is formed by the positively charged surfactant head group and the negatively charged cell surface [66]. The hydrophobic interactions then take place where the hydrophobic tail ends of the surfactant interlock with the cell membrane forming micelles, which result in extracellular disruption [51].

The positive impact of higher PUFA content observed here in the presence of surfactant from SDAF harvest, would improve the economics of this biotechnology by doubling as an agent for cell biomass harvest and in stabilizing the essential long chain PUFA improving FAME yields. In lipid nanoparticle technology, solid lipid nanoparticle and nanostructured lipid carriers incorporate the use of surfactants to stabilize the lipid particles [67]. The presence of surfactant carried through from the recovered biomass should also be assessed in applications that utilize surfactants for downstream processing as this would improve the economics of the process.

3.5. Future prospects and direction

The downstream harvesting of *Schizochytrium* sp. biomass has seldomly been discussed in techniques other than centrifugation, although photoautotrophic microalgae harvesting has been well investigated and reported as one of the major bottlenecks for this biotechnology [7–10]. *Schizochytrium* sp. is a key producer of single cell essential oils such as DHA and DPA [3] and produces highly concentrated biomass, which has been primarily collected using centrifugation. However, centrifugation techniques are energy intensive which negatively impact the overall economics of *Schizochytrium* sp. processing. Effective centrifugation techniques consume an energy of at least 1 kWh/m^3 [59]. However, SDAF only requires 0.003–0.015 kWh/m^3 for operation [19,22,62], and has been demonstrated in this study, effective in harvesting *Schizochytrium* DT3 with a 91 % recovery and a concentration factor of 19 times. Although a secondary dewatering step such as centrifugation maybe required to further concentrate the cells if drying is required, the volume is significantly reduced which will utilize a fraction of the energy consumption that would otherwise be required by centrifugation. Research efforts need to focus on the downstream processing techniques to improve the economic feasibility of this biotechnology.

Further studies using surfactant aided dispersed air flotation for the harvest of biomass to recover other value-added products, are also vital to assess the impact of surfactants on the recovery. CTAB is considered a food-grade chemical and biological agent and has been used in various applications [27]. In this study, the presence of surfactant from the harvesting process also assisted in stabilizing the long chain PUFA, doubling as an agent aiding in the harvest and lipid stabilization. Identification of such interactions in extracting other by-products where surfactants can aid in downstream processes, would further improve the downstream economics. Research in the use of surfactant aided dispersed air flotation biomass for lipid based nanoparticle technology applications, should also be investigated as solid lipid nanoparticle and nanostructured lipid carriers incorporate the use of surfactants to stabilize the lipid particles [67].

More studies using this technology need to be instigated using fermented biomass, to better understand the parameters influencing high biomass cell concentration and the concentration of surfactant together with the air flow rate. Furthermore, scale-up studies are also needed to identify the optimal ratio of surfactant to biomass, to enable its use in large scale-up bioreactor studies. Surfactant aided dispersed air flotation is advantageous over other techniques, as it can be easily adapted into large scale bioreactors which are already equipped with gas sparger units [68].

4. Conclusions

CTAB assisted dispersed air flotation proved effective in harvesting the highly dense *Schizochytrium* DT3 culture, using Response Surface Methodology. The *R* and *CF* were significantly influenced by the surfactant addition and the air flow rate, however, the volume only effected the *CF*. The optimized conditions attributed to a *R* and *CF* of 91 % and 19 times, respectively. Although a secondary dewatering step maybe necessary to further concentrate the biomass, this technique utilizes 70 times less energy as compared to conventional centrifugation, reducing the volume significantly. Furthermore, the presence of surfactant in the harvested biomass resulted in improvements in the PUFA recovery, particularly DPA and DHA. This finding is significant as this technique can easily be adapted into existing bioreactors which are already equipped with aeriation spargers. Future studies should instigate the effects of CTAB on the downstream extraction of other bioactive molecules, and assess the reusability of the residual media to reduce the addition of CTAB in subsequent runs in order to limit wastewater discharge into the environment.

CRediT authorship contribution statement

Mariam Alhattab: Writing – original draft, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Munish Puri:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Supplementary material is attached

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2024.103512.

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