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ORIGINAL PAPER

Oil extraction from microalga *Nannochloropsis* sp. with isopropyl alcohol

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Abstract An efficient process for fractioning microalgae oil and non-lipid biomass was developed. Isopropyl alcohol (IPA) was used to extract oil from *Nannochloropsis* sp. at 80 °C, leaving the majority of non-lipid biomass in the solid fraction. The effectiveness of extraction with or without a dewatering pretreatment (DW) was compared. Effects of dewatering time and solvent ratio, IPA concentration, IPA refluxing time, and sonication pretreatment on the oil and biomass yields were studied. The dewatering conditions with a high water-to-alcohol ratio (W/A = 2:1) and mild mixing (1 min gentle shaking) had 14 % less oil loss in the DW fraction than that with a low water-to-alcohol ratio (W/A = 1:1) and vigorous mixing (30 min and 300 rpm mixing). Sonication resulted in 14–26 % more oil loss in the DW fraction when compared to intact cell treatment. Without dewatering, 85 % of the total oil from intact cells was extracted by a single extraction using 70 % (wt) IPA aqueous solution. The 88 and 95 % IPA treatments extracted similar percentages of oil to that of the 70 % IPA, but used two- and fivefold more solvent. The amount of oil extracted from broken cells increased with increasing IPA concentrations. An effective extraction can be completed in 30 min. On a 100-g (wet matter) scale, the 70 % IPA achieved 92 % oil yield and 93 % non-lipid biomass yield.

Keywords Dewatering · Isopropyl alcohol · Microalgae · Oil extraction · Sonication

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Introduction

Microalgae are promising non-food feedstocks for biofuel production because of their potentially high per-acre oil productivity, minimal land space and environmental requirement, and the ability to utilize solar energy, carbon dioxide, and a wide variety of water sources for their growth [1]. While tremendous efforts have been given to the selection of high oil yield algae strains and improvement of cultivation conditions, the downstream processing is critically important for fully utilizing this new generation of biorenewable resources. Downstream processing includes harvesting, dewatering, oil extraction, and fuel conversion. The oil extraction methods currently used for microalgae are based on the knowledge of oilseed processing. Hexane extraction, for example, is a well-developed industrial scale oil extraction technique for soybeans and other types of oilseeds, but is not suitable for most of the photosynthetic microalgae because of the high polar lipid content in the algae. *Nannochloropsis* sp., for example, contains 37 % polar lipids [2, 3]. The polar lipids in algae can be a high value co-product for food and nutraceutical applications, which would potentially benefit the algae biofuel industry if they were extracted and fractionated from the non-polar lipids. Moreover, the water in algae slurry significantly reduces the extraction efficiency when a non-polar solvent is used. Therefore, drying before oil extraction is necessary but it will increase the production costs considerably. Other organic solvents or their combinations such as chloroform–methanol can completely extract oils but is often used on a laboratory bench scale and the solvent is toxic. Attempts to use accelerated solvent extraction, supercritical fluid, and subcritical water to extract oil from algae have improved the extraction efficiency [4–6]. However, these technologies have not gained

Table 1 Physical properties of ethanol and isopropanol

	Boiling point (°C)	Latent heat of vaporization (cal/g)	Specific heat (cal/g °C)	Water azeotrope	
				Water (% wt)	Boiling point (°C)
Ethanol	78.3	204	0.61	4	78.2
Isopropanol (IPA)	82.5	159	0.60	12	80.4

Data from Johnson and Lucas [7]

popularity because of the high energy requirement in the operations. There is an urgent need for developing scalable and energy-efficient oil extraction technologies to fully utilize algae biomass for biofuel production, and for applications of their co-products.

Alcohols have long been regarded as attractive alternative solvents to hexane for oil extraction [7]. Ethanol, for example, is particularly efficient for extracting oil that has a high percentage of polar lipids such as egg yolk lipids [8]. Oil solubility in alcohols depends on temperature and water content in the alcohol or water contained in the oil-bearing materials. Oil solubility is high at elevated temperature and low when the mixture is cooled, which enables the oil recovery by phase separation instead of the energy-intensive evaporation under reduced pressure. Previous works showed that 95 % (by vol) ethanol could extract 96 and 100 % of oil from *Nannochloropsis* sp. and *Schizochytrium limacinum*, respectively [9].

Using isopropyl alcohol (or isopropanol, IPA) to extract oil from oilseed was investigated long ago [7]. A comparison of the physical properties of IPA with those of ethanol is shown in Table 1. The lower latent heat of vaporization of IPA (159 cal/g) means that it needs less heat or energy to evaporate than ethanol (204 cal/g). The azeotropic mixture of IPA–water can accommodate more water (12 %) than that of ethanol–water (4 %), indicating that less IPA will be needed when using the azeotrope as the oil extraction solvent, and more water can be removed during distillation. Early studies on cottonseed oil extraction with alcohols showed that oil solubility in the azeotrope of IPA–water at its boiling temperature 80 °C is 23 %, which was considerably higher than that in the azeotrope of ethanol–water (5 %) [7]. All the aforementioned advantages have suggested that IPA may be an excellent solvent for algae oil extraction.

Another advantage of using ethanol to extract oil from microalgae is that ethanol can precipitate proteins, allowing the efficient recovery of non-lipid biomass for other value-added applications [9]. However, it is unknown how effective IPA may be in precipitating non-lipid biomass of algae.

The overall objective of this study was to evaluate the feasibility of using IPA to extract algae oil from wet materials and concurrently allowing the recovery of the highest amount of protein or non-lipid biomass. Since mechanical disruption of the cell wall barrier may facilitate solvent penetration, the effect of sonication pretreatment on oil extraction efficiency by IPA was studied. IPA concentration used for the extraction, number of extractions, incubation time, and pre-extraction dewatering conditions were examined with broken and intact cells. A fivefold scale-up of IPA extraction was also conducted to test scalability. *Nannochloropsis* was used because it is one of the photosynthetic microalga that has been extensively studied for its lipid composition and oil extraction. It has relatively high oil content, about 25 % of dry cells, and is attractive for biofuel production.

Materials and Methods

Microalga *Nannochloropsis* sp. was purchased from Seambiotic Ltd. (Tel Aviv, Israel) as a frozen paste containing 15.7–17.5 % solids and 3.1 % (as-is) ash. Solid content was determined by weighing after a convection oven drying at 110 °C for 5 h. Total ash was determined by ashing the dry biomass at 550 °C overnight (AOAC Official Method 923.03). The reagent-grade solvents (IPA, chloroform, and methanol) were obtained from Fisher Scientific (Pittsburgh, PA).

Standard procedure of oil extraction using IPA

The thawed algae paste (20 g, 15.7–17.5 % solids) was mixed with IPA at ambient temperature (ca. 23 °C) to desired IPA concentrations. The system was refluxed at 80 °C with constant stirring at 300 rpm. After 1 h, the hot mixture was immediately centrifuged at 3,000×*g* for 10 min using an IEC Centra CL3 centrifuge (Thermo Fisher Scientific Inc., MA). The supernatant (IPA_1 fraction) was separated from the solids by decanting and it was analyzed for oil content. The solids (with about 35–40 % water) were dispersed in 30 g of IPA (to make an 88 % IPA concentration) and refluxed at 80 °C for 30 min, followed by another centrifugation under the same aforementioned conditions. Then another supernatant (IPA_2 fraction) was obtained. The residual solid is referred to as the cake fraction.

Effect of IPA Concentration on Oil Extraction Efficiency

The effect of IPA concentration used in hot extraction was studied with the "standard procedure" without the

dewatering step. Four IPA concentrations in aqueous solution were used during the first hot extraction, i.e., 50, 70, 88, and 95 % wt. The 88 % IPA solution was the azeotropic mixture with water.

Effect of Dewatering Conditions on Oil Extraction Efficiency

A dewatering step before the standard extraction procedure was adapted from the procedure reported by Wang and Wang [9] with modifications. The effect of the ratio of water to alcohol (W/A) and mixing intensity during dewatering on the extraction efficiency with intact and broken (i.e., sonicated) cells was evaluated. The algae paste was first mixed with IPA at W/A ratios of 1 or 2 (by wt) at ambient temperature (23 °C) in a 50-mL conical centrifuge tube. Two types of mixing were applied to the algae-IPA mixture, mild mixing (hand-held gentle swirling for 1 min) and vigorous mixing (centrifuge tubes horizontally placed on an orbital shaker at 200 rpm for 30 min). After the mixing, the algae paste was centrifuged at 6,000×*g* for 10 min (Legend XL centrifuge, Thermo Fisher Scientific Inc., MA). The supernatant is referred to as the dewatering (DW) fraction. The wet solid was then subjected to the standard extraction procedure by using the 88 % IPA concentration for both the first and second extractions.

Effect of Sonication Pretreatment on IPA Extraction Efficiency

The influence of cell disruption on IPA extraction efficiency was examined by comparing IPA extraction on sonicated cells with that on intact cells. The thawed algae paste was sonicated for 4 min with a relaxation of 2 min after each 30-s pulse using a laboratory ultrasonicator XL (Misonix, Newtown, CT) equipped with a probe (0.5″ tip) set at an amplitude of 10, and 550 W/20 kHz. The algae paste was kept in an ice bath to minimize the effect of heat produced by sonication on algae oil quality or protein denaturation. The ruptured cells were confirmed by microscopic observations. Sonicated algae paste was extracted with the standard procedure using 50, 70, 88, and 95 % IPA concentration. Dewatering conditions as described earlier were also studied by using sonicated algae.

Effect of Refluxing Time on Oil Extraction Efficiency

Effect of refluxing time during the first IPA extraction on the oil extraction of sonicated and intact algae cells was investigated by using the standard procedure with 70 % IPA concentration without the dewatering step. The refluxing time for the extraction was 0.5, 1, and 2 h.

Oil Extraction on a 100-g Scale

Algae paste (100 g) was sonicated and then subjected to the standard procedure with 70 % IPA without dewatering. The wet solids after first centrifugation were mixed with 130 g of IPA (to make an 88 % IPA concentration) for a second extraction at 80 °C for 0.5 h. The oil content in IPA_1 and IPA_2 was quantified. The residual oil in cake was included in the calculation of cake biomass yield and not quantified separately. Four replicates were done.

Oil Quantification Procedure

Oil in IPA_1 and IPA_2 Fractions

The IPA and water in supernatants obtained from the IPA extractions were removed by rotary evaporation at 50 °C. The dried oil extract was subjected to Folch wash [10] with minor modifications to remove non-lipid materials. The dried extract was dissolved in 12 mL of chloroform/methanol (2:1 by vol), followed by the addition of 3 mL of water and vigorous mixing for 30 s. Then phase separation of the mixture was allowed at ambient temperature (23 °C) for 20 min. Since the oil extracted from *Nannochloropsis* was dark green, which made the observation of phase separation very difficult, centrifugation at 1,800×*g* for 2.5 min using an IEC Centra CL3 (Thermo Fisher Scientific Inc., MA) ensured a complete phase separation. The upper water layer was collected for non-lipid mass determination. The solvent in the lower chloroform phase was removed by rotary evaporation at 45 °C. Then 10 mL of IPA was added to remove the residual water by rotary evaporation. The resulting oil was redissolved in 10 mL of chloroform/methanol (3:1 by vol) and filtered through a PTFE membrane filter with 0.45-μm pore size. The solvent in the final oil was evaporated under a stream of nitrogen in a 40 °C water bath then dried overnight in a vacuum oven at 23 °C. The purpose of using a rotary evaporator and chloroform/methanol in this step was only for oil quantification purposes, not for practical oil recovery on a production scale.

Oil in Cake Fraction

The cake or solid residue after IPA extraction was left in the fume hood in an open container to evaporate the residual IPA. The dried cake was then ground with a mortar and pestle. The fine powder was mixed with 50 mL of chloroform/methanol (2:1 by vol) with continuous stirring at 300 rpm for 2 h, and then the mixture was filtered through #1 Whatman filter paper. The solids were resuspended in 50 mL of fresh chloroform/methanol (2:1 by vol) for a second extraction under the same conditions. The

two filtrates were combined and the solvent was removed by rotary evaporation. The dried extract was then subjected to the same Folch wash procedure as described in the previous section.

Total Algae Oil Content Determination

The algae paste was lyophilized and then ground with a mortar and pestle. The fine powder (2 g) was mixed with 75 mL of chloroform/methanol (2:1 by vol) for 2 h at ambient temperature (ca. 23 °C). The mixture was filtered and the solid residue was subjected to a second extraction as above. Then the two filtrates were combined and purified by Folch wash. The total oil content (dry weight basis) obtained by chloroform/methanol extraction was 24.54 %, which was used to calculate the oil yield by IPA extraction as follows: Oil yield (%) = 100 × (g of oil in the extract fraction determined)/(g of algae paste × % solids × 0.2454). Oil mass balance (calculated as the percentage of oil extracted in a fraction relative to the total extracted oil, also referred to as the relative oil yield) was primarily used for the discussion on the comparison of the effects of various extraction parameters on oil extraction efficiency.

Statistical Analysis

All treatments were conducted in duplicates unless otherwise specified. Our preliminary studies showed that the standard extraction procedure used in this study gave results with low variations. The algae paste from the same package was used to complete this study to eliminate batch-to-batch variation. Data were analyzed by ANOVA with SAS (Version 9.1, SAS Institute Inc. Cary, NC, USA) to test significant difference among treatments at $p = 0.05$. The range value was calculated as the difference between the two replicates and presented when appropriate.

Results and Discussion

Effect of IPA Concentration During First Extraction on Oil Extraction Efficiency

To study the effect of IPA concentration on oil extraction efficiency, 50, 70, 88, and 95 % (by wt) IPA were used. Although the oil may become completely miscible with anhydrous IPA when the temperature is greater than 25 °C [7], it is economically impractical to dry algae cells for industrial scale processing. The 95 % IPA concentration was chosen to represent an extremely high IPA concentration condition and to compare with the previous work by Wang and Wang [9] who used 95 % ethanol to extract oil from the same microalga. Lower IPA concentrations, 50–88 %, were used to evaluate how extraction efficiency was affected by using less IPA to accommodate the water contained in algae paste. Ideally most of the algae oil should be extracted by one extraction and recovered from one IPA extract. The second extraction (IPA_2 fraction) as described in the "standard procedure" was solely to evaluate whether more oil can be extracted by increasing the number of extractions, i.e., whether the residual oil was IPA-extractable. No attempt was made to optimize the parameters for the second extraction. The difference between single and double extractions is discussed later.

The highest % oil in IPA_1, about 89 % of total extracted, was obtained from the treatment with 88 % IPA, which was the IPA–water azeotrope, when intact cells were used (Fig. 1a). However, the oil % in IPA_1 fraction (relative to total oil extracted) of the 95 and 70 % treatments were 86 and 85 %, respectively, and not significantly different from the 88 % IPA treatment with $p > 0.05$. The reason that 70 % IPA also achieved high extraction yields may be attributed to the high polar lipid content in *Nannochloropsis*, which increases the total oil solubility in the extraction solvent. The 50 % IPA extracted much less oil (58.3 %) during the first extraction than any other IPA concentrations. When the algal cells were sonicated before the extraction, the amount of oil extracted increased with increasing IPA concentrations (Fig. 1b). Regardless of sonication, the 70, 88, and 95 % IPA extracted 82–92 % of total oil from *Nannochloropsis*, which is a higher value than most of the microalgae oil extraction yields surveyed by Mercer and Armenta [11].

Effect of Dewatering Conditions on Oil Extraction Efficiency

Dewatering before the hot IPA extraction can substantially reduce the amount of alcohol used in the subsequent oil extraction when a high concentration of alcohol is required. In the current industrial practices, the harvested microalgae that have 0.1–0.7 % dry mass are usually dewatered by flocculation and centrifugation. The thick algae paste obtained after this initial dewatering, such as the algae used in this study, typically has 10–20 % solids. Further concentration is difficult and not economically feasible. Adding IPA to algae paste lowers the density of the liquid, and thus makes it easier to separate the liquid from algae biomass by centrifugation. It is also possible that the alcohol used in dewatering can reduce the negative surface charge of algae, which facilitates the subsequent extraction [12]. About 65–71 % (an average value from our experiments) of the water in *Nannochloropsis* algae paste (with about 15 % initial solid content) could be removed by such a dewatering step using an equal volume of alcohol to water

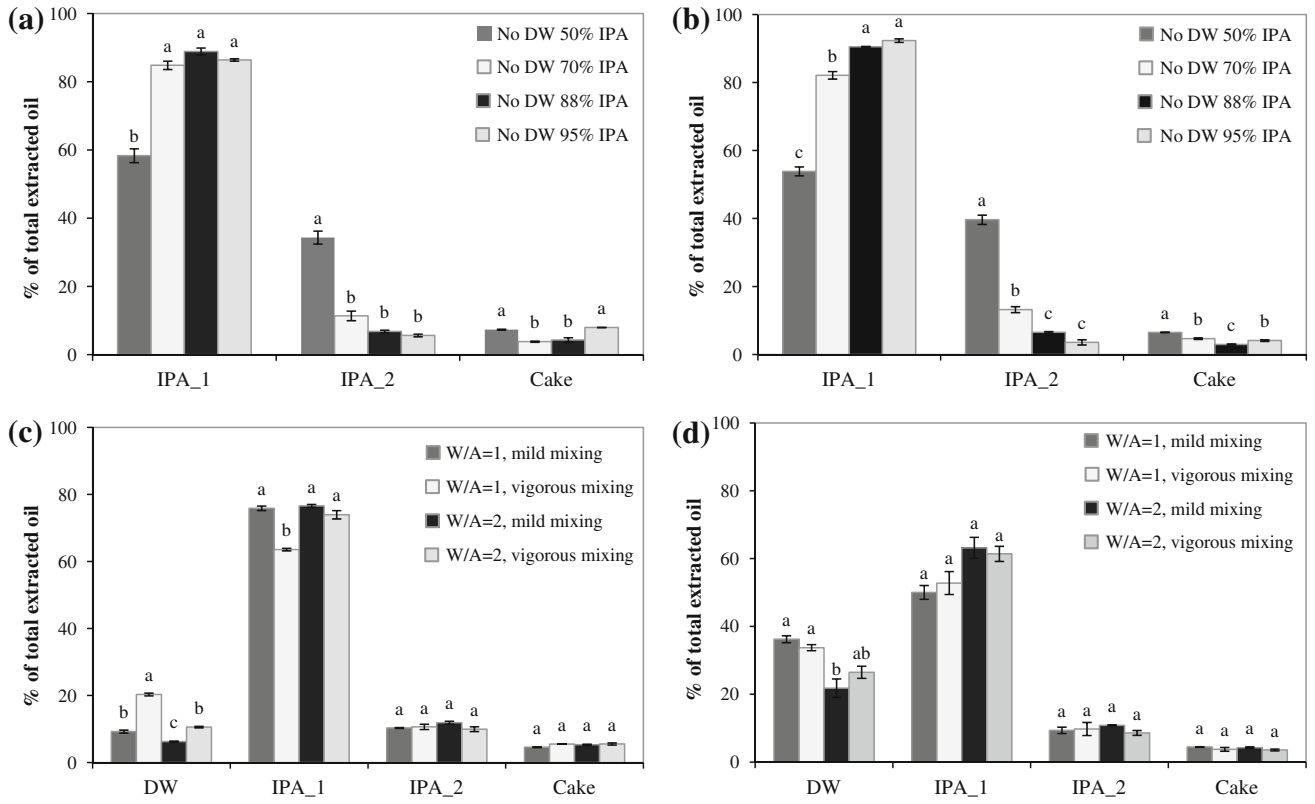

Fig. 1 Oil mass balance (% of total extracted oil) in fractions from IPA extractions of intact cells without dewatering (**a**), sonicated cells without dewatering (**b**), intact cells with dewatering (**c**), and sonicated cells with dewatering (**d**). The values with *different letters* within each fraction group are significantly different with $p < 0.05$. *DW*, dewatering; *W/A*, ratio of water to alcohol. The *error bar* represents the range value of two replicates

or half of the volume of water. However, some oil was lost in the dewatering fraction because the alcohol might have caused cell lysis, at least partially, and thus released some oil before oil extraction. The oil % in the DW fraction varied with the dewatering conditions (Fig. 1c, d).

Working with intact cells, vigorous and mild mixing resulted in 20.3 and 9.2 % oil loss (of total oil extracted) in the DW fraction, respectively, when the W/A ratio was 1 (Fig. 1c). When the W/A ratio was 2, the amount of oil that was leached in the DW fraction decreased by 32.4 and 47.9 % with mild and vigorous mixing, respectively. The vigorous mixing might have disrupted a small number of algae cells and therefore released more oil. A low W/A ratio formed a less polar solvent system, facilitating the dissolution of the released oil.

With broken algae cells, the mixing intensity had less effect on oil % in DW than the ratio of W/A (Fig. 1d). The oil loss from vigorous or mild mixing was not significantly different when the ratio of W/A was 1. With a W/A ratio of 2, the oil loss in the DW fraction was much less with both mixing conditions than those from a W/A ratio of 1; the reductions of oil content in the DW were 14.4 and 21.5 % with mild and vigorous mixing, respectively. The data suggest that the cell breakage had released oil in the algae paste, so the mixing intensity played a lesser role in the amount of oil loss in the DW fraction. The polarity of the dewatering solvent system primarily affected the oil loss in the DW fraction. Wang and Wang [9] applied dewatering as a pretreatment to the ethanol extraction of *S. limacinum* and had less oil loss (about 5 % [9]) in the DW fraction than ours. However, the dewatering mixing condition used in their study was unknown. And the oil in *S. limacinum*, a heterotrophic species, was found to be much easier to extract than *Nannochloropsis* sp., a photosynthetic type [9].

The oil % in IPA_1 fraction obtained from the treatments using vigorous and mild mixing at W/A = 2 and mild mixing at W/A = 1 with intact cells were about the same, which were in the range of 73.9–76.6 % of total extracted oil. Vigorous mixing at W/A = 1 had a significantly lower oil content in the IPA_1 fraction (63.5 %) than the other treatments because of its high oil loss in the DW fraction. With broken cells, the large variations in the oil % of IPA_1 fraction made the difference among various treatments insignificant ($p > 0.05$). Regardless of the mixing intensity, the oil % of IPA_1 fraction of W/A = 2 treatments (61.4 % from vigorous mixing and 63.1 % from mild mixing) seemed to be higher than those from W/A = 1 (52.8 % from vigorous mixing and 50.0 % from

mild mixing). The oil % values in IPA_2 and cake fraction were not affected by the dewatering conditions for both intact and broken cells.

The oil loss in the DW fractions will increase the processing cost for recovering such oil and is not desirable. This problem can be partly relieved by using mild mixing or a high W/A ratio, which minimized the oil released or lost during dewatering when intact cells are used. If the algae cells are pretreated by cell lysis, oil loss in the dewatering step will become significant.

Effect of Sonication Pretreatment on Oil Extraction Efficiency

Various mechanical disruption methods for algae cells have been studied [11, 13–15]. The degree of cell disruption usually determined how well the solvent can penetrate through the cell matrix and dissolve the lipids. *Nannochloropsis* sp. is known to have a very rigid cell wall, which is difficult to disrupt.

The sonication treatment disrupted the integrity of the algae cell wall and thus released part of the oil before IPA extraction or dewatering. The highest oil % of about 92.3 % (of total extracted oil) in IPA_1 fraction among all the treatments without dewatering was obtained from the sonicated cells by the 95 % IPA extraction. Using 95 % ethanol, Wang and Wang [9] obtained 68 % of total extracted oil from the same microalga in the first extract. Without dewatering, 88 and 95 % IPA extracted 1.9 and 6.9 % more oil, respectively, from sonicated cells than from intact cells (Fig. 1a, b). However, such a small improvement was not statistically significant with $p > 0.05$. For this particular alga, cell breakage seems unnecessary for oil extraction using IPA.

Regardless of dewatering conditions, sonicated algae generally had more oil loss (22–36 %) in the DW fraction than intact cells (6–20 %). The greatest difference in oil loss between intact and sonicated cells was found in the treatment with W/A at 1 and mild mixing, i.e., 9.2 % (intact cells) versus 36.2 % (sonicated cells). Because more oil was lost in the DW fraction, the oil % of IPA_1 fraction from sonicated algae was generally lower than that from intact cells under the same dewatering conditions. Therefore, sonication prior to the extraction and dewatering should not be both used in the extraction procedure. Alternatively, sonication may be applied to the cells after dewatering.

Effect of Refluxing Time During First Extraction on Oil Extraction Efficiency

The refluxing time of the first extraction at 0.5, 1, and 2 h had little influence on the oil mass balance among the three IPA fractions from both the intact and broken cells. The oil contents in IPA_1, IPA_2, and the cake fractions of intact cells were averaged to be 85.7 ± 1.0, 10.9 ± 0.7, and 3.4 ± 0.4 %, respectively, for the three different refluxing times, and were almost the same as those from sonicated cells (85.8 ± 0.9, 10.9 ± 0.4, and 3.3 ± 0.5 %, respectively). It is possible to further shorten the refluxing time without affecting the extraction performance. An earlier study of oil extraction from rice bran with IPA showed that 10 min was sufficient for the extraction (76 % yield) at their testing conditions even though their maximum oil yield is much lower than ours [16].

Effect of Sequential Extraction on Oil Extraction Efficiency

Two sequential IPA extractions were used throughout the study in order to determine how much more oil could be extracted with a second extraction. The experimental data can be used for further economic analysis on whether it is valuable to conduct a second extraction in order to completely extract the oil from microalgae. The effect of two sequential IPA extractions versus a single extraction was examined by comparing the total oil % of both IPA_1 and IPA_2 to the oil % from only IPA_1 fraction.

Regardless of sonication, the second extraction recovered 9–12 % of total extracted oil when dewatering was used (Fig. 1c, d). Dewatering conditions had a minimal effect on the oil % in IPA_2 fractions. When algae were directly subjected to hot IPA extraction without dewatering, the IPA_2 fraction contained 4–13 % of total extracted oil (Fig. 1a, b). The double extractions with 50 % IPA extracted 94 % (IPA_1 + IPA_2) of total extracted oil, which was not significantly different from the double extractions with 70, 88, or 95 % IPA treatments (averaged 96 % of total extracted oil). However, about 40 % of the total extracted oil was obtained by the second extraction, which was not desirable.

Total Oil Yield and the Amount of IPA Used

Oil yield values (calculated from theoretical oil content) of various treatments are shown in Table 2. In spite of the large variation, the double extractions all had higher yields compared to the single extraction, and the difference ranged from 4 to 37 %. In the single extraction, 50 % IPA had significantly lower oil yield than the other IPA concentrations regardless of sonication. The effects of IPA concentration on the oil yield generally agreed with those concluded from the relative oil yield (oil mass balance). With sonicated cells, most of the oil originally enclosed in the rigid cell walls was assumed to be available for extraction owing to the cell breakage. So the extraction

Table 2 Effect of IPA concentration, sonication, and extraction times on the oil extraction yield

	Sonicated cells		Intact cells	
	Single extraction	Double extraction	Single extraction	Double extraction
IPA%, without dewatering				
50	50.4 (2.4)[b]	87.4 (0.2)[a]	60.6 (10.5)[b]	96.2 (10.3)[a]
70	94.2 (10.0)[a]	109.4 (14.0)[a]	98.4 (12.7)[a]	111.5 (10.8)[a]
88	89.7 (0.1)[a]	96.2 (0.3)[a]	100.7 (16.5)[a]	108.4 (16.7)[a]
95	107.2 (22.4)[a]	111.2 (21.4)[a]	79.7 (1.0)[ab]	84.9 (1.8)[a]
Dewatering conditions				
W/A = 1, mild mixing	49.1 (0.4)[x]	58.3 (3.1)[x]	83.7 (7.1)[x]	95.0 (7.6)[x]
W/A = 1, vigorous mixing	51.4 (7.1)[x]	60.9 (3.4)[x]	59.0 (5.7)[y]	68.9 (5.1)[y]
W/A = 2, mild mixing	71.3 (15.1)[x]	83.5 (16.1)[x]	74.1 (1.8)[xy]	85.6 (3.1)[xy]
W/A = 2, vigorous mixing	64.2 (3.0)[x]	73.3 (5.5)[x]	86.4 (8.9)[x]	97.7 (8.0)[x]

Oil extraction yield was the ratio of actual oil extracted by IPA to total oil content determined by chloroform/methanol extraction. Yields of single and double extractions were from the oil % of IPA_1 and oil % of (IPA_1 + IPA_2) relative to total oil determined by chloroform/methanol extraction, respectively. Values in parentheses are the range values of two replicates

W/A, ratio of water to alcohol

[a,b] Means with different superscript letters within each column of treatments without dewatering are significantly different with $p < 0.05$

[x,y] Means with different superscript letters within each column of treatments with dewatering are significantly different with $p < 0.05$

Table 3 Average weight of IPA used per gram of dry cell in various IPA extractions

	No dewatering				Dewatering + 88 % IPA extraction	
	50 % IPA	70 % IPA	88 % IPA	95 % IPA	W/A = 1	W/A = 2
IPA used, g/g of dry cells	16	23	48	114	27	25

W/A ratio of water to alcohol

efficiency was primarily dependent on the oil solubility in the IPA solution, which increases with the percentage of IPA concentration. However, with intact cells, the extraction efficiency not only depended on the oil solubility but also on the ability of solvent to penetrate the algae cell wall. This may be one of the reasons that the 95 % IPA did not extract more oil than 88 or 70 % IPA when intact cells were used. The large variation of the oil yields as seen in Table 2 likely resulted from the inaccurate estimation of moisture content of the material used for each replicate. The solid content of algae was only measured a few times during the experiments because of the limited amount of sample. The measured values of solid content varied from 15.7 to 17.5 % reflecting the heterogeneous nature of the paste, which may have resulted in errors in oil yield calculations of the various treatments that were carried out in a randomized order.

The amount of IPA used in the treatment with 70 % IPA concentration (without dewatering) was almost the same as that used in treatments involving the dewatering step, about 23–25 g of IPA per gram of dry cell (Table 3), which was three times less than that used in Wang and Wang's two-stage ethanol extraction [9]. Although 70 % IPA extracted slightly less oil (in terms of oil % of total extracted) than the 88 or 96 % IPA when sonicated cells were used, it only used half and one-fifth of the volume of IPA of 88 and 95 % concentration, respectively. Thus, the 70 % IPA extraction may be more attractive from the perspective of production cost. The ratio of solvent to solid may be further reduced if less water is present in the algae sample.

Effect of Dewatering, Sonication, IPA Concentration on Non-Lipid Biomass Recovery

The co-precipitation of non-lipid materials by IPA increased with increasing IPA concentration. When dewatering was not used, the non-lipid content in cake fractions increased with IPA concentration, which indicate that less non-lipids was extracted into IPA_1 and IPA_2 fractions when the solvent polarity decreased (Fig. 2a, b). The 95 % IPA extraction (20 g scale) precipitated the most non-lipid biomass in the cake fraction, about 77 % of total. Compared with the 95 % ethanol extraction of *Nannochloropsis* sp. by Wang and Wang [9], the yield of biomass in the cake fraction of the 20-g scale with 95 % IPA extraction was lower than theirs at the 5-g scale (84 %) but higher than theirs at the 50-g scale (65 %). Dewatering conditions had little effect on non-lipids mass balance when the IPA concentrations used in the extraction were the same (Fig. 2c, d). Ideally, IPA should extract only oil from the algae and precipitate all the non-lipids in the cake fraction. The

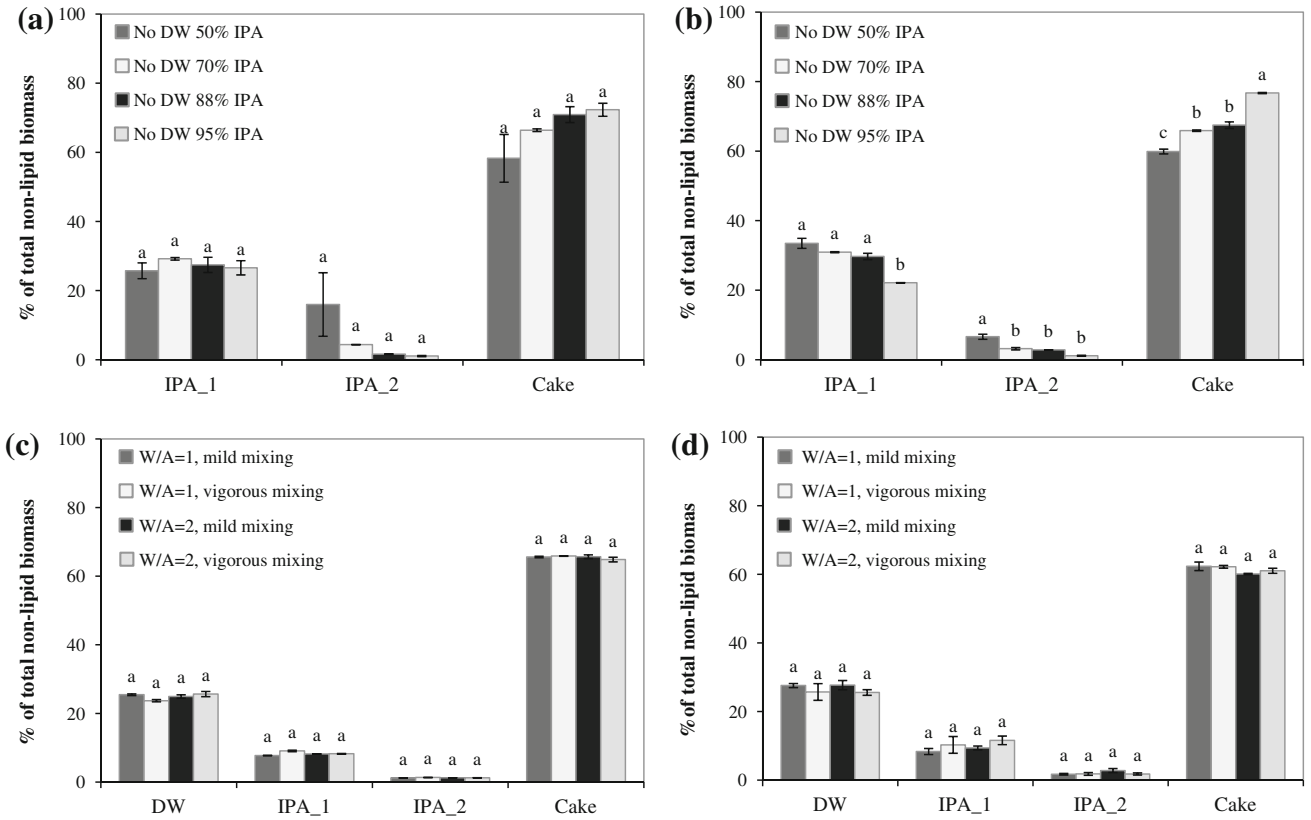

Fig. 2 Non-lipid biomass mass balance (% of total extracted non-lipids) in fractions of IPA extractions of intact cells without dewatering (**a**), sonicated cells without dewatering (**b**), intact cells with dewatering (**c**) and sonicated cells with dewatering (**d**). The values with *different letters* within each fraction group are significantly different with $p < 0.05$. *W/A* ratio of water to alcohol. The *error bar* represents the range value of two replicates

simultaneous extraction of 22–33 % of non-lipids biomass with oil was undesirable and this may be improved by parameter optimization or post-extraction fractionation.

The 100-g Scale-Up Oil Extraction

The 100-g scale-up extractions achieved satisfactory oil yields. The oil yields from single and two sequential extractions with 70 % IPA were 91.6 ± 3.7 % and 101.6 ± 3.9 %, respectively ($n = 4$). The yield of non-lipid biomass (from cake fraction only) was 92.8 ± 3.0 %. The oil and non-lipid biomass yields of the same alga (from the same commercial source) using 95 % ethanol extraction on a 50-g wet cell paste scale were 68 and 65 %, respectively [9], both of which were significantly lower than those obtained from IPA extraction. Other published data on the algae oil extraction were mostly based on the extraction of dry matter. For instance, up to 98.6 % total oil yield of a triple extraction from *Phaeodactylum tricornutum* was obtained using 96 % ethanol on dry cells [6]. Molina Grima et al. [17] reported oil yield from marine microalga *Isochrysis galbana galbana* dry biomass with various mixed solvent, and their highest yield was 93.8 % on a 5-g scale. Starting with wet cells, for example, only 14 % oil was extracted from *Chlorococcum* sp. using hexane–isopropanol co-solvent [18]. Wang and Wang [9] obtained considerably lower non-lipid biomass yield with a larger scale extraction (50 g) and attributed it to the incomplete cell breakage by sonication. On a larger scale, IPA extraction seems to be more efficient in precipitating non-lipids in the cake fraction than using ethanol for oil extraction and protein precipitation.

Conclusion

The elimination of sonication and dewatering steps and the low solvent usage make the 70 % IPA extraction a promising technology to efficiently extract oil from microalgae, particularly for algae that have high polar lipid content. The 100-g scale 70 % IPA extraction with about fivefold reduction in alcohol usage extracted 50 % more oil and precipitated 43 % more non-lipid biomass than the 50-g scale 95 % ethanol extraction, which suggests that IPA is superior to ethanol in extracting oil from this microalga. It is also possible that the partitioning of algae neutral and

polar lipids in the IPA fractions is different, thus enabling a simultaneous lipid fractionation during the extraction. Such information will be presented in our future study. Further investigations on oil recovery using other microalgae species, solvent recycling, and oil quality after IPA extraction are still needed.

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