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Short communication

Microalgae wet extraction using N-ethyl butylamine for fatty acid production

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Abstract

Microalgae are considered a promising feedstock for the production of food ingredients, cosmetics, pharmaceutical products and biofuels. The energy intensity of drying and cell breaking of algae and solvent recovery afterwards hindered the route of algae biorefinery. In this work the influences of freeze drying and cell breaking to the extraction efficiency of crude lipid yield and fatty acid yield were investigated. Results showed that drying and cell breaking are not necessary for N-ethyl butylamine extraction, because good yields were obtained without. Crude lipid yield and fatty acid yield using N-ethyl butylamine were comparable with Bligh & Dyer extraction, making N-ethyl butylamine a candidate for further development of an energy efficient lipid extraction technology for non-broken microalgae.

Keywords: Microalgae; Lipids; Extraction; Switchable solvent; Secondary amine

1. Introduction

Microalgae are considered to be one of the most promising feedstocks in recent years. There are several advantages of algae over other traditional crops, such as rapid growth rate, high productivity, less competition with arable land and freshwater, high CO_2 consumption rate, etc. [1]. Algae primary comprise lipids, proteins, carbohydrates and are widely used in the area of biofuels [2], pharmaceuticals [3] and cosmetics [4], etc.

Lipid extraction is one of the main topics in the research on algae biorefinery processes. Organic solvent extraction and supercritical fluid extraction are the most commonly used methods in algae lipid extraction. Organic solvent extraction has the benefits of inexpensive solvents and high lipid recovery yield [5]. Supercritical CO_2 ($scCO_2$) extraction is considered as an efficient, 'green' and mild extraction method for lipid extraction [6]. Both the methods have their own advantages but also some drawbacks. Chemicals used in organic solvent extraction such as hexane are highly flammable and toxic and the solvent recovery is energy intensive [7]. $ScCO_2$ extraction requires high pressure equipment which is difficult to scale up because of the combination of high pressure equipment with dry solids handling and leads to high operating cost [8].

A method named CO_2 switchable solvent extraction which can achieve lipid extraction, separation and solvent recovery by simply changing solvent hydrophilicity followed by phase splitting, aroused the interest of many researchers in recent years [1,9-12]. In such a process, the CO_2-switchable solvent extracts the lipids from the algae, after which the solvent with the lipids is isolated through simple phase separation. Then, the lipid containing solvent is bubbled with CO_2 to induce a phase splitting. After separation of the lipid and solvent phases, N_2-bubbling, optionally combined with heating may regenerate the solvent. It is proven that switchable solvent extraction is a very promising method for extracting lipids

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from algae for use in energy applications [10]. N-ethyl butylamine (EBA) was selected in this study because of the high lipid extraction efficiency, and in particular with regard to possible energy efficient lipid extraction, the capability to extract from wet, non-broken algae is highly interesting [1].

Algae lipids can be classified into two categories: (1) lipids that contain fatty acids which comprise free fatty acids, acylglycerols, phospholipids and glycolipids, and (2) lipids that do not contain fatty acids, such as hydrocarbons, sterols, ketones, pigments (carotenes and chlorophylls) [13]. Current production techniques of biodiesel and health care products for preventing or treating several diseases from algae are using lipids containing fatty acids as raw material [13,14].

The maximum yield of lipids for the various extraction techniques may vary, and may be dependent on the conditions, i.e. pretreatment procedures. Preferably, pretreatment is limited as much as possible, to reduce processing costs. For extraction methods, it is thus highly important to know whether or not any pretreatment such as cell breaking is necessary, and therefore in this paper, the crude lipid yield, as well as the fatty acid yield of algae was studied. With this study, the influence of freeze drying and cell breaking to the extraction efficiency of crude lipid yield and fatty acid yield was evaluated.

2. Experimental approach

2.1. Preparation and characterization of algae solutions

Algae of the strain *Neochloris oleoabundans* were obtained from AlgaePARC. Algae paste was mixed with water to get ~5 wt.% algae slurry that can be used in extraction. The water content in algae slurry was determined by weighing a sample before and after drying at 105 °C for 24 h. The broken fresh algae slurries were prepared by 24 h bead milling. To create the freeze dried algae powder, algae paste was treated by a lyophilizer.

2.2. Extraction and recovery of lipids from algae

Extraction of lipid from algae slurries was done according to the original Bligh & Dyer (B&D) method [15]. Each 20 g algae sample (5 wt.%) is mixed for 8 min with a mixture of 48 mL methanol and 24 mL chloroform. To the mixture is then added another 24 mL chloroform and after mixing for 1 min, 24 mL water is added and mixing continued for another 1 min. The homogenate is centrifuged and the chloroform layer containing the algae oil was collected. Also prolonged extraction procedures at several extraction times were applied to ensure extraction equilibrium. All experiments were performed in duplo.

For measurements with EBA, 20 g of algae slurries were extracted with EBA for 10 min, 30 min, 1 h, and 2 h till 24 h. After the extraction experiments, the mixtures were centrifuged and the amine layer containing the algae lipids was collected. An equal amount of H_2O was added to the collected organic layer to improve the phase separation and switching efficiency. CO_2 was bubbled in a flow rate of 2 VVM for 60 min, during which the solvent switched into the hydrophilic form. Chloroform was used to recover the lipid layer due to the small scale of experiments. The two phases thus created were separated by centrifugation (9000 rpm, 5 min) and the total amount of the extracted product was measured gravimetrically and reported as percentage on algae dry weight basis (defined as crude lipid yield). All experiments were performed in duplo. The reported error bars correspond to an accuracy of ±2.5% of the yield, which is the averaged relative standard deviation of all experiments.

2.3. Lipid transesterification and GC-MS analysis

The algae lipid extracts were analysed by GC-MS on total fatty acids (TFAs) after transesterification of the triglycerides into the corresponding fatty acid methyl esters (FAMEs). About 5 mg of lipid extract were put in a glass centrifuge tube with 3 mL methanol containing 5% (v/v) sulfuric acid and magnetically stirred at 70 °C for 3 h. Then the sample was cooled down to room temperature and 2 mL MilliQ water and 2 mL n-hexane were added followed by 40 μL of methyl nonadecanoate (10 mg/L) as internal standard. The sample was vortexed for 5 s and mixed for 15 min. After centrifuging at 3000 rpm for 5 min, the hexane (top) phase containing FAMEs was separated to a fresh glass tube. Then 2 mL MilliQ water was added to wash it. The sample was vortexed for 5 s and centrifuged for 5 min at 3000 rpm. The upper layer (hexane containing FAMEs) 1–2 mL was collected and filtrated into a GC bottle.

GC–MS analyses were performed using a 7890 A Agilent HP gas chromatograph equipped with an Varian CP 9154 capillary column (60 m, 250 μm i.d., 0.25 μm film thickness), connected to a 5975C Agilent HP quadrupole mass spectrometer. The injection temperature was 250 °C and the detector temperature was 280 °C. Helium was used as carrier gas at constant pressure of 2.28 bar. Mass spectra were recorded under electron ionization (70 eV) at a frequency of 2.5 scan per second within the range 12–600 m/z. The oven was programmed as follows: 50 °C for 4 min, 100 °C/min to 200 °C, 3 °C/min to 228.5 °C, 1 °C/min to 231.5 °C, 3 °C/min to 243.5 °C, 1 °C/min to 246.5 °C, and then 3 °C/min to 280 °C where the temperature was held for 4 min. The total analytical time was about 45 min.

3. Results and discussion

3.1. To dry, or not to dry

B&D extraction method was taken as a reference in this research. Different lipid yields were obtained when different algae samples (~5 wt.% algae slurry or freeze dried algae) were used for extraction. Fig. 1 shows that the crude lipid yield of *N. oleoabundans* wet paste was 13.8 wt.%. Comparing with extraction from freeze dried algae (14.5 wt.% crude lipid yield), less lipid was extracted out from wet algae sample. In these two experiments, the algae samples used were in different

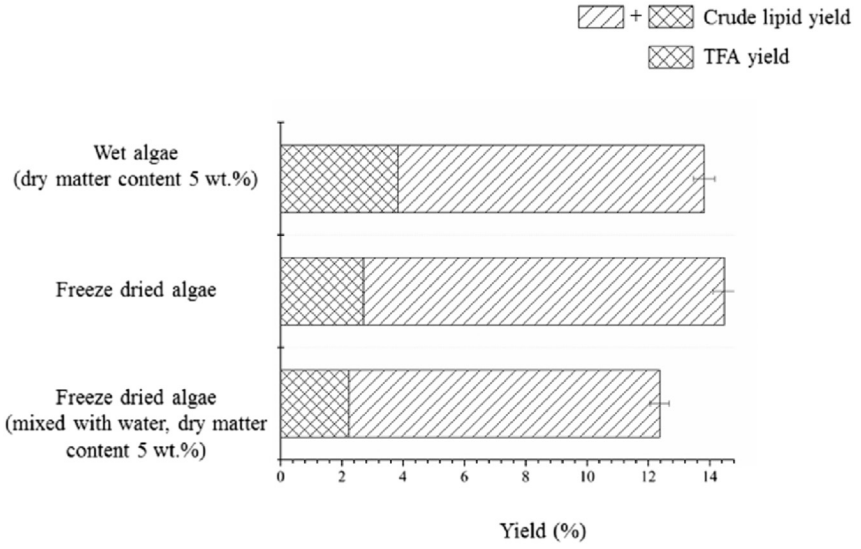

Fig. 1. Crude lipid yield and TFA yield of *Neochloris oleoabundans* samples in different condition.

conditions: with and without lyophilisation. Another difference between these two experiments is that no water is involved in the extraction step when freeze dried algae were used. Therefore, a third experiment was carried out. Freeze dried algae were first mixed with water to form 5 wt.% algae slurry and then used for extraction. If lyophilisation could weaken the algae cell therefore enhance the lipid yield, a higher lipid yield than 13.8 wt.% would be expected. However, it turned out that the crude lipid yield was 12.4 wt.%. These results showed that the main reason which caused lower crude lipid yield is water not the freeze drying operation. Since the lipids containing fatty acids are particularly the species of interest in this work, the total fatty acid (TFA) yields of these three samples were also measured. The results in Fig. 1 show that the highest TFA yield was obtained from the wet algae extraction due to the highest TFA fraction in crude lipid among those three samples. All these results lead to the conclusion that it is wise to do the extraction with wet algae paste instead of dried sample to get a higher TFA yield.

3.2. To break, or not to break

Besides drying, cell breaking is another energy intensive step in algae biorefinery processes. A series of experiments was carried out to compare the extraction from non-broken and broken algae. Several extraction times was applied to study the time required to ensure extraction equilibrium. The results are shown in Fig. 2. For non-broken algae, the maximum crude lipid yield (13.9 wt.% of dry algae) was reached after 120 min extraction. For broken algae, the highest crude lipid yield (15.3 wt.% of dry algae) was obtained at 60 min and slowly went down with longer extraction time. The TFA yield in dry algae keeps constant while the crude lipid yield decreases after long extraction time (120 min). In the broken algae slurry, most substances contained in algae cell were released and mixed. Some degradation may happen to the lipids. The carboxylate end of fatty acid molecules bonded to different head groups (e.g. an uncharged head group or a charged head group) can form different type of lipids (e.g. neutral lipid or polar lipid) [13]. Free

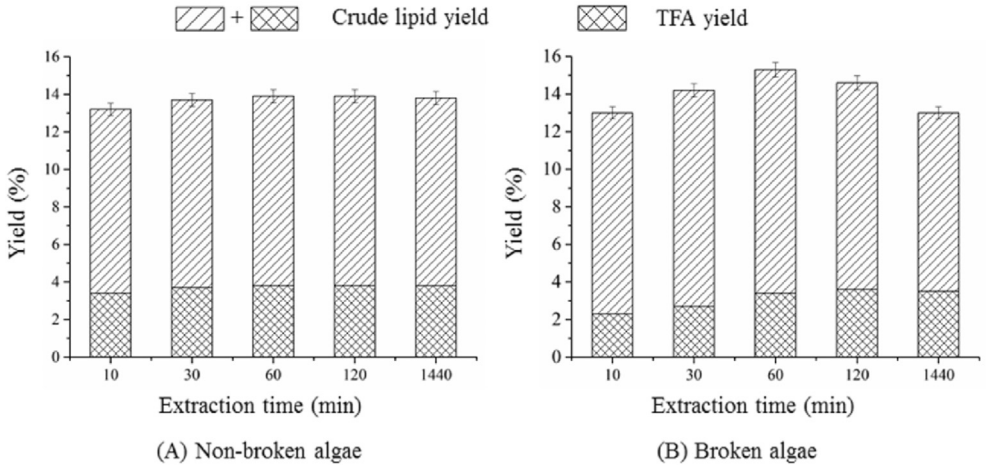

Fig. 2. Results of crude lipid yield and TFA yield of *Neochloris oleoabundans* (5% dw) extracted by B&D method.

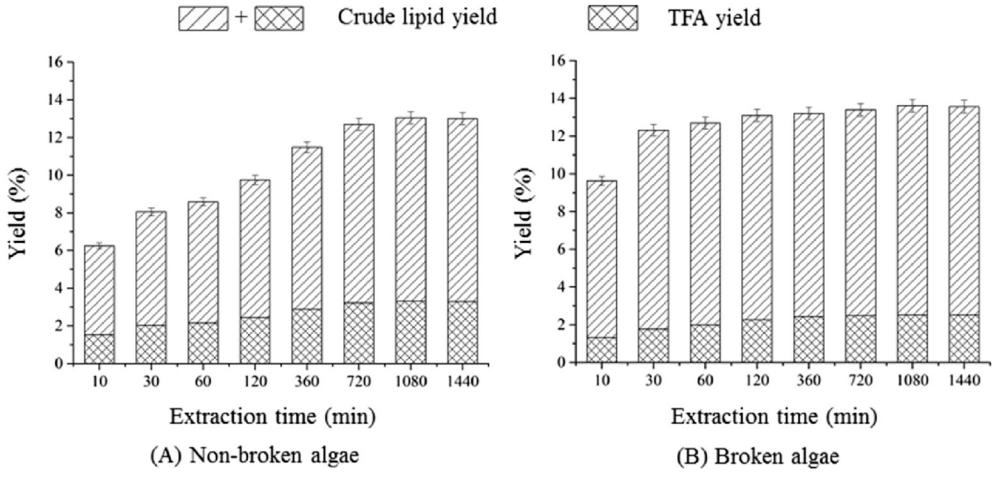

Fig. 3. Results of crude lipid yield and TFA yield of *Neochloris oleoabundans* (5% dw) extracted by EBA method.

fatty acids maybe released from the head groups of lipids when the degradation process happened. These remained in the extracted product. Some of the head groups (e.g. phosphate or sugar complex) due to their high polarity went to the water phase and caused the decrease of crude lipid yield if longer extraction time was applied. Although the crude lipid yield for broken algae is a little higher than for non-broken algae, the TFA yields of 3.6 wt.% and 3.8 wt.% (in algae dry weight) for broken algae and non-broken algae are almost the same. So the B&D 120 min extraction from non-broken algae results will be taken as a reference in further studies.

Both non-broken and broken algae were extracted by EBA at 22 °C with solvent/feed ratio of 1:1 (w/w) while different extraction times were applied. The results in Fig. 3 show that for non-broken algae, the crude lipid yield increased gradually with time and reached equilibrium (13.0 wt.%) at 18 h. And for broken algae, the increase of crude lipid yield happened mostly in the first 2 h. This is because the cell breaking step released the lipids which were inside the algae cell and made them easier to contact with EBA. Although lipid extraction from broken algae was faster and yield was slightly higher than from non-broken algae, remarkably the TFA yield of non-broken algae (3.3 wt.%) was higher than broken algae (2.5 wt.%) and also comparable with B&D extraction results. From the results in Fig. 4 it is visible that the fatty acid compositions of lipids from EBA extraction were constant over extraction time and more unsaturated fatty acids presented in the lipids extracted from non-broken algae. These results show the possibility of extracting lipid from non-broken algae while using EBA as extractant and show that cell breaking prior to extraction might affect the lipid product composition.

Although EBA could already get results comparable with B&D while extracting lipids from non-broken algae in a single stage, more extraction steps may even increase the lipid yield. Therefore, when the non-broken algae were extracted by EBA for 18 h and B&D method for 2 h, a second round of extraction was applied to the extracted algae. The results are illustrated in Fig. 5. After two times extraction, the crude lipid yield of EBA and B&D extraction increased to 16.2 wt.% and 15.3 wt.% respectively. There was also small increase of TFA yield. Fig. 6 shows that the TFA compositions of lipids from the first time and second time extraction are almost constant. Only a little more unsaturated fatty acids appeared in the second time extracted lipids. All these results indicated there were some lipids left in the algae cell after extraction which can be highly recovered by multi-step extraction. Energy requirement should be balanced between extraction cycles and lipid yield while choosing the solvent/feed ratio in real industry process.

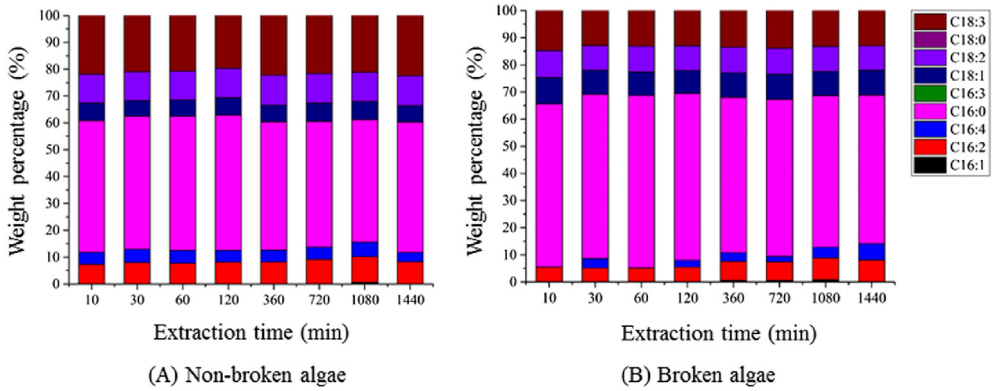

Fig. 4. TFAs compositions of lipids from non-broken and broken *Neochloris oleoabundans* (5% dw) extracted by EBA method.

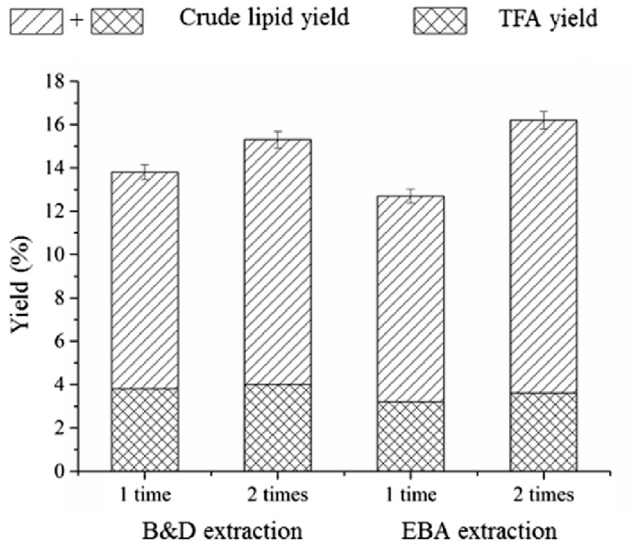

Fig. 5. Results of crude lipid yield and TFA yield of non-broken *Neochloris oleoabundans* (5% dw) extracted by B&D and EBA method for 1 time and 2 times.

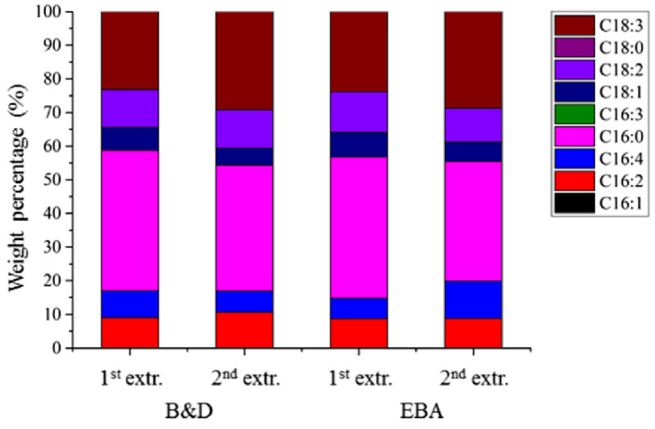

Fig. 6. TFAs compositions of lipids from non-broken *Neochloris oleoabundans* (5% dw) extracted by B&D and EBA method for the 1st time and 2nd time.

4. Conclusions

In this work, extractions using wet algae or freeze dried algae as starting material were investigated. Extraction with wet algae was preferred to get higher TFA yield. Cell breaking increased the crude lipid yield to a certain extent. But extraction from non-broken algae resulted in higher TFA yield. EBA extraction from non-broken wet algae provided promising results of fatty acid production from microalgae.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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