Switchable Solvent for Lipid Extraction from Microalgae

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2018

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This thesis is performed within the AlgaePARC Biorefinery program with financial support from the Netherlands' Ministry of Economic Affairs in the framework of the TKI BioBased Economy under contract nr. TKIBE01009.

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Designed by: Ying Du

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ISBN: 978-90-365-4573-0

DOI: 10.3990/1.9789036545730

URL: <https://doi.org/10.3990/1.9789036545730>

SWITCHABLE SOLVENT FOR LIPID EXTRACTION FROM MICROALGAE

DISSERTATION

to obtain
the degree of doctor at the University of Twente,
on the authority of the rector magnificus,
prof.dr. T.T.M. Palstra,
on account of the decision of the graduation committee,
to be publicly defended
on Friday the 13th of July 2018 at 16.45 hours

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To my family

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Summary

Microalgae are considered one of the most promising sustainable feedstocks for lipid extraction to produce food ingredients, cosmetics, pharmaceutical products and biofuels. Reasons for the increased attention for microalgae in recent years is their rapid growth rate, and thus high productivity and high CO₂ fixation rate, the absence of competition for arable land and (to lesser extent for) freshwater with terrestrial crops. Microalgae occur in a wide range of species, rich in proteins, lipids, carbohydrates, having the ability to produce high-valuable compounds, often lipid-based. For large scale production, however, developments on cost reduction are necessary. Next to the costs for cultivation, the energy intensity of drying and milling of algae prior to lipid extraction and of solvent recovery afterwards is a major obstacle. In the work described in this thesis it was aimed to find/evaluate a novel, effective, switchable solvent system for direct lipid extraction from (unbroken) microalgae in (concentrated) aqueous solutions.

In **Chapter 3**, several switchable solvent candidates were investigated on their lipid extraction performance and the polarity switching and phase splitting ability upon contacting with CO₂. Secondary amine N-ethyl butyl amine (EBA) was selected for lipid extraction from aqueous slurries of fresh, unbroken *Neochloris Oleoabundans* microalgae because of its good performance on both switchability and lipid extraction performance. Moreover, this solvent allows the recovery of the lipid and of the extraction solvent via a simple phase splitting step in the presence of water, induced by contacting with pure CO₂ at atmospheric pressure and ambient temperature. EBA reacts with CO₂ in presence of water and forms EBA carbamate salts and EBA bicarbonate salts. The binary mixture EBA/water can form one single liquid phase, immiscible with e.g. sunflower lipid oil, even after converting only 25% of the initial amount of EBA. This latter aspect showed the possibility of lipid recovery from a semi-switched EBA solution. This semi-switching simultaneously prevents gel formation which might occur at near complete conversion of EBA into carbamate/bicarbonate salts. Solvent recovery after lipid separation is achieved by nitrogen stripping at a slightly elevated temperature (50-90°C), switching EBA back into its apolar form.

Extractions using wet and freeze dried algae as starting material were investigated for both Bligh & Dyer (B&D) extraction, commonly used as analytical technique to determine the total lipid content, and for EBA extraction. Extraction with wet algae using EBA was found to result in a higher total fatty acid (TFA) yield. Cell breaking increased the crude lipid yield to a certain extent for both methods. But extraction from non-broken algae resulted in higher TFA yield when using EBA. Therefore, for the strain *Neochloris Oleoabundans*, drying and cell breaking were not necessary for lipid extraction using EBA, while crude lipid yields and

fatty acid yields were comparable with B&D extraction.

In **Chapter 4**, the lipid extraction yield from non-broken *Neochloris oleoabundans* slurries (~5% dry weight) using EBA was maximized for both single stage extractions, and for multistage extractions. The *Neochloris oleoabundans* microalgae were cultivated in fresh water (FW) and artificial seawater (ASW) under stressed and non-stressed conditions. The study showed that for wet algae a cell disruption step was not needed, although a considerable longer extraction time (12-18 h) was required to achieve the maximum yield at room temperature (22°C) and a solvent/feed ratio of 1:1 (w/w). Multiple extraction stages show that, depending on solvent to feed ratio, a second or even third extraction stage can be beneficial. For fresh water cultivated, non-stressed, non-broken *Neochloris oleoabundans* 13.1 wt.% of lipid extraction yield (based on dry algae mass) was obtained in a single stage extraction while 22.1 wt.% of lipid extraction yield was achieved after 4 times extraction.

For FW-stressed *Neochloris oleoabundans*, in one stage extraction the lipid extraction yield was 47.0 wt.% (all based on algae dry weight) of which 52.9 wt.% total fatty acids. A maximum yield of 61.3 wt.% was reached after four stages of extraction. After two extractions already 56.7 wt.% was obtained, suggesting that two stages might be optimal. The fatty acid profile of *Neochloris oleoabundans* was dominated by palmitic (C16:0), hexadecadienoic (C16:2), hexadecatetraenoic (C16:4), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids, and no significant differences in this fatty acid profile were found between the extraction methods and extraction stages tested.

The results in this work illustrate that the combination of stressing the freshwater cultivated microalgae and applying EBA as solvent in a single stage or multiple stages of extraction is very promising in the development of an energy efficient lipid extraction technology targeting non-broken, wet microalgae.

The study in **Chapter 5** advanced the understanding on the equilibrium extraction of lipid from FW-stressed *Neochloris oleoabundans*. With the hypothesis that after extraction, the algae cells were completely filled with the organic solvent phase, having the same composition as the organic phase outside the cells, the model was successfully fitted to the experimental crude lipid yields of the four stage extractions at various solvent to feed ratios. By modelling it was found that nearly all fatty acids were released from the cell material, but not all is recovered due to the organic phase remaining inside the cell. This mechanism also explains the incomplete lipid recovery in a single extraction stage. The extraction yields estimated by the model showed good agreement with those obtained in experiments. The developed model can predict the amount of crude lipid being recovered from any stage of a multistage extraction process. The parameters of this model may need to be adjusted before the model can be applied to other algae, or other solvent. For common applied solvent to feed

ratios, two extraction stages are sufficient for recovering most of the lipid containing fatty acid.

In **Chapter 6**, an alternative solvent switching method (in comparison to CO₂ switching) for lipid separation and solvent recovery was investigated. The lower critical solution temperature (LCST) behavior of EBA-water mixtures and temperature-dependent phase behavior in the presence of lipid oil were studied. It was proven that the temperature responsive partitioning of EBA over aqueous and apolar phases can be used for oil recovery and also for lipid recovery from microalgae, when cycling the mixture temperature (indicatively) between 4°C and 20°C. The temperature at which LCST-like behavior is observed increases slightly with the oil content. The maximum EBA concentration which can be used for oil separation is 50 wt.%. The oil recovery efficiency can reach more than 90% when EBA fraction in the aqueous phase is below 0.3 wt./wt. In order to get an efficient oil recovery, the lipid concentration with respect to the organic phase should be more than 5 wt.%. A process for wet lipid extraction from microalgae with EBA, and then using the temperature responsive partitioning behavior of EBA for lipid recovery and subsequently for solvent regeneration was proposed.

Research on lipid extraction in a microalgae biorefinery context has been mainly targeting oil content enhancement, cell disruption method investigation, extraction efficiency improvement, etc. There has not been so much attention on the treatment of biorefinery residuals, such as raffinate streams and microalgae cells after lipid extraction. The treatment of raffinate and microalgae cells after wet lipid extraction is important for the viability of this microalgae biorefinery process because solvent loss will cause pollution to the environment and increase the process cost. In **Chapter 7**, several solvents/solvent combinations were evaluated for EBA recovery from both the raffinate and from the residual microalgae cells after extraction.

Dodecane was selected to recover EBA from the raffinate after lipid extraction because of its lower energy usage. Besides the EBA loss in the raffinate, there was also EBA loss in the algal cells residue, both inside and outside the cells. More than 85% of this EBA loss can be recovered by using simply water in one step extraction with a solvent/feed ratio 20:1. EBA was most likely completely removed from algal cells residue. The EBA that was not recovered was because of evaporative losses during the manual operation procedures in the lab, which one should be able to prevent in a large scale process. The EBA from the wash water and the raffinate streams were combined and a liquid-liquid extraction process using dodecane as extractant was applied to extract and recover the EBA from this aqueous stream. After extracting the EBA, the EBA was recovered from the dodecane solvent by distillation. These extraction and distillation processes were simulated and optimized using Aspen Plus V8.8. When pure dodecane was used as extractant, an extraction column should have 24

stages with solvent to feed ratio 1:1.8 (w/w) in order to meet the requirement for waste water at the point of discharge (740 mg/L). In this study, the solvents will be reused after distillation, for which the distillation targets are set at a distillate that contains 99.9 wt.% EBA and a bottoms product that contains 99.9 wt.% dodecane. When this regenerated 99.9 wt.% dodecane is used as extractant, the extraction column has to be at least 39 stages in order to have the raffinate that can meet legal requirements at the point of discharge. The RadFrac block in Aspen Plus was selected for the distillation calculation and optimization, resulting in a heat duty of 2.50 MJ/kg dry algae in the reboiler (Q_{reb}) and -0.55 MJ/kg dry algae cooling duty for condenser (Q_{cond}).

In **Chapter 8**, the two process options studied in this thesis for wet lipid extraction from microalgae with EBA were evaluated, with focus on their energy use. The difference between these two processes is that for switching for lipid recovery and solvent regeneration, one method uses CO_2 to transform the solvent to the hydrophilic state and the other method uses temperature as the stimulus. Process designs have been made for both options to calculate and compare the energy flows. Results showed that the energy usage due to “EBA carbamate loss” accounts for more than 80% (49.7 MJ/kg lipid) of the overall energy requirement (61.7 MJ/kg lipid), making the use of CO_2 switching for energy production via lipid extraction unviable, unless this carbamate reversion is solved. More work on the realization of complete carbamate reversion is therefore needed for this option.

A much lower energy usage (12.4 MJ/kg lipid) and a clear positive energy balance (22.4 MJ/kg lipid) for lipid extraction was achieved with the temperature switching process, making this a very promising method for extracting lipid from algae for use in energy applications.

Finally, the advantages, weaknesses and challenges of using EBA as switchable solvent for lipid extraction from wet microalgae slurries, as microalgae biorefinery extraction technology, are discussed.

Samenvatting

Microalgen worden beschouwd als een van de meest veelbelovende duurzame grondstoffen voor lipide-extractie voor de voedingsmiddelenindustrie, cosmetica, farmaceutische producten en biobrandstoffen. Redenen voor de toegenomen aandacht voor microalgen in de afgelopen jaren zijn hun hoge groeisnelheid, de bijbehorende grote opbrengst per hectare en de hoge CO₂ opname. Ook is er geen directe competitie met landbouwgewassen voor landbouwgrond en (in mindere mate) zoetwater. Microalgen komen voor in een breed scala aan soorten en zijn rijk aan eiwitten, lipiden en koolhydraten. Daarnaast hebben ze het vermogen om hoogwaardige verbindingen te produceren, vaak op basis van lipiden. Voor grootschalige productie zijn echter nog grote ontwikkelingen op het gebied van kostenreductie noodzakelijk. Naast de teeltkosten is het energieverbruik voor het winnen van de lipide olie uit de microalgen een belangrijke kostenpost. Hierbij spelen drogen en vermalen van algen, voorafgaand aan lipide-extractie, en het energie verbruik voor het terugwinnen van de lipide oliën uit het extractiemiddel een belangrijke rol. Recentelijk, staan schakelbare extractiemiddelen, wiens karakter kan worden geschakeld van lipofiel naar hydrofiel en weer terug, in de belangstelling vanwege de mogelijkheid tot eenvoudige lipide olie-extractiemiddel scheiding. Het werk beschreven in dit proefschrift heeft als doel om een nieuw, effectief en schakelbaar oplosmiddelsysteem te vinden en evalueren voor directe lipide-extractie uit (ongebroken) microalgen in (geconcentreerde) waterige oplossingen.

In **Hoofdstuk 3** worden verschillende schakelbare extractiemiddelen onderzocht op hun prestaties in lipide extractie, de polariteitswisseling en het fase-splitsingsvermogen bij contact met CO₂. Op basis van vergelijkend onderzoek wordt het secundair amine N-ethylbutylamine (EBA) geselecteerd voor lipide-extractie uit waterige slurries van verse, ongebroken *Neochloris Oleoabundans* microalgen vanwege de goede prestaties op zowel schakelbaarheid als lipide-extractie. Bovendien maakt dit oplosmiddel de winning van het lipide en van het extractieoplosmiddel mogelijk via een eenvoudige fasescheiding in aanwezigheid van water. De omzetting van lipofiel naar hydrofiel wordt geïnduceerd door contact met CO₂ en werkt al bij atmosferische druk en omgevingstemperatuur. EBA reageert met CO₂ in aanwezigheid van water en vormt EBA-carbamaatzouten en EBA-bicarbonaatzouten. In aanwezigheid van CO₂ kan het binaire mengsel EBA/water een enkele vloeibare fase vormen, niet mengbaar met lipide oliën zoals b.v. zonnebloemolie, zelfs na het omzetten van slechts 25% van de oorspronkelijke hoeveelheid EBA. Dit laatste aspect toont de mogelijkheid tot het terugwinnen van de lipiden uit een onvolledig omgezette EBA-oplossing, hetgeen mogelijk energie bespaart en gelvorming voorkomt, welke kan ontstaan bij volledige conversie van EBA naar de hydrofiële carbamaat / bicarbonaatzouten, wat de

procesvoering bemoeilijkt. Het terugwinnen van het EBA oplosmiddel (na lipide scheiding) uit de waterfase wordt bereikt met stikstof de CO₂ weer uit de oplossing te strippen bij een enigszins verhoogde temperatuur (50-90°C), waardoor EBA weer apolair wordt en zich als separate fase afscheidt.

Extracties met natte of gevriesdroogde algen als uitgangsmateriaal worden onderzocht voor zowel Bligh & Dyer (B&D) extractie, een algemeen gebruikte analytische techniek om het totale lipidegehalte te bepalen, en EBA-extractie. Extractie met EBA uit een natte algen-slurrie bleek te resulteren in een hogere totale vetzuur opbrengst. Door het breken van de celwand nam de ruwe lipide-opbrengst voor beide methodes tot op zekere hoogte toe. Maar extractie uit niet-gebroken algen resulteerde in een hogere totale vetzuur opbrengst in het geval van EBA als extractiemiddel. Voor *Neochloris Oleoabundans* blijkt het drogen en breken van de celwand niet noodzakelijk voor lipide-extractie met behulp van EBA, terwijl ruwe lipide opbrengst en vetzuuropbrengst vergelijkbaar waren met die voor B&D extractie.

In **Hoofdstuk 4** wordt de lipide opbrengst door extractie van niet-gebroken *Neochloris Oleoabundans* slurries (~ 5% drooggewicht) met behulp van EBA gemaximaliseerd voor zowel enkeltraps extracties als voor meertrapsextracties. De *Neochloris Oleoabundans* is gekweekt in zowel zoet water (ZW) als kunstmatig zeewater (KZW) onder zowel gestreste (S) als niet-gestreste (NS) omstandigheden. Het onderzoek toont aan dat voor natte algen er geen cel breek stap nodig is, hoewel er wel een aanzienlijk langere extractietijd (12-18 uur) nodig was en een oplosmiddel/toevoerverhouding van 1:1 (w/w) om de maximale opbrengst bij kamertemperatuur (22°C) te behalen. Meerdere extractiestappen tonen aan dat, afhankelijk van de verhouding tussen oplosmiddel en voeding, een tweede of zelfs derde extractiestap voordelig kan zijn. Voor zoetwater gekweekte-, niet-gestreste- (ZW-NS) en ongebroken *Neochloris Oleoabundans* werd 13.1 wt.% lipide-extractieopbrengst (op basis van droge algenmassa) verkregen in een extractie met een enkele stap, terwijl 22.1 wt.% lipide-extractieopbrengst werd bereikt na een viertrapsextractie.

Voor zoetwater-gestreste (ZW-S) *Neochloris Oleoabundans* bedroeg de extractieopbrengst van de lipiden in één fase-extractie 47.0 wt.% (alles op basis van drooggewicht van algen), waarvan 52.9 wt.% totale vetzuren. Een maximale opbrengst van 61.3 wt.% werd bereikt na vier extractiestappen. Na twee extracties werd al 56.7 wt.% verkregen, wat suggereert dat twee trappen optimaal zouden kunnen zijn. Het vetzuurprofiel van *Neochloris Oleoabundans* werd gedomineerd door palmitinezuur (C16:0), hexadecadienzuur (C16:2), hexadecatetraeenzuur (C16:4), oliezuur (C18:1), linol (C18:2) en linoleen (C18:3) -zuren. Er werden geen significante verschillen gevonden in de vetzuurprofielen tussen de extractiewerkwijzen en de geteste extractiestadia.

De resultaten in dit werk illustreren dat de combinatie van het stressen van de zoetwater

gekweekte microalgen en het toepassen van EBA als oplosmiddel, in één of meerdere fasen van extractie veelbelovend is in de ontwikkeling van een energie-efficiënte lipide-extractietechnologie gericht op niet-gebroken, natte microalgen.

De studie in **Hoofdstuk 5** leidt tot een beter begrip van de evenwichtsextractie van lipide uit ZW-S *Neochloris Oleoabundans*. Met de hypothese dat na extractie de algencellen volledig waren gevuld met het organische extractie, met dezelfde samenstelling als de organische fase buiten de cellen, werd het model met succes toegepast voor het beschrijven van de experimentele ruwe lipide-opbrengsten van de viertrapsextracties bij verschillende oplosmiddel-algenslurrie verhoudingen. Door modellering werd gevonden dat vrijwel alle vetzuren uit het celmateriaal vrijkomen, maar niet alles werd teruggewonnen omdat er wat organische fase in de cel achterbleef. Dit mechanisme verklaart ook de onvolledige extractie van de lipides in één enkele extractiestap. De extractierendementen, zoals voorspeld door het model, vertoonden een goede overeenkomst met die zoals verkregen in de experimenten. Het ontwikkelde model kan de hoeveelheid ruw lipide voorspellen die wordt teruggewonnen tijdens elke fase van een meertraps extractieproces. De parameters van dit model moeten mogelijk worden aangepast voordat het model op andere algen of een ander oplosmiddel kan worden toegepast. Voor gebruikelijke toegepaste verhoudingen tussen oplosmiddel en voeding zijn twee extractiestappen voldoende om overgrote deel van de lipides en vetzuren te extraheren.

In **Hoofdstuk 6** word, in vergelijking met het schakelen onder invloed van CO₂, een alternatieve polair-apolair schakelmethode voor het oplosmiddel onderzocht. Het zogeheten LCST-gedrag (lower critical solution temperature) van EBA-watremengsels is het temperatuurafhankelijk fasegedrag, dat in afwezigheid- en in aanwezigheid van een lipide-olie nader is bestudeerd, met het oog op zowel lipide scheiding uit het oplosmiddel als op terugwinning van het oplosmiddel uit water. Uit de experimenten is gebleken dat de temperatuurgevoelige verdeling van EBA over de waterige en de apolaire fase kan worden gebruikt voor lipide olie productie uit een waterige slurrie van microalgen, wanneer de temperatuur van het mengsel (indicatief) tussen 4°C en 20°C wordt gecirculeerd. De temperatuur waarbij het LCST-achtig gedrag wordt waargenomen, neemt enigszins toe met het oliegehalte in het mengsel. De maximale EBA-concentratie die kan worden gebruikt voor oliescheiding is 50 wt.%. De efficiëntie van de olieterugwinning kan meer dan 90% bereiken wanneer de EBA gewichtsfractie in waterfase lager is dan 0.3. Om een efficiënte oliewinning te verkrijgen, moet de lipide fractie in de organische fase meer dan 5 gewichtsprocent bedragen. Op basis van de verkregen resultaten wordt een nieuw proces voorgesteld voor de lipide-extractie met EBA uit een waterige slurrie van microalgen, gevolgd door een temperatuur geïnduceerde omschakeling van EBA voor het afscheiden van de lipide olie en, in de regeneratiestap, voor het terugwinnen van EBA uit de waterfase.

Onderzoek naar lipide-winning in een microalgen bioraffinage context heeft zich voornamelijk gericht op de verbetering van het oliegehalte van de algen, onderzoek naar het (af-)breken van de celwand, de verbetering van de extractie-efficiëntie enz. Voor de behandeling van reststromen uit bioraffinage, zoals de raffinaatstromen en de microalgen cellen na lipiden extractie is veel minder aandacht geweest. De behandeling van raffinaat- en microalgencellen na een natte lipide-extractie is belangrijk voor de levensvatbaarheid van het voorgesteld proces, omdat een verlies aan oplosmiddel zowel kosten verhoogt als leidt tot ongewenste emissies.

In **Hoofdstuk 7** worden verschillende oplosmiddelen en oplosmiddel combinaties beoordeeld op geschiktheid voor EBA terugwinning uit zowel het raffinaat als uit de resterende microalgencellen na extractie. Op basis van energieverbruik is uiteindelijk dodecaan geselecteerd om EBA terug te winnen uit het raffinaat na lipide extractie. Naast het EBA-verlies in het raffinaat was er ook verlies van EBA in de algencellen, zowel binnen als buiten de cellen. Meer dan 85% van dit EBA-verlies kan worden teruggewonnen door eenvoudigweg water in één-stapsextractie met een oplosmiddel/toevoerverhouding van 20:1 te gebruiken. EBA werd hoogstwaarschijnlijk volledig verwijderd uit algencellen. Verdampingsverliezen tijdens de handmatige procedures in het laboratorium maakten een volledige sluiting van de EBA balans niet mogelijk. Deze verdampingsverliezen zijn naar alle waarschijnlijk te voorkomen in een grootschalige uitvoering van het proces. De EBA terugwinning uit het waswater van de algencellen kan worden gecombineerd met die van de raffinaatstroom in een gezamenlijk vloeistof-vloeistof-extractieproces waarbij dodecaan als extractiemiddel wordt toegepast om het EBA uit deze waterige stroom te extraheren en terug te winnen. Na het extractie kan het EBA door destillatie weer van het dodecaan extractiemiddel worden afgescheiden en gerecycled. Deze extractie- en destillatieprocessen zijn gesimuleerd en geoptimaliseerd met processimulatie software Aspen Plus V8.8. Wanneer zuiver dodecaan wordt gebruikt als extractiemiddel, zou een extractiekolom 24 trappen moeten hebben bij een verhouding van oplosmiddel tot voeding van 1: 1.8 (w/w) om te voldoen aan de vereiste voor afvalwater op het punt van lozing (740 mg/L). Het teruggewonnen oplosmiddel kan na de destillatie opnieuw worden gebruikt. Wanneer bij de destillatie als doel wordt gesteld dat het destillaat 99.9 wt.% zuiver EBA is en het bodemproduct 99.9 wt.% dodecaan bevat, moet de extractiekolom door het recyclen ten minste 39 trappen zijn om te voldoen aan de wettelijke vereisten voor zuivering van de waterstroom op het punt van lozing. Voor de destillatie berekeningen in AspenPlus is gebruik gemaakt van het RadFrac-blok in Aspen Plus, waarmee de optimale operatie condities zijn bepaald, leidend tot een warmtevraag in de reboiler (Q_{reb}) van 2.50 MJ / kg en tot een koelvraag voor de condensor (Q_{cond}) van -0.55 MJ / kg droge algen.

In **Hoofdstuk 8** worden de twee procesopties die in dit proefschrift zijn bestudeerd voor natte

lipide-extractie van microalgen met EBA naast elkaar gezet en vergeleken op basis van energieverbruik. Het verschil tussen deze twee processen is dat voor het transformeren naar de hydrofiele toestand in de eerste methode CO₂ wordt gebruikt en in de andere methode de temperatuur als stimulus wordt gebruikt. Voor beide opties zijn procesontwerpen gemaakt om de energiestromen te berekenen en te vergelijken. De resultaten toonden aan dat voor het schakelen met behulp van CO₂ het energieverbruik als gevolg van "EBA carbamaat verlies" goed is voor meer dan 80% (49.7 MJ/kg lipide) van de totale energiebehoefte (61.7 MJ/kg lipide). Deze energiebehoefte maakt het gebruik van CO₂ voor de schakeling van het oplosmiddel onrendabel voor netto energieproductie, tenzij de EBA carbamaat conversie energiezuinig en (nagenoeg) compleet kan worden uitgevoerd. Vervolgonderzoek aan het CO₂ schakelen moet zich dan ook in eerste instantie toeleggen op dit aspect.

Daarentegen werd een veel lager energieverbruik (12.4 MJ/kg lipide) en een duidelijke positieve energiebalans (22.4 MJ/kg lipide) voor lipide-extractie gevonden voor het proces waarin het oplosmiddel schakelt onder invloed van temperatuursverandering. Deze procesoptie is daarmee een veelbelovende methode is voor het extraheren van lipide uit natte algen slurries voor gebruik in energietoepassingen.

Ten slotte worden de voordelen, tekortkomingen en uitdagingen besproken van het gebruik van EBA als schakelbaar oplosmiddel voor lipide-extractie uit natte microalgen slurries en als bioraffinage technologie.

Chapter 1

Introduction

Abstract

The research described in this thesis deals with the application of so-called switchable solvents for direct lipid extraction from microalgae in (concentrated) aqueous solutions. In this introduction chapter, the research context is presented. Subsequently, a brief overview of the methods for wet extraction of algae lipid using traditional organic solvents, supercritical CO₂ and using CO₂ switchable solvents is presented. Lastly, the scope and outline of this thesis are given.

1 Research context

The use of fossil fuels is at this moment an integral part of human daily life, enabling materials production, transportation, heating and cooking, electricity production etc. Due to the increasing world-wide use of energy, fuels and materials on the one hand and the finite reserves on the other hand the prices are expected to rise significantly in the next decades. In addition, and probably even more important nowadays, the associated anthropogenic CO₂-emissions when burning of fossil fuels are a serious concern [1]. Hence, the worldwide recognized mission of finding abundant, affordable and sustainable liquid fuel alternatives to fossil energy sources has become very important. In the past few decades, the search for sustainable energy supply has evolved rapidly all over the world. In many countries, renewable energy sources such as wind power, solar photovoltaic energy, tidal energy, geothermal energy and energy from biomass are now increasingly being used as part of the energy demand. In the research of Lam and Lee [2], it was predicted that in the near future, as an alternative renewable energy, biofuel will play a more important role in energy structure of the world. Among other liquid fuels such as bioethanol [3] and biobutanol [4], that are bio-based alternatives for petroleum, biodiesel is currently recognized as a promising bio-based alternative to fossil based diesel fuel. The main advantages of biodiesel over fossil diesel are that it can provide a significant reduction of greenhouse gas emissions and its use can be adapted to current transportation systems with almost no additional modification [5, 6].

Among other potential sources, algae are particularly interesting as biofuel feedstock. A comparison of microalgae with other biodiesel feedstock is given in Table 1.1. The oil content of algae (10-70 wt.%) is comparable to other biodiesel feedstock and it can be influenced by varying growth conditions [7]. However, as algae grow rapidly they can offer high oil yields and high biodiesel productivity per hectare of cultivation [8]. The land use for growing algae is much less than for the other crops. Besides above advantages, algae can be cultivated in waste water, produced water or saline water on non-arable land, thereby reducing competition with arable land, limited freshwater and nutrients used for conventional agriculture [9, 10]. Algae can recycle carbon much faster than other crops making use of CO₂-rich flue emissions from stationary sources, including power plants and other industrial emitters [11] as well as combined with direct air capture [12] and algae cultivation does not need herbicides nor pesticides [7]. All in all, cultivation of microalgae for its lipid content might contribute to a more sustainable, carbon-neutral energy supply for the future.

Table 1.1 Comparison of microalgae with other biodiesel feedstock.

Plant / source	Oil content (wt.% oil)	Oil yield (t/ha year)	Land use (m ² year/kg biodiesel)	Source
Corn/Maize	44	0.17	66	[13]
Soybean	18	0.64	18	[13]
Jatropha	28	0.74	15	[13]
Sunflower	40	1.07	11	[13]
Palm oil	36	5.37	2	[13]
Microalgae	25	>10	< 1	

However, there are also limitations in using algae. Since they grow in water it is difficult to obtain a good business case due to the high energy costs for obtaining the oil from the very dilute aqueous algae slurries/solutions. Algae dewatering is the most energy intensive step in the process of extracting oil from algae. Although it is not infeasible to use solar drying [14], this method relies on the sunlight which is limited in some countries at certain time of a year. Furthermore, this process is time consuming and requires a large area and leads to a high water consumption. An alternative way of drying algae was studied by Sander and Murthy [15]. In their research, 69% of the entire energy input was provided by burning natural gas as fuel for drying algae. Another strategy involves concentration of the algae, followed by a wet extraction, see Figure 1.1.

Figure 1.1 Strategies for lipid extraction from dry and wet algae.

Organic solvent extraction and supercritical fluid extraction are the most common methods being used for algae lipid extraction. Organic solvent extraction is widely used since the chemical solvents are relatively inexpensive and high lipid recovery yields can be achieved [16]. Supercritical CO₂ (scCO₂) extraction, is seen as an efficient, ‘green’ and mild extraction method for complete extraction of lipid compounds [17]. Both the methods have their own advantages but also some drawbacks. The drawbacks of using organic solvents such as hexane are the high flammability and toxic properties, and another important issue is the energy intensive solvent recovery [18]. Using scCO₂ is expensive due to the high pressure equipment and operating cost and is more difficult to scale up because of the combination of high pressure equipment with dry solids handling [19].

Although liquid solvent extraction is an energy efficient technology in itself, the common solvent regeneration and lipid recovery technologies such as distillation, evaporation and stripping are energy intensive. A novel method for lipid recovery from the extraction solvent, based on phase splitting, might offer an energy efficient alternative. This phase splitting is induced by changing the nature of the solvent or process conditions. CO₂ switchable solvents, first reported by Philip G. Jessop and co-authors [20], show great potential in this field. CO₂ switchable solvents are liquids that can be converted from a non-ionic (hydrophobic) form to an ionic (hydrophilic) form by contacting with CO₂. This process can be reversed by reversing the reaction, e.g. via stripping the solution with N₂. Switchable solvents can be advantageous as media for reactions, extractions or separations [21] especially when in a multi-step chemical process solvents are used for a specific reaction step which must be completely removed before the next step is carried out [22].

2 Lipid extraction from wet microalgae biomass

2.1 Organic solvent extraction with traditional solvents

Organic solvent extraction is generally based on the concept of “like dissolves like”. Several solvents have been proposed for the extraction of algae lipid, such as methanol/chloroform, hexane/isopropanol, hexane/ethanol, dichloromethane/ethanol etc. In those co-solvent systems, the polar alcohols disrupt the hydrogen bonding and electrostatic forces between the membrane-associated polar lipid and protein and make it porous. This enables the non-polar solvent (e.g. chloroform, hexane) to enter the cell and interact with the hydrophobic neutral lipid [23]. In other cases, pure solvents as 1-butanol, ethanol, hexane etc. have been tested. However, the extraction performances of pure alcohols are never more than 90% of the yield obtained by the Bligh & Dyer (B&D) method [23]. The methods and typical results of lipid extraction from wet algae using traditional organic solvents are summarized in Table 1.2.

Table 1.2 The methods and results of lipid extraction from wet algae using traditional organic solvent.

Solvent	Algae species	Conditions			Maximum final total lipid yield (wt.% of dry algae biomass)	Reference
		State of algae biomass at start of extraction	Enhanced method	Extraction temperature (°C)		
hexane	<i>Scenedesmus dimorphus</i>	concentrated	bead-beater	not specified	29.7	[23]
	<i>Chlorella protothecoides</i>	concentrated	bead-beater	not specified	23.5	[23]
	<i>Chlorococcum sp.</i>	water content = 70%	-	not specified	1	[24]
	<i>Nannochloropsis gaditana</i>	water content = 86%	high-pressure homogenization	20-22	10.9	[25]
	<i>Nannochloropsis salina</i>	20 g/L	hydrodynamic cavitation with 0.89% sulfuric acid	not specified	45.5	[26]
hexane:2-propanol ratio = 2:3 (v/v)	<i>Chaetoceros muelleri</i>	water content = 85%	-	near boiling point	87% of yield from five-step water/methanol/chloroform extraction	[27]

Solvent	Algae species	Conditions			Maximum final total lipid yield (wt.% of dry algae biomass)	Reference
		State of algae biomass at start of extraction	Enhanced method	Extraction temperature (°C)		
hexane:methanol ratio = 7:3 (v/v)	<i>Chlamydomonas reinhardtii</i>	concentrated	osmotic shock (50–100 g/L of NaCl)	not specified	20	[28]
ethanol	<i>Chaetoceros muelleri</i>	water content = 85%	-	near atmospheric boiling point	82% of yield from five-step water/methanol/chloroform extraction	[27]
	<i>Chlorella sp. 725</i>	water content = 90%	-	26	33.31	[29]
	<i>Chlorella sp. 442</i>	water content = 89%	-	26	~ 30	[29]
	<i>Picochlorum sp. 802</i>	water content = 90%	-	26	~ 30	[29]
	<i>Nannochloropsis oculata</i>	water content = 40.5%	microwave	not specified	5.2	[30]
ethanol:hexane ratio = 1:1 (v/v)	<i>Scenedesmus dimorphus</i>	concentrated	wet milling	not specified	25.3	[23]

Solvent	Algae species	Conditions			Maximum final total lipid yield (wt.% of dry algae biomass)	Reference
		State of algae biomass at start of extraction	Enhanced method	Extraction temperature (°C)		
	<i>Chlorella protothecoides</i>	concentrated	bead-beater	not specified	18.8	[23]
water:ethanol:hexane = 1:18:7.3 (v/v/v)	<i>Auxenochlorella protothecoides</i>	water content = 90%	pulsed electric field	not specified	36	[31]
chloroform:methanol ratio = 2:1 (v/v)	<i>Botryococcus braunii</i>	concentrated	-	not specified	28.6	[32]
ethyl acetate:methanol:water ratio = 15:5:9 (v/v/v)	<i>Ankistrodesmus falcatus</i>	1.2-2.0 g/L	-	not specified	0.22 ± 0.07	[33]
	<i>Ankistrodesmus falcatus</i>	1.2-2.0 g/L	pulsed electric field	not specified	0.51 ± 0.13	[33]
dichloroethane:ethanol ratio = 1:1 (v/v)	<i>Cladophora sp.</i>	concentrated	-	not specified	~ 37	[34]
dichloroethane:methanol ratio = 1:1 (v/v)	<i>Cladophora sp.</i>	concentrated	-	not specified	~ 40	[34]
dimethyl ether	<i>Microcystis</i>	water content = 91%	-	20	40.1	[35]

Solvent	Algae species	Conditions			Maximum final total lipid yield (wt.% of dry algae biomass)	Reference
		State of algae biomass at start of extraction	Enhanced method	Extraction temperature (°C)		
1,2-dimethoxyethane:water ratio = 6.5:1 (v/v) ethanol: dipotassium hydrogen phosphate ratio = 3:5 (w/w)	<i>Botryococcus braunii</i>	wet algae biomass	-	not specified	96% of maximum yield from control extractions	[36]
	<i>Chlorella spp.</i>	water content = 98.75%	-	70	69 ± 2% of maximum yield from control extractions	[37]
1-butanol	<i>Chaetoceros muelleri</i>	water content = 85%	-	near boiling point	94% of maximum yield from control extractions	[27]
	<i>Monoraphidium minutum</i>	water content = 85%	-	near boiling point	81% of maximum yield from control extractions	[27]
p-cymene	<i>Nannochloropsis oculata</i>	20% dry weight paste	-	heating	21.45 ± 2.64	[38]
α-pinene	<i>Dunaliella salina</i>	20% dry weight paste	-	heating	3.29 ± 0.05	[38]

2.2 Supercritical fluid extraction

An extraction method popular for extracting valuables from biomass material is the use of supercritical fluid extraction. Several supercritical fluids have been investigated for biodiesel production, such as CO₂, methanol and ethanol [40-42]. Although supercritical methanol and supercritical ethanol can convert lipid compounds in algae biomass directly into biodiesel, the operation temperature (about 260°C) is much higher than that for scCO₂ (31.2 °C), which will significantly increase the energy usage. Due to the low critical temperature of CO₂, thermal degradation of products is not observed, which is another advantage of scCO₂ for extraction of high valuable, thermo-sensitive compounds [43]. ScCO₂ is considered as an important alternative for lipid extraction with organic solvents since CO₂ is non-toxic, non-flammable, relatively chemically inert and it produces a solvent-free crude lipid fraction.

Several studies on lipid extraction from algae for biodiesel using scCO₂ have been conducted in recent years. For example, in one study, lipid from freeze dried *Chlorella vulgaris* biomass was extracted using scCO₂ at temperatures of 40 and 55°C and pressures up to 35 MPa [44]. It was found that by increasing the pressure from 20 MPa to 35 MPa, the lipid yield was increased from 4% to 5% with whole algae and from 5% to 13.3% with crushed algae. Cell disruption led to a significant higher lipid yield (8%) at the same conditions. However, the maximum extraction yield using scCO₂ is still significantly lower than when using the B&D method result (24.5 wt.% of dry biomass) [44]. In the study by Andrich, the effect of operating conditions on the kinetics of the supercritical fluid extraction (SFE) on process yields and on the fatty acid composition of lipid extracts was examined [45]. They found in their research that the yields for scCO₂ and n-hexane are comparable, but extraction using scCO₂ proved to be much faster. Couto et al. found that scCO₂ extraction is suitable for the production of value added products from *C. cohnii* that are directed towards pharmaceutical purposes because of the high Docosahexaenoic acid (DHA) percentage of the obtained total fatty acids[46]. However, compared with the total lipid yield obtained using the B&D method, the scCO₂ extraction yield was less than one third. In general it is found that scCO₂ extraction is more selective towards neutral lipid. The optimal extraction condition in terms of maximized oil production using scCO₂ extraction from microalgae *Desmodesmus sp.* was found by Garcia Alba [47]. At 50°C and a pressure of at least 35 MPa an oil with an acylglycerol content of more than 90 wt.% (mainly triacylglycerols) was obtained, having a very low nitrogen content, and a high higher heating value of 37 MJ/kg [47]. In the study of Cheng et al., the efficiency of *Pavlova sp.* lipid extraction using organic solvents and supercritical CO₂ was compared [48]. The highest Fatty Acid Methyl Ester (FAME) extraction yield was obtained by the scCO₂ extraction. Furthermore, the influence of drying method, pressure and temperature on extraction yields and kinetics were studied in the work of Crampon et al. and Pohndorf et al. [49, 50].

The scCO₂ extraction studies mentioned above used lyophilized algae or dry algae powder as starting material. Besides those studies, a few studies reported on scCO₂ extractions using wet algae. Soh and Zimmerman tested the impact of algae water content to extraction efficiency. They used *Scenedesmus dimorphus* which was frozen, and compared with centrifuged algae or lyophilized algae to which water was added incrementally [51]. Their results suggest that scCO₂ extraction can possibly be used for wet algae and the quantity and profile of FAME produced did not change with the water content [51]. In the study of Taher et al., the lipid was extracted from enzymatically disrupted wet sample of *Scenedesmus sp.* The scCO₂ extraction was able to obtain a lipid extraction yield of 12.5% while the highest lipid extraction yield of 16.6% was achieved using hexane [52]. In the study of Hernández et al., scCO₂ showed to be the most efficient methodology to extract fatty acids from microalgae for biodiesel production compared to Soxhlet and Kochert method [53]. The performance of scCO₂ extraction was compared with scCO₂ doped with 20% (w/w) ethanol in the study of Nobre et al. [54] It was found that the addition of ethanol was able to enhance the yield from 33 g lipid/100 g dry biomass to 45 g lipid/100 g dry biomass [54]. Garcia Alba found that the oil yield decreased when increasing the water content of *Desmodium sp.* algae slurry [47]. Also the FAME feed content of the oil also decreased with the increase in water content [47]. In the study of Crampon et al., *N. oculata* microalgae samples containing 4-20 wt.% water were used in scCO₂ extraction [55]. It was found that the presence of water up to 20 wt.% has no influence on the oil extraction yields [55]. In the study of Halim et al., the performance of scCO₂ extraction of lipid from dewatered *Chlorococcum sp.* paste (30 wt.% DM) was examined [25]. The maximum lipid yield (7.1 wt.% relative to dry biomass) was obtained at temperatures of 60°C and pressures of 30 MPa. One important observation here is that the total lipid yield of wet extraction was higher than for the extraction of dry algae powder (5.8 wt.% to dry biomass) [25]. In their experiments, the wet algae were used directly after centrifugation, without further treatment, and the dry algae were dried in an oven and then grinded into powder by a ring mill [25]. The positive role of water was said to contribute to the extraction because of its swelling of the cellular matrix and its acting as a polar co-solvent. In the studies of Garcia Alba [47], Halim et al. [47] and Crampon et al. [55], opposite results of the water influence in scCO₂ extraction have been reported. It seems effect of water highly dependent on the type of microalgae used. However, there is still the potential that by using scCO₂ extraction, the energy used for the drying and milling step could be avoided.

2.3 CO₂ switchable solvents for extraction and separation

Switchable solvents [20] were first reported by Philip G. Jessop, Charles Liotta and their co-authors from Queen's University, in Kingston, Ontario and Georgia Institute of Technology in Atlanta. Switchable solvents are liquids that can be converted from one form to another, where the two forms differ in their physical properties such as conductivity, polarity, solubilizing capability or viscosity [56]. As media for reactions, extractions or separations, switchable solvents are advantageous compared to conventional solvents. In a multi-step chemical process, each step may require a particular solvent for optimal activity. Solvents used must then be completely removed before the next step is carried out. This solvent-solute separation is often energy intensive and increases production costs and environmental waste over the entire process [22].

Switchable solvents have proven to be useful for certain applications where the processes of reaction and separation were simplified, e.g. in the polymerization of styrene [22]. In the case of lipid extraction from microalgae in (concentrated) aqueous solutions, the high affinity of the switchable solvent system towards non-polar compounds is exploited to extract oil from algae, while the polar form of the switchable solvent (obtained after contacting with CO₂) is used to recover oil from the switchable solvent after the induced solvent-lipid phase separation. The switchable solvent cycle is completed by transforming the switchable solvent to the non-polar form by removing the CO₂ by N₂ stripping, with- or without heating. The concept is shown in Figure 1.2.

Figure 1.2 Concept of lipid extraction from algae solution using switchable solvent.

2.3.1 Two-component switchable solvent systems

The first switchable solvent system was reported by Jessop et al. [20] and consisted of an equimolar mixture of an alcohol and an amidine such as DBU (1,8-diazabicyclo-[5.4.0]-undec-7-ene).

Scheme 1.1 Switchable mechanism of alcohol / amidine (guanidine) mixtures. [20, 22] (A) Protonation equilibrium of DBU in the presence of an alcohol and carbon dioxide. (B) The different polarity of each liquid under the two conditions is illustrated by the miscibility of decane with the hexanol/DBU mixture under nitrogen, a decane phase separates out once the solvent mixture becomes polar in the presence of CO_2 . This process is reversible.

Exposure of a 1:1 mixture of the binary liquids—namely DBU and 1-hexanol—to gaseous CO_2 at one atmosphere and at room temperature converts the DBU and 1-hexanol into the ions DBUH^+ and RCO_3^- , and with that, the liquid mixture obtains the character of an ionic liquid (see Scheme 1.1A) [20]. Key properties, such as polarity, viscosity, miscibility and conductivity, change significantly. This conversion is reversed by removing the CO_2 via the use of an inert stripping gas, such as N_2 or argon [20]. For a more rapid reaction, elevated temperature (50°C) may be applied for shifting the equilibrium reaction with CO_2 [20]. The polarity changes are demonstrated by testing the solubility of decane—a nonpolar compound—in each of the liquids. Decane is found to be miscible with the liquid under a N_2 atmosphere but not with the one under a CO_2 atmosphere (see Scheme 1.1B) [20].

The reaction with CO_2 causes a marked increase in the viscosity of the liquid, which does depend on the choice of the alcohol [20]. The choice of the alcohol is therefore critical, as for

proper liquid-liquid operation a limited viscosity is desired. DBU alkyl carbonate salts, prepared by contacting CO₂ with the equimolar mixtures of DBU and ethanol, methanol, or water are solids at room temperature. However, DBU salts with longer alkyl chains are liquids at room temperature, and are therefore more suitable for the extraction process. Also the polarity switch of the switchable solvent system is influenced by the length of the alkyl chain. Using shorter alcohols gives a greater difference between the polarities of the ionic and neutral forms of the solvent [22].

In the research by Samorì et al., the DBU/octanol system exhibited a better extraction yield than n-hexane, both with dried and wet algae samples (7.8% and 5.6% hydrocarbons yield respectively) [57]. The best results were obtained when the equimolar mixture DBU/octanol was used to extract freeze-dried algae at 60°C for 4 h ($16 \pm 2\%$ total hydrocarbons yield). The extraction of lipid from algae cultures (0.8 g algal dry weight/L) with the equimolar mixture DBU/octanol at room temperature for 24 h gave $8.2 \pm 1\%$ hydrocarbon yield. While these solvents were shown to be able to extract oil from microalgae and be separable from the oil without distillation, both the switchable solvents and the algae had to be rigorously dried to prevent the unwanted formation of the (solid) bicarbonate salt of DBU. For large scale application, the feasibility of these DBU based systems is therefore questionable.

Amine/amidine (guanidine) mixtures form another type of switchable solvent that can be triggered by CO₂ and N₂. Compared with alcohol/amidine (guanidine) mixtures, they are less water sensitive [58]. In the study by Yamata et al. primary amines were chosen because they react more readily with CO₂ than secondary (or tertiary) amines [59]. After CO₂ exposure, the polarity of the solvents exhibits a marked change. This polarity is measured using the absorption spectrum of the solvatochromatic dye Nile Red as measured by a UV spectrophotometer. After mixing the solvent with Nile Red, the wavelength for maximum absorbance, which is indicative of the solvent polarity, can be determined.

For example, at room temperature, the equimolar mixture of compound **4** (Scheme 1.2) and compound **5** is a molecular liquid. Through spectroscopic measurements it is found that its 423 nm absorption maximum indicates an environment slightly less polar than toluene ($\lambda_{\text{max}} = 425$ nm) [59]. After CO₂ exposure, it converts to the ionic liquid **6**, and the absorption maximum is shifted to 438 nm, indicating an environment more polar than acetone ($\lambda_{\text{max}} = 433$ nm) and less polar than N,N-dimethylformamide (DMF, $\lambda_{\text{max}} = 446$ nm) [59]. After stripping with N₂, the ionic liquid **6** switches back to the molecular form **4** and the polarity changes accordingly[59].

Scheme 1.2 Switching mechanism of aliphatic primary amines/ amidine (guanidine) mixtures. [59]

2.3.2 Single component switchable solvent systems

The two-component switchable solvent systems proposed by Jessop et al. [20] rely on the equimolar composition, making the systems more difficult to control. A single component system is therefore more preferable. In the following sub-sections, such systems, which were proposed in literature after the first appearance of the two compound systems, are discussed.

2.3.2.1 Amidines

Switchable hydrophilicity solvents (SHS) form a special class of single component switchable solvents. Upon switching they not only change their polarity, but also can switch from having poor miscibility with water to having excellent miscibility with water. CO_2 at atmospheric pressure is used to switch the solvent to its hydrophilic form, and air and/or heat can be used to switch it back again [60]. N,N,N'-tributylpentanamidine was reported as a suitable SHS.

Scheme 1.3 Switching mechanism of switchable hydrophilicity solvent N,N,N'-tributylpentanamidine. [60]

In conventional extraction processes, the solvent/product separation is an energy intensive process. Besides the large necessary energy input, distillation or evaporation requires the use of a volatile solvent, with the disadvantage of vapour losses and resulting emissions to the

environment. The SHS can, in principle, be used for the extraction of low-polarity organic products, followed by the removal of the solvent from the product by carbonated water, hence without distillation [60]. The SHS, separated from water by switching the solvent back to the hydrophobic state, can then be reused [60].

For comparison, the current industrial process for obtaining soybean oil involves extracting the oil using hexane and then removing the hexane by distillation. In the research of Sheenhan et al. [61], the soybean extraction has a solvent : bean mass ratio of 1.2 and the reported hexane losses are 2.4 kg per ton of flaked beans processed. SHS make it possible to extract soybean oil from soybeans, and then to separate the solvent from the extracted oil without distillation, by solvent switching using CO₂ and water [62].

2.3.2.2 Secondary amines

Within the class of secondary amines, some compounds can function as switchable solvent using CO₂ as a trigger. Secondary amines are cheaper than amidines and have a significantly lower polarity form. Moreover, secondary amine switchable solvents are less sensitive than DBU/Alcohol systems to small amounts of water. They react with CO₂ to form carbamate salts via carbamic acids (see Scheme 1.4).

Scheme 1.4 Switching mechanism of secondary amines. [63]

For a solvent system to be classified as a switchable solvent, the carbamate salt and the amine must be liquids and exhibit a significant change in polarity. Most liquid amines (including primary alkyl amines, allyl amine, piperidine, pyrrolidine, and benzyl amine) are converted into solid carbamates [63], whereas some secondary amines yield room temperature liquid salts. Light secondary amines—such as ethyl methyl amine, di-ethylamine, and methyl-propyl amine—are less preferred, because these amines are very volatile and highly flammable. Four liquid amines that potentially could be used as switchable solvent have been reported: N-ethyl butyl amine (EBA), N-ethyl propyl amine (EPA), di-propyl amine (DPA), and benzyl methyl amine (BMA) [63].

Du et al. proposed a process for lipid extraction from wet algae using secondary amines [64]. The proposed process configuration is illustrated in Figure 1.3. First, the oil is extracted from the algae using the switchable solvent in its lower polarity (lipophilic) form. The solvent phase with the extracted lipid oil is then phase-separated from the aqueous stream containing the algae residue. Then, by contacting the separated solvent phase with CO₂, the solvent is switched into its more polar, ionic form, indicated in the figure as “switching forward” and phase-separates from the apolar lipid oil. The solvent is then converted back to its original

state (“switching backward”) by N_2 stripping at elevated temperature. Key enabling steps in this process are (1) uptake rate of CO_2 and the extent of the reaction of CO_2 with the solvent in the liquid mixture to change the solvent from lipophilic to lipophobic state and (2) the rate and extent of the back-switching reaction. To increase the rates and shifting equilibria, water could be added (or not totally removed from the previous stage), and the solvent might be heated up. Additional points of attention are the solubility of the solvent in water (which might affect algae cultivation when recycling the untreated water) and the solubility of the solvent in the lipid oil phase. For practical purposes, if a portion of the switchable solvent ends up in the aqueous phase after extraction, this may introduce a significant problem if the aqueous phase would be re-used for growing again algae since many alkyl amines are toxic even at low concentrations [65]. It would also imply that post-treatment is needed if the water is to be discharged to the environment. Thus solvent recovery is mandatory before recycling or discharging the aqueous phase. It should be noted that when a significant fraction of the solvent leaves the process being dissolved in the product oil, this also requires an additional recovery operation (e.g. washing with water) to limit the loss of solvent and the contamination of the oil product. Therefore, a set of selected solvents was tested for the desired phase behaviour, i.e. immiscibility with water under extraction conditions and immiscibility with oil in the presence of CO_2 at atmospheric pressure, next to their extraction performance to harvest lipid oil from microalgae slurries. The results showed that EBA and DPA have good switching behaviour upon contacting with CO_2 [64].

Figure 1.3 Process scheme of switchable solvent extraction of lipid from wet microalgae. [64]

2.3.2.3 Tertiary amines

The use of tertiary amines has been proposed as another type of switchable hydrophilicity solvent (SHS), easier to prepare and (often) commercially available, in contrast to the amidine system reported previously [66]. Again, these tertiary amines are hydrophobic solvents that have very low miscibility with water under a nitrogen atmosphere, but become

hydrophilic in the presence of CO₂ at atmospheric pressure. The change in miscibility is caused by a chemical reaction of the CO₂, water and the SHS, giving a water-soluble bicarbonate salt of the protonated SHS. The switching mechanism of tertiary amines is illustrated in Scheme 1.5.

Scheme 1.5 Switching mechanism of tertiary amines. [66]

By stripping with e.g. N₂ or air through the [NR₃H][O₂COH]/water (single phase) mixture the reaction is reversed and CO₂ is removed via the gas phase. Consequently, the mixture splits again into two liquid phases. Operating at elevated temperature helps to shift the equilibrium to the side of CO₂ and the hydrophobic tertiary amine. Due to the reaction stoichiometry an equimolar amount of H₂O is required, in contrast to e.g. primary and secondary amine systems. Tertiary amines are normally less reactive towards CO₂ than secondary amines, which may lead to longer reaction times needed to switch the solvent, but require less energy input to reverse the reaction.

The tertiary amine N, N-dimethyl cyclohexyl amine (DMCHA) was reported for extraction of lipid from microalgae. In the research published by Jessop et al., the freeze-dried algae *Botryococcus braunii* and DMCHA were used in the experiments [67]. The amount of crude lipid extracted with DMCHA and isolated from the solvent was up to 22% of the dry cell weight when the extraction was performed at 60 to 80°C and the residual amine content in the extracts was 18-24 wt.% [67]. Extractions performed at room temperature yielded up to 19% crude lipid and the residual amine content in these extracts was about 4-8 wt.% [67]. However, 4-8 wt.% residual amine in the lipid extract is still a lot, which indicates that additional recovery operation is needed [67]. Samorì et al. used DMCHA for extracting and recovering lipid directly from wet algae samples (about 80% water content) and directly from cultures (biomass concentration around 2 g/L) of three microalgae strains: *Nannochloropsis gaditana*, *Tetraselmis suecica*, and *Desmodesmus communis* [65]. This work is remarkable, since this was the first time that extraction of lipid from dilute cultivation media without any treatment for cell disruption was reported. The total lipid contents expressed on algal dry weight basis obtained through DMCHA extraction of wet samples (50 mg/mL, 24 h extraction) of *D.communis*, *N.gaditana* and *T.suecica* were 29.2 ± 0.9%, 57.9 ± 1.3% and 31.9 ± 1.5% respectively, which was comparable with extraction yields using the B&D lipid extraction method, commonly applied for analytical purpose [65].

Summarizing, from the few works reported on extracting lipid from algae by switchable solvents [64, 65, 67], promising results have been obtained with regard to the amount of lipid extracted when compared with traditional analytical extraction methods, such as the B&D method. However, these laboratory results do not elucidate mechanisms nor reflect on large

scale processes and on potential benefits of using these solvents at large scale still not much is known. Furthermore, for a sustainable use of switchable solvents in extraction of lipid from wet algae feed streams, the method and conditions of solvent recovery from both the aqueous raffinate and from the lipid fraction after or during back-switching needs to be developed, since the solubility of these switchable solvents in their neutral form in the water phase is not negligible.

3 Scope and outline of the thesis

This work aims to find/evaluate an effective switchable solvent system to extract lipid from microalgae directly from (concentrated) aqueous solutions. Scope of this thesis is illustrated in Figure 1.4.

Figure 1.4 Scope of the thesis.

In **Chapter 2**, all materials, experimental procedures and analytical equipment used in this thesis to perform all the experiments are presented.

The principle of using switchable solvents for lipid extraction from wet algae solutions and lipid recovery by solvent switching is proven in **Chapter 3**. First, several switchable solvent candidates are investigated for their lipid extraction performance. The polarity switching and phase splitting ability are examined upon contacting with CO₂. The selected solvent for this study should have good performance on both switchability and extraction performance. Then the influences of freeze drying and cell breaking on the extraction efficiency for crude lipid yield and fatty acid yield are investigated. The results are compared with B&D method [68] which is commonly taken as a reference.

In Chapter 4, the extraction yield of lipid from non-broken *Neochloris oleoabundans* slurries (~5% dry weight) is maximized for EBA as switchable extraction solvent. Experimental process parameters as e.g. cell breaking, extraction time, extraction temperature, solvent/feed ratio and extraction stages that affect the extraction performance are investigated. The optimized extraction is also applied on fresh water (FW)-stressed *Neochloris oleoabundans* targeting maximizing the lipid yield.

In Chapter 5, a model is developed that describes the equilibrium state of lipid extraction from FW-stressed *Neochloris oleoabundans* algae slurry using EBA as solvent. The model should provide a qualitative insight in the extraction process and should be able to predict the amount of crude lipid being recovered from FW-stressed *Neochloris oleoabundans* at any stage of a multistage EBA extraction process. For this purpose extraction experiments using different solvent to feed ratios are carried out. An extraction model based on experimental results or visual observations is proposed and cross-checked against the generated experimental data to detect the validity of the proposed model. Finally, the reliability of the newly developed model is discussed based on the sensitivity analysis of the assumed parameters.

Besides the CO₂ triggered switching, an alternative for recovering lipid from the extraction solvent is proposed in **Chapter 6**. In this method the lower critical solution temperature (LCST) behavior of EBA is used as stimulus for switching hydrophilicity, instead of using CO₂. First, the LCST behavior of EBA in binary mixtures with water is investigated, then also in ternary systems with the presence of oil the temperature-responsive partitioning behavior of EBA is investigated. Then the possibility of using the LCST behavior of EBA for oil recovery and also for lipid recovery from microalgae is investigated. The influences of oil concentration and EBA fraction in water are also investigated. Finally, a microalgae lipid extraction process which utilize the LCST behavior of EBA for lipid separation and solvent recovery is proposed.

Chapter 7 focuses on the EBA recovery from both the raffinate and from the residual microalgae cells after extraction. The work describes the evaluation of several solvents/solvent combinations for EBA recovery. Subsequently, by using a flow sheeting program (Aspen Plus V8.8), a liquid-liquid extraction process followed by a distillation process for recovery of EBA is modelled and optimized.

In Chapter 8, the use of EBA for wet extraction of microalgae lipid is evaluated on an energy usage basis. Two processes are designed, one using CO₂ switching and the other one using temperature as the stimulus for switching and solvent regeneration. The energy usage for these two process is calculated and compared, to identify the most beneficial option for algae lipid recovery from microalgae, including solvent recovery. In the end, the advantages, weaknesses and challenges of using switchable solvent for lipid extraction from wet microalgae are pointed out.

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Chapter 2

Materials & experimental approach

Abstract

This chapter contains all the information concerning the materials, experimental procedures and analytical equipment used to perform all the experiments and analyses presented in this thesis. Definitions and calculation methods are also introduced.

1 Materials

Algae of the strain *Phaeodactylum tricornutum*, *Neochloris oleoabundans* (fresh water cultivated and marine cultivated in artificial seawater) were obtained from AlgaePARC (NL). The *Neochloris oleoabundans* were cultivated both in non-stressed form and stressed under nitrogen limitation conditions at AlgaePARC (NL). Algae paste was mixed with water to get ~5 wt.% algae slurry that can be used in extraction. The water content in the algae slurries 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 ilShin TFD5503 freezedryer at -55°C and 5 mTorr for 24 h.

The chemicals used for the experiments described in this thesis are listed in Table 2.1.

Table 2.1 Chemicals used in experiments.

Chemical	Formula	Distributor	Purity
Ethylenediamine	C ₂ H ₈ N ₂	Sigma-Aldrich	≥ 99%
1,3-Diaminopropane	C ₃ H ₁₀ N ₂	Aldrich	≥ 99%
Pentylamine	C ₅ H ₁₃ N	Sigma-Aldrich	≥ 99.5%
Hexylamine	C ₆ H ₁₅ N	Aldrich	99%
Diethylenetriamine	C ₄ H ₁₃ N ₃	Sigma-Aldrich	99%
N,N-diisopropylethylenediamine	C ₈ H ₂₀ N ₂	Aldrich	99%
N-Ethylbutylamine (EBA)	C ₆ H ₁₅ N	Aldrich	≥ 98.0%
Dipropylamine (DPA)	C ₆ H ₁₅ N	Aldrich	99%
Dibutylamine (DBA)	C ₈ H ₁₉ N	Aldrich	≥ 99.5%
Tripropylamine (TPA)	C ₉ H ₂₁ N	Aldrich	≥ 99.8%
2-(Dibutylamino)ethanol (DBAE)	C ₁₀ H ₂₃ NO	Aldrich	99%

Chemical	Formula	Distributor	Purity
N,N-Dimethylcyclohexylamine (DMCHA)	C ₈ H ₁₇ N	Aldrich	99%
1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU)	C ₉ H ₁₆ N ₂	Fluka	≥ 99.0%
1-Octanol	C ₈ H ₁₈ O	Sigma-Aldrich	≥ 99%
1-Hexanol	C ₆ H ₁₄ O	Sigma-Aldrich	≥ 99%
Nile Red	C ₂₀ H ₁₈ N ₂ O ₂	Sigma	≥ 98%
Hexane	C ₆ H ₁₄	Sigma-Aldrich	≥ 95%
Cyclohexane	C ₆ H ₁₂	Sigma-Aldrich	99.5%
Toluene	C ₇ H ₈	Aldrich	99.8%
2-Propanol	C ₃ H ₈ O	Sigma	≥ 99.5%
Acetic Acid	C ₂ H ₄ O ₂	Sigma-Aldrich	≥ 99.7%
Chloroform	CHCl ₃	Sigma-Aldrich	≥ 99.9%
Methanol	CH ₃ OH	Fluka	≥ 99.9%
Acetone	C ₃ H ₆ O	Sigma-Aldrich	≥ 99.5%
Heptane	C ₇ H ₁₆	Sigma-Aldrich	99%
Dodecane	C ₁₂ H ₂₆	Sigma-Aldrich	≥ 99%
Hexadecane	C ₁₆ H ₃₄	Sigma-Aldrich	99%
Dichloromethane (DMC)	CH ₂ Cl ₂	Sigma-Aldrich	≥ 99.8%
Ethanol	C ₂ H ₆ O	Merck	≥ 99.8%

Chemical	Formula	Distributor	Purity
Methyl nonadecanoate	C ₂₀ H ₄₀ O ₂	Fluka	≥ 99.5%
Sulfuric acid	H ₂ SO ₄	Sigma-Aldrich	95.0-98.0%
FAME column evaluation mix	-	Supelco	1000 µg/mL each component in methylene chloride, analytical standard
Silica gel	SiO ₂	Sigma-Aldrich	high-purity grade (Davisil Grade 633), pore size 60 Å, 200-425 mesh particle size
TLC silica gel 60	-	Merck	-
Copper (II) sulfate	CuSO ₄	Fluka	≥ 98.0%
Orcinol	C ₇ H ₈ O ₂	Aldrich	97%
Ammonium molybdate	N ₂ H ₈ MoO ₄	Aldrich	99.98% trace metals basis
Tin(II) chloride	SnCl ₂	Fluka	98.0%
Glycerol	C ₃ H ₈ O ₃	Sigma	≥ 99.0%
Hydrochloric acid solution	HCl	Fluka	Reag. Ph. Eur.
Acetonitrile-d ₃	C ₂ H ₃ N	Sigma-Aldrich	99.8%

2 Polarity measurements

Polarity of the solvents was measured using the solvatochromatic dye Nile Red, using a Hach DR 5000 UV-Vis Laboratory Spectrophotometer. Approximately 1.5 mL of the sample was placed in a 1 cm glass cuvette. Nile Red was added at a suitable concentration (about 1.5×10^{-7} M) to give an absorbance between 0.5 and 1.0. Spectra were acquired periodically to follow the change in $\lambda_{\max}$, the wavelength for maximum absorbance is obtained, which is indicative of the normalized solvent polarity parameter E_N^T [1].

The E_N^T values of switchable solvents were estimated by determining the $\lambda_{\max}$ value and comparing this with the measurements for a set of liquids used for calibration. The latter results are presented below in Figure 2.1 and correlated via Eq. 2-1.

$$E_N^T = 0.0164 \cdot \lambda_{\max} - 8.4777 \quad (R^2 = 0.9705) \quad \text{Eq. 2-1}$$

Figure 2.1 Correlation between the E_N^T value and measured $\lambda_{\max}$.

3 Solvent switching

3.1 CO₂ induced solvent switching

The switchable solvents were brought in contact with CO₂ by bubbling pure CO₂ at atmospheric pressure for 30 minutes to bring the solvents to the high polarity form and then switched back to the lower polarity form by removing the CO₂ through bubbling with N₂. Optionally, additional heating to 50°C-90°C was applied for up to 2 h. Samples were taken every several minutes and measured by UV-Vis. The experiments were performed at least twice for each switchable solvent, the reported polarity index values are the average of the two values obtained. The deviation between the individual measurements of $\lambda_{\max}$ is normally less than 1 nm.

To study lipid and solvent recovery, a mixture was made of sunflower oil (mimicking algal lipid), the switchable solvent under consideration and, in most cases, water in a 1:3:4 v/v/v ratio. With this experiment, the possibility to recover the solvents of interest by an induced phase separation of the extracted oil and the solvent were investigated. Initially, several experiments were conducted without the addition of water. However, the high viscosity of switched solvent often prevented a facile phase separation and the addition of some water was found to greatly enhance the switching and phase separation processes. Each phase was analyzed by FT-IR after any treatment like CO₂ bubbling or heating.

3.2 Temperature induced solvent switching

The temperature induced solvent switching, which was due to the lower critical solution temperature (LCST) behavior of EBA in water, was investigated in two ways: (1) visually (to assess the number of liquid phases present) and (2) by pH/Karl Fischer (KF) measurement.

(1) Several EBA-water mixtures with EBA mass fraction from 0.02 to 0.95 were prepared and sealed in 20 mL glass vials. The samples were totally immersed in a cooling bath (Temperature control F-32, Julabo) which was cooled down to 0°C. The samples were shaken until reaching equilibrium (forming one homogenous mixture). Then the temperature was increased slowly and the cloud points, indicating a phase separation, were observed.

(2) Samples with EBA mass fraction of 0.5 were prepared, sealed in 20 mL glass vials, and cooled down to 0°C to form one phase. Then the samples were increased to several different temperatures. Each aqueous phase and organic phase was analyzed by acid titration and KF measurement respectively. Each experiment was carried out at least three times. The error bars represent the standard deviation over the three measurements in all figures.

To investigate the LCST behavior of EBA in water in the presence of oil, different amount of sunflower oil was added to EBA-water mixture. The cloud point was measured visually as described above.

4 Solvent recovery from extraction residue

4.1 EBA recovery from raffinate after lipid extraction

An EBA saturated aqueous solution was prepared by adding a surplus of EBA to water. The solution was mixed for 24 h to ensure distribution equilibrium and then settled for the formation of two phases. The bottom phase was the aqueous phase saturated with EBA.

Different solvents like cyclohexane, hexane, heptane, dodecane, hexadecane and dichloromethane were mixed and brought into contact with this prepared aqueous phase separately. Different solvent to feed ratio (1:10, 1:5, 1:2 and 1:1) and agitation time (from 1 min to 60 min) were tested. The EBA amount in aqueous phase before and after mixing was measured by pH titration.

4.2 EBA recovery from microalgae paste after lipid extraction

The EBA content of algae paste after lipid extraction was measured by a drying/distillation experiment and followed by GC-MS measurement. A known amount of algal cells residue after lipid extraction and filtration were transferred into a 50 mL flask for distillation (see Figure 2.2). The flask was heated up to 125°C in an oil bath at atmospheric pressure. The vapors were collected in a condenser which was cooled in ice. The amount of liquid collected was measured gravimetrically. The liquid sample was diluted in acetone in the ratio of 1:20 and then measured by GC-MS for its EBA content. Each experiment was performed at least four times.

Figure 2.2 The setup used for distillation.

GC–MS analyses were performed using a 7890A 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 a 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: 45°C for 4 min, ramps of 5°C /min to 280°C where the temperature was held for 10 min. The total analysis time was about 61 min.

Standard solutions from 1 to 80 wt.% of EBA in demineralized water were prepared. The prepared samples were then diluted in acetone in the ratio of 1:20 for calibration.

For EBA recovery, algae paste after lipid extraction and filtration were mixed with different solvent: cyclohexane, cyclohexane + water, water and ice water for more than 18 h. The solvent/feed ratio was 10:1 when cyclohexane was used and the ratio was changed to 20:1 when cyclohexane + water (1:1 w/w), water and ice water were used. EBA concentration in water was measured by titration. EBA concentration in cyclohexane was measured by GC–MS and then calculated based on the calibration.

4.3 EBA recovery calculation

Hydrochloric acid was used as titrant solution in this work. The reactions between HCl and EBA are described by Eq. 2-2 and Eq. 2-3.

From equation Eq. 2-2 it is clear that the molar amount of EBA in the sample equals the moles of added HCl, up to the titration point.

However, titration only measures the un-protonated EBA in aqueous solution. It should be noted that EBA is a base and tends to be partly protonated in water. Therefore, also amount of protonated EBA in the aqueous solution must be determined. Before the titration starts, in aqueous solution, there is an equilibrium of base dissociation can be written as:

Since $EBAH^+$ has the same concentration as OH^- , $[EBAH^+]$ can be calculated by Eq. 2-5 and Eq. 2-6 when the pH value of the solution is measured.

$$pH + pOH = 14 \quad \text{Eq. 2-5}$$

$$[EBAH^+] = [OH^-] = 10^{-pOH} \quad \text{Eq. 2-6}$$

The amount of EBA in protonated form (m_{EBAH^+}) can be calculated by

$$m_{EBAH^+} = [EBAH^+] \cdot M_{EBAH^+} \cdot \frac{m_{solvent}}{\rho_{solvent}} \quad \text{Eq. 2-7}$$

where M_{EBAH^+} is the molecular weight of $EBAH^+$, $m_{solvent}$ and $\rho_{solvent}$ are the weight and density of the aqueous solution after extraction, respectively.

Since m_{EBAH^+} is insignificant (< 0.1 wt.%) compared to the amount of aqueous solution to which the small sample of EBA containing phase was added, the approximation $\rho_{solvent} \approx \rho_{H_2O}$ can be used.

The total amount of EBA in aqueous solution (m_{EBA}^{total}) is the summation of protonated and un-protonated EBA amount.

$$m_{EBA}^{total} = m_{EBA} + m_{EBAH^+} \quad \text{Eq. 2-8}$$

where m_{EBA} is the amount of un-pronated EBA in solution as determined by titration.

5 Extraction and recovery of lipid from microalgae

5.1 Bligh & Dyer (B&D) extraction

Extraction of lipid from algae slurries was done according to the original B&D method [2]. Each 20 g algae sample (5 wt.%) is mixed for 8 minutes 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 minute, 24 mL water is added and mixing is continued for another 1 minute. The mixture was centrifuged and the chloroform layer containing the algae oil was collected. Prolonged extraction procedures at several extraction times (30 min, 1 h, 2 h and 24 h) were applied to ensure extraction equilibrium. All experiments were performed at least twice.

5.2 Switchable solvent extraction

For switchable solvent extraction, 20 g of algae slurries were extracted with varying amounts of switchable solvent for more than 18 h under continuous stirring. After the extraction, the mixtures were centrifuged and the organic layer containing the algal lipid was isolated. When a second extraction was applied to the extracted algae, the amine layer containing the algal lipid was isolated and replaced by an equal amount of fresh solvent. This procedure was applied multiple times to achieve up to four extraction stages. To assist the lipid recovery from the isolated organic layers, equal amounts of water were added, improving the phase separation as a result of improving the switching efficiency.

During the switching procedure, CO₂ was bubbled at a flow rate of 2 VVM (volume per volume per minute) for 60 min, during which the solvent switched into its hydrophilic form. A small amount of chloroform was used to recover the lipid layer with a syringe, due to the small scale of experiments. Please note that this step is not required when working with larger volumes. The two phases thus created were separated by centrifugation (9000 rpm, 5 min) and the total amount of the extracted product was measured gravimetrically (after evaporating the chloroform) and reported as percentage lipid on algae dry weight basis (defined as crude lipid yield). All experiments were performed at least twice. The reported error bars correspond to an accuracy of $\pm 2.8\%$ yield, which is the averaged relative standard deviation of all experiments (determined over more than 110 similar extraction experiments).

6 Lipid characterization techniques

6.1 Column Chromatography

Silica gel column chromatography was used to separate lipid extracts into three fractions, neutral lipid (NL), glycolipid (GL) and phospholipid (PL). The separation was achieved on a chromatographic column (1×15 cm) packed with silica gel (200–425 mesh), and the NL, GL and PL fractions were obtained by sequential elution of chloroform (200 mL), acetone (400 mL) and methanol (200 mL), according to the procedure reported in the literature [3], using 100–200 mg of lipid. The fractions were collected and measured gravimetrically after evaporation of the solvent.

In this research, lipid fractions from *Phaeodactylum tricornutum* and *Neochloris oleoabundans* were fractionated. A clear separation in three different fractions was obtained, as illustrated in Figure 2.3. After calculation, it was found that the content of NL, GL and PL (% in lipid) was in line with the results presented in literature [4] (see Table 2.2). It was concluded that this silica gel column chromatography method is able to separate lipid from microalgae into three fractions, and further analysis of the fractions was carried out.

Figure 2.3 Fractionation results of column chromatography for lipid from (A) *Phaeodactylum tricornutum* and (B) *Neochloris oleoabundans*.

Table 2.2 Weight percentage of fractions in lipid (%) from different microalgae.

Name of the microalgae	NL	GL	PL	Data source
<i>Desmodesmus communis</i>	9	57.9	33.1	[4]
<i>Nannochloropsis gaditana</i>	16	27.9	56.1	[4]
<i>Tetraselmis suecica</i>	11.0	50.0	39.0	[4]
<i>Phaeodactylum tricornutum</i>	16.1	41.6	42.3	This work
<i>Neochloris oleoabundans</i>	8.8	36.9	54.3	This work

6.2 Thin Layer Chromatography (TLC)

TLC experiments were carried out at Wageningen University with the help of dr. L.A.M. (Ben) van den Broek.

Samples were dissolved in chloroform/methanol 2:1 (v/v) and diluted to ~50 mg/mL. Then 2 μ L sample was applied to the TLC plate and the front of the elution solvent (chloroform/methanol/water 65:25:4 v/v/v) was allowed to run until 1 cm from the top. The plate was sprayed with three different reagents to visualize all lipid, phospholipid and glycolipid.

General lipid was visualized with 10% (w/v) copper (II) sulfate in 8% (v/v) sulfuric acid. The plates were sprayed with this reagent and incubated at 140°C for 10 minutes, after which the lipid spots became brown, grey or black.

Glycolipid was determined by 0.2% (w/v) orcinol in 70% (v/v) sulfuric acid. The plates were sprayed and heated at 100°C for 5 minutes. Spots containing sugars appear pink-violet on a white background.

Phospholipid was detected according to the method described in literature [5, 6]. The solution was made by dissolving 2.5 g ammonium molybdate in 66% (v/v) sulfuric acid. The second solution was made by dissolving 626 mg tin(II) chloride in 25 mL glycerol followed by a 10-fold dilution with water. These solutions were mixed in a ratio of 1:1 (v/v). To receive a spray reagent for phospholipid visualization, the final solution was mixed with water in a ratio of 10:2 (v/v). This spray reagent reacts with phospholipid immediately, without heating, to give blue spots on a white ground.

A TLC fractionated result of lipid from *Neochloris oleoabundans* is shown in Figure 2.4. All

the lipid spots were visualized under general staining (Figure 2.4 (B)). *Neochloris oleoabundans* lipid (N.o) contain neutral lipid (NL), phospholipid (PL) and glycolipid (GL). After glycolipid staining (Figure 2.4 (C)) and phospholipid staining (Figure 2.4 (D)), nether pink-violet nor blue color appeared in NL fraction, indicating the NL fraction contains only NL. Figure 2.4 (D) shows that PL were only found in the PL fraction. However, Figure 2.4 (C) shows that GL were present in both the GL and the PL fraction, which means the separation of GL is not complete.

(A) No staining (B) General staining (C) Glycolipid staining (D) Phospholipid staining

Figure 2.4 TLC fractionated result of lipid from *Neochloris oleoabundans*. NL is neutral lipid fraction; GL is glycolipid fraction; PL is phospholipid fraction; N.o is *Neochloris oleoabundans* lipid.

6.3 Lipid transesterification

About 5 mg of lipid extract was 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 allowed to cool 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 and transferred 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), typically 1-2 mL, was collected and filtrated into a GC bottle.

6.4 Gas Chromatography-Mass Spectrometry (GC-MS)

The algae lipid extracts were analyzed by GC-MS on total fatty acids (TFAs) after transesterification of the lipid which contain fatty acids into the corresponding fatty acid methyl esters (FAMES).

GC-MS analyses were performed using a 7890A 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 a 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, ramps of 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 analysis time was about 45 min. The relative response factors used for the quantification were obtained by injecting solutions of known amounts of methyl nonadecanoate and commercial mixtures of FAMES.

The TFA yield is defined as:

$$TFA \text{ yield (\%)} = \frac{m_{TFA}}{m_{dry \text{ algae}}} \times 100\% \quad \text{Eq. 2-9}$$

In this research, TFA fraction in crude lipid is also used for evaluation and it is defined as:

$$TFA \text{ fraction in crude lipid (\%)} = \frac{m_{TFA}}{m_{crude \text{ lipid}}} \times 100\% \quad \text{Eq. 2-10}$$

7 Other characterization techniques

7.1 Fourier Transform-Infrared Spectroscopy (FT-IR)

FT-IR analyses were performed at room temperature with a Bruker Tensor 27 spectrometer equipped with an Attenuated total reflection system (ATR) and a deuterated triglycine sulphate detector (DTGS). Absorbance was measured in the range 650 to 4000 cm^{-1} with an spectral resolution of 4 cm^{-1} , and 16 scans were performed. Afterwards, the spectra were baseline corrected and normalized.

7.2 Karl-Fischer (KF)

The water concentration in the liquid was determined by KF titration using a Metrohm 787 KF Titrino. Hydranal Composite 5 was used as titrant and a solution of ethanol: dichloromethane (3:1, v/v) was used as a solvent.

7.3 pH titration

The amine content in the solution was determined by Metrohm 785 DMP Titrino, which connected with Metrohm 806 Exchange unit. HCl (0.1 M) was used as titrant.

7.4 ^{13}C Nuclear Magnetic Resonance (^{13}C -NMR)

Quantitative ^{13}C -NMR measurements were performed with a Bruker 400 MHz Ascend NMR spectrometer. Samples were prepared by dissolving approximately 0.05-0.1 g of sample in 1.0 mL of acetonitrile. Measurements were performed using “inverse gate decoupling” method with 5 seconds of relaxation time and 1024 scans. The acetonitrile signal was used as internal reference.

Manual integration of the spectra was performed with MestReNova software (version 6.1.1-6384). When present, solvent peak of acetonitrile was manually integrated and subtracted from the corresponding region. The integration regions were defined according to Böttinger et al. [7] Jessop et al. [8] and Kortunov et al. [9] who used ^{13}C -NMR to study amine solutions upon reacting with CO_2 . The ^{13}C chemical shift of pure EBA and its salts are shown in Table 2.3.

Table 2.3 ^{13}C chemical shift of pure EBA and its salts.

Carbon atom	$\delta \text{ } ^{13}\text{C-NMR}$ (ppm)
15.46
44.35
49.81
32.59
20.68
14.07
$\left[\text{R}_2\text{NH}_2^{\oplus} \right] \left[\text{O}^{\ominus} \text{C}(\text{O})\text{COH} \right]$	161
$\left[\text{R}_2\text{NH}_2^{\oplus} \right] \left[\text{O}^{\ominus} \text{C}(\text{O})\text{CNR}_2 \right]$	163.6

7.5 Elemental Analysis (EA)

The elemental composition of nitrogen, carbon, and hydrogen (in wt.%) in the dry algae and crude lipid product was determined using an Elemental Analyser Thermo Scientific Flash 2000. Oxygen content was calculated by difference. Duplicated analyses were conducted for each sample and if the difference was in $\pm 1\%$, the results were accepted and average values were taken. If the results were further away from each other, more measurements were taken to improve the accuracy of the results. The elemental composition results are shown in Table 2.4.

Table 2.4 Elemental analysis of dry algae and lipid obtained from fresh water stressed *Neochloris oleoabundans*.

Sample	N (wt.%)	C (wt.%)	H (wt.%)	O (wt.%)
fresh water stressed <i>Neochloris oleoabundans</i> dry biomass	4.8	55.8	8.0	31.4
EBA extracted lipid from fresh water stressed <i>Neochloris oleoabundans</i>	1.4	72.7	9.5	16.4

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Chapter 3

Opportunities for switchable solvents in lipid extraction from microalgae

Abstract

Lipid from algal biomass may provide renewable fuel and chemical feedstock in large quantities. The energy intensity of drying and milling of algae prior to extraction and of solvent recovery afterwards is a major obstacle. The objective of this study is to use switchable solvents to extract oil directly from wet microalgae slurries without the need for drying and milling, and subsequently recover the extracted oil and solvent by phase splitting, using CO₂ as trigger. In this chapter several switchable solvent candidates were investigated for lipid extraction, for polarity switching and for phase splitting ability upon contacting with CO₂. N-ethylbutylamine (EBA) was selected for further study because of the good performance on both switchability and extraction performance. The influences of freeze drying and cell breaking on the extraction efficiency of crude lipid yield and fatty acid yield were investigated. For the strain *Neochloris Oleoabundans*, drying and cell breaking were not necessary for lipid extraction using EBA, while crude lipid yields and fatty acid yields were comparable with Bligh & Dyer extraction, making EBA a candidate for further development of an energy efficient lipid extraction technology for non-broken microalgae slurries.

1 Introduction

Sustainable renewable fuel alternatives to fossil energy sources are highly required to reduce the impact on the environment [1]. Currently, a strong focus on gas as alternative fossil energy source is dominating energy research in the oil and gas sector [2]. However, gas is also not an option for the longer term since the reserves/production (R/P) ratio of the world's natural gas is around 52.8 years [3]. Therefore, next to the strong developments in the gas-to-liquids technology, abundant, affordable, and sustainable liquid fuel alternatives to fossil energy sources are needed to avert an impending energy crisis. Among other potential sustainable energy sources, algae are particularly interesting as biofuel feedstock. The advantages of algae over traditional terrestrial crops as a source of transportation biofuels include (1) algae grow rapidly and algae productivity can offer high biomass yields per acre of cultivation [4], (2) algae can be cultivated in waste water, produced water, or saline water on non-arable land, thereby reducing competition with arable land, limited freshwater and nutrients used for conventional agriculture [5, 6], (3) algae can recycle carbon much faster than other crops from CO₂-rich flue emissions from stationary sources, including power plants and other industrial emitters [7], (4) algae cultivation does not need herbicides or pesticides [8], (5) the growth rate and lipid content of algae can be controlled by varying growth conditions [8].

Several process steps are needed to produce liquid biofuels from algae, such as algal cultivation, harvesting and dewatering, extraction and fractionation, fuel conversion, distribution and utilization, etc. To produce this algal oil efficiently and economically viable, a considerable investment in technological development is still needed. Significant progress is made in recent years, especially in the areas of algae cultivation, harvesting and extraction. For the cultivation of algae, the effects of pH, nitrogen, CO₂ concentration, aeration and light intensity have been examined by various authors [9-12] and several cultivation systems have been tested. For the extraction of oil from microalgae, different process strategies may be utilized. For example, algae could be harvested, dried, milled and the oil can be extracted from the dried biomass. Another strategy involves concentration of the algae, followed by a wet extraction. The extraction from wet algae could be done *in situ* without disrupting the algal cells, or alternatively extraction could be improved by destroying the wet cell integrity prior or during extraction, e.g. through wet milling, ultrasonic treatment or using a solvent that destroys the cell walls (this may even be a supercritical solvent) [13].

Liquid extraction is a very energy efficient separation technology in itself. However, solvent regeneration is needed as a second operation and often the efficiency of the extraction and the energy requirements of the recovery operation are trading off, i.e. the higher the affinity of the solvent for the solute, the more difficult it is to recover the solvent. Common recovery technologies that could yield the product in a pure form are crystallization, distillation, evaporation and stripping, all thermal processes that are not necessarily energy efficient.

A recovery method based on phase splitting might offer an energy efficient alternative. This phase splitting could be induced by changing the nature of the solvent. CO₂-switchable solvents which were first reported by Philip G. Jessop and coworkers [14] and introduced in chapter 1 would be a good option.

CO₂-switchable solvents are all amines and among them are primary, secondary and tertiary amines, including as well amidines and guanidines. Among them, the primary and secondary amines have not been investigated for extraction of lipid from algae, but could contain interesting molecules that could act as extraction solvent. Molecular property constraints for the solvents include a limited volatility, low viscosity and a low water solubility, at least in the more apolar form. Therefore, short chain primary amines were left out of consideration and our studies was further limited to some secondary amines. For comparison, the DBU-based switchable solvents were included in our studies. Besides the solvent screening, the possibility of lipid extraction from wet algae slurry by CO₂-switchable solvents is also investigated in this chapter, by evaluating extraction efficiency, with and without cell disruption, and lipid/solvent recovery via CO₂ induced phase separation.

2 Results and discussion

2.1 Solvent screening

For the concept envisaged, the desired solvent(s) should not be too volatile in view of evaporative losses and not be too viscous, which would hinder effective and rapid extraction and phase splitting. Primary amines and diamines that were initially considered but found too volatile to be used in practice or became too viscous during the phase switching were rejected in an early stage of the work. The solvents considered included ethylenediamine, 1-3-diaminopropane, pentylamine, hexylamine, diethylenetriamine and N,N-diisopropylethylenediamine. Besides the volatility and viscosity, miscibility which relates to solvent losses to water in the extraction phase, should also be considered. Additionally, the price of solvent also plays an important role in solvent selection since this research aims for industrial scale application. After considering all the above factors, the selected solvents that will be further investigated are listed in Table 3.1.

Table 3.1 Solubility (wt.%) of pre-selected solvents at 20°C.

Solvent name and abbreviation	Structure	Solvent in water	Water in solvent
N-ethylbutylamine (EBA)		5.06	30.7
Dipropylamine (DPA)		5.30	21.38
Dibutylamine (DBA)		0.57	6.36
Tripropylamine (TPA)		0.02	0.11
2-(dibutylamino)ethanol (DBAE)		0.44	5.94
N,N-dimethylcyclohexylamine (DMCHA)		1.62	21.4

A switching test was carried out for each solvent to see how well they could change their miscibility with water under introduction and removal of CO₂. Experiments were carried out at room temperature. Each solvent was mixed with water (v/v=1:1) to form a biphasic mixture. CO₂ was bubbled through the mixture with a flow rate of 15 VVM. The system was considered switched when a complete macroscopic switch from two liquid phases to a single phase was observed. Table 3.2 summarizes the time required for the switching forward of investigated solvents. In all these cases complete switching was achieved.

Table 3.2 Results of switching forward.

Solvent	Time for switching forward (min)
EBA	1
DPA	1
DBAE	> 120
DMCHA	60

In addition to the list in Table 3.2, two other solvents DBA and TPA were rejected due to their bad switching property. Under the introduction of CO₂, DBA formed a gel solution with water.

TPA showed a very slow change – roughly 1.5 mL (out of 7 mL) of TPA moved to the aqueous phase after 2 h CO₂ bubbling.

The behavior of TPA may stem from two of its characteristics: steric effect of three bulky propyl groups around nitrogen atom and the extremely low miscibility between water and the original solvent. As described in the introduction, it has been suggested that the reaction between tertiary amine and carbon dioxide also requires water as one of its reagents. The low miscibility between TPA and water, therefore greatly reduces the possibility of the three reagents to meet each other. The steric effect of three propyl groups also makes it difficult for other reagents to reach the reactive lone electron pair of nitrogen atom. On the other hand, another liquid tertiary amine, DBAE shows better switching property compared to TPA though they share, to some extent, the same steric hindrance property. An explanation can be drawn out from the better solubility between DBAE and water, which is at least 20 times higher than in case of TPA and water at room temperature [15, 16].

Besides the switchability, another key property of the desired solvent is the performance for lipid extraction from wet, preferably non-broken, microalgae. Thus extraction experiments were carried out to compare the extractability of EBA, DPA, DBAE and DMCHA. Those results were compared with Bligh & Dyer (B&D) method [17] which was commonly taken as a reference. The results are shown in Figure 3.1.

Figure 3.1 Lipid yields calculated on algae dry weight basis, obtained with EBA, DPA, DBAE, DMCHA and B&D method from non-broken and broken *Phaeodactylum tricornutum* algae samples, after 24 h extraction.

The extraction performance of switchable solvents was investigated by performing extraction experiments at room temperature. Four switchable solvents were mixed with non-broken or broken algae for 24 h to reach an equilibration extraction. From the results it is visible that those four switchable solvents were able to extract some lipid from the algae, and breaking the algae resulted in an increase of extraction yield. However, comparing the extraction yields of DBAE and DMCHA with B&D solvent 24 h extraction, the extraction yields for DBAE and DMCHA were quite low. This may be related to their larger molecular sizes comparing with EBA and DPA. In contrast to DPA which has a symmetrical structure, an EBA molecule has an ethyl and a butyl group which may result in a better compatibility with water and lipid respectively and therefore lead to the higher lipid extraction yield of EBA. It is a remarkable result that the EBA extraction yields for both non-broken and broken microalgae are comparable to the B&D extraction yield, which is often seen as the lab-analysis standard for lipid content determination. And the difference between the yield of non-broken algae and broken algae is less than 1%, which points to a possible application where the energy intensive drying and grinding steps may be omitted. Therefore, in the following work, EBA as the best candidate under evaluation will be studied further.

2.2 Switching properties of EBA

2.2.1 Polarity change of EBA

The two switchable solvent systems of Figure 3.2 (B) can be considered as two different types: 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU)/Alcohol binary solvent systems and secondary amines as single compound systems. In the DBU/Alcohol system, protonation of DBU in the presence of an alcohol and carbon dioxide is reversed when CO₂ is removed. Secondary amines react with CO₂ to form carbamate salts. This is expected to change the solvent phase from nearly immiscible to miscible with water and may also change the solvent from lipophilic to lipophobic, which is investigated in this work. The DBU/Alcohol system is known to be sensitive to the presence of water [18] and hence not directly suitable for the extraction of lipid from wet microalgae slurries. Nevertheless, it was used in this work to act as a reference system on the phase switching behavior, as the DBU/Alcohol system was the first and most used system to demonstrate this phenomenon.

The reversibility between the polar and the non-polar form of the secondary amines as switchable solvents was first confirmed by measuring their polarity as indicated by the UV-Vis technique, while CO₂ and N₂ were bubbled through the solution. In order to quantify the polarity, the solvatochromatic dye Nile Red was used, and several commonly used solvents of which the normalized solvent polarity parameter E_N^T has been published [19] were used to develop a correlation between the observed $\lambda_{\max}$ and the E_N^T value.

Figure 3.2 Polarity of switchable solvents as a function of time during treatment with CO_2 followed by N_2 and heating.

Figure 3.2 shows that the polarity rose when CO_2 was bubbled through the solution, and it dropped upon N_2 addition. To speed up the reverse reaction, the switching back was done at a slightly elevated temperature of 50°C . The polarity change of EBA has the same trend as DBU/1-hexanol system. Compared with DBU/1-hexanol, EBA has a significantly less polar low-polarity form, which is beneficial for extraction of apolar lipid.

2.2.2 EBA switching in presence of water

Since the aim of this project is to extract lipid from wet algae slurry, the switching performance of EBA in presence of water was investigated. Pure CO_2 was bubbled through the mixture of EBA and water at ambient conditions. The binary mixtures all formed one phase within 5 min after contacting with CO_2 in a flow rate of 5 VVM (Table 3.3). The formation of one phase is because that secondary amines react with CO_2 to form carbamate salts via carbamic acids [20] which is soluble in water (Scheme 3.1 (A)).

Table 3.3 Switching forward results of EBA in water.

EBA : water (w/w)	5:1	2:1	1:1	1:2	1:5
Time to get one phase (min)	4	5	5	3	2

Scheme 3.1 Switching mechanism of secondary amines. [20]

The conversion percentage of EBA in an EBA-water (1:1 w/w) mixture was measured by titration. The results in Table 3.4 indicates that the binary mixture can form one phase even with 25% EBA conversion. This showed the possibility of lipid recovery from semi-switched EBA solution.

Table 3.4 Conversion percentage of EBA when reacts with CO₂.

Time (min)	5	15	30
EBA conversion (%)	25	48	69

Moreover, gel formation was observed when CO₂ was applied to the EBA-water mixture for a longer time (more than 1 h). CO₂ was bubbled to the mixture for 2 h to get complete gel formation and then separated to solid and liquid phase by vacuum filtration (Figure 3.3) and identified by ¹³C NMR. Based on the research of Böttinger et al. [21] and Vanderveen et al. [22], the peak associated with the bicarbonate ion appears near 160-162 ppm, while the peak associated with the carbamate ion appears near 163-165 ppm. The ¹³C NMR spectra of reacted EBA (both solid and liquid phase) are shown in Figure 3.4. The liquid phase spectrum contain peak at 163.8 ppm, indicating the presence of carbamate ion, while the spectra of the solid phase contains peak near 161.5 ppm, indicating the presence of bicarbonate ions. The composition of the mixture above is illustrated in Figure 3.5. It can be seen from the results that 9.9 wt.% of the total mixture was EBA. That means 78.1 wt.% EBA converted to EBA carbamate (3/4) or bicarbonate salt (1/4) after 2 h CO₂ stripping. EBA bicarbonate which is the main compound in solid phase accounted for 12.5 wt.% of the total mixture weight.

Since the precipitation typically occurs with a long time CO₂ supply, more CO₂ enlarges the chance that the reaction shown in Scheme 3.1 (B) occurs. This reaction requires water to participate, which results in the increase of viscosity and even gel formation. It was experimentally evaluated that the gel formation can be avoided when the EBA to water ratio is decreased to 1:10. However, if more CO₂ is chemically bound, the energy demand of back

switching process will increase. Considering the results above, it is wise to keep the EBA-water at a relatively high ratio and do an incomplete switching to recover the lipid.

Figure 3.3 Gel formation and solid liquid separation of EBA/water mixture after 2 h CO_2 bubbling.

Figure 3.4 ^{13}C NMR spectra of reacted EBA (both solid and liquid phase).

Figure 3.5 Composition of EBA-water (1:1 w/w) after 2 h CO₂ bubbling.

2.2.3 EBA switching in presence of oil

To investigate the possibility to recover the solvents from the lipid by an induced phase splitting, mixtures were made of sunflower oil (mimicking algal lipid) and the solvents. Pure CO₂ was bubbled through the mixture at ambient conditions. The binary mixtures of sunflower oil and solvents formed one phase and after contacting with CO₂ no phase separation occurred, there was still one phase.

Next to the binary oil / solvent mixtures, also the effect of (possibly co-extracted-) water was investigated in some experiments by adding water to the mixture. In these ternary mixtures, there were two phases, one organic phase and an aqueous phase. It could be easily observed that the amine solvent transferred to the aqueous phase upon contacting with CO₂ and that the sunflower oil was left on the top. It was therefore concluded that a certain amount of water contributes (or even is essential) for the phase separation, which is in contrast with the DBU/Alcohol systems. A further insight in the composition of the liquid phase before- and after switching was obtained by FT-IR analysis and shown in Figure 3.6.

Figure 3.6 The switchability and phase transfer of EBA monitored by FT-IR.

In the ternary mixture, first sunflower oil, amine and H_2O were shaken together in a 1:3:4 v/v/v ratio at room temperature, giving a biphasic mixture. The two phases were analyzed by FT-IR, showing that the upper phase was oil, amine and a small amount of water (Figure 3.6 (A)) and the lower phase was water (Figure 3.6 (B)). After CO_2 was bubbled through the biphasic mixture, the amount of upper phase decreased while the lower phase increased. The FT-IR spectra proved that the upper phase was oil (Figure 3.6 (C)) and the lower phase contained the carbamate species and water (Figure 3.6 (D)). Then, the upper layer (oil) was removed with a syringe and the lower phase was evaluated separately for solvent recovery by bubbling N_2 through the mixture to remove the CO_2 . It was found that the mixture split into two liquid phases. FT-IR spectra showed that the upper phase was the recovered secondary amine solvent with a little bit of water (Figure 3.6 (E)), while the lower phase still contained some of the carbamate salts of amine (Figure 3.6 (F)). This may imply that the reaction was not completely reversed, and the lost EBA need to be recovered further. This conversion of the aqueous solution of the carbamate salts of the amine solvent to the biphasic mixture of water and amine could be accelerated by heating the solution to 90°C while stripping N_2 .

2.3 Process concept for lipid extraction from microalgae with a secondary amine solvent

The proposed process is illustrated in Figure 3.7. First, the oil is extracted from the algae using the switchable solvent in its lower polarity (lipophilic) form. Then by contacting the solvent with CO_2 , the solvent is switched into its more polar, ionic (“lipophobic”) form and phase-separates from the apolar lipid oil. The solvent can be returned in its original state by flushing N_2 through the solvent. Key steps in this process are (1) uptake of CO_2 and reaction of CO_2 with the solvent in the liquid mixture to change the solvent from lipophilic to lipophobic and (2) the rate of the back-switching reaction. To increase these rates, water could be added (or not totally removed from the previous stage), and the solvent might be heated up. Additional points of attention are the solubility of the solvent in water (which might affect algae cultivation when recycling the water untreated) and the solubility in the lipid oil phase. For practical purposes, if a portion of the switchable solvent ends up in the aqueous phase after extraction, this may not be considered as a significant problem because the aqueous phase would be re-used. However, if a significant fraction of the solvent leaves the process dissolved in the oil, this requires an additional recovery operation (e.g. washing with water) to limit the loss of solvent and the contamination of the oil product. Therefore, the selected solvent EBA (see Figure 3.2 (B)) was tested for the desired phase behavior: immiscibility with water under extraction conditions and immiscibility with oil in the presence of CO_2 at (no more than) atmospheric pressure.

Figure 3.7 Process concept for extraction of lipid from algae with a secondary amine solvent, followed by CO_2 -induced phase splitting for solvent recovery and N_2 -induced back-switching.

2.4 Lipid extraction from microalgae

2.4.1 To dry, or not to dry

The B&D extraction method was taken as a reference in this research. It is important to investigate the influence of sample type and extraction time to the lipid yield, and then choose a benchmark for further research. Different lipid yields were obtained when different algae samples (~5 wt.% algae slurry or freeze dried algae) were used for extraction. Figure 3.8 shows that the crude lipid yield of *Neochloris Oleoabundans* wet paste was 13.8 ± 0.3 wt.%. Comparing with extraction from freeze dried algae (14.5 ± 0.4 wt.% crude lipid yield), less lipid was extracted out from wet algae sample. In these two experiments, the algae samples used were in different conditions: with and without lyophilisation. Another difference between these two types of experiments is that no water is involved in the extraction step when freeze dried algae were used. Water was added at the end only for phase separation and the final volume ratio of chloroform/methanol/H₂O was 2/2/1. Therefore, a third type of experiment was carried out. Freeze dried algae were first mixed with water to form 5 wt.% algae slurry and then used for extraction. The volume ratio of chloroform/methanol/H₂O was 2/2/0.8 during extraction and 2/2/1.8 in the end. 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 ± 0.3 wt.%. These results showed that the main reason which caused lower crude lipid yield is water, and not the freeze drying operation. Since the lipid containing fatty acids is particularly interesting in this work, the total fatty acid (TFA) yields of these three samples were also measured. The results in Figure 3.8 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 B&D extraction with wet algae paste instead of dried sample to get a higher TFA yield.

Figure 3.8 Crude lipid yield and TFA yield of *Neochloris Oleoabundans* samples in different condition obtained by B&D extraction.

2.4.2 To break, or not to break

Besides drying, cell breaking is another energy intensive step in the algae biorefinery. A series of experiments was carried out to compare the extraction from non-broken and broken algae. Several extraction times were applied to ensure extraction equilibrium. The results are shown in Figure 3.9. 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 keep constant while the crude lipid yield decrease after long extraction time (120 min). In the broken algae slurry, most substance contained in algae cell were released and mixed. Some degradation may happen to the lipid. The carboxylate end of fatty acid molecules, when bonded to different head groups (e.g. an uncharged head group or a charged head group) can form different type of lipid (e.g. neutral lipid or polar lipid) [23]. Free fatty acids maybe released from the head groups of lipid 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 may have contributed to the observed decrease of crude lipid yield when longer extraction time applied. Although the crude lipid yield of broken algae is higher than non-broken algae, the TFA yields 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.

Figure 3.9 Crude lipid yield and TFA yield of *Neochloris Oleoabundans* (5% dw) extracted by B&D method.

Both non-broken and broken algae were extracted by EBA at 22°C with solvent/feed ratio of 1:1 (w/w) for 24 h to ensure extraction equilibrium. The extraction results of crude lipid and TFA yield were compared with B&D method and shown in Figure 3.10 (A). It can be seen from the results that EBA can extract as much crude lipid as B&D extraction. Extraction using from broken algae got slightly higher crude lipid yield 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 Figure 3.10 (B) it is visible that the crude lipid extracted by EBA has similar composition as B&D extraction. More unsaturated fatty acids presented in the lipid extracted from non-broken algae. The different fatty acid fractions between lipid from non-broken and broken algae show that cell breaking prior to extraction might affect the lipid product composition.

These results show the possibility of extracting lipid from non-broken algae while using EBA as extractant. It points to a possible application where the energy intensive drying and grinding steps may be omitted.

Figure 3.10 (A) Crude lipid yield and TFA yield of *Neochloris Oleoabundans* (5% dw) extracted by EBA method and (B) TFAs compositions of the extracted lipid.

3 Conclusions

In this chapter, secondary amine EBA was selected as the most promising candidate for lipid extraction from aqueous slurries of fresh, unbroken *Neochloris Oleoabundans* microalgae because of the good switchability and extractability. Moreover, this solvent allows the recovery of the lipid and of the extraction solvent via a simple and energy efficient phase splitting step in the presence of water, induced by contacting with pure CO₂ at atmospheric pressure and ambient temperature. Solvent recovery is achieved by nitrogen stripping at a slightly elevated temperature (50-90°C).

Extractions using wet or freeze dried algae as starting material were investigated for both

B&D extraction and EBA extraction. 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 an outlook to a cost and energy efficient lipid recovery from microalgae.

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Chapter 4

Optimizing wet lipid extraction from whole cell *Neochloris oleoabundans* using multiple extraction stages

Abstract

The extraction yield of lipid from non-broken *Neochloris oleoabundans* slurries (~5% dry weight) was maximized for N-ethylbutylamine (EBA) as switchable extraction solvent, for single step- and multiple extraction stages. The *Neochloris oleoabundans* microalgae were cultivated in fresh water and artificial seawater under stressed and non-stressed conditions.

In the study, experimental parameters that affect the extraction were investigated. The study showed that with wet algae (at least) 18 h extraction time was required for maximum yield at room temperature and a solvent/feed ratio of 1:1 (w/w). Multiple extraction stages show that, depending on solvent to feed ratio, a second or even third extraction stage can be beneficial. For fresh water (FW), non-stressed, non-broken *Neochloris oleoabundans* 13.1 wt.% of lipid extraction yield (based on dry algae mass) was achieved. The lipid extraction yield of FW-stressed *Neochloris oleoabundans* was 47.0 wt.% of which 52.9 wt.% total fatty acids. For wet lipid extraction, and especially for non-broken algae, the EBA solvent system was found to outperform the Bligh & Dyer reference method. The yield after four stages of extraction for the FW-stressed *Neochloris Oleoabundans* was as high as 61.3 wt.% lipid, an almost 5 times higher yield than for non-stressed algae with a single extraction step.

The results in this work illustrate that the combination of stressing the freshwater cultivated microalgae and applying EBA as solvent in multiple stages of extraction is very promising in the development of an energy efficient lipid extraction technology targeting non-broken, wet microalgae.

1 Introduction

Microalgae have been considered as one of the most promising sustainable feedstocks in recent years. Algae primarily comprise of lipid, proteins, carbohydrates and are widely used in the area of biofuels [1], pharmaceuticals [2] and cosmetics [3], food and feed [4]. Microalgae are receiving increasing attention, due to their rapid growth rate, and thus high productivity, less competition with arable land and freshwater as compared to other crops, and high CO₂ consumption rate [5]. Several process steps are needed to produce products from algae, such as algal cultivation, harvesting and dewatering, extraction and fractionation, distribution and utilization.

Lipid extraction is one of the main topics in the research on algae biorefinery processes. Organic solvent extraction has the benefits of inexpensive solvents and high lipid recovery yield [6]. Supercritical CO₂ (scCO₂) extraction is considered as an efficient and “green” extraction method for lipid extraction [7]. Both the methods have their own advantages but also some drawbacks. The chemicals used in organic solvent extraction such as hexane are often highly flammable and toxic, and the solvent recovery is energy intensive [8]. ScCO₂ 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 [9]. Moreover, these methods generally require drying of the biomass [10-15], which accounts for more than 70% of the energy that can be produced by algae [16].

A method named CO₂-switchable solvent extraction aroused the interest of many researchers in recent years [5, 16-19]. With this technology lipid can be extracted, after which solvent recovery is accomplished by switching the solvent hydrophilicity with CO₂, which induces phase splitting. In the research by Samorì et al., it was found that switchable solvents with the DBU/1-octanol system exhibited a better extraction yield than n-hexane, both with dried and wet *Botryococcus braunii* samples (7.8 wt.% and 5.6 wt.% hydrocarbons yield respectively) [19]. The tertiary amine N, N-dimethylcyclohexylamine (DMCHA) was also reported for extraction of lipid from microalgae. In the research of Jessop et al., the lipid extraction yields of lyophilized *Botryococcus braunii* were 19 wt.% (room temperature) and 22 wt.% (60 to 80°C) of the algal dry weight [17] while using DMCHA as extractant. Samorì et al. used DMCHA for extracting and recovering lipid directly from wet algae samples (about 80% water content) of three microalgae strains: *Nannochloropsis gaditana*, *Tetraselmis suecica*, and *Desmodesmus communis* and obtained 29.2 wt.%, 57.9 wt.% and 31.9 wt.% lipid yield respectively [18]. In the work of Du et al., secondary amines N-ethylbutylamine (EBA) and dipropylamine (DPA) were found to be able to extract 16.8 wt.% and 15.4 wt.% lipid respectively, from aqueous slurries of fresh, non-broken microalgae *Desmodesmus sp.* [5]. An energy evaluation showed that the switchable solvent extraction method is a very promising method for extracting lipid from algae for use in energy applications [16]. The net

energy yield increases 5 to 6 times when the lipid yield increases from 10 wt.% to 45 wt.% [16]. This increase may be achieved by stressing the algae.

In this chapter, multistage lipid extraction from both non-stressed and stressed (fresh water (FW) stressed and artificial sea water (ASW) stressed) non-broken *Neochloris oleoabundans* slurry (~5% dry weight) using secondary amine EBA was studied. Lipid yields of EBA extraction were compared with the Bligh & Dyer (B&D) lipid extraction method, commonly applied for analytical purpose. The fatty acid composition of the extracted lipid was analyzed and compared. The extraction was optimized using various parameters, such as cell disruption, extraction time, extraction temperature and solvent to feed ratio to maximize the lipid yield and total fatty acid yield.

2 Results and discussion

2.1 Effect of cell breaking and time

The B&D extraction method was taken as a reference method in this research. Several different extraction times were applied to study the required time to reach the extraction equilibrium. The results are shown in Figure 4.1 (A). For both non-broken algae and broken algae, the crude lipid extraction yield is comparable at approximately 13 wt.% of the dry mass for lower extraction times. Over time, from 10 minutes to 24 hour, the maximum crude lipid yield for non-broken hardly increased (max 13.9 wt.% of dry algae), while for the broken algae the yield increased slightly in the first 60 min. to 15.3 wt.% of dry algae.

Apparently, from the broken algae some compounds are extracted, that are not extractable in the case of non-broken algae. After 60 minutes, the yield slowly went down with prolonged extraction time to fall eventually back to the 13 wt.% dry mass that was initially also extracted. It may be that breaking the algae enables disclosure of some extractable compounds, that at longer extraction times do (partially) decompose or evaporate, but at this point in time there is no true understanding of this observed effect, which is a clear and significant trend in view of measurement accuracy. Overall, it can be concluded that both the crude lipid yield and the TFA yields from broken algae and from non-broken algae are comparable. The B&D 120 min extraction from non-broken algae results will be taken as a reference in further studies.

Figure 4.1 Crude lipid yield (in percentage of the dry algae mass) and TFA yield of non-stressed *Neochloris oleoabundans* extracted by (A) B&D extraction and (B) EBA extraction.

Both non-broken and broken algae (from the same batch of algal biomass) were also extracted by EBA at 22°C with solvent/feed ratio of 1:1 (w/w), again applying different extraction times. The results in Figure 4.1 (B) show that for non-broken algae, the crude lipid yield increased gradually with time and reached the equilibrium (~13 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 lipid which were inside the algae cell and made them easier to be extracted by EBA. However, the higher crude lipid yield did not result in higher TFA yield.

The TFA yield of non-broken algae was approximately 3 wt.% and slightly higher than broken algae (~2.5 wt.%). From the results of TFA fraction in crude lipid shown in Table 4.1, it can be seen that the TFA fraction in crude lipid of non-broken algae kept constant while that of broken algae increased with extraction and reached equilibrium in 3 hours. The low TFA fraction in the total crude lipid of broken algae in the first 3 h was not due to less extraction of lipid containing fatty acids, but due to high non-fatty acids lipid yield which was caused by the lipid released from cell breaking. This also shows that there is no benefit to break the algae cells if fatty acid is considered as the main product. From the results in Figure 4.2 it is visible that the fatty acid compositions of lipid from EBA extractions were constant with increasing extraction time. Although there are some slight differences, the composition is in both cases comparable. When compared to the B&D method, it shows that the maximum crude lipid yield and TFA yield using EBA is comparable, but the time required with this solvent is longer. From this it can be concluded that with the EBA solvent, it is not necessary to break the algae, which is an energy intensive step. However when non-broken algae are used as feedstock for lipid, considerable longer extraction times should be accounted for.

Table 4.1 TFA fraction in crude lipid of non-stressed *Neochloris oleoabundans* extracted by EBA method. ^a

Extraction time (min)	TFA fraction in crude lipid (%)	
	Non-broken	Broken
10	24.5	13.8
30	25.3	14.4
60	25.1	15.7
120	25.0	17.3
360	25.1	18.4
720	25.4	18.4
1080	25.3	18.5
1440	25.3	18.5

^a Results are mean $\pm$ standard deviation (SD), $n \geq 2$, $SD \leq 1.3$.

Figure 4.2 TFA compositions of lipid from (A) non-broken and (B) broken non-stressed *Neochloris oleoabundans* extracted by EBA method.

2.2 Effect of extraction temperature

Non-broken *Neochloris oleoabundans* were extracted by EBA for 18 h with solvent/feed ratio of 1:1 (w/w) at different temperatures to study the effect. Crude lipid yield and TFA yield obtained at 22°C and 50°C were summarized in Figure 4.3. The lipid extraction yield was 13.0 wt.% at 22°C. When the extraction temperature was increased to 50°C, a higher lipid extraction yield of 14.3 wt.% could be achieved. The higher temperature may contribute to the distribution of the lipid to the EBA phase. The lipid that are largely present in cell membranes are supramolecular structures. Keeping these structures in tact is more difficult at higher temperature, thus it becomes more easy to extract single molecules from the supramolecular aggregates. At the meantime, there was almost no change of the TFA yield when increasing the extraction temperature. There are more unsaturated fatty acid present in the lipid of 22°C extraction than 50°C extraction. Considering the energy input and output, there are no obvious benefits of doing the extraction at an elevated temperature. Hence, an

extraction temperature of 22°C (room temperature) was chosen for further use in this study.

Figure 4.3 Crude lipid yield, TFA yield and TFA compositions of non-stressed *Neochloris oleoabundans* extracted by EBA at 22°C and 50°C.

2.3 Effect of solvent/feed ratio

Lipid extraction experiments using broken *Neochloris oleoabundans* (5% dw) at 22°C were also carried out with different solvent/feed ratios (2:1, 1:1, 1:2 and 1:5 w/w). After 18 h of extraction, the highest crude lipid yield (14.2 wt.%) and TFA yield in algae dry weight (3.6 wt.%) were obtained at the highest solvent/feed ratio of 2:1 (see Figure 4.4). Lowering the solvent/feed ratio resulted in a continuously lowered crude lipid yield. An equilibrium validation experiment using 72 h extraction time was done for the solvent/feed ratio of 1:5, showing that indeed, equilibrium was reached after 18 h extraction (7.3 wt.% $\pm$ 0.2 wt.%). The TFA compositions were compared for all tested solvent/feed ratios and are shown in Figure 4.5. The results indicate that the lipid obtained from different solvent/feed ratio had exactly the same FAME profiles. Thus the solvent/feed ratio only influences the lipid extraction yield and not the lipid composition. Solvent/feed ratios higher than 2:1 were not studied because the highest lipid yields at the ratios of 1:1 and 1:2 are already very close, suggesting that a higher solvent/feed ratio will not lead to much higher lipid extraction yields. Because more solvent also means a higher heat duty in the lipid recovery from the solvent, from energy balance point of view, a solvent/feed ratio of 1:1 was chosen in this study.

Figure 4.4 Crude lipid yield and TFA yield of non-stressed *Neochloris oleoabundans* extracted by EBA method at different solvent/feed ratio.

Figure 4.5 FAME compositions of lipid from non-stressed *Neochloris oleoabundans* extracted by EBA method at different solvent/feed ratio.

2.4 Multistage extraction

To investigate whether higher yields could be obtained than in a single stage extractions, multiple extraction steps were applied to the same batch of algal biomass. After an EBA-extraction of non-broken algae for 18 h (and for B&D after 2 h), a second extraction was applied to the extracted algae. The solvent layer was separated after centrifuging, removed and replaced by an equal amount of fresh solvent. This procedure was applied multiple times to achieve four extraction stages. The results obtained for various S/F ratios and for the B&D method are illustrated in Figure 4.6. In all cases, the cumulative crude lipid yield increased significantly from the second to the fourth stage. The highest crude lipid yield (22.1 wt.%) obtained in EBA 1:1 ratio extraction was 6.7 wt.% higher than the B&D 4 stages extraction (15.4 wt.%). Because of the limited amount of recovered lipid in the third and fourth stages, only the lipid from first and second extractions were analyzed by GC-MS. The TFA compositions are presented in Figure 4.7. The fatty acid profile of *Neochloris oleoabundans* was dominated by palmitic (C16:0), hexadecadienoic (C16:2), hexadecatetraenoic (C16:4), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids, and no significant differences were found between the extraction methods and extraction stages tested.

Lower solvent/feed ratios resulted in lower crude lipid yields. This is not only valid for single extraction experiments, but was also found for the multistage extractions. Part of the reduced yields may be caused by losses of EBA remaining inside the algae cell after extraction. Assuming there is a distribution of lipid over the mixture inside the cell and the EBA phase, it will show that the more solvent is used, the lower the equilibrium concentrations in both the cells and in the bulk EBA phase, and the less lipid is lost remaining inside the algae cell. This might at least partly explain the results showed in Figure 4.8. For further elucidation, an extraction starting with a solvent/feed ratio of 1:5 followed by a second extraction with solvent/feed ratio of 1:1 was carried out. In the second extraction step, it was found that 5.2 wt.% lipid was extracted. This was more than the corresponding second extraction lipid yield of both combinations “1:1 + 1:1” (4.7 wt.%) and “1:5 + 1:5” (1.4 wt.%).

The results above further prove that there is always some lipid material left in the algae cell after an extraction. Thus a simple phase distribution model is not sufficient to describe the extraction process. It also shows clearly, and from Figure 4.6 especially for the EBA-extraction method, that the overall yield of lipid could be further increased significantly by applying a multistage extraction.

Figure 4.6 Crude lipid yield for non-stressed *Neochloris oleoabundans* extracted by EBA method for multistage extractions.

Figure 4.7 TFA compositions of lipid from non-stressed *Neochloris oleoabundans* obtained by EBA 1st and 2nd extraction.

Figure 4.8 Crude lipid yield of non-stressed *Neochloris oleoabundans* extracted by EBA method for different solvent/feed ratio combinations.

2.5 Lipid extraction from stressed *Neochloris oleoabundans*

From the results above it can be found that EBA has good performance in lipid extraction from wet, non-broken algae. The algae used in pervious study were cultivated in fresh water without stressing. To investigate to what extent this procedure is applicable to *Neochloris oleoabundans* cultivated in different ways, the crude lipid yield and TFA yield were determined for both the B&D extraction and for the EBA extraction for stressed and non-stressed algae, cultivated in fresh water and artificial sea water. The results are summarized in Figure 4.9. Compared with extraction from non-stressed *Neochloris oleoabundans*, a much higher crude lipid yield and TFA yield were obtained from freshwater cultivated-, stressed (“FW-stressed”) algae for both B&D and EBA extraction. Surprisingly, EBA can extract 80% more lipid than via the B&D method, while the TFA fraction of the crude lipid products is similar in these two cases. The results indicate that EBA has an excellent performance for lipid extraction from non-broken, FW-stressed algae.

The lipid yield obtained from the artificial seawater cultivated-, stressed algae (“ASW-stressed”) algae was even less than from (FW-) non-stressed algae. Especially the lipid extraction yield of the B&D method (for non-broken ASW-stressed algae) was only 1.3 wt.% which was much less than expected. Therefore, also an extraction of lipid from broken ASW-

stressed algae was carried out. With broken cells, the extraction yield was much higher than with non-broken cells for the ASW-stressed algae, suggesting that the lipid extraction from ASW-stressed algae was limited by the fact that the cells were intact. Different culture media and culture conditions influence the properties of the cell and cell wall. Microscope images were taken from the cells and displayed in Figure 4.10. As can be seen in Figure 4.10, compared to non-stressed algae, the stressed algae cells are more transparent because of the higher lipid content. FW-stressed algae have a smaller cell size than the ASW-stressed algae. Algae cultivated in ASW have thicker cell wall which make the extraction difficult. Cell disruption is needed for ASW-stressed in order to get competitive lipid extraction yield. Thus, the FW-stressed algae, with the highest lipid yield and no need to be dried, nor to be broken prior to extraction, are preferred.

The fatty acid compositions of the lipid obtained from the B&D and EBA extraction were determined (Figure 4.11). No significant difference was observed, meaning that the lipid fractions from B&D and EBA extraction contained roughly the same fatty acids.

Figure 4.9 Crude lipid yield and TFA yield of non-stressed, FW-stressed, ASW-stressed and ASW-stressed (broken) *Neochloris oleoabundans* extracted by B&D and EBA method The “Non-stressed”, “FW-stressed” and “ASW-stressed” series were measured for non-broken algae.

Non-stressed

FW-stressed

ASW-stressed (broken)

ASW-stressed

Figure 4.10 Light microscope images of non-stressed, FW-stressed, ASW-stressed and ASW-stressed (broken) *Neochloris oleoabundans* prior to extraction.

Figure 4.11 TFA compositions of lipid from non-stressed, FW-stressed, ASW-stressed and ASW-stressed (broken) *Neochloris oleoabundans* extracted by B&D and EBA method.

The four stage extraction procedure was also applied to non-broken FW-stressed and non-broken ASW-stressed *Neochloris oleoabundans*. The results are presented in Figure 4.12 (A). After four times extraction, the lipid extraction yield of ASW-stressed algae (24.3 wt.%) only accounts for 60% of the single stage extraction yield of ASW-stressed broken algae (39.6 wt.%). This makes extraction from non-broken, ASW-stressed algae not very attractive. For FW-stressed algae, two extraction steps extract more than 92% of the total lipid obtained after 4 times extraction (61.3 wt.%), which is considered sufficient. Adding more extraction stages will require more solvent and more processing time, leading to more energy usage and lower production capacity, while the additional yield is limited. Also it can be found from the results in Figure 4.12 (B) that the TFA content in crude lipid is lower in the second extraction stage. It therefore seems that two extraction stages is optimal.

Figure 4.12 Crude lipid yield (A) and TFA compositions (B) of non-stressed, FW-stressed and ASW-stressed *Neochloris oleoabundans* extracted by EBA method for multistage extractions.

3 Conclusions

In this chapter, extraction conditions for using EBA to extract lipid from *Neochloris oleoabundans* were optimized for both single stage extractions, and for multistage extractions. It was shown that the EBA extraction is very suitable for high lipid contents, such as observed in stressed fresh water algae *Neochloris oleoabundans*, and cell disruption was not needed, although a considerable longer extraction time (18 h) was required for non-broken cells. Room temperature (22°C) and solvent/feed ratio 1:1 (w/w) were selected as optimal conditions with respect to lipid yield (47 wt.% in a single stage for fresh water stressed algae). Multiple extractions increased the crude lipid yield, reaching for fresh water stressed *Neochloris oleoabundans* a maximum yield of 61.3 wt.% after four extractions. After two extractions already 57 wt.% was obtained, suggesting that two stages should be sufficient. This study shows that by using both algae stressing techniques and an effective lipid extraction technology for non-broken wet microalgae high lipid yields can be realised.

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Chapter 5

Multistage wet lipid extraction from fresh water stressed *Neochloris oleoabundans* slurry – Experiments and Modelling

Abstract

Algae are considered an important renewable feedstock for lipid extraction to produce biofuels. Algae strain *Neochloris oleoabundans* used in this research can yield a high lipid content under stressed conditions. N-ethylbutylamine (EBA) as a switchable solvent has previously shown outstanding performance on energy efficient lipid extraction from non-broken wet algae slurry. In this work, a model was developed that describes the equilibrium state of lipid extraction from fresh water (FW)-stressed *Neochloris oleoabundans* algae slurry using EBA as solvent. When assuming that the cell interior is almost completely filled with the solvent phase during extraction, the model estimated extraction yields showed good agreement with those obtained in experiments. The developed model can predict the amount of crude lipid being recovered from any stage of a multistage extraction process.

1 Introduction

The global demand for energy is rapidly increasing with increasing human population, urbanization and modernization [1]. Hence, abundant, affordable and sustainable liquid fuel alternatives to fossil energy sources are necessary, especially in order to reduce the impact on the environment [2]. Biomass based energy production systems can partly replace the presently employed energy systems.

Microalgae as an important feedstock for biofuels are receiving increasing attention [3-7]. They have rapid growth rate, and thus high productivity, less competition with arable land and freshwater as compared to other crops and a high CO₂ fixation rate [8]. The algae *Neochloris oleoabundans* used in this research is a freshwater species that has been shown capable of producing 35-54% lipid of algae dry weight [9]. *Neochloris oleoabundans* was stressed to improve the lipid content under different growth conditions, such as other nitrogen sources [10], nitrogen starvation [9, 11, 12], mixotrophy (the use of phototrophy and heterotrophy in combination) [13], pH and salt concentration [14]. The research findings provide an interesting outlook on its application as alternative feedstock for biofuel production.

Lipid extraction is one of the main topics in the research of algae biorefinery process. Organic solvent extraction [15-25] and supercritical fluid extraction [21, 26-34] are the most common methods being used for algae lipid extraction. In recent years, a method named CO₂-switchable solvent extraction aroused the interest of many researchers [8, 35-39]. With this technology lipid can be extracted, after which solvent recovery is accomplished by switching the solvent hydrophilicity with CO₂, thereby inducing phase splitting. These studies also showed the possibility of extracting lipid from wet algae slurries, hence without the need for harvesting and drying the algal biomass prior to extraction. In the work of Chapter 4, it was found that using N-ethylbutylamine (EBA) for lipid extraction from fresh water (FW) stressed *Neochloris oleoabundans* reached extraction equilibrium within 18 h and the lipid extraction yield in that study was 47.0 wt.%. It was also found that the yield after four stages of extraction for the FW-stressed *Neochloris oleoabundans* was as high as 61.3 wt.% lipid. However, why not all lipid was recovered during the first extraction step, hence: incomplete extraction, is still unknown. Also in literature very little attention has been devoted to the equilibrium status of algal lipid extraction and to the possibility and potential of multistage extraction.

In this chapter, we aim to develop a model that describes the equilibrium state of lipid extraction using EBA from wet microalgae slurry. The model should provide a qualitative insight in the extraction process, and be able to predict the amount of crude lipid being recovered from FW-stressed *Neochloris oleoabundans* at any stage of a multistage EBA

extraction process. For this purpose extraction experiments of using different solvent to feed ratio were carried out. Several model assumptions were made based on experimental results or visual observations and cross-checked against the generated experimental data to detect the validity of the proposed model. Sensitivity analysis of the assumed parameters further proves the reliability of the newly developed model, discussed in this work.

2 Results and discussion

2.1 Model assumptions

The modelling approach to describe the multistage lipid extraction is based on some experimental observations that are discussed prior to setting up the model in order to make the approach more comprehensive. It was found in the results of lipid extraction from fresh water (FW) stressed *Neochloris oleoabundans* that the extraction reached equilibrium within 18 h (Chapter 4), and by applying multiple extraction steps to the same batch of algal biomass, the lipid yield could be increased, as is shown in Figure 5.1. Two extraction stages extract more than 92% of the total lipid obtained after 4 times extraction (61.3 wt.%). Because of the limited amount of recovered lipid in the third and fourth stages, only the lipid from first and second extractions were analyzed by GC-MS. It can be found from the results in Figure 5.2 that although the TFA fraction in crude lipid was lower in the second extraction stage, the composition of the fatty acids is very similar, if not identical.

Figure 5.1 Crude lipid yield of *Neochloris oleoabundans* extracted by EBA method for multistage extractions.

Figure 5.2 TFA composition, TFA fraction in crude lipid and TFA yield of lipid from *Neochloris oleoabundans* obtained by EBA 1st and 2nd extraction (S/F=1:1).

From the light microscope images of FW-stressed *Neochloris oleoabundans* at different extraction times (Figure 5.3) it can be seen that the extraction did not change the shape of the

algae cells, and even after 24 h, clear cells similarly of shape as compared to the cells before extraction can be identified. However, it is also clearly visible that the chloroplast of algae cell, which is the dark green part, shrank during the extraction process and became a tiny part (“dot”) at the end of extraction. In Figure 5.4, it can be observed that the algae cells just after being contacted with solvent, thus before significant extraction took place, move to the bottom of the tube during centrifugation, while algae cells after extraction (24 h) stayed between the organic layer and aqueous layer during centrifugation. This indicates that the density of algae cells changed during extraction. Based on this observation, it can be hypothesized that organic solvent went into the cells after extraction. After isolation of the EBA-layer and recovery of the lipid from the solvent, the lipid that are solubilized in the solvent that is still inside the cell walls is not measured in the yield calculation for the first extraction step, but the composition of these lipid resembles the composition of the lipid isolated after the first extraction step. A significant amount of these lipid is then liberated from the cells during the second extraction step, and results in the similarity between the lipid compositions in both extraction steps. The model was developed to account for this effect, in order to accurately predict the extraction yields in the various extraction steps.

Figure 5.3 Light microscope images of FW-stressed *Neochloris oleoabundans* at different extraction time.

Figure 5.4 (a) algae slurry and (b) algae slurry mixed with EBA for 24 h after centrifugation (9000 rpm, 5 min).

To estimate the total lipid content ($Y_{lipid,tot}$), as shown in Figure 5.5, an empirical exponential curve was fitted to the experimental results which was shown in Figure 5.1. Algae have a finite amount of lipid, so it is assumed that when the number of steps would be increased to 10 steps, all the lipid should be extracted. Based on this hypothesis, the $Y_{lipid,total}$ is calculated to be 64.0 wt.% of the algae dry weight.

Figure 5.5 Crude lipid yield of *Neochloris oleoabundans* extracted by EBA method in different stages.

Figure 5.6 A schematic diagram of the crude lipid extraction in equilibrium state.

The model should describe the equilibrium states of lipid extraction using EBA from wet microalgae slurry, and the parameters that may be used in calculation are illustrated in Figure 5.6. The following assumptions are made:

- 1) The cells are assumed to be spherical with a constant volume $V_{cell, in}$ before and after extraction.
- 2) The total volume of algae cells ($V_{cell, tot}$) was assumed to be the same as the volume of algae paste after centrifugation.

The algae solution was centrifuged for 10 min at 9000 rpm to get an algae paste. The algae paste was dried at 105°C for 24 h to determine the algae content, $C_{algae\ paste}$ was found to be 20.6 wt.% $\pm$ 0.5 wt.%.

- 3) The algae in the extraction mixture are assumed to have an initial crude lipid amount $m_{lipid, tot}$. The dark green part inside the cell is chloroplast which contains lipid and shrinks during extraction as shown in Figure 5.3. When the extraction reaches equilibrium, the volume of chloroplast is assumed to be negligible.
- 4) It is assumed that in each extraction stages, different amount of lipid will be released from cellular material. The ratio of lipid released from cellular material in stage i ($m_{lipid, rel, i}$, $i = 1, 2, 3, 4$ is the stage number) to the total lipid amount ($m_{lipid, tot}$) is defined as:

$$R_{rel, i} = \frac{m_{lipid, rel, i}}{m_{lipid, tot}} \quad \text{Eq. 5-1}$$

- 5) The cell wall is not selectively permeable for solvent and lipid. Therefore it is assumed that at equilibrium state, the solvent phase inside and outside the cells has the same composition and the crude lipid concentration in the solvent phase inside the cell ($C_{lipid, in}$) and outside ($C_{lipid, out}$) are also the same.
- 6) Organic solvent goes into the cells during extraction. This assumption was made

based on the density change of algae paste before and after extraction. However, it is uncertain if there is any aqueous phase inside the cells at equilibrium and we did not see a possibility to measure this under the current experimental conditions. So it is assumed that at equilibrium state, the ratio of organic phase volume ($V_{cell,org}$) inside the cell to the total volume inside the cell ($V_{cell,in}$) is:

$$R_{vol,i} = \frac{V_{cell,org,i}}{V_{cell,in}} \quad \text{Eq. 5-2}$$

where i is the stage number, $i = 1, 2, 3, 4$.

- 7) In each stage, only the solvent outside cells can be separated for lipid recovery. In subsequent stages, the remaining solvent containing lipid inside cells is washed out.

2.2 Modelling

The model is developed for 4 stages extraction of lipid from FW-stressed *Neochloris oleoabundans* slurry with ~5 wt.% algae content ($C_{algae\ slurry}$). At the start of an extraction, EBA is added to the algae slurry. Directly after mixing, two liquid phases are formed: one organic phase and one aqueous phase, each of them containing both EBA and water because of the partial mutual solubility. The weight of organic phase and aqueous can be calculated by using the equations below.

$$S_{water\ in\ EBA} = \frac{m_{water,org}}{m_{EBA,org} + m_{water,org}} \quad \text{Eq. 5-3}$$

$$m_{water,org} = \frac{S_{water\ in\ EBA} \times m_{EBA,org}}{1 - S_{water\ in\ EBA}} \quad \text{Eq. 5-4}$$

$S_{water\ in\ EBA}$ is the solubility of water in EBA. $m_{water,org}$ is the mass of water in organic phase. $m_{EBA,org}$ is the mass of EBA in organic phase.

$$S_{EBA\ in\ water} = \frac{m_{EBA,aq}}{m_{EBA,aq} + m_{water,aq}} \quad \text{Eq. 5-5}$$

$$m_{EBA,aq} = \frac{S_{EBA\ in\ water} \times m_{water,aq}}{1 - S_{EBA\ in\ water}} \quad \text{Eq. 5-6}$$

$S_{EBA \text{ in water}}$ is the solubility of EBA in water. $m_{EBA, aq}$ is the mass of EBA in aqueous phase. $m_{water, aq}$ is the mass of water in aqueous phase.

$$m_{EBA} = m_{EBA, org} + m_{EBA, aq} \quad \text{Eq. 5-7}$$

$$m_{water} = m_{water, aq} + m_{water, org} \quad \text{Eq. 5-8}$$

m_{EBA} is the mass of EBA in total. m_{water} is the mass of water in total.

$$m_{EBA, org} = \frac{m_{EBA} \times (1 - S_{EBA \text{ in water}}) \times (1 - S_{water \text{ in EBA}}) - m_{water} \times S_{EBA \text{ in water}} \times (1 - S_{water \text{ in EBA}})}{1 - S_{water \text{ in EBA}} - S_{EBA \text{ in water}}} \quad \text{Eq. 5-9}$$

$$m_{water, aq} = \frac{m_{water} \times (1 - S_{EBA \text{ in water}}) \times (1 - S_{water \text{ in EBA}}) - m_{EBA} \times S_{water \text{ in EBA}} \times (1 - S_{EBA \text{ in water}})}{1 - S_{water \text{ in EBA}} - S_{EBA \text{ in water}}} \quad \text{Eq. 5-10}$$

$$m_{org} = m_{EBA, org} + m_{water, org} \quad \text{Eq. 5-11}$$

$$m_{aq} = m_{water, aq} + m_{EBA, aq} \quad \text{Eq. 5-12}$$

m_{org} is the mass of organic phase. m_{aq} is the mass of aqueous phase. Here the mass of lipid is considered as negligible.

It was checked experimentally that the total volume didn't change significantly with mixing EBA and water together. So the density of organic phase (ρ_{org}) and aqueous phase (ρ_{aq}) can be approximated as below:

$$\rho_{org} = \frac{m_{org}}{\frac{m_{EBA, org}}{\rho_{EBA}} + \frac{m_{water, org}}{\rho_{water}}} \quad \text{Eq. 5-13}$$

$$\rho_{aq} = \frac{m_{aq}}{\frac{m_{water, aq}}{\rho_{water}} + \frac{m_{EBA, aq}}{\rho_{EBA}}} \quad \text{Eq. 5-14}$$

where the ρ_{EBA} and ρ_{water} are the densities of pure EBA and water respectively.

The total amount of lipid ($m_{lipid,tot}$) is calculated by

$$m_{lipid,tot} = m_{dry\ algae} \times Y_{lipid,tot} \quad \text{Eq. 5-15}$$

Besides the lipid, there are some parts of the algae that cannot be extracted by EBA ($m_{non-extractable}$).

$$m_{non-extractable} = m_{dry\ algae} - m_{lipid,tot} \quad \text{Eq. 5-16}$$

Total volume of the algae cells $V_{cell,total}$ is calculated by

$$V_{cell,tot} = \frac{m_{dry\ algae}}{C_{algae\ paste} \times \rho_{algae}} \quad \text{Eq. 5-17}$$

where $m_{dry\ algae}$ is the dry weight of algae added in the system, $C_{algae\ paste}$ is the algae content of algae paste, and ρ_{algae} is the average density of algae cells. Based on our observation that the cells of algae *Neochloris oleoabundans* flocculate very slowly without centrifugation, the density ρ_{algae} is assumed to be the same as the density of water.

The volume inside the cells ($V_{cell,in}$) is calculated by the equation below:

$$V_{cell,in} = V_{cell,tot} - \frac{m_{non-extractable}}{\rho_{algae}} \quad \text{Eq. 5-18}$$

Where, due to lack of additional information, the density of non-extractable is assumed to be the same as the average density of algae.

Since it is assumed that at equilibrium, the algae cells are filled with the same mixture as the organic phase, the volume of organic solution outside the cells ($V_{org,out,i}$) is calculated by

$$V_{org,out,i} = V_{org,i} - R_{vol,i} \times V_{cell,in} \quad \text{Eq. 5-19}$$

The amount of lipid released from cellular material in stage i is calculated by

$$m_{lipid,rel,i} = R_{rel,i} \times m_{lipid,tot} \quad \text{Eq. 5-20}$$

where $R_{rel,i}$ which is defined in Eq. 5-1 is the ratio of lipid released from cellular material in stage i to the total lipid amount. And $m_{lipid,tot}$ is the total lipid amount.

The lipid concentration is calculated by

$$C_{lipid} = \frac{m_{lipid,rel}}{V_{org}} \quad \text{Eq. 5-21}$$

The amount of extracted lipid $m_{lipid,extr}$ is calculated by

$$m_{lipid,extr} = C_{lipid} \times V_{org,out} \quad \text{Eq. 5-22}$$

Therefore the lipid yield is calculated by

$$Y_{lipid} = \frac{m_{lipid,extr}}{m_{dry\ algae}} \times 100\% \quad \text{Eq. 5-23}$$

The un-extracted lipid amount in extraction stage i is named $m_{lipid,unextr,i}$.

$$m_{lipid,unextr,i} = m_{lipid,unextr,i-1} - m_{lipid,extr,i} \quad \text{Eq. 5-24}$$

For the first stage extraction, the $m_{lipid,unextr,i-1}$ is the total lipid amount in algae $m_{lipid,tot}$. $m_{lipid,unextr,i}$ contains two parts: one part is the lipid that hasn't dissolved into the organic solvent ($m_{lipid,unextr,non-free,i}$), the other part is the lipid fraction that did dissolve in the organic solvent but is still present in the organic phase inside the cell wall ($m_{lipid,unextr,free,i}$).

$$m_{lipid,unextr,free,i} = R_{vol,i} \times V_{cell,in} \times C_{lipid,i} \quad \text{Eq. 5-25}$$

After the first stage extraction, the organic layer was separated, some makeup water ($m_{water,makeup}$) was added to the remaining aqueous phase and algae residue to make it the same weight as the starting feed ($m_{algae\ slurry}$).

$$m_{water,makeup,i} = m_{algae\ slurry} - m_{aq,i-1} - m_{non-extractable} - R_{vol,i-1} \times V_{cell,in} \times \rho_{org} \quad \text{Eq. 5-26}$$

Then, fresh EBA (m_{EBA}) was added and the second stage extraction started.

The lipid extracted in the second stage extraction was formed by part of the lipid which was already in the organic solvent inside the algal cells during previous extraction stage ($m_{lipid,unextr,free,i-1}$) and part of the lipid that was released from cellular material during the second stage extraction ($m_{lipid,rel,i}$).

In $m_{lipid,extra,i}$, some of the lipid was released during the first stage extraction $m_{lipid,unextr,free,from\ stage\ 1,i-1}$. In the first stage extraction, $m_{lipid,unextr,free,from\ stage\ 1}$ is the same as $m_{lipid,unextr,free}$. In the subsequent extraction stages, $m_{lipid,extr,from\ stage\ 1,i}$ can be calculated by

$$m_{lipid,extr,from\ stage\ 1,i} = m_{lipid,unextr,free,from\ stage\ 1,i-1} \times \frac{m_{lipid,extr,i}}{m_{lipid,unextr,free,i-1} + m_{lipid,rel,i}} \quad \text{Eq. 5-27}$$

The remaining lipid which was from stage 1 ($m_{lipid,unextr,free,from\ stage\ 1,i}$) can be

calculated by

$$m_{lipid,unextr,free,from\ stage\ 1,i} = m_{lipid,unextr,free,from\ stage\ 1,i-1} - m_{lipid,extr,from\ stage\ 1,i}$$

Eq. 5-28

The key parameter $R_{vol,i}$ (the ratio of organic phase volume inside the cell to the total volume inside the cell) and $R_{rel,i}$ (ratio of lipid released from cellular material in stage i to the total lipid amount) in this model were identified based on the minimization of difference between estimated and experimental lipid yield, representing the absolute error. Three groups of experiment results were used in the error calculation. The solvent to feed ratio was the same for four extraction stages which was 1:1 in experiment 1 and 1:2 in experiment 2. In experiment 3, the solvent to feed ratio was 2:1 for stage 1, 1:2 for stage 2, 1:1 for stage 3 and 4, respectively. The absolute error between estimated and experimental lipid yield at stage i were calculated by:

$$Error_i = \sum_{n=1}^3 |Y_{lipid,est,i} - Y_{lipid,exp,i}|_n$$

Eq. 5-29

where $Y_{lipid,est,i}$ and $Y_{lipid,exp,i}$ is the estimated and experimental lipid yield at stage i respectively. And n is the number of experiment group.

The effect of $R_{vol,i}$ and $R_{rel,i}$ to $Error$ at each extraction stage is illustrated in Figure 5.7.

Figure 5.7 Effect of $R_{vol,i}$ and $R_{rel,i}$ to *Error* (absolute error between estimated and experimental lipid yield) at (a) stage 1, (b) stage 2, (c) stage 3 and (d) stage 4. The point where *Error* reached minimum value is marked by ★.

The optimum value of $R_{vol,i}$ and $R_{rel,i}$ was taken when *Error* reached the minimum. From the calculation results shown in Figure 5.7 it can be observed that in all four stage extraction, the optimum value of $R_{vol,i}$ is 1, which means that the cells were filled with 100% organic phase at equilibrium state. The optimum values of $R_{rel,i}$ ($i=1, 2, 3, 4$) are listed below:

$$R_{rel,1}=87.5\%$$

$$R_{rel,2}=3.7\%$$

$$R_{rel,3}=2.3\%$$

$$R_{rel,4}=2.2\%$$

The proposed model was used to predict the crude lipid yield for a four stages extraction when the solvent to feed ratio was 1:1. The estimated model results for the four stages lipid extraction from FW-stressed *Neochloris oleoabundans* using EBA are listed in Table 5.1.

Table 5.1 Estimated results of 4 stages lipid extraction from FW-stressed *Neochloris oleoabundans* using EBA (S/F=1:1).

Source	Name	Unit	Stage 1	Stage 2	Stage 3	Stage 4
Input	S/F	-	1:1	1:1	1:1	1:1
Model – Eq. 5-23	Y_{lipid}	-	47.1%	9.5%	2.7%	1.6%
Model – Eq. 5-20	$m_{lipid,rel}$	g	0.6683	0.0283	0.0176	0.0168
Model – Eq. 5-21	C_{lipid}	g/mL	0.0191	0.0037	0.0011	0.0006
Model – Eq. 5-22	$m_{lipid,extr}$	g	0.5623	0.1137	0.0322	0.0192
Model – Eq. 5-24	$m_{lipid,unextr}$	g	0.2015	0.6501	0.7316	0.7446
Model – Eq. 5-25	$m_{lipid,unextr,free}$	g	0.1060	0.0206	0.0059	0.0035
Model – Eq. 5-27	$m_{lipid,extr,from\ stage\ 1}$	g	0.5623	0.0898	0.0137	0.0021
Model – result	$m_{lipid,extr,total}$	g	0.7275			
Model – result	$m_{lipid,rel,total}$	g	0.7310			
Model – result	$\frac{m_{lipid,extr,from\ stage\ 1}}{m_{lipid,extr}}$	-	100.0%	79.0%	42.6%	11.1%
Model – result	$\frac{\sum_{i=1}^n m_{lipid,extr,from\ stage\ 1}}{m_{lipid,rel,stage\ 1}}$	-	84.1%	97.6%	99.6%	99.9%

From calculation it can be found that in the second stage extraction, 79% of the extracted lipid was released already in the first stage (but remained in the solvent phase inside the cells). Because of the limited amount of recovered lipid in the third and fourth stages, only the lipid from first and second extractions were analyzed by GC-MS. The total fatty acid (TFA) compositions are presented in Figure 5.2. The fatty acid profile of *Neochloris oleoabundans* was dominated by palmitic (C16:0), hexadecadienoic (C16:2), oleic (C18:1), linoleic (C18:2), stearic (C18:0) and linolenic (C18:3) acids, and (as will now be clear because of the 79% mentioned above) no significant differences were found between the first and second extraction stage.

The TFA yield in crude lipid of first stage extraction (52.9 wt.%) was much higher than the second stage extraction (38.3 wt.%). The extracted crude lipid from second stage was formed by the lipid released from both first stage and second stage extraction. Taking the error into account, the TFA yield in second stage released lipid was calculated to be 0 to 1 wt.%, suggesting that (near) all TFA is released in the first stage (but not all is recovered!). This also confirms that the lipid released in first and second stage had different compositions which may result in different extractability. Most of the lipid that contained fatty acid were released in the first stage extraction. About 99.9 wt.% of the lipid from first stage release was extracted after four stages while more than 97.5 wt.% was extracted in two stages. It was therefore concluded that two extraction stages is sufficient for TFA recovery, when using the same 1:1 S/F ratio.

Besides the four stages extraction experiment which solvent to feed ratio was 1:1, another two experiments were carried out in which the solvent to feed ratio was 2:1 for one experiment and changed for each stage (2:1 for stage 1, 1:2 for stage 2, 1:1 for stage 3 and 4) for another. The model results are presented in Table 5.2 and Table 5.3.

Table 5.2 Estimated results of 4 stages lipid extraction from FW-stressed *Neochloris oleoabundans* using EBA (S/F=1:2).

Source	Name	Unit	Stage 1	Stage 2	Stage 3	Stage 4
Input	S/F	-	1:2	1:2	1:2	1:2
Model – Eq. 5-23	Y_{lipid}	-	38.1%	14.4%	5.2%	2.5%
Model – Eq. 5-20	$m_{lipid,rel}$	g	0.6381	0.0270	0.0168	0.0160
Model – Eq. 5-21	C_{lipid}	g/mL	0.0386	0.0127	0.0047	0.0023
Model – Eq. 5-22	$m_{lipid,extr}$	g	0.4339	0.1642	0.0591	0.0287
Model – Eq. 5-24	$m_{lipid,unextr}$	g	0.2953	0.5651	0.6702	0.7005
Model – Eq. 5-25	$m_{lipid,unextr,free}$	g	0.2042	0.0670	0.0247	0.0120
Model – Eq. 5-27	$m_{lipid,extr,from\ stage\ 1}$	g	0.4339	0.1450	0.0417	0.0123
Model – result	$m_{lipid,extr,total}$	g	0.6859			
Model – result	$m_{lipid,rel,total}$	g	0.6979			
Model – result	$\frac{m_{lipid,extr,from\ stage\ 1}}{m_{lipid,extr}}$	-	100.0%	88.3%	70.6%	42.8%
Model – result	$\frac{\sum_{i=1}^n m_{lipid,extr,from\ stage\ 1}}{m_{lipid,rel,stage\ 1}}$	-	68.0%	90.7%	97.3%	99.2%

Table 5.3 Estimation results of 4 stages lipid extraction from FW-stressed *Neochloris oleoabundans* using EBA while solvent to feed ratio was different for each stage.

Source	Name	Unit	Stage 1	Stage 2	Stage 3	Stage 4
Input	S/F	-	2:1	1:2	1:1	1:1
Model – Eq. 5-23	Y_{lipid}	-	51.8%	4.5%	3.0%	1.6%
Model – Eq. 5-20	$m_{lipid,rel}$	g	0.6381	0.0270	0.0168	0.0160
Model – Eq. 5-21	C_{lipid}	g/mL	0.0089	0.0044	0.0011	0.0006
Model – Eq. 5-22	$m_{lipid,extr}$	g	0.5910	0.0509	0.0342	0.0186
Model – Eq. 5-24	$m_{lipid,unextr}$	g	0.1383	0.6784	0.6951	0.7106
Model – Eq. 5-25	$m_{lipid,unextr,free}$	g	0.0471	0.0232	0.0058	0.0032
Model – Eq. 5-27	$m_{lipid,extr,from\ stage\ 1}$	g	0.5910	0.0323	0.0126	0.0018
Model – result	$m_{lipid,extr,total}$	g	0.6947			
Model – result	$m_{lipid,rel,total}$	g	0.6979			
Model – result	$\frac{m_{lipid,extr,from\ stage\ 1}}{m_{lipid,extr}}$	-	100.0%	63.6%	36.9%	9.8%
Model – result	$\frac{\sum_{i=1}^n m_{lipid,extr,from\ stage\ 1}}{m_{lipid,rel,stage\ 1}}$	-	92.6%	97.7%	99.7%	100.0%

2.3 Experimental validation of the extraction model

Figure 5.8 shows both the experimental and model estimated values of crude lipid yield of four extraction stages. For both the experiments at constant S/F (Figure 5.8 (a) and (b)) and those with changing S/F per stage (Figure 5.8 (c)), the model estimated values were shown to be in very good agreement with those obtained in experiments. The correlation between estimated and experimental values was excellent. This is clearly illustrated by the parity plot provided in Figure 5.8 (d). When the solvent to feed ratio was 2:1, 93% of the lipid released from cellular material in stage 1 was extracted, while only 68% for solvent to feed ratio 1:2. Since most of the TFA were released in the first stage, the more solvent used, the more TFA were recovered from first stage extraction.

Figure 5.8 Estimated and experimental values of crude lipid yield of *Neochloris oleoabundans* extracted by EBA method for multistage extractions, (a) S/F=1:1, (b) S/F=1:2, (c) S/F=2:1 for stage 1, 1:2 for stage 2, 1:1 for stage 3 and 4, (d) parity plot.

The extracted lipid was analyzed by GC-MS when the solvent to feed ratio was 1:2 for four stages and 2:1, 1:2, 1:1 and 1:1 for stage 1 to 4 respectively. The TFA analysis results in Figure 5.9 show that when the solvent to feed ratio was 1:2, the TFA yield in crude lipid of first and second stage extraction are 53.9 wt.% and 47.1 wt.% respectively. When the solvent to feed ratio was 2:1 for first stage and 1:2 for second stage, the TFA yield in crude lipid of first and second stage extraction are 53.3 wt.% and 33.6 wt.% respectively. Neither the solvent to feed ratio nor the extraction stages had influence on the TFA composition. TFA content in crude lipid in the second extraction stage was lower than the first. After calculation, it was found out that the lipid released from cellular material in second extraction stage contains little/no fatty acid, which was the same as the results earlier when the solvent to feed ratio was 1:1. If the lipid released in the second stage was assumed containing no fatty acid, the estimated value of TFA yield in crude lipid extracted in second stage was equal to the measured value. The proposed model was thus found to successfully describe the extraction equilibrium using EBA for lipid extraction from FW-stressed *Neochloris oleoabundans*. Unfortunately, direct experimental evidence of the mechanism, especially the presence of the organic phase inside the cells, was not possible. However, the good fit of model data with experimental findings, in combination with the sensitivity study, shows that our assumptions are not in conflict with the data and likely to be a fair representation of the actual extraction mechanism.

Figure 5.9 TFA composition, TFA fraction in crude lipid and TFA yield of lipid from *Neochloris oleoabundans* obtained by EBA 1st and 2nd extraction (a) S/F=1:2, (b) S/F=2:1 for stage 1 and S/F=1:2 for stage 2.

2.4 Sensitivity analysis

The model discussed and successfully applied in the above section contains several parameter values and assumptions. As different assumptions and different parameter values assumed may lead to different estimated values, a sensitivity analysis towards several key assumptions made is considered valuable. Therefore a simulation was performed for the crude lipid yields of the four extraction stages with changing the volume of algae cells.

The parameter $C_{algae\ paste}$ is the algae content of algae paste, which was used for calculating the volume of algae cells. Before extraction, the $C_{algae\ paste}$ was around 20 wt.% and this value was used for the modelling. Whether the volume of algae cells changed during extraction is difficult to identify experimentally and still not known. So the crude lipid yields of the four extraction stages at different $C_{algae\ paste}$ were simulated and showed in Figure 5.10. The crude lipid yield of first stage extraction increased when higher $C_{algae\ paste}$ applied. This is because higher $C_{algae\ paste}$ means lower water content in the algae paste, also means the volume of algae cells are smaller. Less organic solvents containing lipid loss into the cells with smaller algae cells volume. The increasing of crude lipid yield in first stage results in the decreasing in second stage because of the finite lipid amount of algae. The influence of $C_{algae\ paste}$ to the crude lipid yield was not very strong when the solvent to feed ratio was 2:1 for first stage extraction. This may because that when more solvent was used for extraction, the lipid concentration in the organic solvent was lower. The difference in the algae cells volume hardly influences the amount of lipid left inside the cells. Considering the experimental results of crude lipid yields in the four stage extraction, it can be concluded that even if the volume of algae cells changes during extraction, the change will be less than 10% of the original volume and this will not cause too much difference for the estimated values.

(a)

(b)

Figure 5.10 Crude lipid yields of four extraction stages at different $C_{algae\ paste}$ when (a) $S/F=1:2$, (b) $S/F=2:1, 1:2, 1:1$ and $1:1$ for stage 1 to 4 respectively.

3 Conclusions

This study advanced our understanding on the equilibrium extraction of lipid from FW-stressed *Neochloris oleoabundans*. With the hypothesis that after extraction, the algae cells were completely filled with the organic solvent phase, having the same composition as the organic phase outside the cells, the model was successfully fitted to the experimental crude lipid yields of the four stage extractions at various solvent to feed ratios. By modelling it was found that nearly all fatty acids were released from the cell material, but not all is recovered due to the organic phase remaining inside the cell. This mechanism also explains the incomplete lipid recovery in a single extraction stage. For common applied solvent to feed ratios, two extraction stages is sufficient for recovering most of the lipid containing fatty acid.

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Chapter 6

Using Lower Critical Solution Temperature (LCST) behavior of N- ethylbutylamine for lipid recovery and solvent regeneration in lipid extraction processes

Abstract

The objective of this study was to investigate an alternative solvent switching method for recovering the lipid by using the lower critical solution temperature (LCST) behavior of N-ethylbutylamine (EBA) instead of the switching with CO₂. In this work the LCST behavior of EBA in water with/without the presence of oil was investigated. It was proven that the temperature responsive partitioning of EBA over aqueous and apolar phases can be used for oil recovery and also for lipid recovery from microalgae and the window of operational conditions with respect to solvent and lipid concentrations was identified. The EBA fraction in water have to be lower than 0.5 wt./wt. in order to form a separate oil layer upon temperature decreasing. The influences of sunflower and of algal oil concentration (from *Neochloris Oleoabundans*) and the EBA fraction in water were investigated. The temperature at which LCST-like behavior is observed increases slightly with the oil content. For microalgae lipid recovery, the EBA fraction in water should be below 0.3 wt./wt. and the lipid concentration in organic phase should be more than 5 wt.% for an efficient oil recovery. Finally, a novel process concept for wet lipid extraction from microalgae with EBA and using the temperature responsive partitioning behavior of EBA for lipid recovery and solvent regeneration was proposed.

1 Introduction

Solvents are important in many industrial reaction processes as they affect reaction rates, phase equilibria and mass transfer efficiency [1, 2]. There is a great interest in an emerging class of solvents, so-called switchable solvents, that respond to external stimuli (e.g. pH, light, heat, magnetic field, etc.) with changes in properties [3-25]. A solvent system that can be switched reversibly from a single, homogeneous liquid into two separate liquid phases enables potentially a wide variety of applications on reaction, extraction and separation. Temperature driven phase transition systems can be classified in to three types, systems with an upper critical solution temperature (UCST), ones with a lower critical solution temperature (LCST) or both [26] (see Figure 6.1). The UCST or LCST is the characteristic temperature that above/below which the binary mixture mix in all proportions and below/above which they form two phases. Mixtures exhibiting UCST [27-29], LCST [30-35], and both UCST and LCST behavior [36, 37] have been reported. So far, the investigation of LCST phase behavior overwhelmingly focused on covalent polymers and much less attention has been paid to low molecular weight compounds which may have practical value for example in extraction process [38].

Figure 6.1 The temperature–composition diagrams which have (A) UCST, (B) LCST and (C) both UCST and LCST.

In a microalgae biorefinery process for extraction of e.g. lipid, it is critical to choose a suitable solvent that not only shows good extraction performance from biomass, but also allows for subsequent separation and recovery. N-ethylbutylamine (EBA) was found to be able to extract lipid from wet, even unbroken, microalgae slurries and have very promising performance [39-41]. Lipid separation and solvent recovery was achieved by CO₂ stripping and heating combined N₂ stripping respectively [39]. From the change in solubility in water with temperature [42], it was additionally found that EBA shows a LCST behavior. It will have a great advantage if temperature can be used as a stimulus in this process, since then no

extra substance will be involved and phase switching can happen over only a small temperature range. To obtain detailed insight into the LCST behavior of EBA, cloud points were determined visually and by pH/Karl Fischer (KF) measurements. The influence of oil present (representing the extracted microalgae lipid in an actual biorefinery process) and the oil recovery efficiency were also investigated. Finally, a microalgae lipid extraction process which utilizes the LCST behavior of EBA for lipid separation and solvent recovery is proposed.

2 Results and discussion

2.1 LCST behavior of EBA

EBA-water mixtures with different EBA mass fraction were prepared to investigate the LCST behavior of EBA in water. The results are illustrated in Figure 6.2. The phase diagram was constructed based on two independent measurement techniques: visually and by using pH/Karl Fischer (KF) measurement. Details about the experimental method can be found in Chapter 2. The results indicate that both methods can be used and yield the same results. Below the line in the phase diagram, there was only one single homogenous liquid phase. Increasing the temperature led to the formation of two phases. These results show the LCST behavior of EBA in water and also the possibility of using this for oil separation.

Figure 6.2 Characteristic LCST behavior of EBA-water binary mixtures.

2.2 LCST behavior of EBA in presence of oil

In order to evaluate if the LCST behavior of EBA, as observed in Figure 6.2, can be used for lipid separation, similar experiments as in the previous sub-section were carried out, but now with the addition of varying amounts of sunflower oil. The temperature induced phase transfer of EBA from the oil phase to the aqueous phase is illustrated in Figure 6.3. To a mixture containing 20 wt.% EBA in water, 5 wt.% oil was added, and the phase behavior at different temperatures is presented in Figure 6.3. A further insight in the composition of each phase at different temperatures was obtained by FT-IR analysis and shown in Figure 6.4. The spectra indicate that at room temperature (22°C), the upper phase of the mixture contained oil and EBA with some water (Figure 6.4 (A)), and the lower phase contained mainly water (Figure 6.4 (B)). After lowering the temperature to 0°C, the amount of upper phase decreased. A clearly visible high peak of the ester bonds of the lipid fraction at 1750 cm^{-1} indicates the presence of oil in the remaining upper phase (Figure 6.4 (C)). Since no signal from water and EBA was observed, it can be concluded that the upper phase was only oil. The spectrum of the lower phase showed the characteristic peaks of water (1650 and 3500 cm^{-1}) which indicated the presence of water, while the other appearing peaks showed the presence of EBA (Figure 6.4 (D)). No oil-specific peak was observed in the lower phase. However, it's not possible to conclude that there is absolutely no oil present, because of the small amount of oil in the sample and the high dilution may lead to a very weak peak of oil (1750 cm^{-1}) and therefore might be covered by the peak at 1650 cm^{-1} . It is expected, however, that the loss of oil to the heavier aqueous phase will be very small. The FT-IR analysis results clearly prove the possibility of using the LCST behavior of EBA in water for (lipid) oil recovery.

Figure 6.3 Temperature induced phase change of EBA-water mixture (20 wt.% EBA) to which 5 wt.% oil was added.

Figure 6.4 The temperature induced phase transfer of EBA-water mixture with oil monitored by FT-IR.

The graphs showing the temperature-dependent switching behavior of the EBA partitioning in EBA-water mixtures to which varying amounts of sunflower oil (2 wt.%, 5 wt.% and 10 wt.%) was added are shown in Figure 6.5. In this figure, the temperature is indicated at which a significant switch in EBA partitioning was observed as function of the initial EBA composition. Based on the LCST-behavior in binary EBA-water mixtures, it was expected that below the critical temperature curve in the diagram, an EBA-water mixture should be formed with an oil layer floating on top. From the observation of experimental results, it turned out that cooling the mixture down to 0°C indeed leads to the formation of a small layer on top mainly consisting of oil, and a large layer containing mainly water and EBA below. However, this temperature-responsive EBA partitioning was only observed for initial EBA concentrations below 50 wt.% (Figure 6.5 Region 1). For initial EBA concentrations above this boundary concentration, only one single phase could be observed (Figure 6.5 Region 2). Decreasing the temperature to below the critical solution temperature induces EBA and water to become completely miscible. The solubility of oil in this water-EBA phase is dependent on the composition. For low EBA concentrations, the phase resembles more the properties of

water, and the system remains in two-phases at equilibrium, whereas at higher EBA concentrations, the nature of the phase becomes more hydrophobic, which results in a higher solubility of the lipid oil, eventually causing the second phase to completely disappear resulting in a single homogenous phase in Region 2. Therefore, only when the EBA concentration is lower than the boundary concentration, this system can be used for lipid separation from the EBA/lipid mixture obtained after lipid extraction. With heating up, the lower phase in Region 1 and the whole sample in Region 2 became cloudy at a certain temperature (cloud point) (Figure 6.5 Region 3).

A different oil content may have an impact on the temperature at which the phase change occurs (cloud point). The LCST-like behavior for EBA-water mixtures with varying amount of added oil is plotted in Figure 6.6. It can be seen that the temperature of cloud points increases slightly with the oil content, especially when the EBA concentration is lower than 50 wt.%.

Figure 6.5 Characteristic LCST-like behavior for EBA-water mixtures to which 2 wt.%, 5 wt.% and 10 wt.% oil was added. The initial EBA-water composition is displayed, and the temperature at which a strong change in the partitioning was observed.

Figure 6.6 Comparison of LCST-like behavior for EBA-water mixture with different oil content.

2.3 Oil recovery by using LCST-like behavior of EBA

It was confirmed in previous sub-section that the partitioning of EBA over aqueous and apolar phases is strongly temperature dependent. It can be utilized for oil separation. Sunflower oil was added to EBA-water mixture, with an overall EBA weight fraction from 0.05 to 0.5. Oil recovery efficiency was measured and the results are shown in Figure 6.7. The higher the oil concentration was, the higher the recovery efficiency became. When oil concentration was 10 wt.%, the recovery efficiency was more than 95%. When the oil concentration was 2 wt.% and 5 wt.%, the recovery efficiency stayed constantly above 90% and decreased when EBA fraction in the initial water-EBA mixture was more than 0.3. The EBA fraction had a larger impact on the oil recovery efficiency of the samples with a lower initial overall oil concentration. Due to EBA migration to the aqueous phase at dropping temperature, the polarity decreased, and at higher EBA fractions, the reducing polarity resulted in a higher solubility of oil in the aqueous phase. The results from this experiment suggests that using the partitioning behavior of EBA can result in an efficient oil recovery, provided that the overall EBA weight fraction in the mixture is relatively low (below 30 wt.%).

Figure 6.7 Oil recovery efficiency using LCST-like behavior of EBA.

2.4 Microalgae lipid recovery

The phase behavior of EBA is shown to facilitate the separation and recovery of lipid oil, as evidenced by the experiments with sunflower oil, which has similarities with the neutral lipid fraction of microalgae lipid oil. The next step is to check for the recovery of complete, and more complex, microalgae lipid oil. After extracting lipid from microalgae with EBA, the organic phase was mixed with water to form a two phase mixture at 22°C (Figure 6.8a). The dark top layer is the EBA-lipid phase. The mixture was cooled to 4°C to dissolve EBA in the water phase, leaving a small lipid layer on top (Figure 6.8b). After collecting the top layer (lipid), the solution was then heated to 22°C; EBA separated from water and formed two phases again (Figure 6.8c). These results confirm that microalgae lipid recovery and solvent regeneration can be achieved by exploiting the LCST behavior of EBA, but also that some more polar compounds (coloring the phases) switch along with the EBA from organic to aqueous phase and vice versa.

Figure 6.8 Recovery of microalgae lipid and solvent regeneration upon changing the temperature between 22°C and 4°C. (a) organic phase after extraction which contains lipid was mixed with water at 22°C; (b) the organic phase-water mixture was cooled to 4°C; (c) after removing the top layer (lipid) of (b), the solution was heated again at 22°C to induce phase separation.

It was known from measurement that the lipid content of the organic phase (Figure 6.8a) was 1.6 ± 0.1 wt.%. Different amounts of water were added to form organic phase-water mixtures with organic phase fractions from 0.05 to 0.5. After lowering the temperature to 4°C, the top layer (lipid) (Figure 6.8b) was collected and measured gravimetrically. The recovery efficiency was calculated from the known amount of used lipid for all samples. Figure 6.9 shows the lipid recovery results for the experiments with 1.6 wt.% lipid in the solvent phase and at varying overall organic phase fraction. For every fraction of the organic phase between 0.05 to 0.5, the recovery efficiency was just around 20%. After cooling down, the phase separation was difficult to see due to the low amount of lipid. Apparently, this technique does not work conveniently for such low concentrations of lipid in the solution (1.6 wt.%).

Figure 6.9 Efficiency of lipid recovery from the organic phase after lipid extraction from microalgae.

Subsequently, temperature switching experiments with higher lipid concentrations were carried out. Algae lipid was mixed with EBA to form organic samples with 5 wt.%, 10 wt.% and 20 wt.% lipid content. Those samples were added into different amounts of water and the organic phase weight fractions were 0.1, 0.2 and 0.3 respectively. The recovered lipid amount was measured and calculated recovery efficiency is shown in Figure 6.9. There was no significant improvement of the lipid recovery efficiency for the organic phase containing 5 wt.% lipid, when compared to previous results. When lipid content in organic phase were 10 wt.% and 20 wt.%, the lipid recovery efficiency after temperature switching can reach up to 70%. The results show that a lower fraction of the organic phase in the overall mixture results in a higher recovery efficiency. This requires a larger amount of water to be added to the extraction phase, which may cause higher operating costs. Therefore, an appropriate lipid content in organic phase and organic phase fraction should be selected based on specific process. These results also provide an opportunity of incomplete lipid recovery when extra lipid is added as starting material to create a situation of high lipid content in the lipid recovery unit and remains in the process after recovering the amount of lipid that comes from microalgae. This situation can also be created initially by recycling the solvent without recovering the lipid and, in steady stage, by recovering only the amount of lipid extracted from the microalgae entering the extraction unit. For example, when the lipid content of the organic phase after extraction is 2 wt.%, it is impossible to recover all the lipid based on the existing results. But, if some lipid is added to increase the lipid content of the organic phase

to 10 wt.%, then the *required* recovery efficiency is only 20% to get all the lipid that comes from microalgae alone. The rest part of lipid will stay in the system and participate in the next lipid recovery cycle. This, and other process considerations will be discussed in the next section.

Figure 6.10 Efficiency of lipid recovery from the organic phase containing 5 wt.%, 10 wt.% and 20 wt.% lipid.

2.5 Proposed lipid extraction process

Based on the results from the study presented in the previous sub-sections, the proposed process of using the LCST behavior of EBA for lipid extraction, recovery and solvent regeneration is illustrated in Figure 6.11.

Figure 6.11 Process concept for wet lipid extraction from microalgae with EBA, followed by temperature induced lipid recovery and solvent regeneration.

First, the lipid is extracted from the microalgae with EBA at room temperature (22°C). In the next step, phase separation, the lipid/EBA organic layer is separated from the algae solution (containing now lipid-extracted microalgae) and is sent to the Lipid Recovery Unit. Then by lowering the temperature to 4°C, EBA transfers to the water phase. The lipid can now easily be recovered as separate liquid phase. EBA can be recovered from the chilled water phase by increasing the temperature to room temperature again. In the Solvent Recovery Unit the EBA extraction solvent is separated from the water and sent to the extraction step for the next cycle, whereas the water is recycled to the Lipid Recovery Unit. Key step in this process is lowering the temperature for lipid recovery by phase separation. To enhance the phase separation and lipid recovery efficiency, some makeup lipid and water are added to maintain a certain lipid concentration and organic phase fraction. Since it is important to keep certain lipid concentration for the recovery, only the amount of lipid that comes from the algae solution will be recovered and the rest will be reused for next cycle, which implies that a complete lipid recovery is not necessary.

It was found in Chapter 5 that it is likely that after lipid extraction the algae paste residue is (at least partly) filled by organic phase. As this would otherwise lead to a significant EBA loss, representing high costs and an undesirable emission to the environment, an additional EBA recovery step from the algae residue is necessary. This will be studied in the following chapter.

3 Conclusions

In this chapter, the LCST behavior of EBA-water mixture was studied, and temperature-dependent phase behavior with oil has been investigated. It was proven that, depending on mixture composition, the temperature-dependent partitioning behavior of EBA can be used for separating the lipid oil as separate liquid phase from EBA-water mixtures by lowering temperature. The maximum EBA concentration which can be used for oil separation is 50 wt.%. The oil recovery efficiency can reach more than 90% when EBA fraction in water is below 0.3 wt./wt. The LCST behavior of EBA can also be used for microalgae lipid recovery. A process for wet lipid extraction from microalgae with EBA, and then using the temperature responsive partitioning behavior of EBA for lipid recovery and solvent regeneration has been proposed. This system is expected to be a cost and energy efficient alternative process configuration to the CO₂ switching system which has been studied in Chapter 3.

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Chapter 7

Recovery of N-ethylbutylamine from raffinate and microalgae cells after wet lipid extraction

Abstract

After the extraction, residual solvent retains dissolved in the liquid raffinate and in residual microalgae cells. The treatment of raffinate and microalgae cells after wet lipid extraction is important for the viability of this microalgae biorefinery process because solvent loss will cause pollution to the environment and increase the process cost. In this study, several solvents/solvent combinations were evaluated for N-ethylbutylamine (EBA) recovery from both the raffinate and from the residual microalgae cells after extraction and subsequently the recovery process is modelled and evaluated for its energy use.

After screening different solvents, water was found to be the best option for recovering the EBA from microalgae cells residue after lipid extraction. The EBA in residual microalgae cells can be removed completely by using only water in a two-step extraction with a solvent/feed ratio 20:1. Afterwards, the extract was joined with the liquid raffinate from the lipid extraction and the EBA was extracted from the combined aqueous streams using dodecane. The required extraction column and distillation column were simulated and optimized using Aspen Plus V8.8, resulting in a distillate that contains 99.9 wt.% EBA (algae extraction solvent) and a bottom product that contains 99.9 wt.% dodecane (solvent for EBA recovery) at a reboiler heat duty of 2.50 MJ/kg dry algae.

1 Introduction

Energy is indispensable in today's world as all activities are driven by energy, most of it coming from fossil fuel. Since the amount of fossil fuel is limited and fossil fuel derived CO₂ emissions should be avoided, renewable energy sources are needed to meet the future needs. Heat and power can be produced by several renewable energy sources such as wind, solar, geothermal and tidal energy, whereas for transportation fuels biomass seems a good option [1, 2]. Microalgae have great potential in generating bioenergy due to the rapid growth rate, high productivity, limited competition with arable land and freshwater, and a high CO₂ consumption rate [3]. Extraction of lipid from microalgae aroused the interest of many researchers around the world. Many different extraction technics have been investigated, such as organic solvent extraction [4-14] and supercritical fluid extraction [10, 15-23]. A method named CO₂-switchable solvent extraction has also been explored [3, 24-28]. N-ethylbutylamine (EBA) has shown outstanding performance as switchable solvent for lipid extraction from microalgae [29, 30].

Research on microalgae biorefinery has been mainly targeting oil content enhancement, cell disruption method investigation, extraction efficiency improvement, etc. There has not been so much attention on the treatment of biorefinery residuals, such as raffinate streams and microalgae cells after lipid extraction. A simplified process diagram for lipid extraction from microalgae with EBA is shown in Figure 7.1. In this example of an extraction process, there is some loss of EBA to the water phase due to its solubility in water when the extraction is performed. The solubility of EBA in water is 5.06 wt.% at 20°C which is significant and cannot be ignored [31]. It was found in Chapter 5 that it is highly possible that after lipid extraction the cells in the algae paste residue are filled by the organic solvent which leads to an additional amount of EBA loss. All these solvent losses will result in a high consumption of EBA and thereby increase the process cost. Besides that, the untreated raffinate and residue will cause emissions to the environment.

Figure 7.1 Simplified process diagram for wet lipid extraction from microalgae with EBA.

Extraction has advantages in large capacity separation of small contents of high-boiling component from aqueous solutions. For the selection of a suitable solvent for extracting the solvent from the aqueous phase, not only the extraction selectivity has to be considered, but also the solubility in the raffinate, ease of handling and regeneration, etc. In this chapter, several solvents/solvent combinations will be evaluated for EBA recovery from the raffinate and microalgae cells residue after lipid extraction. By using a flow sheeting program (Aspen Plus V8.8), a liquid-liquid extraction followed by a distillation process for recovery of EBA from the EBA-water binary system and then regenerated from extractant was simulated and optimized. With this, the energy required for EBA solvent recovery after wet lipid extraction from microalgae can be determined.

2 Results and discussion

2.1 EBA recovery from raffinate after lipid extraction

EBA is a hydrophobic solvent ($\log P = 1.79$) [32] with a boiling point of 108°C , for which recovery from the aqueous raffinate is proposed via extraction by another, more a-polar solvent. Several solvents listed in Table 7.1 were investigated and the results are shown in Figure 7.2. Among all those solvents, dichloromethane (DCM) showed the best performance. However, it was rejected because of the (eco)toxicity, low boiling point and high solubility in water, making it less attractive for large scale operation. Cyclohexane, hexane and heptane showed similar extraction performance which was represented by the EBA recovery

efficiency (defined in Eq. 7-1). However, considering the health hazards of hexane to human body, cyclohexane and heptane are good options as extractant for EBA recovery from the raffinate after lipid extraction. Compared with hexane and heptane, cyclohexane was selected for the lower specific heat capacity and the lower boiling point (in view of required heating utility).

$$\eta_{EBA\ recovery} = \frac{m_{EBA_recovered}}{m_{EBA_total}} \times 100\% \quad \text{Eq. 7-1}$$

$\eta_{EBA\ recovery}$ is the EBA recovery efficiency, $m_{EBA_recovered}$ and m_{EBA_total} are the mass of recovered EBA by extraction (g) and the total EBA amount (g) in the solution before extraction.

Table 7.1 Solvent candidate properties for EBA recovery from water.

Solvent	Cyclohexane	Hexane	Heptane	Dodecane	Hexadecane	DCM
Boiling temperature (°C)	80.55	67.85	97.85	216.20	286.90	39.85
Solubility in water at 25°C (mg/L)	55.00	9.50	2.93	3.70×10^{-3}	2.10×10^{-5}	13200
ΔH_{vap} (kJ/kg)	393.30	359.71	359.25	361.16	359.24	330.00
Specific heat capacity (kJ/(kg·°C))	1.85	3.08	2.24	2.21	2.26	1.21

Figure 7.2 Single stage extraction efficiency for EBA recovery from water using different solvents.

The results in Figure 7.2 indicate that the more solvent used, the more EBA can be recovered. For cyclohexane, the EBA recovery efficiencies were 90.4 %, 85.7%, 69.1% and 63.2% when the solvent/feed ratio were 1:1, 1:2, 1:5 and 1:10 respectively. Dodecane and hexadecane had a bit lower extraction efficiency. However, because dodecane and hexadecane have higher boiling points than EBA (108°C), instead of distilling all the solvent, EBA can be distilled for recovery. The amount of EBA was much less compared with the extraction solvent. Therefore, the energy used for solvent recovery can be significantly reduced by using a higher boiling extraction solvent. For this reason, dodecane and hexadecane were also selected for further evaluation.

Although the extraction efficiency can be improved by increasing solvent/feed ratio, the concentration of EBA in extractant then lowers. Higher solvent/feed ratio means a larger amount of solvent, which will result in a higher heat duty for solvent regeneration. Therefore, it is important to reduce the extractant flow while ensuring the extraction efficiency. When the extraction takes place in a countercurrent arrangement, the extraction factor E (defined in Eq. 7-2) have to be >1 in order to get a higher extraction efficiency with larger extraction stage by looking at the Eq. 7-3 [33].

$$E = K \cdot S/F \quad \text{Eq. 7-2}$$

K is the distribution coefficient defined in terms of mass ratio and S/F is the solvent to feed ratio used in extraction.

$$\frac{x_R}{x_F} = \frac{E-1}{E^{N+1}-1} \quad \text{Eq. 7-3}$$

x_R and x_F are the mass fraction of EBA in the raffinate and feed, N is the number of stages.

The fraction of solute that is not extracted is given by Eq. 7-3, where it is clear that the larger the extraction factor, the greater the extent to which the solute is extracted. Large values of E result from large values of the distribution coefficient K , or large solvent to feed ratio [33]. For cyclohexane, dodecane and hexadecane as extractant, the values of K were obtained from Aspen Plus based on experimental data. The minimum solvent to feed ratio S/F_{min} for each solvent shown in Table 7.2 was estimated based on $E > 1$. Detailed study of solvent/feed ratio selection will be carried out in Section 2.3.

Table 7.2 The minimum solvent to feed ratio when cyclohexane, dodecane and hexadecane was used as extractant.

Solvent	Cyclohexane	Dodecane	Hexadecane
K	6.2	1.8	1.4
S/F_{min}	1:6	1:1.8	1:1.4

Experimentally, the effect of agitation time to the EBA recovery efficiency of cyclohexane was also checked and the results are shown in Figure 7.3. It turns out that for cyclohexane, there is no influence of agitation time to the EBA recovery efficiency within the range of study. Hence, the recoveries measured and reported here are determined by phase equilibria.

Figure 7.3 Effect of agitation time to the EBA recovery efficiency of cyclohexane.

2.2 EBA recovery from microalgae paste after lipid extraction

During extraction, the solvent breaks the cell membrane and enters the cells to extract lipid [34]. As can be seen on the microscope image presented in Figure 5.4, the algae cell walls stayed intact after lipid extraction. Therefore, after lipid extraction, it is highly possible that there is a significant amount of EBA lost to residual algal cells, both inside and outside the cells. Also the liquid around algae cells of the residue contains EBA as well. The loss of solvent to algae residues was determined by a “distillation” experiment of the algae cells residue after lipid extraction. The total liquid content of the algae paste (obtained by filtration after extraction) was 92.0 ± 0.0 wt.%. After determining the liquid composition by GC-MS, it was found that EBA counts for 15.2 ± 1.0 wt.% of the total mass of the wet residual paste while the remaining liquid was water. Distilling EBA from the wet algae paste, although unconventional, is thus a feasible method. However, it was hypothesized to be more efficient to follow a two-step approach, where in the first step EBA is “rinsed out of algae cells” with a liquid and in the second step EBA is recovered from the rinsing liquid.

Four different solvent/combinations: cyclohexane, cyclohexane + water, water and ice water were tested for EBA recovery from algae cells residue by rinsing. Cyclohexane was selected based on the good performance in EBA recovery from raffinate after lipid extraction. The combination of cyclohexane and water was used since EBA interacts more with organic solvent. And the algae cell wall is hydrophilic, which is water-attracting. Therefore, water may help the organic solvent to get out of algae cells. The ratio of cyclohexane/water was 1:1

(w/w). At room temperature (20°C), the solubility of EBA in water is ~5 wt.%, therefore, the option to use water as wash solvent was also investigated for recovery. The amount of used water was twenty times of the algae residue to avoid significant equilibrium limitations. In addition, the use of ice water was tested since EBA is completely miscible with ice water due to the lower critical solution temperature (LCST) behavior as studied in Chapter 6. The solvent/feed ratio was 10:1 when cyclohexane was used and the ratio change to 20:1 when cyclohexane + water (1:1 w/w), water and ice water were used. Results for EBA recovery efficiency of the four tested solvents are shown in Figure 7.4.

Figure 7.4 Efficiency of EBA recovery from algae paste using different solvents. Different colors in the last bar presents the distribution of recovered EBA over water and cyclohexane.

Figure 7.4 shows that all the used solvents have relatively good recovery performance for a single stage extraction. The EBA recovery efficiency is similar (more than 85 wt.%) for using ice water, water and the combination of cyclohexane and water. Using the combination of cyclohexane and water recovered more EBA than only using cyclohexane as extractant. Water also shows a higher recovery efficiency than the pure organic solvent cyclohexane. This may be because the solvent to feed ratio for cyclohexane is lower than the other solvents. Due to the large solvent to feed ratio, the concentration of EBA in solvent was below the solubility limit. This extraction process is similar as a washing out step. The EBA and solvent are mixed, and part of this mixture is inside the cells and most of the mixture is outside the cells. The outside part of EBA thus can be recovered. Therefore, below the solubility limit,

EBA recovery efficiency relates to the amount of solvent used for extraction. The extraction model which was developed in Chapter 5 can be applied here. This model can also explain the reason that using water has comparable result with using ice water and the combination of cyclohexane and water. Since lowering the water temperature didn't increase the EBA recovery efficiency, the use of ice water is not necessary. Using cyclohexane - water will lead to an extra separation step of these two solvents. Hence, based on the above results, water was selected as the solvent for EBA recovery from microalgae paste after lipid extraction. An additional advantage is that this water phase can be treated similarly (or even simultaneously) with the aqueous phase raffinate for reclaiming the EBA.

After water was selected as the extractant, another set of experiments was carried out to study the influence of solvent/feed ratio and extraction steps. Four different solvent/feed ratios were used, which were 20:1, 15:1, 10:1 and 5:1. After mixing and liquid solid separation, fresh water was added into the algae paste residue for the next extraction step. Three extraction steps were performed. The results are shown in Figure 7.5.

Figure 7.5 Effect of solvent to feed ratio and extraction steps on the EBA recovery efficiency from algae paste.

In one extraction step, the more solvent used, the more EBA was recovered. This trend didn't change when more extraction steps were performed. The lower solvent/feed ratio is, the more extraction steps are needed. When the solvent/feed ratio was 20:1, more than 85% EBA was recovered in one extraction step. More extraction steps had no obvious help in improving the

recovery efficiency. In order to get similar recovery efficiency, more than three extraction steps were required when solvent/feed ratio was 5:1. Therefore, it is wise to use solvent/feed ratio 20:1 for EBA recovery from algae paste residue.

A distillation was carried out to remove any residual EBA from the algae residue, monitored by GC-MS after three extraction steps by water were carried out to check for the presence and the composition of liquid in the algae residue. No EBA was found in the distillate fraction. This result indicates that the EBA was most likely completely removed from algae residue. Therefore, the unrecovered EBA must be lost during handling. The EBA evaporation rate (during handling) was tested in the fume hood and found to be $1155 \pm 82 \text{ g/m}^2\cdot\text{h}$ at 20°C . Several steps e.g. weighting, mixing, centrifuging and vacuum filtration were involved in the experiment of EBA recovery from algae paste residue. Vacuum filtration supposed to be the step that lost most part of the EBA since it was operated openly in the fume hood and took the longest time. A funnel with a diameter of 0.08 m was used for vacuum filtration during experiment which means $5.8 \pm 0.4 \text{ g}$ EBA evaporated in that funnel in one hour. In our case, about 0.3 g EBA was lost in the extraction process. It is very likely that this EBA loss was due to the evaporation in the operation procedure. This loss should be possible to be prevented by operating the process in a closed system.

2.3 Conceptual process design

Both the aqueous raffinate phase after lipid extraction and the aqueous extract phase that is obtained after washing out EBA from the residual algae cells contain EBA. This EBA can be recovered by liquid-liquid extraction using a hydrophobic solvent such as cyclohexane, dodecane or hexadecane as explored in Section 2.1. The aim of this section is to make a conceptual process design and use it to estimate heat duties for processes with different solvents. The comparison is done by simulation and optimization of the liquid-liquid extraction process followed by a distillation process for recovery of EBA. All the work was developed using Aspen Plus V8.8.

The nonrandom, two-liquid (NRTL) model was used to describe the phase equilibrium of the EBA-water mixture which is a non-ideal chemical system. Aspen Plus was used for the regression of liquid-liquid equilibrium data which were presented in Figure 6.1 to determine binary interaction coefficients for the NRTL model. The regressed parameters are listed in Table 7.3. The simulated phase diagram of the EBA-water binary system is presented in Figure 7.6 and compared with experimental results, from which it can be concluded that the phase equilibria of EBA-water binary system can be modelled properly using the NRTL model with the binary coefficients listed in Table 7.3. These binary coefficients of EBA-water binary system will therefore be used for further simulation.

Table 7.3 NRTL binary parameters of EBA-water system.

Component i	H ₂ O
Component j	EBA
A _{ij}	4.56
A _{ji}	12.56
B _{ij}	111.14
B _{ji}	-3960.38
α	0.40

Figure 7.6 Phase diagram of EBA-water binary system constructed from the NRTL model with parameters as listed in Table 7.3 and experimental results.

For the liquid-liquid extraction process, an extraction column will be applied. Before considering multistage countercurrent operation, single stage extraction simulation results with different solvent/feed ratio were compared with experimental single stage results when cyclohexane, dodecane and hexadecane were used as extractant (Figure 7.7). The NRTL binary parameters presented in Table 7.3 and Table 7.4 were used for simulation. The NRTL binary parameters of EBA-sunflower oil were regressed by Aspen Plus with the ternary liquid-liquid equilibrium data of the system water-EBA-sunflower oil (experimentally determined in Chapter 6). It was assumed that for describing the binary interaction between EBA-cyclohexane, EBA-dodecane and EBA-hexadecane, the binary interaction parameters of EBA-sunflower oil could be applied. The binary interaction parameters between water-

cyclohexane, water-dodecane and water-hexadecane were taken from the Aspen Plus databank “APV88 LLE-ASPEN” in version 8.8.

The simulation results show good agreement with experimental results when the solvent/feed ratio is 1:1. The difference between simulation results and experimental results increases as the solvent/feed ratio decreases. This recovery efficiency difference may be due to the interaction parameters difference between water-EBA-sunflower oil system and water-EBA-cyclohexane/dodecane/hexadecane system. The results indicate that the designed column is highly likely to meet the separation requirements, since the simulated EBA recovery efficiency was at the conservative side; i.e. lower than the experimental result when solvent/feed ratio was lower than 1.

Figure 7.7 Comparison of single stage extraction experimental results and simulation results of EBA recovery efficiency using cyclohexane, dodecane and hexadecane.

Table 7.4 NRTL binary parameters used in extraction simulation.

Component i Component j	H ₂ O Cyclohexane ^a	H ₂ O Dodecane ^a	H ₂ O Hexadecane ^a	EBA Sunflower oil
A _{ij}	13.14	23.43	28.22	0
A _{ji}	-10.46	-6.09	-5.45	0
B _{ij}	-1066.98	-2638.14	-3920.97	51.39
B _{ji}	4954.90	3794.11	3588.23	169.67
α	0.20	0.20	0.20	0.3

^a NRTL binary interaction parameters for water-cyclohexane, water-dodecane and water-hexadecane were taken from the Aspen Plus databank “APV88 LLE-ASPEN” in version 8.8.

Figure 7.8 Process diagram for EBA recovery from raffinate and microalgae after wet lipid extraction.

After lipid extraction, there are two streams that have to be treated for EBA recovery. One is the extraction raffinate (#5) and the other one is lipid depleted algae (#1) (see Figure 7.8). First EBA in lipid depleted algae is washed out with water and the aqueous extract (#4) will then be combined with the extraction raffinate (#5) and extracted by an extractant. In this section, only the process in the dashed box in Figure 7.8 is studied.

There are strict regulations for the maximum chemical oxygen demand (COD) of wastewaters before they can be discharged to the environment. COD is the amount of oxygen required to oxidize an organic compound to carbon dioxide, ammonia, and water. The

following expression was used to calculate the COD concentration.

Based on the Waste Water Ordinance [35], the COD concentration for chemical industry waste water at the site of occurrence depends on the COD concentration at the site occurrence of waste water. Therefore, the COD concentration of #4 and #5 should be known. The mass balance of the solvents used in extracting EBA from raffinate and microalgae cells after wet lipid extraction is calculated and shown in Table 7.5. All the calculations were based on processing 1 kg of dry algae biomass.

Table 7.5 Mass flows of the solvents used in extracting EBA from raffinate and microalgae cells after wet lipid extraction normalized to the dry algae mass.

Stream	#1	#2	#3	#4	#5
Lipid depleted algae (kg/kg dry algae)	0.53	-	0.53	-	-
Water (kg/kg dry algae)	5.09	132.50	2.04	135.55	84.24
EBA (kg/kg dry algae)	1.01	-	-	1.01	4.49
COD (mg/L)	-	-	-	2.1×10^4	1.4×10^5

Based on previous work, 20 kg of EBA is needed for lipid extraction from 20 kg algae slurry (5 wt.%) which contains 1 kg of dry biomass [30]. The lipid extraction yield is 47 wt.% of the microalgae dry weight after one step extraction [30]. Therefore, 0.53 kg lipid depleted dry biomass is in #1 and leaves the system via #3. For calculation, it was assumed there is no loss of EBA in the process. It was found in Section 2.2 that the EBA counts for 15.2 ± 1.0 wt.% of the total mass of the wet residual paste while the rest liquid was water. To extract EBA from the algae residual paste, water (#2) was added in a solvent to feed ratio 20:1. The water content in #3 was measured to be 79.4 wt.%. The rest part of water together with EBA goes to #4.

When temperature is used as the trigger for EBA solvent switching after lipid extraction, the EBA fraction in water for switching should be below 0.3 based on the study in Chapter 6. Therefore, 62.1 kg water is added to the liquid after lipid extraction to help switching forward. After back switching, the aqueous phase (#5) contains 84.2 kg of water and 4.5 kg of EBA.

After calculation, it is found that the COD of #4 and #5 is 2.1×10^4 mg/L and 1.4×10^5 mg/L respectively. For waste water flows whose COD concentration at the site of occurrence of waste water is a) more than 50000 mg/L, a COD concentration of 25000 mg/L shall apply; b) more than 750 mg/L, a COD concentration equivalent to a 90% reduction in COD shall apply [35]. The higher standard was selected for safety reasons. This means that the COD concentration has to be lower than 2100 mg/L to meet the requirement for waste water disposal at the point of discharge. And that COD concentration corresponds to a EBA concentration of 740 mg/L.

Figure 7.9 Process concept for EBA extraction from water by organic extractant.

A countercurrent extraction process (Figure 7.9) was employed by adding #4 and #5 from the top and extractant from the bottom. Different solvent/feed ratios were evaluated in a process simulator, using cyclohexane, dodecane and hexadecane as extractant, respectively, for a varying number (2-25) of extraction stages. Similar results were found when using #4, #5 or a mixture of #4 and #5 as inlet stream. Figure 7.10 presents the simulated results of EBA content in raffinate (water) using #5 alone, since #5 has a higher EBA content and the extraction condition is more stringent. The horizontal line at 740 mg/L is the requirement for waste water at the point of discharge. The lower the solvent to feed ratio is, the more extraction stages are required. The cost of building an extraction column and solvent recovery belong to the CAPEX and OPEX of the process expense respectively. The solvent to feed ratio and number of stages in a extraction column should be considered comprehensively in the design. Figure 7.10 (A) shows that the EBA content can meet the point of discharge constraint with all six evaluated solvent to feed ratios in the calculated 25 extraction stages. Since less solvent in an extraction process will reduce the recovery heat duty and equipment size, a lower solvent to feed ratio is preferred, on the condition that the number of stages remains reasonable. With cyclohexane as extractant, a 19 stages column with solvent to feed

ratio 1:6 will be a good option for EBA recovery. From Figure 7.10 (B) and (C), a 24 stages column with solvent to feed ratio 1:1.8 for dodecane and a 18 stages column with solvent to feed ratio 1:1.3 for hexadecane were selected.

Figure 7.10 Simulation results of EBA content in raffinate at different solvent to feed ratios and extraction stages when using (A) cyclohexane, (B) dodecane and (C) hexadecane as extractant.

Considering the results exhibited in Figure 7.10, the energy cost for EBA recovery from 1 kg of contaminated water using cyclohexane, dodecane and hexadecane was calculated and shown in Table 7.6. The results indicate that without heat exchanger, cyclohexane with a solvent to feed ratio 1:6 in a 19 stages extraction column has the best performance. However, when the feed stream is heated via heat exchanger with a temperature difference of 10°C before entering the distillation column, the best option is using dodecane as extractant in a 24 stages extraction column for EBA recovery from contaminated water due to the lowest energy cost (0.0396 MJ/kg contaminated water).

Table 7.6 Energy cost (MJ/kg contaminated water) for EBA recovery using cyclohexane, dodecane and hexadecane in extraction columns.

Solvent	Cyclohexane	Dodecane	Hexadecane
Solvent to feed ratio	1:6	1:1.8	1:1.3
Number of stages	19	24	18
Energy use without heat exchanger [MJ/kg contaminated water]	0.0957	0.1353	0.1804
Energy use with heat exchanger [MJ/kg contaminated water]	0.0845	0.0396	0.0447

The extraction column is designed under the condition that pure organic solvent is used as extractant. However, when a distillation is applied for regenerating dodecane and EBA from the extract #9 (in Figure 7.8), the distillate and bottoms product are not pure single solvent anymore. This difference may influence the design of extraction column. In this study, the distillation target was set as a distillate that contains 99.9 wt.% EBA and a bottoms product that contains 99.9 wt.% dodecane. When this regenerated 99.9 wt.% dodecane is used as extractant, the extraction column has to be at least 39 stages in order to have the raffinate that can meet the point of discharge.

The RadFrac block in Aspen Plus was selected for the distillation calculation. The distillation column operates at 1 bar and has #9 as the feed. A thermodynamic description for the distillation system was developed using the NRTL model with the binary interaction parameters for EBA and water shown in Table 7.3. A sensitivity analysis was carried out to investigate the required number of equilibrium stages (NTS), number of stages above feed (NFS) and reflux ratio (RR). While varying the NTS (from 2 to 20) and NFS (from 2 to 19), RR was optimized in order to meet the distillation target. The results are shown in Figure 7.11. It is shown that increasing the NTS reduces the value of RR. There is only a slight change of RR when NTS is above 12, more NTS will only cause to increase the capital costs. It was found that the minimum reflux ratio is around 0.18. Since for most commercial operations the optimal operating reflux ratios are between 1.1 and 1.3 times the minimum reflux ratio [33, 36], 1.2 times minimum reflux was selected as a first approximation. Therefore, a column for which RR is 0.22 (rounded to 0.2 for the convenience of operation), NTS is 11 is the optimum for this system. The feed stream (20°C) should be added above the 7th stage. After applying a heat exchanger with a temperature difference of 10°C, the heat duties of reboiler (Q_{reb}) and condenser (Q_{cond}) are 2.50 MJ/kg and -0.55 MJ/kg dry algae, respectively.

Figure 7.11 Calculated results of NTS and RR for which the distillation purity target is reached.

3 Conclusions

In this chapter, dodecane was selected to recover EBA from the raffinate after lipid extraction because of its lower energy usage. Besides the EBA loss in the raffinate, there was also EBA loss in the algal cells residue, both inside and outside the cells. More than 85% of this EBA loss can be recovered by using only water in one step extraction with a solvent/feed ratio 20:1. EBA was most likely completely removed from algal cells residue. The EBA that was not recovered was because of the evaporation during operation procedure in the lab, which should be prevented in a large scale process. The EBA from the wash water and the raffinate streams were combined and a liquid-liquid extraction process was applied to extract the EBA from this aqueous stream. After extracting the EBA, the EBA was recovered from the solvent by distillation, the extraction and distillation process were simulated and optimized using Aspen Plus V8.8. Dodecane was selected as the solvent due to the low energy usage. It was found that an extraction column should have 24 stages with solvent to feed ratio 1:1.8 (w/w). The optimum design of the distillation column is NTS = 11, RR = 0.2 with the feed added above the 7th stage. The heat duties of reboiler (Q_{reb}) and condenser (Q_{cond}) are 2.50 MJ/kg and -0.55 MJ/kg dry algae, respectively.

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Chapter 8

Lipid extraction from wet microalgae using EBA as switchable solvent: process evaluation and outlook

Abstract

Microalgae are considered a promising feedstock for the production of food ingredients, cosmetics, pharmaceutical products and biofuels. Next to the costs for cultivation, this route is especially hindered by the energy intensity of drying algae prior to extraction and solvent recovery afterwards. In this work two process options for wet lipid extraction from microalgae with N-ethylbutylamine (EBA) are evaluated. The difference between these two processes is that for switching and solvent regeneration, one uses CO₂ to transform the solvent to hydrophilic state and the other one uses temperature as the stimulus. Process designs have been made for both options to calculate and compare the energy flows. Results show that the overall energy requirement of lipid recovery using CO₂ switching is 61.7 MJ/kg lipid, which is mostly due to losses of EBA carbamate. This is far beyond acceptable since the energy content of 1 kg of lipid is only 34.8 MJ. In contrast to this, lipid recovery using temperature switching costs only 12.4 MJ per kg lipid, resulting in a significant positive energy balance of 22.4 MJ/kg lipid. This makes the temperature switching process a very promising method for extracting lipid from algae for use in energy applications. Finally, the extraction technology developed is put into perspective by discussing the advantages, weaknesses and challenges of using EBA as switchable solvent for lipid extraction from wet microalgae.

1 Introduction

Microalgae are an important resource for a large number of biotechnology areas, including biofuels [1], pharmaceuticals [2] and cosmetics [3]. Because of the advantages of algae over other traditional crops, such as rapid growth rate, high productivity, less competition with arable land and freshwater, and a high CO₂ consumption rate [4], large scale microalgae culture as source for renewable energy is getting more attention, albeit that for fuel applications this is not yet competitive on cost-price with current fossil resources [5]. In the overall process to obtain valuable products from microalgae, several steps are needed, such as cultivation, harvesting, dewatering, extraction and utilization. This study focuses mainly on the lipid extraction and solvent regeneration.

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 [6]. Supercritical CO₂ (scCO₂) extraction is considered as an effective, “green” and mild extraction method for lipid extraction [7]. 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 [8]. ScCO₂ 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 [9]. A method named “switchable solvents extraction” which can achieve lipid extraction, separation and solvent recovery by simply changing the solvent hydrophilicity followed by phase splitting, aroused the interest of many researchers in recent years [4, 10-15].

Two processes for wet lipid extraction from microalgae with a switchable solvent i.e. N-ethylbutylamine (EBA) are evaluated on an energy usage basis in this chapter. The differences in these two processes is that one uses CO₂ to induce the phase splitting for lipid recovery and use N₂ stripping combined with heating to induce the back switching for solvent regeneration. The other process uses temperature to induce phase splitting for lipid recovery and solvent regeneration. The two processes are hereafter named “CO₂ switching” and “temperature switching” respectively. A comparison between these two processes is made including conceptual process designs and Sankey diagrams to present the energy flows.

2 Process design

The process schemes and description of lipid extraction from wet microalgae by EBA with CO₂ and temperature induced switching are presented in this section. For the design of these two processes, information of process characteristics has been used that was obtained with experimental work using fresh water (FW) stressed *Neochloris oleoabundans*. as described in earlier chapters of this thesis.

Figure 8.1 shows the process scheme for wet lipid extraction from microalgae with EBA, followed by CO₂ induced phase splitting for lipid recovery and N₂ stripping with heating to induce back switching for solvent regeneration. The algae feed is used directly after harvesting and concentration, without further pretreatment (no cell disruption). The algae concentration after processing by Evodos centrifugal equipment is around 20 wt.% to 30 wt.%, whereas in earlier chapters also more dilute streams of 5 wt.% have been applied, and it was found that concentrations higher than 10 wt.% are impractical in solvent extraction. However, dewatering up to a concentration as high as 20 wt.% might be beneficial for nutrient recovery, even though it seems incomprehensible to remove first water and then add water again to reduce the concentration to the practical maximum of 10 wt.%. To study on the effect of the concentration after dewatering on the overall process requirements, a sensitivity study about using algae slurries with concentrations ranging from 5 wt.% to 20 wt.% as starting material is carried out in the section of results and discussion. First, the algae slurry (Figure 8.1 #1) is mixed with EBA (Figure 8.1 #3) for lipid extraction (in Figure 8.1 unit B1). Some water (Figure 8.1 #2) is added if the algae concentration of #1 is higher than 10 wt.%. After this, the mixture (Figure 8.1 #4) is transferred (Figure 8.1 unit B2) to separate the liquid (Figure 8.1 #6) and solid phase (Figure 8.1 #5). The solid phase i.e. lipid depleted algae are removed for further treatment. For startup of the process, a small amount of water must be added to the liquid phase to control the water to EBA ratio for the switching step (Figure 8.1 unit B3). During steady-state operation this is no longer needed. Next, CO₂ (Figure 8.1 #7) is contacted with the organic phase, in the presence of water, to switch the solvent into its hydrophilic form. As a result the EBA will be transferred from the original solvent/lipid phase to the aqueous phase. As the extracted lipid remains hydrophobic, the lipid (Figure 8.1 #8) will phase separate and form a separate layer in the settler, which can be collected. Then the bottom phase (Figure 8.1 #9), containing water and switched solvent in its hydrophilic form, can be separated and flushed with N₂ (Figure 8.1 #10, #11) in combination with heating in order to switch back the solvent by removing CO₂ (Figure 8.1 #12) from the reaction equilibrium (in Figure 8.1 unit B4). This CO₂ can be recovered and reused, but this is left out of consideration here.

In the study of Chapter 3 it was found that EBA reacts with CO₂ to form carbamate salt and bicarbonate salt. However, it was also discovered that after back switching (restoring the two-

phase system), the carbamate salt was not converted completely. Therefore, a separate carbamate conversion step (Figure 8.1 unit B5) is needed to convert the remaining carbamate salt back to EBA from the organic phase (Figure 8.1 #13) and aqueous phase (Figure 8.1 #15) before recycling to the system. The solvent thus recovered can be collected as separate, hydrophobic phase (Figure 8.1 #14) and reused for extraction, while the water phase can be reused in the extraction (Figure 8.1 #16) and also forward switching step (Figure 8.1 #17).

In Chapter 7 it was found that a considerable portion of the EBA used for extraction is located inside the lipid extracted algal biomass residue, which was separated after extraction. Therefore, water (Figure 8.1 #21) should be used to wash the algae cells and rinse out the EBA (in Figure 8.1 unit B6). The aqueous extract (Figure 8.1 #19) is then mixed with an immiscible organic extractant i.e. dodecane (Figure 8.1 #20) to remove EBA from water (in Figure 8.1 unit B7). The water can be reused in the washing (Figure 8.1 #21) and forward switching step (Figure 8.1 #22). A distillation step (Figure 8.1 unit B8) separates the recovered EBA as distillate (Figure 8.1 #26) from the bottom product dodecane (Figure 8.1 #25). Both recovered solvent fractions (EBA and dodecane) can be reused in their respective extraction steps.

Figure 8.2 shows the process scheme for wet lipid extraction from microalgae with EBA, followed by temperature induced phase splitting for lipid recovery and solvent regeneration. In this temperature switching process most process steps are the same as for the CO₂ switching process. The difference is that for the forward switching step for lipid recovery (Figure 8.2 unit B3), the solution temperature is lowered to ca. 4°C (instead of CO₂ stripping). EBA dissolves completely in water at 4°C, leaving a lipid layer (Figure 8.2 #7) on top, which can be collected. After collecting the lipid phase, the solution (Figure 8.2 #9) is then reheated to ambient conditions (i.e. 22°C). EBA then separates from water and the system forms two phases again (Figure 8.2 unit B4). The recovered EBA (Figure 8.2 #9) and water (Figure 8.2 #10, #11) can be reused directly in the extraction step and forward switching step respectively, since there was no other compound formed during the switching step. The treatment of the lipid depleted algae was identical to that for the CO₂ switching process.

Figure 8.1 Process scheme for wet lipid extraction from microalgae with EBA, followed by CO₂ induced phase splitting for lipid recovery and N₂-heating induced back switching for solvent regeneration.

Figure 8.2 Process scheme for wet lipid extraction from microalgae with EBA, followed by temperature induced phase splitting for lipid recovery and solvent regeneration.

3 Energy calculation methods and approaches

The unit operations in the process schemes displayed in Figure 8.1 and Figure 8.2 were evaluated to make an estimation of the energy usage in the wet lipid extraction from microalgae with EBA by both the CO₂ switching and temperature switching process.

As there are many algae species in nature, the variety in lipid content and composition is large. In this study, FW-stressed (fresh-water cultivated and stressed by nitrogen deprivation) *Neochloris oleoabundans* was selected since it has a relatively high lipid content and was provided by consortium partner AlgaePARC (Wageningen, NL), where cost-effective and sustainable microalgae production methods are being developed. The annual productivity of dry biomass can reach 30 ton/ha using a tubular cultivation system. All numbers are calculated based on the processing of 1 kg of dry microalgae at ambient temperature (22°C).

For wet lipid extraction from microalgae with EBA, a 5 wt.% algae solution was used in the studies of previous chapters. For both 5 and 10 wt.% algae slurries, a similar lipid extraction yield was found for non-stressed *Neochloris oleoabundans* algae (13.1 wt.% ± 0.2 wt.%, for the 5 wt.% slurry) and (13.0 wt.% ± 0.04 wt.% for the 10 wt.% slurry). This equal extraction performance is assumed to be also valid for the FW-stressed *Neochloris oleoabundans* algae. Doubling the algae mass concentration from 5 wt.% to 10 wt.% reduces the amount of water in the extraction by half, thereby reducing the energy usage significantly, whereas the difference of energy used for concentrating algae slurry to 5 wt.% (0.32 MJ/kg dry algae) and 10 wt.% (0.36 MJ/kg dry algae) is very small. A 10 wt.% algae solution is therefore selected as feed concentration for this process. In Figure 8.1 in extraction unit *BL*, water is added to the algae slurry to reach this algae concentration and then mixed with EBA with a mass ratio of 1:1 (algae solution/EBA) for 18 h to allow for complete extraction without cell breaking. The energy usage for extraction $E_{\text{extraction}}$ is calculated by Eq. 8-1, where τ is the residence time (h), $m_{\text{extractor}}$ is the total mass within in the extractor (kg) and $E_{\text{extractor,kg}}$ is the unit energy usage of extraction (W/kg). The interfacial tension of EBA-water is expected to be in the same range as dipropylamine-water (< 10 mN/m), since they are both secondary amine and have the same number of carbon atoms [16]. This low interfacial tension facilitates the phase dispersion and enables a column without (or very low) energy input for mixing to be selected as extraction equipment [17]. Therefore, for this specific energy usage $E_{\text{extractor,kg}}$ a (still conservative) value of 0.1 W/kg is used. This value is somewhat below a typical value of 1 W/kg for stirred vessels [18], but seems more than reasonable considering the slow settling rate of the phases (from lab experience: about several minutes for EBA-water and hours for EBA - algae slurries).

$$E_{\text{extraction}} = \tau \cdot m_{\text{extractor}} \cdot E_{\text{extractor,kg}} \quad \text{Eq. 8-1}$$

After extraction, the solids (lipid depleted algae) are separated by pressure filtration (Figure 8.1, unit B2), thereby assuming an energy usage of 0.5 kWh/m³. The separated liquid phase containing water, EBA and lipid is sent to the switching forward step: lipid recovery unit B3.

Based on the result found in Chapter 3, for CO₂ switching (Figure 8.1 unit B3), some water is added to control the mass ratio of EBA/water at 1:1. Then CO₂ is introduced to the mixture at ambient conditions at a flow rate of 5 VVM for 5 min. A stirred contactor is selected to ensure a good gas-liquid contacting to transfer the CO₂ to the liquid phase. The energy usage is calculated by Eq. 8-1. About 25% EBA reacts with CO₂ or CO₂ and water to form carbamate salt and bicarbonate salt. The molar percentage of EBACOO⁻ and HCO₃⁻ is 64% and 36% based on the ¹³C-NMR determination (Chapter 3). As a result of the changing composition due to the reaction, both reacted and unreacted EBA dissolve in the water phase, leaving lipid on top. A lipid yield of 47 wt.% (on dry algae basis) is assumed based on the study in Chapter 4. After collecting the lipid, the bottom liquid phase which contains water, EBA and EBA salt is reconverted by heating to 90°C under stripping with N₂ gas (at 2 VVM for 90 min) in order to remove CO₂ from the reaction equilibrium (Figure 8.1, unit B4). The heat capacity of EBA is assumed to be the same as dipropyl amine which is 2.5 kJ/(kg·K) [19]. Since they are both secondary amine and have the same number of carbon atoms, this seems a reasonable assumption. A heat exchanger is used between the incoming and outgoing streams to minimize the energy usage. It is assumed that the heat can be exchanged with an approach temperature of 3°C. Besides the energy required for heating up, the heat used for breaking the bond between CO₂ and amine group must be considered. The binding energy of primary amines as e.g. mono ethanol amine (MEA) is around 80 kJ/mole CO₂, which is higher than for secondary and tertiary amines [20]. The binding energy for carbamate formation (EBAH⁺ with EBACOO⁻) and bicarbonate formation (HCO₃⁻) are assumed to be 70 kJ/mol and 40 kJ/mol respectively [21], in the same range as reported by Kim et al [22]. Under the regeneration conditions tested and assumed in the process, almost all the bicarbonate salt converts back to EBA, while the conversion of EBA carbamate salt is 70%.

For temperature switching (Figure 8.2, lipid recovery unit B3), some water is added to maintain the mass ratio of EBA/water at 4:1. Some extra lipid is also added as starting material to create a situation of 10 wt.% lipid concentration in the lipid recovery unit (Figure 8.2 unit B3). This EBA/water ratio and lipid concentration are selected for an efficient lipid recovery based on the study of Chapter 6. The mixture is lowered to 4°C to let EBA dissolve in water and leave a lipid phase on top. In this case, based on results from Chapter 6, the lipid recovery efficiency is set to be 50% of the total lipid in the system. Only the amount of lipid that extracted from the microalgae (Figure 8.2 #1) entering the extraction unit is separated out (Figure 8.2 #7) while the remaining part of recovered lipid stays in the process. After lipid collection, the hydrophilic mixture (Figure 8.2 #8) is then heated to 22°C (Figure 8.2,

back switching unit *B4*). A heat exchanger with a temperature difference of 3°C is also applied here to reduce the energy usage.

For EBA recovery from lipid depleted algae, water is first used to rinse the EBA from algae cells. The added water is 20 times of the mass of the lipid depleted algae. EBA is removed completely from algal cells residue. Subsequently, in a next step, dodecane is used to extract the EBA from water. It was found in the study of Chapter 7 that this liquid-liquid extraction should be operated in an extraction column that has 39 stages with dodecane to (water and EBA) ratio of 1:1.8 (w/w). After extraction, the EBA content in water is reduced to 736 mg/L, and the extracted EBA is dissolved in dodecane. A distillation column is implemented to separate EBA from dodecane for reuse. The optimum design is a distillation column that has 11 equilibrium stages, with the feed added above the 7th stage. The reflux ratio is 0.2. With this distillation column, a separation of a distillate that contains 99.9 wt.% EBA and a bottoms product that contains 99.9 wt.% dodecane is achieved. The energy requirement of the extraction column and distillation column, scaled to the biomass intake, was estimated using Aspen Plus to be respectively 0.5 MJ/kg dry algae and 3.5 MJ/kg dry algae.

The elemental composition of the dry *Neochloris oleoabundans* sample and of the extracted lipid was determined using an elemental analyser. For the energy calculation, the Higher Heating Value (HHV) was calculated based on Boie's formula (Eq. 8-2) [4].

$$\text{HHV}_{\text{Boie}} = 0.3516 \cdot C + 1.16225 \cdot H - 0.1109 \cdot O + 0.0628 \cdot N \quad \text{Eq. 8-2}$$

The HHV of input materials and products used for this study are listed in Table 8.1.

Table 8.1 HHV of input materials and products used for this study.

Item	HHV (MJ/kg)
Dry <i>Neochloris oleoabundans</i>	25.8 ± 0.3 ^a
Extracted lipid	34.8 ± 0.4 ^a
EBA	43.2
[EBAH ⁺][EBACOO ⁻]	39.3
[EBAH ⁺][HCO ₃ ⁻]	21.9
Dodecane	47.6

^a The value was calculated based on elemental analysis results (Chapter 2).

4 Results and discussion

As explained in the previous section, the process involves a starting paste of 20 wt.% *Neochloris oleoabundans*, assuming a 47 wt.% lipid yield of dry algae biomass. Sankey diagrams for wet lipid extraction from microalgae with EBA by CO₂ switching and temperature switching are shown in Figure 8.3 and Figure 8.4. The width of the arrows shown is proportional to the energy of the stream involved.

The main energy factor in the temperature switching extraction process is the EBA recovery step (9.0 MJ/kg lipid) which includes the operations *B5* (EBA extraction from algae paste by water), *B6* (EBA extraction from water by cyclohexane) and *B7* (EBA and cyclohexane regeneration by distillation) in Figure 8.2. The EBA amount in #5 is 2.14 kg/kg lipid. This is a significant amount and must be recovered from both an energy and environmental point of view. The extraction and distillation column were designed and optimized in Aspen Plus. There seems, however, little opportunity for improvement here. For the purpose of bioenergy production only, one might consider producing pyrolysis oil and/or bio gas from the residual lipid extracted algae to make the energy balance much more favorable [23] .

Another large energy consumer is the switching step (2.7 MJ/kg lipid), in which temperature switching requires more energy than CO₂ switching. Although for CO₂ switching, the temperature difference for forward and backward switching is more than 50°C larger than the one for temperature switching, this difference does not exist anymore with the use of heat exchanger (aiming at 3°C approach temperature). For temperature switching, a large amount of water was added to maintain the EBA concentration in the desired operating window in order to get relatively high lipid recovery yield. It should be noted (as upside potential) that the energy for reheating to ambient conditions can probably easily be obtained by heat exchange with ambient air, the waste water stream or the incoming algae slurry.

Figure 8.3 Sankey diagram of wet lipid extraction from microalgae with EBA by CO₂ switching. All numbers are based on the processing of 1 kg of dry microalgae.

Figure 8.4 Sankey diagram of wet lipid extraction from microalgae with EBA by temperature switching. All numbers are based on the processing of 1 kg of dry microalgae.

The most impacting contributions for the energy usage for producing 1 kg of lipid via both CO₂ switching and temperature switching extraction process are evaluated and compared in Figure 8.5.

Figure 8.5 Energy requirements in MJ/kg lipid produced for CO₂ switching and temperature switching extraction process.

In Figure 8.5, different types of energy e.g. thermal energy (heating and cooling), electrical energy (stirring, pumping) and chemical energy are plotted in one single bar. It is realized that the efficiency of the different forms of energy is not the same, but since CO₂ switching and temperature switching extraction process use similar type of energy in each step, this kind of combination will not influence the final result negatively. It can be noticed that CO₂ switching and temperature switching extraction process use similar amount of energy for extraction, separation, EBA recovery and solvent loss.

In a sensitivity study it was evaluated whether performing the extraction in a two stage extraction process with a total lower solvent to feed ratio (EBA/algae slurry) is beneficial, based on the extraction model developed in Chapter 5. When the total solvent to feed ratio is larger than 0.7, a higher lipid extraction yield is obtained with two stages extraction. After calculating this trade off of energy usage vs. energy production for different solvent to feed ratios it seems that a two stages extraction is better. With a two stages extraction, the net energy yield (lipid production energy minus total energy usage) has an improvement of 0.88

MJ/kg lipid and 0.97 MJ/kg lipid for CO₂ switching and temperature switching respectively, when the total solvent to feed ratio is 1. However, the time factor should also be considered since a two stages extraction means that the mixing time and extraction equipment is doubled.

In this process, algae slurries with concentrations ranging from 5 wt.% to 20 wt.% can be used as starting material. During harvesting and dewatering more energy is required for concentrating the algae slurry to a higher concentration. But the total amount of material in the process is reduced, therefore the energy used in the other following steps will also be reduced. When 5 wt.%, 10 wt.% and 20 wt.% algae slurry are used as starting material, the detailed energy usage in each step of temperature switching process is compared and the results are listed in Table 8.2. When the algae slurry starting concentration is 20 wt.%, it will be diluted to 10 wt.% which is the maximum concentration for extraction in this process. Comparing with 20 wt.%, 0.12 MJ/kg lipid and 0.04 MJ/kg lipid energy in the centrifuge step can be saved when 5 wt.%, 10 wt.% algae slurry is used as starting material, respectively. However, starting with 5 wt.% or 10 wt.% algae slurry will increase the amount to the waste water treatment. As a consequence, the energy for EBA recovery increases from 9.04 MJ/kg lipid to 10.84 MJ/kg lipid and 9.71 MJ/kg lipid for using 5 wt.% and 10 wt.% algae slurry respectively. Moreover, starting with 5 wt.% algae slurry also increases the process inventory, and hence increases equipment sizes, which makes this scenario even more unfavorable. Therefore, despite redilution, it is better to start with 20 wt.% algae slurry than 5 or 10 wt.%.

Table 8.2 Energy requirements in MJ/kg lipid produced for temperature switching extraction process when 5 wt.%, 10 wt.% and 20 wt.% algae slurry are used as starting material.

Algae slurry starting concentration	5 wt.%	10 wt.%	20 wt.%
Centrifuge	0.68	0.76	0.80
Lipid extraction	0.66	0.33	0.33
Separation	0.18	0.09	0.09
Switching	5.72	2.74	2.74
EBA recovery	10.84	9.71	9.04
Solvent loss	0.23	0.23	0.23
Total	17.62	13.09	12.42

In previous work, at the start of the development this wet lipid extraction process using switchable solvents, an energy calculation has been made for the CO₂ switching process [11]. The calculations presented at that time showed that CO₂ switchable solvent extraction is potentially a very promising method for extracting lipid from microalgae. The total energy requirement was calculated to be 19.8 MJ/kg lipid. Now, after having studied the process in much more detail, the pros and cons of the different process steps are much more clear.

The main difference with previous results is the potential EBA carbamate loss. The newly developed option of temperature switching avoids this issue. Temperature switching does not involve carbamate formation, thus no EBA carbamate loss nor extra energy for carbamate reconversion. The energy usage due to “EBA carbamate loss” accounts for more than 80% (49.7 MJ/kg lipid) of the overall energy requirement for the CO₂ switching process. Such a high energy loss (via carbamate) makes the use of CO₂ switching for energy production via lipid extraction unviable. This “EBA carbamate loss” was due to the uncomplete conversion of EBA carbamate salt during back switching (stream #16 in Figure 8.1). From experimental measurements it is known that almost all of the EBA bicarbonate salt converted back to EBA, while the recovery of EBA carbamate salt was 70 wt.%. And 90 wt.% of the unconverted EBA carbamate salt was situated in the organic phase. All the unconverted EBA-carbamate was considered as EBA carbamate loss in Figure 8.5. This EBA carbamate loss has to be minimized in order to lower the energy usage to such a level that the process becomes feasible. Figure 8.6 shows the effect of EBA carbamate salt recovery to the total energy usage of the CO₂ switching extraction process. It is clear that the energy usage decreases significantly by increasing the recovery of EBA carbamate salt. In order to get a positive net energy yield, at least 87 wt.% of EBA carbamate salt must be converted back to EBA. A better recovery during back switching should be achieved with a higher temperature and/or lower pressure. Ion exchange membranes might be applied in the carbamate conversion step since they are claimed to be efficient tools for the separation of ionic species and successfully applied for treating industrial effluents [24]. Due to the lack of information and time limitation, the energy usage for carbamate conversion could not be included in the calculation and is shown as “to be defined” in Figure 8.3. The energy usage due to EBA carbamate loss and conversion has to be lower than 22.8 MJ/kg of lipid, in order to get a positive net energy yield. Only when the EBA carbamate salt can be 100% converted to EBA, and the energy used for conversion is less than 0.06 MJ/kg of lipid, the CO₂ switching method becomes more favorable than temperature switching. Otherwise, the temperature-switch option is the more promising one.

Figure 8.6 Effect of EBA carbamate salt recovery in back switching of the CO₂ switching extraction process.

5 Conclusions

In this chapter, two process options for wet lipid extraction from microalgae with EBA were designed, one using CO₂ for switching the solvent hydrophilicity during lipid recovery and the other one using a temperature step for the same purpose. The energy flows were calculated and compared. Results showed that the use of CO₂ switching is not viable if the objective is energy production via lipid extraction, as the energy usage due to “EBA carbamate loss” accounts for more than 80% (49.7 MJ/kg lipid) of the overall energy requirement (61.7 MJ/kg lipid).

A much lower energy usage (12.4 MJ/kg lipid) and positive energy balance for lipid extraction was achieved with the temperature switching process, making this a very promising method for extracting lipid from algae for use in energy applications.

6 Outlook

In the work described in this thesis it was demonstrated that the application of switchable solvents for direct lipid extraction from (unbroken) microalgae in (concentrated) aqueous solutions is technically feasible with remarkable lipid extraction yield of up to 0.613 kg/kg dry algae. Microalgae in concentrated aqueous solution was selected as starting material instead of dried and broken algae to minimize the energy usage, since dewatering and drying are very energy intensive steps in conventional solvent based processes of extracting lipid from (broken) microalgae. Moreover, cell breaking was also proven to be unnecessary to achieve an efficient lipid extraction for the lipid rich *Neochloris Oleoabundans*. investigated in this work. EBA extraction from non-broken, wet algae provides an outlook to a cost and energy efficient lipid recovery from microalgae.

An advantage of using EBA is that the extraction process is simple and can be performed at mild conditions. Simply mixing the solvent with algae slurry leads to an effective transfer from the lipid in the algae to the solvent. Lipid separation and solvent recovery can be achieved just by “switching” the solvent and removing the lipid layer. The process can be operated continuously. It is expected that the lipid extraction and lipid recovery (switching-) processes are relatively easy to scale up and without requiring high investment costs.

For the switching process two options were compared. Switching hydrophilicity with CO₂ as reagent results in the formation of EBA carbamate salt. Upon relatively mild regeneration conditions the original two-phase system could be restored. However, upon NMR inspection only 70% of the carbamate salts formed were converted back under the back switching condition studied in this work. The major fraction of unconverted EBA carbamate salt situated in the organic phase. This will result in an accumulation and upon recycling to the extraction step probably a carbamate loss via the aqueous effluent. More research is needed on the reaction of EBA with CO₂ to have a better understanding of the reaction kinetics, selectivity of EBA bicarbonate salt and recovery of the carbamate salt. To make the CO₂ switching industrially feasible, the recovery of EBA carbamate salt must be improved and/or an efficient purification method must be investigated.

The use of temperature switching for lipid separation and solvent recovery can avoid the losses of EBA via the carbamate salt form. In this way, by just reducing the temperature from ambient temperature (22°C) to 4°C, EBA switched from organic phase to aqueous phase and vice versa. A separated lipid layer was formed at 4°C and therefore can be easily phase separated. As this switching method does not require the introduction of any other substances and uses milder conditions for switching compared with CO₂ switching, this method is therefore to be preferred. Screening other solvents for this type of phase-behavior might lead to more (and potentially even better) solvent candidates.

A weakness of the lipid extraction using EBA is the number of process steps induced by the solvent recovery. Part of the success of EBA as extraction solvent is due to its ability to penetrate the algae cells. For solvent recovery from the wet algae paste after lipid extraction, there is a significant amount of EBA residues inside, this part of EBA must now be recovered, here proposed by washing the algae paste residue with water. The EBA that dissolved in water was then recovered by dodecane extraction followed by distillation. For the whole process of using EBA for microalgae wet lipid extraction, besides extraction, switching and back switching, now also liquid-solid extraction, liquid-liquid extraction (EBA recovery) and distillation (EBA/dodecane separation) are needed. An alternative option that could be considered (and potentially optimized) is the distillation of EBA out of the wet algae paste directly (used in Chapter 7 to determine the EBA content of the wet algae paste). This distillation option could become attractive (energy wise) if a high degree of heat recovery (above 75%) can be realized, e.g. using a heat pump. More in depth study including energy and investment costs is needed for economic optimization.

Besides lipid extraction from microalgae, switchable solvents might find their way in several other applications. A switchable solvent is possibly a good replacement for hexane in other oil extraction applications (e.g. soybean oil extraction [25]). Switchable solvents were also used in micro-extraction of palladium, Cd (II) and mercury in environmental samples [26-28]. Nitro aromatic compounds were determined quantitatively by switchable-hydrophilicity solvent-based micro-extraction combined with gas chromatography [29]. In these studies, amine as switchable solvent first react with CO₂ and the formed hydrophilic solution was used for extraction [26-29]. It allows the extraction of the extracts in a homogeneous aqueous phase without dispersive solvent which is the main advantage of using switchable solvents in the metal extraction. A switchable hydrophilicity solvents N,N-dimethylcyclohexylamine was used for polyethylene and aluminium recovery from food aseptic packaging [30]. Another switchable solvent was used for diesel extraction from oil-based drill cuttings [31] and also used for phenols extraction from lignin microwave-pyrolysis oil [32]. It is interesting to investigate if switchable solvents could also be used for the upgrading (fractionation) of biomass derived pyrolysis oil and hydrothermal liquefaction oil. However, all the studies above only considered the formation of carbonate/bicarbonate salt when switchable solvent reacted with CO₂. As this study has shown, attention should be paid to the possible loss (and hence: recovery-) of any carbamate during the process and on the applicability of temperature switching in these applications.

The intriguing phase behavior of switchable solvents as promising extraction solvent raised the interests of many researchers. However, there has not been so much attention on the application of switchable solvents on consumer/producer acceptance in the area of pharmaceuticals, cosmetics, food and feed. The acceptance from both social and legal side

of the use of organic solvents is a serious issue to be tackled prior to further upscaling and deployment. The use of hexane for food applications (e.g. in decaffeination) shows that this is not impossible on beforehand. Therefore, studies with regard to completeness of solvent removal from product stream and quantification of emissions are most likely needed and highly recommended for those applications.

As mentioned above, there are still many challenges before using switchable solvent for lipid extraction from microalgae becomes an economically feasible commercial process. More technological improvements on extraction equipment technology, increasing solvent recovery efficiency, preventing environment pollution, mini-plant demonstration and further reduction of process costs are required and recommended.

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Appendix

Table A.1 Mass balance of steady-state operation of wet lipid extraction with EBA by CO₂ switching for processing 1 kg (dry weight) of FW-stressed *Neochloris Oleoabundans*.

Unit [kg]	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12
Lipid	0.47	0	0	0.47	0	0.47	0	0.47	0	0	0	0
Lipid depleted dry biomass	0.53	0	0	0.53	0.53	0	0	0	0	0	0	0
Water	4.00	0	0	9.00	5.09	3.91	0	0	9.13	0	0	0
EBA	0	0	0.54	10.00	1.01	8.99	0	0	6.91	0	0	0
EBA carbamate	0	0	0	0	0	0	0	0	2.19	0	0	0
EBA bicarbonate	0	0	0	0	0	0	0	0	0.82	0	0	0
CO ₂	0	0	0	0	0	0	0.61	0	0	0	0	0.49
N ₂	0	0	0	0	0	0	0	0	0	9.99	9.99	0
Dodecane	0	0	0	1.01×10 ⁻³	0	1.01×10 ⁻³	0	1.01×10 ⁻³	0	0	0	0
Total	5.00	0	0.54	20.00	6.63	13.38	0.61	0.47	19.05	9.99	9.99	0.49

(Table A.1 continued)

Unit [kg]	#13	#14	#15	#16	#17	#18	#19	#20	#21
Lipid	0	0	0	0	0	0	0	0	0
Lipid depleted dry biomass	0	0	0	0	0	0.53	0	0	0
Water	3.71	3.71	0	1.29	4.22	2.04	135.55	0	132.50
EBA	8.39	8.39	0	6.85×10^{-2}	0.22	0	1.10	0	9.17×10^{-2}
EBA carbamate	0.66	0	0.66	0	0	0	0	0	0
EBA bicarbonate	0	0	0	0	0	0	0	0	0
CO ₂	0	0	0	0	0	0	0	0	0
N ₂	0	0	0	0	0	0	0	0	0
Dodecane	0	0	0	0	0	0	0	1.01×10^{-3}	0
Total	12.76	12.10	0.66	1.35	4.44	2.57	136.64	1.01×10^{-3}	132.59

(Table A.1 continued)

Unit [kg]	#22	#23	#24	#25	#26
Lipid	0	0	0	0	0
Lipid depleted dry biomass	0	0	0	0	0
Water	1.09	1.96	0	0	0
EBA	7.53×10^{-4}	1.35×10^{-3}	1.08	7.60×10^{-2}	1.00
EBA carbamate	0	0	0	0	0
EBA bicarbonate	0	0	0	0	0
CO ₂	0	0	0	0	0
N ₂	0	0	0	0	0
Dodecane	0	0	75.96	75.95	1.01×10^{-3}
Total	1.09	1.96	77.04	76.03	1.01

Table A.2 Mass balance of steady-state operation of wet lipid extraction with EBA by temperature switching for processing 1 kg (dry weight) of FW-stressed *Neochloris Oleoabundans*.

	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10
Lipid	0.47	0	0	1.00	0	1.00	0.47	0.50	0.50	0
Lipid depleted dry biomass	0.53	0	0	0.53	0.53	0	0	0	0	0
Water	4.00	0	0	9.00	5.09	3.91	0	44.37	3.96	1.04
EBA	0	0	1.35×10^{-3}	10.00	1.01	8.99	0	11.09	8.94	5.54×10^{-2}
Dodecane	0	0	0	1.01×10^{-3}	0	1.01×10^{-3}	1.01×10^{-3}	0	0	0
Total	5.00	0	1.35×10^{-3}	20.53	6.63	13.91	0.47	55.96	13.40	1.10

(Table A.2 continued)

	#11	#12	#13	#14	#15	#16	#17	#18	#19	#20
Lipid	0	0	0	0	0	0	0	0	0	0
Lipid depleted dry biomass	0	0.53	0	0	0	0	0	0	0	0
Water	39.37	2.04	135.55	0	132.50	1.09	1.96	0	0	0
EBA	2.10	0	1.10	0	9.17×10^{-2}	7.53×10^{-2}	1.35×10^{-3}	1.08	7.60×10^{-2}	1.00
Dodecane	0	0	0	1.01×10^{-3}	0	0	0	75.96	75.95	1.01×10^{-3}
Total	41.47	2.57	136.64	1.01×10^{-3}	132.59	1.09	1.96	77.04	76.03	1.01

List of publications

Y. Du, B. Schuur, S.R.A. Kersten, D.W.F. Brilman (2018). *Multistage wet lipid extraction from fresh water stressed Neochloris oleoabundans slurry – Experiments and modelling*. Algal Research, 31: 21-30.

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In preparation

Y. Du, B. Schuur, V. Cyprichová, K. Hoppe, D.W.F. Brilman, *Swing strategies for solvent recovery in wet lipid extraction processes*.

Publications prior to PhD thesis

Y. Du, X. Tao, K. Shi and Y. Li (2010). *Degradation of lignite model compounds by the action of white rot fungi*. Mining Science and Technology (China) 20(1): 76-81.

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J. Yao, **Y. Du** and L. Xiao (2007). *Application of microwave - assisted for active substances extraction*. Journal of Biology 24(6): 61-63.

Acknowledgements

Seven years ago, I came to the Netherlands and started a whole new journey of my life. A journey which filled with great opportunities and fantastic challenges. I enjoyed the years I spent working at the University of Twente very much because I have developed so much during these years. Besides accomplishing a doctoral degree, I have also gained a lot in other aspects of life, which makes all the efforts become worthwhile. I would like to express my gratitude to all the people that accompanied and supported me throughout the years of my PhD.

First of all, I would like to thank my supervisors Sascha Kersten and Wim Brilman and co-supervisor Boelo Schuur. Thank you for giving me the opportunity to work in the Sustainable Process Technology (SPT) research group. Without your support, this thesis would not have been completed. Sascha, thank you for guiding me in completing my PhD work with your critical questions and invaluable suggestions. Wim, thank you for giving me the opportunities and also on everything that you have done for me. You were always patient, positive and also encouraging me through many valleys of my PhD. I really enjoyed the time working with you. Boelo, thank you for your unlimited ideas and thoughtful guidance throughout my entire PhD study. I have learned a lot from you in separation technologies.

I would also like to extend my gratitude to the rest of my graduation committee members: Prof.dr.ir. J.W.M. Hilgenkamp, Prof.dr.ir. A. Nijmeijer, Dr.ir. W.M. de Vos from the University of Twente, Prof.dr.ing. M.H.M. Eppink from Wageningen University and Research, Prof.dr.-ing. I. Smirnova from Hamburg University of Technology and Dr. C. Samorì from University of Bologna. Thank you for your effort in reviewing this thesis. It is a great honor to have all of you being part of my defense committee. Special thanks to Chiara, thank you for teaching me the transesterification and GC analysis of lipid from microalgae. Your help has taken me to the first step of my research. Thank you and Cristian for your thoughtful arrangements when I was in Ravenna. It was a great and unforgettable time.

I would like to thank all the partners in the AlgaePARC Biorefinery project. Thanks for your help and valuable discussions. I really appreciate to work with you. Special thanks to Ben, I have learned so much knowledge about the lipid analysis during our discussions. Also thank you for helping me with the TLC experiments.

Not to forget, many thanks to the China Scholarship Council (CSC) which funded the first two years of my study in the Netherlands as a joint PhD student. Without this I would not have gotten the opportunity of being a PhD candidate in the University of Twente. I would like to thank my former supervisor Prof. Tao Xiuxiang from China University of Mining and Technology. Thank you for your great support and suggestions when I was both in China and

the Netherlands. I would like to thank my friends in China: Xiaochuan, Xiaoling, He Huan, Chen Hui, Kaiyi, Xu Ning, Jinyan, Cai Pei, Jinzhou, Shujie, Yuhang and Keyan. Thank you for helping me during my graduation at China University of Mining and Technology.

I would like to express my gratitude to all the staff members in SPT group. Firstly to all technicians, Erna, Benno, Karst and Johan. Erna, thank you for introducing me to all analytical equipment. I have learned a lot which will also be very useful in my future work. Karst and Johan, thank you for solving all the technical problems. Benno, thank you for building those handy tools for me, especially the mixing-heating setup which saved me lots of effort on the experiment. You were always willing to help and your tremendous knowledge made me think that you can make any setup. Yvonne, thank you for helping me with all kinds of forms, emails and daily supports. Louis, thank you for all the information and advices about ASPEN Plus software.

Special thanks to Muthia and Pushkar for being my paranymphs and for your support on the defense preparations. Muthia, we have experienced pregnancy together. Thank you for all the pleasant discussions and your company which made me feel not alone. Thank you for helping me to solve the problems which I encountered during the use of ASPEN Plus. Pushkar, you are always enthusiastic and willing to help. Thank you for all the help and information in the use of GC-MS.

I would also like to thank all my colleagues for the wonderful time I have experienced in the SPT group. I like all the activities that we had together. Roel, you are my first officemate and learned me my first Dutch sentence: hoge pieken, diepe dalen. Pavlina, thank you for passing me the working place which I enjoyed a lot in the past years. Laura, thank you for sharing all the articles and experiences about microalgae. Mathijs, I enjoyed our attempts to grow microalgae outside the high pressure lab. That was a memorable summer! Rens, you are a patient teacher who explained me those calculations in FTV and showed me how to supervise students in the practicum. Maria, I enjoyed very much our talk about nice food and our dream restaurants (your Spanish restaurant and my Chinese restaurant). Xiaohua, we learned swimming and tennis together, travelled together to the northern Europe, shared happiness and also worries, there were so many memorable moments. Stijn, thank you for all your help and suggestions for my research and Dutch Inburgeringsexamens. Michal and Ehsan, thanks for your help with HPLC analysis and many valuable discussions. Guus, Maarten, David, Prasad, Jose, Nick, Carolien, Shushil and Sam, thank you for helping me rapidly to adapt to the new environment when I first arrived to the Netherlands. Martin, Thomas, Lisette, Rick, Dion and Chiel, thank you for answering all my Dutch questions and helping me with the Dutch exams. Marek, Sandra, Stevia, Varsha, Qian, Daniel, Natalia, Surika, Lucia, Ozge, Vincent, Anne, Juraj, Thimo, Michel, and many others, thank you all for the friendly and welcoming atmosphere in the group. I will never forget all the pleasure moments that we have spent together.

I also would like to express my gratitude to my students Khoa, Mila, Chiel, Kevin, Rosa, Veronika, Brian, Timo, Simon and Laura. Your works have broadened my knowledge and they were very important to my research. Special thanks to Kevin and Veronika, you both made a very important contribution to this work. The work would not have been done so much without your help. I wish you all a life full of success!

I would like to thank all other friends whom I met in Enschede and other cities in the Netherlands. Wang Lei, you are my first friend in the Netherlands. We both lived in the ITC hotel and later became roommate for half year. We went to work together, had dinner together, travelled together. You are just like me sister who will always be there for me. Lulu, you always seemed to be muddleheaded but always brings us joy. Jingwei and Zhonghua, you cared for me like both a sister and a brother, encouraged me and accompanied me when I was sad or confused. Thank you guys for making me strong. Junwen and Ye Liang, you both are such a lovely couple. I hope that when we are old, we can still go camping together and travel everywhere. Wai-Ying and Yean-Sheng, thank you for being always thoughtful and willing to help. Thanks to Jiahui, Liu Yao, Liu Fei, Yanting, Hairong and Weiyu, Yali, Zhao Jie, Yingnan, Jia Yin, Honglin, Minmin, Aijie, Wang Yi, Meiru, Bram, Xichen, Yujie, Leilei, Xiaobin, Youwen, Mengdi, Dongfang, Lin Lin, Wang Jun, Xinling, Xu Hong, Xiaolin, Wang Rong, Kenan, Xingwu, Hainan, Zhuola, Lixian, Cheng Wei, Zhao Yong, Haihan, Chen Wei, Xueling, Binlong, Xiaokui, Xuelong, Wenlong, Jinfeng, Amin, Tao Yin, Gao Peng, Ma Song, Yaxing, Liu Feng and all others. It is hard to name them all, but I do keep all the memories with me. Thanks all for sharing great times together, dinner, BBQ, badminton, travelling and many more.

My heartfelt gratitude to my family. My dear parents, Du Baishu and Wang Chunyan, thank you for raising me up, being so open-minded and also for always trusting me in my decisions. Without your support and encouragement, I would not have reached this point of my life. Thank you for everything you have done for me, papa and mama, I love you! I would also like to express my thanks to my parents in law Chang Yunjin and Yuan Kerong. Thanks for your understanding and support during these years. Lantian, my dearest husband, words cannot express my gratitude for everything you have done for me. I am deeply grateful to have you in my life. You are like sunshine, making my life brighter, warmer and more colorful. Thank you for always making me feel happy, supporting me and encouraging me. I hope and believe we will always trust, admire and love each other. Last but not least, I would like to thank my beloved daughter, Yifan. You are my precious, my angel. Thank you for bringing me infinite power and endless joy!

July, 2018

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ISBN:978-90-365-4573-0