Hydrothermal liquefaction within a microalgae biorefinery

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DISSERTATION

von

M.Sc. Bingfeng Guo

aus Shenyang China

Erstgutachter: Prof. Dr. Nicolaus Dahmen

Zweitgutachter: Prof. Dr.-Ing. Clemens Posten

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Declaration

I declare that this thesis represents my own work and that I have written this thesis independently by myself, without the use of other documents or sources beyond those stated in the references.

Karlsruhe,

Bingfeng Guo

Preamble

Some chapters of this thesis have been published or are currently under preparation for peer-reviewed articles. They describe my major research work. The text of these chapters is partially identical to the content of the publications. Layout, citation style, figures and formatting have been modified and adjusted to the style of this thesis. Chapters that contain contents of previously published work or publication in progress are as follows:

Chapter 3 with the subject “Hydrothermal liquefaction of residual microalgae biomass after pulsed electric field assisted valuables extraction”.

Chapter 4 with the subject “Aqueous product recycle from continuous hydrothermal liquefaction of Chlorella vulgaris for algae cultivation” (under preparation).

Chapter 5 with the subject “Hydrothermal liquefaction of *Chlorella vulgaris* and *Nannochloropsis gaditana* in a continuous stirred tank reactor and hydrotreating of biocrude by nickel catalysts”.

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List of publications

Peer-reviewed original research papers

Silve, A., Papachristou, I., Wüstner, R., Sträßner, R., Schirmer, M., Leber, K., Guo, B., Interrante, L., Frey, W. (2018). Extraction of lipids from wet microalga Auxenochlorella protothecoides using pulsed electric field treatment and ethanol-hexane blends. *Algal research*, *29*, 212-222.

Guo, B., Walter, V., Hornung, U., & Dahmen, N. (2019). Hydrothermal liquefaction of Chlorella vulgaris and Nannochloropsis gaditana in a continuous stirred tank reactor and hydrotreating of biocrude by nickel catalysts. *Fuel Processing Technology*, *191*, 168-180.

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Guo, B. A mechanism study of microalgae hydrothermal liquefaction by means of pyrolysis-gas chromatography-mass spectrometry and thermogravimetric analysis. Talk at 2nd International Conference on Marine Biomass as Renewable Energy in Glasgow 2018.

Conference poster presentations

Guo, B., Hornung, U., Frey, W., Dahmen, N. Biorefinery of microalgae by means of hydrothermal liquefaction. Poster at status seminar of Bioeconomy Research Program in Stuttgart 2015.

Guo, B., Hornung, U., Frey, W., Dahmen, N. Hydrothermal liquefaction of raw and components-extracted microalgae with assist of pulsed electric field pretreatment. Poster at 2nd International Bioeconmy Congress in Stuttgart 2017.

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Abstract

In the last decade, microalgae have been considered as a promising feedstock for biofuel production, due to their fast growth rate and ability to accumulate various valuable biocomponents such as lipids, protein and carbohydrates. Besides, high-quality agricultural land is not required for the cultivation of microalgae.

Biorefining of microalgae means a value chain to obtain biofuels, energy and high-value products by means of biomass transformation and process equipment. For this purpose, up-stream processes regarding the microalgae cultivation, photobioreactor design, valuable components extraction have been performed in Karlsruhe institute of technology (KIT). For down-stream processing of microalgae biomass, hydrothermal liquefaction (HTL) is considered as one of the most suitable thermochemical techniques. HTL is conducted in water at temperature between 250 ℃ and 370 ℃ and, high pressures between 10 and 25 MPa, utilizing the special properties of water at these conditions, microalgae biomass is converted into an organic and an aqueous liquid phase as well as solids as by-product. The biocrude as main product with a similar energy value to fossil petroleum can be used as renewable feedstock for upgrading to fuel components or co-processed in fossil-based refineries. The gas and the aqueous product are recyclable for further microalgae cultivation and the solid product exhibits the potential to be used as biofertilizer. Therefore, together with other research groups at KIT, a complete biorefinery of microalgae route has been proposed with HTL as the core down-stream technology, facilitating co-production of valuable chemicals from extraction and bioenergy.

However, microalgae biofuel production via HTL is still lacking of commercial competiveness against fossil fuels. To better understand the existing obstacles, the state of art in microalgae biofuel production is firstly presented, including algae strain selection, cultivation, harvesting, pretreatment, conversion by HTL, biocrude upgrading and recovery of the aqueous product. The stated development and technical obstacles in these major steps will be the motivation of this thesis.

The aim of this thesis is to investigate the optimization possibilities in the down-stream production via HTL as the core conversion technique within the microalgae biorefinery

concept. Specifically, three key processing steps have been investigated in depth in this thesis. A techno-economic assessment is also performed for the full process.

- Microalgae pretreatment for efficient, valuables extraction before HTL processing and residual biomass HTL behavior.
- Product separation in a continuous HTL process and recovery of nutrients from aqueous product.
- Catalytic upgrading of biocrude from continuous HTL production.
- Techno-economic assessment of the overall process.

The efficient extraction of valuable products from microalgae and utilization of the residual biomass for biofuel production are expected to bring economic benefits to the microalgae biorefineries. Pulsed electric field (PEF) treatment has been proposed as a promising pretreatment for microalgae wet extraction. A combination of PEF assisted valuables extraction from microalgae and HTL of the residual biomass is investigated for the first time. The microalgae strains *Auxenochlorella protothecoides*, *Chlorella vulgaris*, and *Scenedesmus almeriensis* were cultivated, harvested, treated by PEF, and then subjected to lipid extraction, protein extraction or extraction of amino acids after enzymatic protein hydrolysis, respectively. The residual biomass obtained from PEF treated and PEF-assisted valuables extraction were subjected to HTL in micro-autoclaves at a temperature of 350 ℃ and a pressure of 25 MPa for 15 min holding time. Product yields and analytical results obtained by ultimate analysis, ^{1}H-nuclear magnetic resonance spectroscopy, Fourier-transform infrared spectroscopy, and gel permeation chromatography show that PEF alone has no significant direct influence on microalgae HTL. In this case, the harsh HTL conditions play a dominating role on the product yields and biocrude quality. However, PEF enhances lipid extraction yield from 4 wt.% to 33 wt.%. Accordingly, biocrude yield decreases from 58 wt.% to 43.2 wt.%. Besides, PEF also boosts protein extraction yield from almost zero to 41.6 wt.% of the total protein content, resulting in an increased biocrude yield of about 2 wt.%. Finally, PEF accelerates the formation of amino acids by enzymatic hydrolysis, improving the extraction efficiency up to 150 % in the first 60 min of the extraction. The extracted residue promises to produce 6 wt.% higher biocrude yield and better quality biocrude with lower nitrogen content. For all these cases, overall mass balances of PEF-assisted valuables extraction and HTL are presented.

Apart from the pretreatment step, continuous hydrothermal liquefaction (cHTL) is also an essential step for microalgae biofuel industrialization. Recycling of the aqueous product (AP) from a cHTL process could contribute to better economics of the microalgae biorefinery. The product separation procedure plays a key role regarding the property of organic biocrude and the composition of the aqueous phase. However, the commonly used separation solvent dichloromethane (DCM) seems to be unfavorable for industrial application. A study is presented regarding the processing of microalgae *Chlorella vulgaris* in a continuous stirred tank reactor at different temperatures (300, 325, 350, 375 and 400 ℃) and at 24 MPa for 15 min holding time. The effect of using DCM in a cHTL product separation procedure is investigated in terms of product yield, biocrude and solid quality as well as cHTL-AP composition. Subsequently, cHTL-AP with purification treatment is evaluated for algae cultivation. Results of cHTL product yields and elemental analysis suggest that 350 ℃ is the optimal temperature for cHTL operation, leading to the highest biocrude plus solid yield and an average of 9 wt.% higher biocrude plus solid yield was achieved by using DCM in cHTL product separation. Analysis of AP by total organic carbon and total nitrogen content, high performance liquid chromatography, inductively coupled plasma optical emission spectroscopy show that at conversion temperatures of 350 and 375 ℃, more nitrogen containing component and other ions were retained in AP. Activated carbon absorption treatment was found to remove undesired toxic components effectively and brought more K, Mg, Na into AP compared to the standard medium, resulting in a better algae growth.

Biocrude obtained from HTL is usually not suited for direct fuel applications because of their high viscosity and undesired hetero-atoms like nitrogen. To make microalgae biocrude from cHTL applicable for fuel applications in combustion engine, a further upgrading step of algae biocrude is required. Therefore, the catalytic upgrading of microalgae raw biocrude produced from continuous HTL is studied. Two strains of microalgae were used for being processed in a continuous stirred tank reactor at 350 ℃ and 24 MPa for 15 min residence time. An average of 36.2 wt.% and 31.5 wt.% biocrude yields were achieved for *Chlorella vulgaris* and *Nannochloropsis gaditana*, respectively. The obtained biocrude was then upgraded by hydrotreating using commercial $NiMo/Al_2O_3$ and NiW/Al_2O_3 catalysts at two temperatures (250 ℃ and 400 ℃) in a batch autoclave reactor for 4 hours. Product distribution, elemental analysis, gas chromatography, gel permeation chromatography, thermogravimetric analysis and nuclear magnetic resonance spectroscopy on upgrading products indicate that upgrading

by both catalysts lead to improved physicochemical fuel properties. During upgrading at 250 ℃, decarbonylation, decarboxylation and repolymerization are the dominant reactions while hydrodeoxygenation and cracking reactions are more promoted at 400 ℃. The gasoline, kerosene and diesel oil fractions in the algae biocrude were increased from 18 wt.% to more than 30 wt.% after catalytic upgrading.

According to the results obtained from previous chapters, a preliminary techno-economic assessment is conducted, based on a microalgae biorefinery pilot plant with an output of 0.5 MW in the form of HTL biocrude. To investigate the impact of PEF treatment and fractionation of valuables on the capital costs of the overall process, three scenarios have been compared. The results indicate, as expected, that the use of microalgae only for the biofuel production is not economically feasible, leading to an annual net loss of 2.615 M€. However, a fractionation step to produce amino acids would significantly reduce the final minimum fuel selling price (MFSP) by over 50%, and the implementation of PEF treatment further brings the MFSP of microalgae biofuel of 0.78 € kg^{-1} closer to a competitive level compared to fossil crude oil (0.37 € kg^{-1}). Assuming that selling microalgae biofuel is possible at the same price of fossil crude oil, the minimum market price of the high-value amino-acid product as biofertilizer is supposed to be 7.43 € L^{-1} for a positive return of capital.

The research questions of the thesis are answered also with drawing conclusions based on the experimental results achieved from the previous chapters. Besides, recommendations and guidelines for future studies have been proposed.

Zusammenfassung

In den letzten Jahrzenten rücken Mikroalgen immer mehr in den Fokus der Forschung. Ihre schnelle Wachstumsrate und die Möglichkeit der Anreicherung verschiedener Biomaterialen wie Lipide, Proteine und Kohlenhydrate machen sie zu einem vielversprechenden Ausgangsmaterial für die Herstellung von Biokraftstoffen. Zudem stellt ihre Kultivierung keine hohen Ansprüche an die landwirtschaftliche Fläche.

Unter der Bioraffinierung von Mikroalgen versteht man eine Wertschöpfungskette zur Gewinnung von Biokraftstoffen, Energie und weiteren hochwertigen Produkten mittels Biomassekonversionstechnologien. Zu diesem Zweck wurden im Karlsruher Institut für Technologie (KIT) eine Reihe von Vorbehandlungsprozessen durchgeführt. Diese beziehen sich weitestgehend auf die Mikroalgenkultivierung, das Design von Photobioreaktoren und die Extraktion wertvoller Komponenten. Die hydrothermale Verflüssigung (HTL) wird für die Weiterverarbeitung der Mikroalgenbiomasse als eine der am besten geeigneten thermochemischen Techniken angesehen. Die Reaktion findet in Wasser bei einer Temperatur zwischen 250 °C und 370 °C und hohen Drücken zwischen 10 und 25 MPa statt. Unter diesen Bedingungen werden die Mikroalgen in eine wässrige flüssige, feste und gasförmige Phase, umgewandelt, wobei die organische flüssige Phase als Biocrude bezeichnet wird. Dieses Hauptprodukt hat einen ähnlichen Energiewert wie fossiles Erdöl und kann direkt zu Kraftstoffkomponenten aufgearbeitet werden oder in Erdölraffinerien zur gemeinsamen Verarbeitung mit Rohöl verwendet werden. Das Gas und das wässrige Produkt können zur weiteren Kultivierung von Mikroalgen recycelt werden, der Feststoff ist ein potenzieller Dünger. Es wurde zusammen mit anderen Forschergruppen am KIT eine vollständige Bioraffinerie auf Mikroalgenbasis mit der HTL als Kernkonversionstechnologie vorgeschlagen, die die gemeinsame Produktion wertvoller Chemikalien aus Extraktion sowie einem Biocrude für Kraftstoffanwendungen ermöglicht.

Bei der Herstellung von Mikroalgen-Biokraftstoffen mittels HTL mangelt es immer noch an wirtschaftlicher Wettbewerbsfähigkeit gegenüber der Nutzung fossiler Brennstoffe. Um die bestehenden Hindernisse besser zu verstehen, wird in dieser Arbeit zunächst der Stand der Technik bei der Herstellung von Mikroalgen-Biokraftstoffen vorgestellt, einschließlich der Auswahl von geeigneten Algenstämmen, deren Kultivierung und Ernte sowie die Vorbehandlung, die Umwandlung durch HTL, sowie dem katalytischen Upgrading der erhaltenen Biocrudes und der Nutzung des wässrigen Nebenprodukts.

Ziel dieser Arbeit ist es, die identifizierten Hemmnisse zu überwinden, daher werden Optimierungsmöglichkeiten in der Downstream-Produktion durch HTL als Kernumwandlungstechnik im Rahmen des Mikroalgen-Bioraffinerie-Konzepts untersucht. Dies betrifft im Wesentlichen die drei in der Folge erwähnten Verarbeitungsschritte sowie eine technisch-ökonomische Bewertung der gesamten Prozesskette.

- Mikroalgenvorbehandlung zur effizienten Wertstoffgewinnung vor der HTL-Konversion und die Biocrude Erzeugung mittels HTL aus der Restbiomasse.
- Produkttrennung in einem kontinuierlichen HTL-Prozess und Rückgewinnung von Nährstoffen aus wässrigen Produkten.
- Katalytische Aufwertung von Biokraftstoffen aus der kontinuierlichen HTL-Produktion.
- Techno-ökonomische Bewertung des Gesamtprozesses.

Die effiziente Gewinnung von Wertprodukten aus Mikroalgen und die Nutzung der Restbiomasse zur Produktion von Biokraftstoffen sind die gemeinsame die Grundlage für eine ökonomisch sinnvolle Bioraffinerie für Mikroalgen. Die Behandlung mit gepulsten elektrischen Feldern (PEF) wurde als vielversprechende Vorbehandlung für die Nassextraktion von Mikroalgen vorgeschlagen. Erstmals wird eine Kombination aus PEF-gestützter Wertstoffgewinnung aus Mikroalgen und HTL der Restbiomasse untersucht. Die Mikroalgenstämme *Auxenochlorella protothecoides*, *Chlorella vulgaris* und *Scenedesmus almeriensis* werden kultiviert, geerntet, mit PEF behandelt und anschließend einer Lipidextraktion, Proteinextraktion oder Extraktion von Aminosäuren nach enzymatischer Proteinhydrolyse unterzogen. Die aus der PEF-unterstützten Wertstoffextraktion erhaltene Restbiomasse wird in Mikroautoklaven bei einer Temperatur von 350 °C und einem Druck von 25 MPa für 15 min Haltezeit einer HTL unterzogen. Produktausbeuten und Analyseergebnisse aus der Elementaranalyse, ^{1}H-Kernresonanzspektroskopie, Fourier-Transform-Infrarotspektroskopie und Gelpermeationschromatographie zeigen, dass PEF allein keinen signifikanten Einfluss auf die HTL von Mikroalgen hat. In diesem Fall spielen die harschen HTL-Bedingungen eine dominierende Rolle für die Produktausbeute und die Qualität der Bioprodukte. PEF erhöht jedoch die Lipidextraktionsausbeute von 4 Gew.-% auf 33 Gew.-%. Dementsprechend sinkt die Ausbeute von Bio-Rohöl von 58 Gew.-% auf 43.2 Gew.-%. Außerdem steigert PEF auch die Ausbeute der Proteinextraktion von nahezu Null auf 41.6 Gew.-% des Gesamtproteingehalts und führt zu einer erhöhten Bio-Rohölausbeute von etwa 2 Gew.-%. Darüber hinaus beschleunigt PEF die Bildung von Aminosäuren durch

enzymatische Hydrolyse und verbessert die Extraktionseffizienz in den ersten 60 Minuten der Extraktion um bis zu 150%. Der extrahierte Rückstand führt einer um 6 Gew.-% höheren Bio-Rohölausbeute mit besserer Qualität durch einen geringerem Stickstoffgehalt. Für all diese Fälle werden die Gesamtmassenbilanzen der PEF-gestützten Wertstoffgewinnung und der HTL dargestellt.

Neben der Vorbehandlung ist die kontinuierliche hydrothermale Verflüssigung (cHTL) ein wesentlicher Schritt für die Anwendung von Mikroalgen-Biokraftstoffen. Das Recycling der wässrigen Produktphase (AP) aus einem cHTL-Prozess wird zu einer besseren Wirtschaftlichkeit der Mikroalgen-Bioraffinerie beitragen. Das Produkttrennungsverfahren spielt eine Schlüsselrolle in Bezug auf die Eigenschaft von organischem Biocrude und der Zusammensetzung der wässrigen Phase. Allerdings scheint das üblicherweise verwendete Extraktionslösungsmittel Dichlormethan (DCM) für die industrielle Anwendung nachteilig. Eine Studie zur Verarbeitung von Mikroalgen *Chlorella vulgaris* in einem kontinuierlichen Rührkesselreaktor bei verschiedenen Temperaturen (300, 325, 350, 375 und 400 °C) und bei 24 MPa für 15 min Haltezeit wird vorgestellt. Der Effekt der Verwendung von DCM in einem cHTL-Produkttrennungsverfahren wird in Bezug auf Produktausbeute, Qualität von Biocrude und Feststoff sowie die cHTL-AP Zusammensetzung untersucht. Anschließend wird cHTL-AP mittels Aktivkohle gereinigt und für die Algenkultivierung bewertet. Die Ergebnisse der cHTL-Produktausbeuten und der Elementaranalyse zeigen, dass 350 °C die optimale Temperatur für den cHTL-Betrieb ist. Diese führt zu der höchsten Biocrude- und Feststoffausbeute bei einer Verweilzeit von 15 min. Durch die Verwendung von DCM bei der cHTL Phasentrennung wird eine durchschnittlich 9 Gew.-% höhere Ausbeute von Biocrude und Feststoff erzielt. Der Gesamtgehalt an organischem Kohlenstoff und Gesamtstickstoffgehalt, sowie die Hochleistungsflüssigchromatographie und die induktiv gekoppelte optische Plasmaemissionsspektroskopie zeigen, dass bei Umwandlungstemperaturen von 350 und 375 °C mehr stickstoffhaltige und andere Ionen in AP vorhanden sind. Es wurde festgestellt, dass die Aktivkohle-Absorptionsbehandlung unerwünschte toxische Komponenten wirksam entfernt und mehr K, Mg, Na in AP im Vergleich zum Standardmedium bewirkt, was zu einem besseren Algenwachstum führt.

Aus HTL gewonnenes Biocrude ist aufgrund seiner hohen Viskosität und unerwünschter Heteroatome wie Stickstoff normalerweise nicht für Direktkraftstoffanwendungen geeignet. Um Mikroalgen-Biocrude aus cHTL für Kraftstoffanwendungen in Verbrennungsmotoren geeignet zu machen, ist ein weiterer Schritt

zur Qualitätsverbesserung für das Algen-Biocrude erforderlich. Daher wird das katalytische Upgrading von Mikroalgen-Rohbiocrude untersucht, die mittels kontinuierlicher HTL hergestellt wird. Zwei Mikroalgenstämme werden in einem kontinuierlichen Rührkesselreaktor bei 350 °C und 24 MPa für 15 Minuten Verweilzeit umgesetzt. Für *Chlorella vulgaris* und *Nannochloropsis gaditana* wurden durchschnittlich 36.2 Gew.-% bzw. 31.5 Gew.-% Ausbeute an Biocrude erzielt. Das erhaltene Biocrude wurde dann durch Hydrotreating unter Verwendung der kommerziellen $NiMo/Al_2O_3$ und NiW/Al_2O_3 Katalysatoren bei zwei Temperaturen (250 °C und 400 °C) in einem diskontinuierlichen Autoklavenreaktor für 4 Stunden veredelt. Produktverteilung, Elementaranalyse, Gaschromatographie, Gelpermeationschromatographie, thermogravimetrische Analyse und Kernresonanzspektroskopie an Upgradingsprodukten zeigen, dass das Upgrading mit beiden Katalysatoren zu verbesserten physikalisch-chemischen Kraftstoffeigenschaften führt. Während des Upgrades bei 250 °C sind Decarbonylierung, Decarboxylierung und Repolymerisation die vorherrschenden Reaktionen, jedoch treten Hydrodesoxygenierungs- und Crackreaktionen bei 400 °C stärker auf. Die Benzin, Kerosin und Dieselölfraktionen in der Algen Biocrude wurden nach dem katalytischen Upgrading von 18 Gew.-% auf mehr als 30 Gew.-% erhöht.

Basierend auf einer Mikroalgen-Bioraffinerie-Pilotanlage mit einer Leistung von 0.5 MW in Form von HTL-Biocrude, wird eine vorläufige technoökonomische Bewertung durchgeführt. Um die Auswirkungen der PEF-Behandlung und der Fraktionierung von Wertstoffe auf die Kapitalkosten des Gesamtprozesses zu untersuchen, wurden drei Szenarien verglichen. Die Ergebnisse deuten erwartungsgemäß darauf hin, dass der alleinige Einsatz von Mikroalgen zur Herstellung von Biokraftstoffen wirtschaftlich nicht vertretbar ist und zu einem Jahresfehlbetrag von 2.615 Mio. € führt. Ein Fraktionierungsschritt zur Herstellung von Aminosäuren würde jedoch den endgültigen Mindestverkaufspreis für Kraftstoffe (MFSP) um mehr als 50% senken. Eine PEF-Behandlung senkt den MFSP für Mikroalgen-Biokraftstoff auf 0.78 € kg^{-1} und liegt damit näher an fossilem Rohöl (0.37 € kg^{-1}). Unter der Annahme, dass der Verkauf von Mikroalgen-Biokraftstoffen zum gleichen Preis wie von fossilem Rohöl möglich ist, beträgt der Mindestmarktpreis für eine positive Kapitalrendite für das Aminosäureprodukt als Biodünger 7.43 € L^{-1}.

Die Forschungsfragen der Dissertation werden mit Schlussfolgerungen beantwortet, die auf den experimentellen Ergebnissen der vorangegangenen Kapitel basieren. Es werden Empfehlungen und Leitlinien für zukünftige Studien vorgeschlagen.

List of abbreviations

ACA Activated carbon absorption

ANOVA Analysis of variance

AP Aqueous product

Ap Auxenochlorella protothecoides

$CDCL_3$ Deuterated chloroform

cHTL Continuous hydrothermal liquefaction

CSTR Continuous stirred tank reactor

Cv Chlorella vulgaris

DCM Dichloromethane

DSC Differential scanning calorimetry

EA Elemental analysis

EPA Environmental protection agency

EU European union

FAME Fatty acid methyl esters

FC Fixed costs

FH Flash hydrolysis

FID Flame ionization detector

FPA Flat panel airlift

FTIR Fourier-transform infrared spectroscopy

GC Gas chromatography

GC-MS Gas chromatography-mass spectrometry

GHG Greenhouse gas

GPC Gel permeation chromatography

HHV Higher heating value

HPLC High-performance liquid chromatography

HTL Hydrothermal liquefaction

ICP-OES Inductively coupled plasma optical emission spectroscopy

KIT Karlsruhe institute of technology

LLG Lab logistics group

MCM Mobil composition of matter

MFSP Minimum fuel selling price

NMR Nuclear magnetic resonance

OD optical density test

PC Pulsed electric field treatment costs

PEF Pulsed electric field

RED Renewable Energy Directive

Sa Scenedesmus almeriensis

SAG Culture collection of algae at Göttingen university

SCWG Supercritical water gasification

SEQHTL Sequential hydrothermal liquefaction

TAGs Triacylglycerides

TAN Total acid number

TC Total carbon

TCD Thermal conductivity detector

TEA Techno-economic analysis

TGA Thermogravimetric analysis

TIC Total inorganic carbon

TMS Tetramethylsilane

TN_b Total nitrogen bound

TOC Total organic carbon

VC Variable costs

XRF X-ray fluorescence

ZSM-5 Zeolite Socony Mobil-5

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Chapter 1. Microalgae biofuel production via hydrothermal liquefaction

1. Microalgae biofuel production via hydrothermal liquefaction

1.1. Introduction

Fossil fuels are the essence of modern civilization. The energy produced from fossil fuels for transportation and electricity has played a vital role in improving human living conditions and promoting advanced technological development over the last one and half-century. In 2018, the global primary energy consumption has reached 13.9 BTon oil equivalent and it grew at a rate of 2.9%, the fastest since 2010. Meanwhile, the carbon emissions grew by 2.0%, also rising at the highest rate during the last seven years (BP Statistical Review of World Energy 2019), reaching a maximum CO_2 concentration of 420 ppm in the atmosphere. It is reasonable to assume that the world is on an unsustainable path: the longer carbon emissions are allowed to rise, the harder and more costly will be the eventual adjustment to net-zero carbon emissions. The concerns about global warming, population growth, oil depletion, and environmental issues have urged a great motivation for research on clean and sustainable energy supplies (Stephens, Ross et al. 2010).

Renewable energy plays a significant role in meeting these challenges. In 2018, the renewable energy grew by 14.5%, accounted for the second-largest increment for energy growth. With the implementation of the Renewable Energy Directive (RED II) – the European Union (EU) is required to generate at least 32% of its energy from renewable sources by 2030. And by 2050, 92% of the EU ́s electricity and nearly half the electricity in the world are expected to come from renewable energy. Therefore, it is believed that renewable energy has an increasingly important influence on human energy consumption in the future.

Together with other types of renewable energy such as wind, solar, hydroelectric, geothermal energy and nuclear, biofuels are also one alternative to reduce dependence on fossil fuels and greenhouse gas (GHG) emissions, promoting the defossilization of transportation fuels and increasing the security of energy supply. Biofuels are fuels produced through contemporary processes from biomass, rather than fuels produced by the very long-winded geological processes involved in the formation of fossil fuels. If the biomass used in the production can regrow quickly, the fuel can be generally considered to be unlimited and

CO_2 neutral to large extent. Apart from their sustainability, biofuels also hold advantages such as (1) their environmental impacts are smaller than that of fossil fuels, reducing GHG emissions; (2) biofuels are adaptable to existing diesel engine designs and perform well in most conditions; (3) they can reduce dependence on oil imports and enhance the local economic security; (4) biofuels also create jobs and have a positive economic impact. Overall, biofuels exhibit great potential in solving current energy and environmental problems.

Amongst various types of biomass, microalgae, as one of the most ancient life forms on earth, are considered a promising feedstock for biofuel production. This is mainly due to the high biomass productivity of microalgae (Tredici 2014) by their high photosynthesis efficiency (3-8% compared to 0.5%-1.5% for terrestrial plants and up to 10% utilization of the solar energy (Huber, Iborra et al. 2006)) and possible high specific area yields (30-100 times more energy per hectare compared to terrestrial plants (Demirbas 2010)). High-quality agricultural land is not required for microalgae, thus not competing with food (Scott, Davey et al. 2010). They show a high tolerance to live in harsh environments, even applicable in wastewater cultivation (Wang, Min et al. 2010, Guo, Liu et al. 2013, Lee, Oh et al. 2014). Besides, their richness in genetic diversity (estimation about 72500 species all around the world (Guiry 2012)) is crucial for the wide range application (easier to find the suitable strain according to local environment). Depending on microalgae stem, they can contain various commercially useful components, such as lipids (Chisti 2007), proteins, polysaccharides, antioxidants and pigments (Rosello Sastre, Csogor et al. 2007), some species of algae are able to accumulate large amounts of triacylglycerides (TAGs), which is a major feedstock for biodiesel, making them interesting as economical feedstock.

Even though growing microalgae biomass is, in fact, more costly than terrestrial crops, the increasing concern about global warming and sustainable resources for fuels and chemicals make microalgae appear as versatile biological cell factories for biofuel production. It was demonstrated that microalgae appear to be the only source of renewable biodiesel that is capable of meeting the global demand for transport fuels (Chisti 2007). Hence, there is a tremendous expansion in the development in the field of the microalgae biofuel during the last decade (Kröger and Müller-Langer 2014).

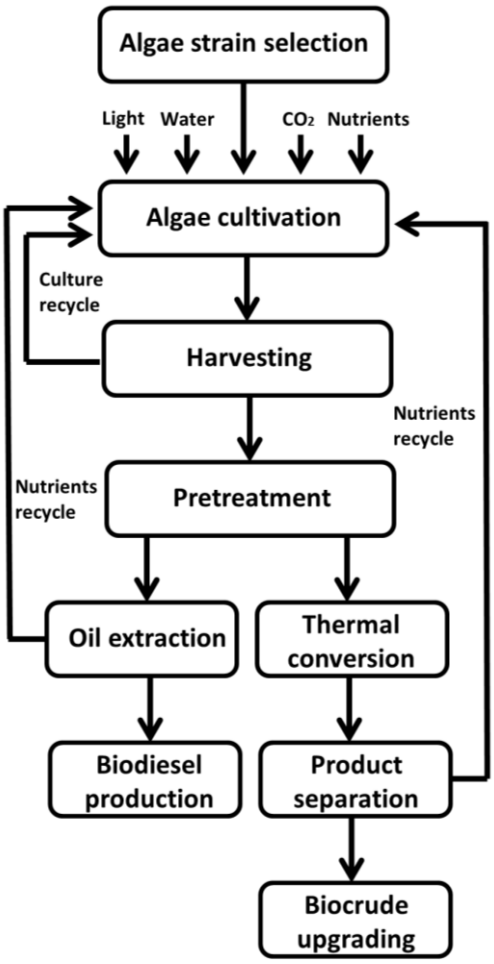

Fig 1.1 Schematic representation of the algae biofuel production processing step (based on (Mata, Martins et al. 2010))

However, until now algae biofuel production is still in its infancy and lack of commercial competitiveness against fossil fuels, the mass production of microalgae biofuel also faces a number of challenges in each processing step.

Fig 1.1 shows a schematic representation of the conventional algae biofuel production key steps, including the selection of suitable microalgae strains, cultivation system and method applied, harvesting techniques, pretreatments (dewatering and cell disruption), oil extraction or thermal conversion, product separation, biocrude intermediate upgrading and recycle of side HTL products. In the next sections, current development and existing problems related to each key processing key step will be presented and discussed.

1.2. Development of microalgae biofuel processing steps

1.2.1. Microalgae strain selection

As the first step for the production of algae biofuel, the selection of algae strain is a key consideration, which can strongly influence the economic viability of the algae biofuel enterprise (Venteris, Wigmosta et al. 2014). Algae are an informal term for a large, diverse group of photosynthetic eukaryotic aquatic organisms. They can be unicellular (microalgae) or multicellular (macroalgae). Microalgae are small in size (1-10 μm), and due to their ancient lineage (about 1.5-2 billion years old), there are more than 72500 species all over the world, whose diversity is much greater than that of land plants. The diversity of microalgae provides a great opportunity but also challenge for the selection, a range of criteria for a suitable biofuel feedstock shall be fulfilled, such as the accumulation of target compounds, high value products, temperature tolerance, photosynthesis capacity, lipid content and quality, and ease

of harvesting (Larkum, Ross et al. 2012). Moreover, the ability to use saline and wastewater is becoming an increasingly important consideration for global production. Fig 1.2 shows the most studied algae strains for biofuels.

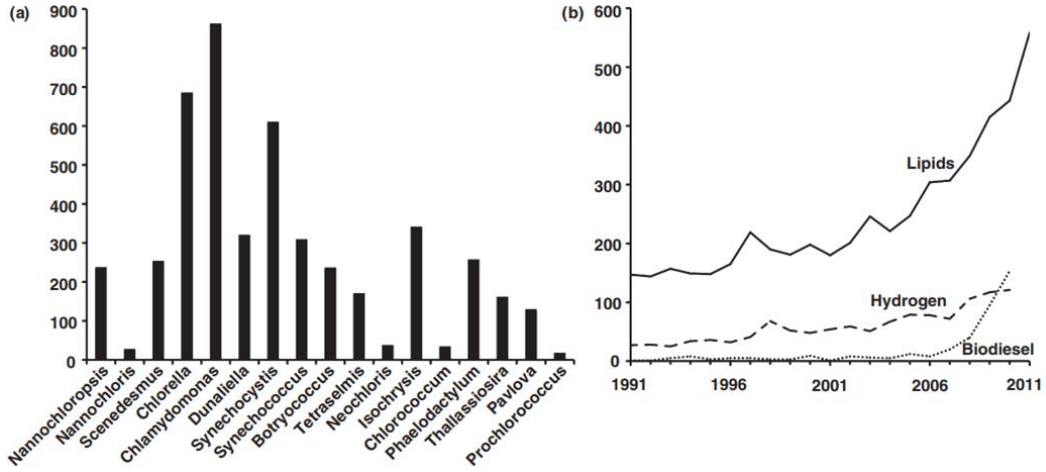

Fig 1.2 Most used algae strain for biofuel purposes from 1991 to 2012 (Larkum, Ross et al. 2012). (a) based on the species (b) publication numbers based on the topics.

Among all the strains, the high lipid content strains are most attractive due to a high concentration of TAGs, which are easily converted to biodiesel (fatty acid methyl esters, FAME), however, a nitrogen starvation condition is usually conducted for accumulating high lipid content, while in such case, the microalgae growth rate will be slowed down, therefore a trade-off and complex balance shall be reached. Besides, with different aquatic, geographical and climatic conditions, different species are supposed to function differently. In addition, the production of high-valued side-stream is becoming a key consideration when selecting strains for microalgae biorefineries. Therefore, unlike other agricultural crops, a targeted selection and domestication of microalgae strains is still in its infancy. Each algae strain still needs careful selection and optimization to increase their lipid productivity and biofuel properties (Duong, Li et al. 2012).

1.2.2. Biomass cultivation

Another important step is the cultivation and biomass production since the growth of algae depends significantly on their cultivation conditions. There are four major types of cultivation conditions for microalgae: photoautotrophic, heterotrophic, mixotrophic and photoheterotrophic, as described as follows:

- Phototrophic cultivation: microalgae only use light as the energy source and inorganic carbon (e.g., carbon dioxide) as the carbon source to form chemical energy through photosynthesis.
- Heterotrophic cultivation: some strains of microalgae are able to grow not only under phototrophic but also use organic carbon when kept in the dark. In this case, organic carbon is used as an energy and carbon source at the same time.
- Mixotrophic cultivation: both organic and inorganic carbon can be used for growth. The algae are able to live under either phototrophic and heterotrophic conditions.
- Photoheterotrophic cultivation: the algae require light as the energy source when using organic compounds as the carbon source.

Heterotrophic growth is believed to provide higher oil productivity, however, the culture of a heterotrophic system can get contaminated easily. Phototrophic cultivation has gained considerable attention because it is easy to scale up with a relatively low operation cost and microalgae are able to convert CO_2 directly from the environment into the oil, making it a carbon-neutral route, while the oil productivity of phototrophic cultivation is usually low. After all, heterotrophic and phototrophic are more promising and more intensive studied for microalgae biofuel applications. A comparison of different cultivation conditions is listed in Table 1.1.

Table 1.1 Comparison of different cultivation conditions (Chen, Yeh et al. 2011).

Cultivation condition	Energy source	Carbon source	Cell density	Reactor scale-up	Cost	Issues associated with scale-up
Phototrophic	Light	Inorganic	Low	Open pond or photobioreactor	Low	Low cell density High condensation cost
Heterotrophic	Organic	Organic	High	Conventional fermentor	Medium	Contamination High equipment cost

Mixotrophic	Light and organic	Inorganic and organic	Medium	Closed photobioreactor	High	Contamination High equipment cost High substrate cost
Photoheterotrophic	Light	Organic	Medium	Closed photobioreactor	High	Contamination High equipment cost High substrate cost

Another issue related to algae cultivation is the culture system. The closed photo-bioreactor systems have the advantages of ability to cultivate under different conditions and prevent contamination. Besides, they have higher areal productivities and avoid loss of water through evaporation, several principles shall be considered such as light saturation and dilution, CO_2-supply and aeration, et al. when designing photo-bioreactors (Posten 2009). Indoor cultivation tend to employ artificial light, while for an open pond system, the sunlight shall be the major energy source. A very dilute algae slurry ranging from 0.05-0.075% dry matter in the open pond systems to 0.3-0.4% for closed systems can be achieved by different types of reactors. However, it is more advisable to trail in larger outdoor cultivation, since more parameters such as irradiation, nutrients, temperature, O_2 and CO_2 content in the culture, pH, salinity, mixing and harvesting techniques in an up-scaling production are of more significance. Careful selection of cultivation parameters for each alga is an important prerequisite to develop economic biofuel production.

1.2.3. Biomass harvesting and dewatering

Generally, the algae harvesting refers to the separation or detachment of algae from its growth medium (Singh and Patidar 2018). Biomass production and harvesting together constitute more than 90% of the total biofuel production cost (Ruiz, Olivieri et al. 2016), of which the fraction referred to harvesting is reported to be at 20%-30% of the total production costs (Barros, Gonçalves et al. 2015). Therefore, the selection of suitable harvesting techniques for different algae strain is another key consideration.

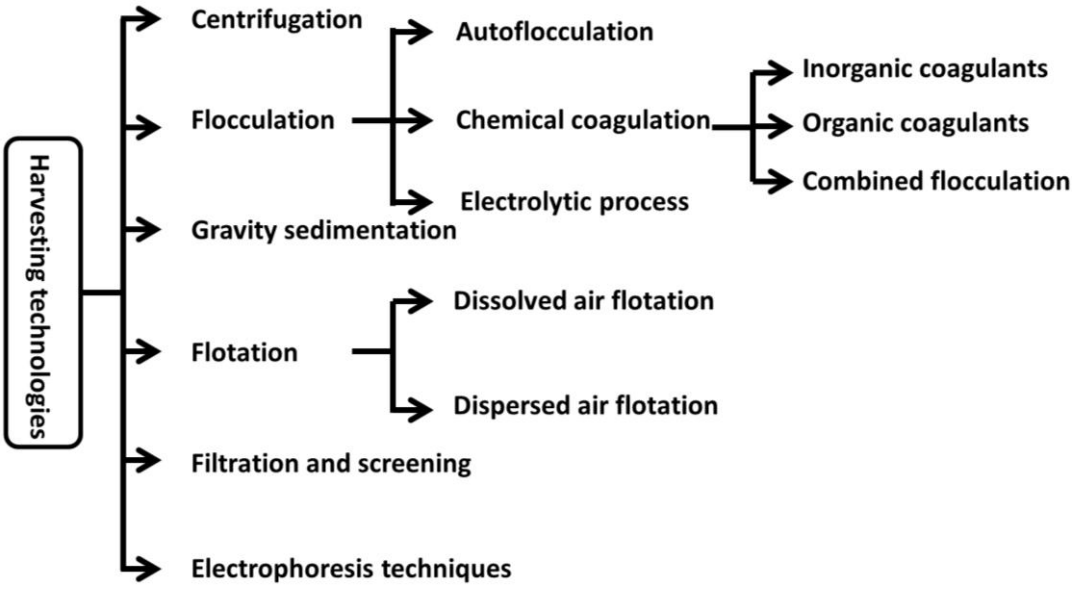

Fig 1.3 Commonly used harvesting techniques for microalgae biomass.

As shown in Fig 1.3, the most common techniques currently applied for the harvesting of microalgae biomass include centrifugation, flocculation, filtration and screening, gravity sedimentation, flotation, and electrophoresis techniques. The selection of the harvesting technique depends very much on the properties of microalgae, such as density, algal cell size or the requirement for the final value product. Some commonly used harvesting techniques are compared in Table 1.2.

Table 1.2 Comparison of different harvesting techniques. (Singh and Patidar 2018)

Harvesting techniques	Advantages	Disadvantages
Coagulation/flocculation	The fast and easy technique Used for large scale Less cell damage Applied to the vast range of species Fewer energy requirements Auto and bioflocculation	Expensive chemicals Highly pH-dependent Difficult to separate the coagulant from harvested biomass Efficiency depends upon the coagulant Culture medium recycling limited Possible microbial contamination

Flotation	Used for large scale Low cost and low space requirement Short operation time	Surfactants needed Ozoflotation is expensive
Electrical processing	Applicable to all microalgal species No chemicals required	Metal electrodes required High energy and equipment costs Metal contamination
Filtration	High recovery efficiency Cost-effective No chemicals required Low energy consumption Low shear stress Water recycles	Slow, requires pressure or vacuum Not suitable for small algae Membrane fouling/clogging and replacement increases operational and maintenance costs High energy consumption
Centrifugation	A fast and effective technique High recovery efficiency Preferred for small scale and laboratory Applicable for all species	Expensive, with high energy requirement High operation and maintenance costs Time-consuming and expensive for large scale Risk of cell destruction

Amongst all, the centrifugation is considered as a common harvesting method for the most types of microalgae. Using a centrifugation force of 500-1000 G, about 80-90% microalgae can be recovered within 2-5 min. Flocculation is a process in which dispersed particles are aggregated together to form large particles for settling. Currently, coagulation and flocculation, centrifugation and filtration are regarded as the most suitable and commonly used methods in microalgae biofuel production. However, there is no single method proved to be effective in harvesting and it is suggested that a combination of methods could result in cost-effective harvesting.

1.2.4. Cell disruption and valuables extraction

In designing a biorefinery, to achieve an optimized balance between product values and energy expenditure for a maximum financial profit is essential (Anastas and Zimmerman

2003). Nowadays, the multi-use of microalgae biomass and obtaining of the high-value product has become serious thinking for economic production. These situations require feedstock pretreatments prior to extraction, such as the concentration of the algae solution and cell disruption to release the intracellular components.

Microalgae cells are small, covered with a relatively thick cell wall. For breaking the cell membrane or cell wall, various mechanical, chemical and biochemical disruption methods are applied, i.e., bead milling, high-pressure homogenization, autoclaving, microwave irradiation, ultrasonication, and enzymatic pretreatment. In general, they can be divided into two main groups, mechanical and non-mechanical methods, as shown in Fig 1.4.

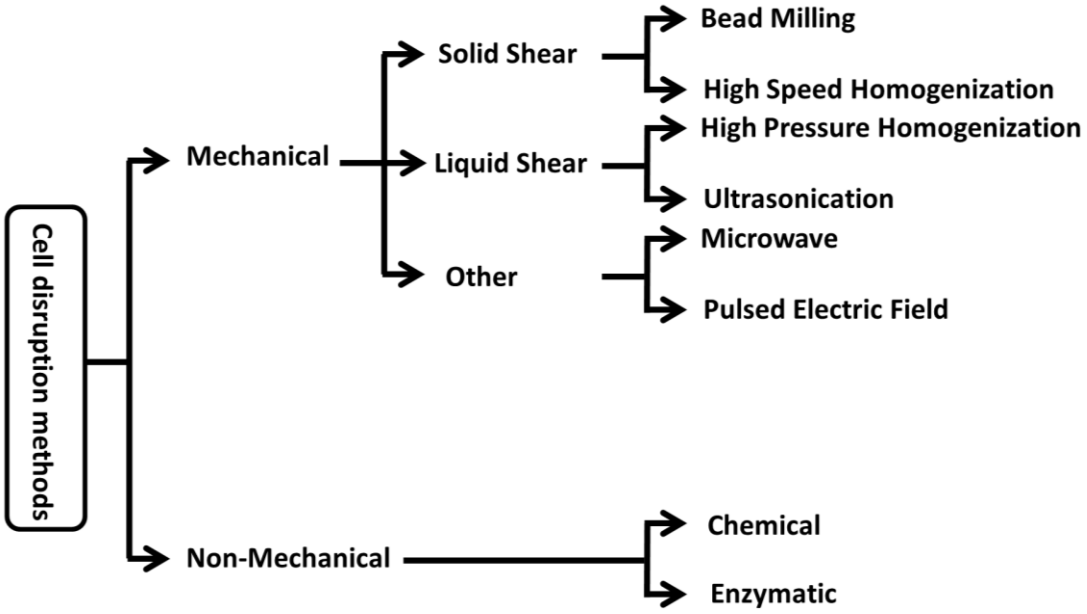

Fig 1.4 The most common cell disruption methods for microalgae. (Gunerken, D'Hondt et al. 2015)

As compared above, the traditional cell disruption methods either make use of heavy machinery, which usually is at the expense of large energy consumption, or involves chemical agents. Besides, the high energy-consuming step often leads to intensive changes to the desired bio components, thus lowering the quality of high-value products. Debris that is generated during the mechanical disruption could be also problematic in later processing. Therefore, it becomes more and more important to extract the intercellular valuables from wet microalgae biomass in a mild and energy-efficient way, which requires more studies.

1.2.5. Conversion to biofuel

As shown in Fig 1.1, the conversion of microalgae to biofuel can be generally divided into two processing routes: (1) lipid extraction and subsequent esterification yield biodiesel of the fatty acid methylether type, plus transesterification (Chisti 2007, Ehimen, Sun et al. 2010); (2) thermochemical conversion of the entire biomass to biocrude. Considering that extracting the algae oil at low cost and at the industrial scale is not yet mature, the second option could provide an economic way into viable and sustainable liquid biofuel. Unlike the route (1), which largely depends on the algae lipid contents, thermochemical route can convert not only lipids but also other organic components (Biller and Ross 2011). The focus of this thesis lays on the thermochemical conversion of whole microalgae.

For the conversion processes, a key element is the water content of algae feedstock, which affects the process conditions greatly, leading to a variety of primary products. The general classification depending on the feedstock state and primary product is given in Table 1.3.

Table 1.3 Thermochemical conversion processes for microalgae (López Barreiro, Prins et al. 2013).

		Feedstock state	
		Wet	Dry
Primary product	Solid	Hydrothermal carbonization	Carbonisation/Torrefaction/Slow pyrolysis
	Liquid	Hydrothermal liquefaction	Fast pyrolysis
	Gas	Hydrothermal gasification	Gasification

Amongst all the conversion processes, hydrothermal liquefaction (HTL) has been considered as a very promising technology (Biller, Sharma et al. 2015, Arvindnarayan, Sivagnana Prabhu et al. 2017). The origins of HTL can be dated back to the 1930s and was initially developed to liquify coal into fuels and it has been used more recently to convert the biomass, agricultural residues, as well as industrial waste into bio-oil.

During HTL process, mostly at temperatures between 250-370 ℃ and 4-25 MPa, the high molecular components in the feedstock will be thermally cracked into smaller fractions

and mainly by the elimination of water and CO_2, compounds with less oxygen are formed, which are less polar and consequently for a separate phase. As an example, phenolic compounds are formed from lignin and from carbohydrates as well. In addition, organic acids and other small and polar compounds are produced, dissolved in water (Kruse and Dahmen 2018). Although pyrolysis oil has some advantages such as lower viscosity, HTL leads to a more competitive product in terms of yield, nitrogen and oxygen content. HTL is also more versatile and can directly convert microalgae into liquid biocrude oil with or without a catalyst, avoiding energy-intensive drying process. Not only lipids but also carbohydrates and proteins can be all converted into biocrude and up to 60 wt.% biocrude yield has been achieved via HTL (Elliott, Biller et al. 2015). Besides, at maximum 85% of the energy in the feedstock can be transferred into products. Additional advantages of the HTL process are relatively low operation temperature and possibly implemented continuous process. Studies on techno-economic analyses and life cycle assessments suggest HTL exhibits a higher economic benefit and lower environmental impact than traditional lipid extraction and transesterification technology (Delrue, Li-Beisson et al. 2013, Liu, Saydah et al. 2013).

However, HTL is still in an early stage of development: the chemical mechanism during HTL reaction is not fully understood and the production costs make this technology not competitive when competing in the fossil dominated commodities market, indicating additional high-value by-product shall be obtained simultaneously with the biofuel product. Meanwhile, investigation of HTL in a continuous mode is necessary for its industrial scale-up, however most of the current studies on microalgae HTL were only conducted in batch reactors, so maintaining at laboratory level. For further process development, there is a lack of continuous processing data, which limits its further industrial production investigation. Table 1.4 summarizes the recent research progress on microalgae continuous HTL (cHTL). Although several groups have made the first exploration on different types of reactors and conversion conditions, research on cHTL of microalgae is still scarce (less than 10 over the last 5 years) and various challenges remain to create competitive commercial cHTL. The general focus of the microalgae continuous studies is on the conversion conditions and biocrude yield, to achieve the highest yields is undoubtedly important for the whole process performance, however, an optimized conditions of cHTL concerns not only biocrude but also the characterization and usage of aqueous phase, which requires further investigation.

Table 1.4 Recent studies on microalgae continuous HTL.

Groups	Algae strains	Reactor types	Temperature	Residence time or capacity	Maximal yields	Main findings related to continuous HTL	Discussion of nitrogen or oxygen
(Jazrawi, Biller et al. 2013)	Chlorella Spirulina (1-10 wt.%)	Pilot Plant	250-350 ℃	3-5 min	41.7 wt.%	Maximal yield may be obtained under shorter residence time under continuous flow hydrothermal processing than batch studies have suggested.	An increase in nitrogen content at higher processing temperature, indicating an increase in the production of biocrude from protein faction. High nitrogen content constitutes a problem for direct usability as a fuel.
(Patel and Hellgardt 2015)	Nannochloropsis	Plug flow reactor	300-380 ℃	0.5-4 min	38 wt.%	Higher biocrude yields may be achieved at even higher temperatures and shorter residence times.	Biocrude from HTL of algae offers better elemental ratios compared to lignin-based fuels, the oxygen and nitrogen content is still problematic.
(Barreiro, Gómez et al. 2015)	Nannochloropsis Scenedesmus (9.1 and 18.2 wt.%)	Continuous stirred-tank reactor	350 ℃	15 min	54.8 ± 3.4 wt.%	Nitrogen and carbon content in biocrude increased at high biomass loading, as well as the HHV, higher loading leads to higher carbon content, 15 min lead to comparable yields.	Higher biomass loadings are positive for deoxygenation and negative for denitrogenation.
(Biller, Sharma et al. 2015)	Chlorella	Continuous flow hydrothermal processing	350 ℃	1.4 and 5.8 min	40 wt.%	HHV of biocrude was approximately 35 MJ/kg, however, the nitrogen content of 6% and oxygen content of 11% render it unsuitable for direct combustion.	The majority of oxygen is shown to be associated with high molecular weight material and can be reduced with further following solvent extraction of the oils while the nitrogen content could only be

							reduced slightly.
(Prapaiwatcharapan, Sunphorka et al. 2015)	Coelastrum	Semi-continuous system	280-360 ℃	2 hour	About 45 wt.%	Using a semi-continuous process, the two steps sequential HTL provided a higher total biocrude yield, less solid residue.	A 20 MPa operating pressure was selected for the second THTL step since it gave the highest biocrude yield and the lowest nitrogen content.
(Mørup, Becker et al. 2015)	General biomass	Continuous reactor system for biomass HTL	350 ℃	15 min	38.9± 3.2 wt.%	A comprehensive description of construction and commissioning of a continuous reactor system for hydrothermal liquefaction of biomass.	The nitrogen content keeps increasing over the course of the experiment, it could be a catalytic effect of the SS316 reactor wall or the ash layer that slowly covers the wall.
(He, Liang et al. 2016)	Oedogonium (2-5 wt%)	Continuous flow pilot-scale reactor (90L/h)	300-350 ℃	3-5 min	25 wt.%	Co-solvent HTL offers a simple and effective means of fractionating HTL biocrude on the basis of polarity.	Solvent oil produced with n-heptane had significantly reduced levels of nitrogen (1.1 wt.%) and oxygen (12.5 wt.%) and possessed relatively low viscosity.
(Wagner, Le et al. 2017)	Scenedesmus Chlorella (5 wt.%)	Continuous flow system (3-7 ml/min)	300-340 ℃	3-7 mL min^{-1}	21.9 wt%	By combining high heating rates with extended reaction times, the continuous system was able to yield significantly enhanced biocrude yields compared to the batch system.	The extended residence time in the continuous flow reactor allowed more denitrogenation reactions to take place than that of the batch.
(Anastasakis, Biller et al. 2018)	Spriulina platensis (16.4 wt.%)	Pilot Plant	350 ℃	100 L h^{-1}	32.9 wt.%	The yields appear to increase with reactor run time. Bio-crude from HTL of Spirulina was mainly composed of palmitic acid, glycerol, heptadecane, and linolelaidic acid.	The oxygen contents in the present study are relatively high, compared to other continuous HTL studies and this could be due to the high heating rate and low residence times employed.

1.2.6. Biocrude upgrading

The other pronounced issue concerning the production of algae biofuel is its further upgrading: the obtained high viscous HTL algal raw biocrude usually has dark brown color and a characteristic odor, containing various oxygenated compounds, carboxylic acids, alcohols, unstable aldehydes, esters, ketones, phenols, guaiacols (Xiu and Shahbazi 2012) and condensed structures like naphthols and benzofurans (Elliott, Biller et al. 2015). It contains significant amounts of nitrogen, oxygen (10-20 wt.%) and sulfur, rendering its high acidic value, worse blending capability and lowers heating value, which is undesirable for storage and transport. The high viscosity (10-20 times more than that of diesel fuel (Bahadar and Bilal Khan 2013)), makes pumping and handling in either industrial settings and direct fuel application difficult. All the aforementioned reasons beckon a further upgrading step. Recent publications on algal biocrude upgrading are presented in Table 1.5. It can be noted that various types of catalyst have been studied, particularly nickel-based catalysts have been widely applied in this area, and in a recent study (Haider, Castello et al. 2018), $NiMo/Al_2O_3$ was reported to reduce 100 % O and 60 % N, reaching 1.80 H/C and a maximum higher heating value (HHV) of 44.38 MJ kg^{-1}. NiW catalyst was also reported as an outstanding hydrodesulfurization catalyst in the petroleum industry (Fang, Ma et al. 2017), which has not yet been investigated for algal biocrude upgrading. In general, hydrodeoxygenation and cracking are the two major routes proposed for biocrude upgrading (Mortensen, Grunwaldt et al. 2011). For such purpose, the application of nickel catalysts is quite common in oil refineries (Ramirez, Brown et al. 2015). Therefore the selection of nickel-based catalyst, processing route and oil performance seem to be a promising research direction.

Table 1.5 Studies on microalgae biocrude catalytic upgrading.

Groups	Applied catalysts	Temperature	Residence time	Pressure	Maximal yields or fuel property improvement	Main findings related to upgrading
(Li and Savage 2013)	HZSM-5	400-500 ℃	0.5-4 hour	4.35 MPa	High yield (75 wt.%) at 400 ℃ with HZSM-5	Reaction temperature had a larger effect on the treated oil composition and gas yield than did the reaction time or the catalyst loading.
(Bai, Duan et al. 2014)	Pt/C,Pd/C,Ru/C,Pt/C, Mo_2C,MoS_2,alumina, CoMo/c-Al_2O_3, Ni/SiO_2–Al_2O_3, HZSM-5, activated carbon, and Raney-Ni	400 ℃	4 hour	6 MPa	Ru/C-Raney-Ni presents the highest oil yield of 77.2 wt.% and the energy yield of 86%.	Compared to catalytic upgrading in n-hexane, upgrading in the presence of water could more effectively control coke formation. Gas production during hydrothermal treatment was insensitive to the catalyst type and seemed to be thermally controlled.
(Biller, Sharma et al. 2015)	CoMo/Al_2O_3 and NiMo/Al_2O_3	350 ℃ and 405 ℃	2 hour	13.8 MPa	Nitrogen content was typically reduced by 60% at 405 ℃ whereas oxygen content was reduced by 85%.	The majority of oxygen is shown to be associated with high molecular weight material and can be reduced further following solvent extraction of the oils while the nitrogen content could only be reduced slightly.
(Duan, Wang et al. 2015)	Ru/C+Mo_2C	400 ℃	4 hour	6 MPa	51.5 wt.% of upgraded oil yield was achieved from crude HTL oil.	All of the upgraded bio-oils have a larger fraction boiling below 350 ℃ than their corresponding crude bio-oils.

(Wang, Adhikari et al. 2016)	Activated carbon-supported Pt, Ru, Ni and Co	350 ℃	4 hour	6.8 MPa	Hydrotreating with all the catalysts increased higher heating value while reducing viscosity and total acid number (TAN) of the biocrude.	Catalyzed hydrotreatment produced a liquid product which showed increased heating value, reduced TAN, viscosity and water content compared to the untreated biocrude.
(López Barreiro, Gómez et al. 2016)	Pt/Al_2O_3 HZSM-5	400 ℃	4 hour	4-9 MPa	About maximum 60 wt.% for both tested strains	Catalysts were not exhibiting a high activity for upgrading under the experimental configuration applied. Many of the effects were caused by the temperature applied rather than by the catalysts.
(Bian, Zhang et al. 2017)	Fe_2O_3/MCM-41	320-350 ℃	2 hour	Not mentioned	Methyl palmitate conversion to 56% and decarboxylation selectivity to 62%	Fe_2O_3/MCM-41 catalyst should improve the pentadecane selectivity of palmitic acid decarboxylation.
(Hosseinpour, Golzary et al. 2017)	H^+ZSM-5	250-500 ℃	1 hour	Not mentioned	Maximum nitrogen removal of 75% at 400 ℃	In the presence of H^+ZSM-5, a higher temperature is more favorable to aromatization than cracking which makes hydrodenitrogenation more difficult. In the absence of a catalyst, H/C ratio showed no substantial trend with temperature which demonstrates the significance of H+ZSM-5 catalyst for cracking.
(Patel, Arcelus-Arrillaga et al. 2017)	Pt/Al_2O_3,Pd/Al_2O_3,Ru/Al_2O_3 and $NiMo/Al_2O_3$	400 ℃	1 hour	7.5 MPa	60 wt.% via $NiMo/Al_2O_3$	Upgraded Biocrude yield of over 60 wt% was achieved with the highest yield gained via $NiMo/Al_2O_3$ catalyzed reaction.
(Xu and Savage 2018)	Pt/C, Ru/C and Mo_2C	400 ℃	1 hour	2.5 MPa	Pt/C provided C (80.9 wt%) and H (10.6 wt%) and heating value (41.6 MJ kg^{-1}), and the lowest N (2.47 wt%), S (0.26 wt%), and O contents (4.8 wt%).	Upgrading of water-soluble biocrude showed that supercritical water treatment, even without catalyst, could be used to effect significant positive changes in the biocrude composition.

(He, Xu et al. 2018)	Pt/C, Pd/C, Ru/C, Pt/C + Pd/C, and CoNiMoW/γ-Al_2O_3	400 ℃	4 hour	3.4 MPa	55.1 wt.%	Both Pt/C and CoNiMoW/γ-Al_2O_3 perform well in the converting high-boiling-point macromolecules into smaller molecular compounds in water-soluble biocrude upgrading.
(Haider, Castello et al. 2018)	NiMo/Al_2O_3	250-400 ℃	2-4 hour	4-8 MPa	Deoxygenation degree of 100% was achieved at 375 ℃, 7 MPa initial H_2 and 3h residence time.	Up to 350 ◦C, the degree of deoxygenation is mainly driven by temperature, whereas the degree of denitrogenation also relies on initial H_2 pressure and temperature-pressure interaction.

1.2.7. Recovery and utilization of HTL aqueous phase

HTL generates different types of products. By utilizing the hot, pressurized water environment, organics in the algae biomass undergo various reactions including hydrolysis, decarboxylation, dehydration, depolymerization and repolymerization, et al. During the reaction, this complex network of reactions generates a four-phase mixture: a biocrude with higher carbon and energy density compared to the feedstock, which can be used as blend fuel after a further upgrading step; a solid residue with potential in fertilizer production; a gaseous product mainly composed of CO_2 and a handful of CO and alkane; and an aqueous product (AP) solution containing organics (alcohols, acids and phenols et al.) and essential nutrients (N, P and K) dissolved.

The AP contains approximately 30 %-60 % of the feedstock carbon and more than 50 % of the nitrogen. Therefore, it has been suggested that biocrude production and HTL wastewater treatment through algae cultivation could be integrated. By this, HTL-AP could be recovered as the water and nutrients for algae cultivation. The effective utilization of the organic-rich AP provides a promising economic benefit for algae biorefinery (Biller, Ross et al. 2012, Du, Hu et al. 2012, Garcia Alba, Torri et al. 2013). Table 1.6 presents recent studies (in the last 5 years) about recovery of nutrients from microalgae HTL-AP.

Table 1.6 Recent studies about the recovery of nutrients from microalgae HTL-AP.

Groups	Algae strains	HTL conditions	Separation and pretreatment of AP	Main findings in nutrients recovery	Main finding related to HTL or cultivation
(Gai, Zhang et al. 2015)	Chlorella. pyrenoidosa (15–35 wt.% loading)	Batch reactor 260–300 ℃, holding time 30–90 min	Filtration (No solvent)	The concentration of total nitrogen gradually increased with the increment of reaction temperature at shorter retention time, while a reverse trend was observed at a longer time. The total nitrogen concentration increased with the increment of retention time at lower temperatures, whereas it decreased slightly when prolonging the retention time at higher temperatures.	GC–MS indicated that the AP contained a higher concentration of organic acids and lower concentration of N&O- heterocyclic compounds when higher energy recoveries were obtained at optimized operating conditions (280 ℃, 60 min, 35 wt.% and 300 ℃, 60 min, 25 wt.%).
(Fushimi, Kakimura et al. 2016)	F. solaris (1 wt.% loading)	autoclave HTL reactor 320 ℃ Residence time 0, 10, 20, 30, 40 and 60 min	Filtration (No solvent)	0.5 wt.% activated carbon significantly enhances total nitrogen recovery in the aqueous phase in HTL. The larger amount of activated carbon does not have a positive effect on total nitrogen recovery.	It is considered the enhancement of total nitrogen recovery is due to i) chemisorption with the organic functional groups on activated carbon surfaces, ii) physisorption of N-containing ions within the pores of activated carbon, or iii) catalytic effect of activated carbon on thermal decomposition of microalgae in HTL.
(Caporgno, Clavero et al. 2016)	Nannochloropsis oculata (20 wt.% loading)	Batch reactor 300 ℃ 10 MPa, holding time 30 min	DCM	The nitrogen content was approximately 7000 mg N/L, and around 68% of the TN was in the form of NH_4^+.	Recycling the aqueous phase generated during HTL to the microalgae cultivation unit reduces the amount of fertilizers for cultivation. The high nutrient content in the aqueous phase is problematic for water discharge; thus, the microalgae cultivation can contribute to accomplishing the regulation for water discharge.
(Shanmugam, Adhikari et al. 2017)	Nannochloropsis sp. (15wt.% loading)	320 ℃ holding time 30 min	Activated carbon pretreatment	The removal of PO_4^{3-} was found to be greater than 99%. The residual PO_4^{3-} concentrations ranged from 2.56 ± 0.11 mg/L to 9.46 ± 0.07 mg/L. On the other hand, NH_4^+-N removal varied between 40 ± 8 to 100 ± 3%.	Both struvite and methane can be produced from bio-oil aqueous phase

(Chen, Zhu et al. 2017)	C. sorokiniana, C. vulgaris and G. sulphuraria. (10 wt.% loading)	Sequential HTL (bomb type reactor) 160 ℃ 0.75-0.79 MPa holding time 20 min	Filtration (No solvent)	C. sorokiniana and C. vulgaris exhibited the ability to hydrolyze polysaccharides, using 77% and 64% of the polysaccharides and removing 94% to 95% of the phosphate, respectively. G. sulphuraria on the other hand, could not use the polysaccharides.	The findings indicate that although being species-dependent, it is possible to reuse the nutrients recovered from SEQHTL of algal biomass for algal culture
(Jin, Oh et al. 2017)	Chlorella sp. KR1 (10 wt.% loading)	high-pressure batch-type reactor 150−300 °C holding time 1h	DCM	The total N content gradually increased with increasing temperature. The P concentration slowly increased with HTL temperature up to 200 ℃ and then decreased at higher temperatures.	In the aqueous phase, carbohydrates could be extracted at mild temperatures (<200 ℃), mainly as polysaccharides. At high temperatures above 200 ℃, however, organic products that are toxic to subsequent bioprocesses (e.g., acetone and phenols) are generated with the consumption of carbohydrates.
(Teymouri, Kumar et al. 2017)	Nannochloropsis gaditana (20.56 wt.% loading)	flash hydrolysis (FH) 280 ℃, holding time 9s	Centrifugation and filtration (No solvent)	The results from elemental composition and XRF spectroscopy analysis also confirmed that FH on Nannochloropsis gaditana efficiently extracted most of the macronutrients such as nitrogen and phosphorous (between 50 and 60 wt %),	The liquid hydrolysate, rich in nutrients, was tested for direct nutrients recycling for algae cultivation. Results show that N. gaditana was able to grow in this medium, even though the release of inorganic ammonium from amino-acids and peptides is necessary.

As presented in Table 1.6, it is noteworthy that most of the recovery studies were performed in batch HTL. Besides, organic separation solvent dichloromethane (DCM) is most frequently used, since it recovers biocrude with the highest yield/energy efficiency and has the lowest energy consumption ratio, compared to other solvents such as acetone or toluene (Watson, Lu et al. 2019). However, organic solvents are expected to not only affect the properties of biocrude (organics initially presented in the aqueous transfer into biocrude phase, resulting in a rise of the biocrude yield at the expense of higher oxygen and nitrogen content (Xu and Savage 2014, López Barreiro, Riede et al. 2015)) but also undermine the reuse ability of AP. The remaining organic solvent in the AP could be harmful for further algae cultivation.

The usage of organic solvent in the commercial production is environmentally-unfriendly. Besides, the effect of organic solvent on the product yields has been investigated in batch experiments to deliver realistic and meaningful data for industrial-scale (Xu and Savage 2014, López Barreiro, Riede et al. 2015), but to which extent, this effect will impact in a continuous procedure is still unknown. The studies of recovery methods for an economically and environmentally viable biocrude extraction applied to continuous HTL is, therefore, very relevant to an industrial implementation that intends to make use of the aqueous phase as the growth medium. The understanding of DCM effect and recycle of aqueous phase from a cHTL process is of great interest for the economics of microalgae biorefinery.

Chapter 2. Aims and outline of the thesis

2. Aims and outline of the thesis

2.1. Aims of the thesis

As discussed in chapter 1, there is still a substantial lack of knowledge regarding the chemistry mechanism during HTL, technical upscaling problems and the practical gap between laboratory data and industrial production in each processing steps, preventing the commercial production of microalgae biofuel. At KIT, microalgae biorefinery concepts are being investigated, as outlined in Fig 2.1. There are research groups investigating the up-stream steps such as algae cultivation (Wagner, Braun et al. 2016, Gille, Trautmann et al. 2019, Wild, Trautmann et al. 2019), nutritional value as animal feed (Wild, Trautmann et al. 2019), algae-derived bioenergy (Klassen, Blifernez-Klassen et al. 2017), and extraction of valuables (Silve, Kian et al. 2018, Akaberi, Gusbeth et al. 2019, Scherer, Krust et al. 2019). Complementary to the activities of other research groups at KIT, the aim of the thesis is to investigate the optimization possibilities in the down-stream production via HTL as the core conversion technique within the microalgae biorefinery concept.

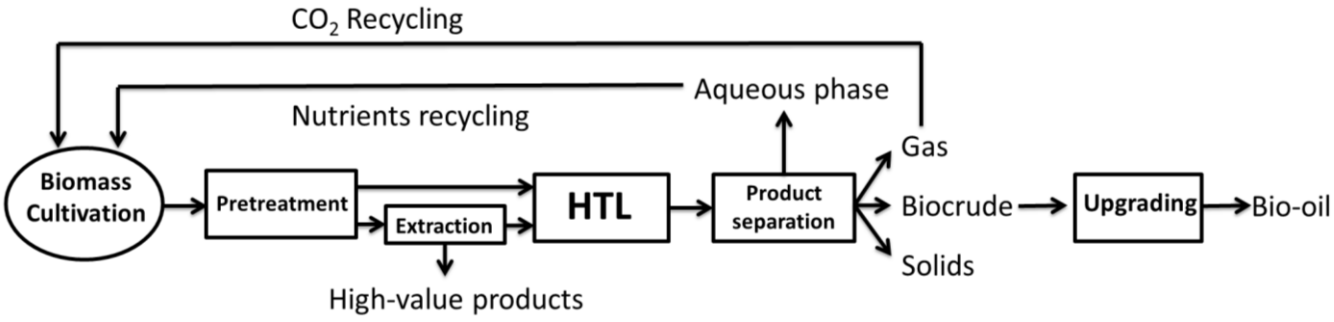

Fig 2.1 Simplified flowchart of the processing steps investigated in the thesis.

Specifically, three processing steps and an overall techno-economic assessment, together with respective research questions are investigated in depth in this thesis.

- Combining pre-treatment and HTL conversion. How can pre-treatment for valuables extraction and HTL for biofuel production be combined efficiently? How to extract valuables from wet microalgae biomass energy-efficiently? How is the HTL behavior of the residual biomass?

- Continuous HTL and product separation step. How is the HTL behavior of microalgae in a continuous mode? How to reuse cHTL-AP for algae cultivation? What is the impact of DCM on a continuous HTL product separation?
- Upgrading step. How is the cHTL and upgrading behavior of different strains microalgae? What are the possibilities to upgrade cHTL biocrude? Which types of reactions take place during the upgrading step?
- Techno-economic assessment. Is it economically viable to realize such a processing chain? What is the recommendation for the commercialization of microalgae biofuel production?

2.2. Outline of the thesis

The outline of the thesis is designed as follows:

Chapter 1 presents a review of current development and existing problems in the microalgae biofuel production pathway, including biomass cultivation, harvesting, pretreatment, conversion methods, product separation, oil further upgrading and recovery of HTL aqueous product. An overview of these steps is presented as the background and motivation of this thesis.

Chapter 2 introduces the motivation and research questions of this thesis and presents an outline of the experiments designed and aims of each chapter.

Chapter 3 investigates an novel cell disruption method, pulsed electric field treatment (PEF treatment), as a pretreatment facilitating valuables extraction (lipid, protein or amino acids) from three types of microalgae (*Auxenochlorella protothecoides*, *Chlorella vulgaris*, and *Scenedesmus almeriensis*) and their residual biomass HTL behavior at 350 ℃, 25 MPa and 15 min in micro autoclave reactor. This work was studied for a potential combination of PEF treatment and HTL technique for more economic utilization of microalgae biomass.

Chapter 4 studies the separation step of HTL products in a continuous system at different temperatures (from 300 ℃ to 400 ℃) with different methods (with/without dichloromethane) and the recycling of aqueous phase for microalgae cultivation. This work focuses on what role of the organic solvent plays on the product separation in the concept of a continuous mode, for delivering the realistic data from laboratory to industrial production. Moreover, to evaluate how good the aqueous phase from continuous HTL can be used for

microalgae cultivation, which could have a strong economic benefit in the overall microalgae biorefinery.

Chapter 5 presents a further study following chapter 4, about the catalytic upgrading of the HTL biocrude produced in a continuous stirred tank reactor. Two strains of microalgae (*Chlorella vulgaris* and *Nannochloropsis gaditana*) were first processed via continuous HTL (at 350 ℃, 24 MPa for 15 min holding time), and then the obtained biocrude was upgraded by hydrotreating using two types of catalysts ($NiMo/Al_2O_3$ and NiW/Al_2O_3) at 250 ℃ and 400 ℃. The upgrading possibilities and possible chemical reactions are investigated.

Chapter 6 uses the information collected from the above studies to modify a techno-economic assessment of the microalgae biorefinery. The economic impact of PEF treatment and extraction of valuables are evaluated.

Chapter 7 presents the conclusions of this thesis. Based on the experimental data, the research questions of this thesis are answered and guideline for future study of microalgae biofuel commercialization is proposed.

Chapter 3. Hydrothermal liquefaction of residual microalgae biomass after pulsed electric field-assisted valuables extraction

Chapter redrafted after:

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Declaration of contributions

HTL experiments and analysis: Bingfeng Guo and Boda Yang.

Analysis, interpretation of the data and draft of manuscript: Bingfeng Guo and Boda Yang.

Lipid extraction experiment: Aude Silve and Ioannis Papachristou.

Protein extraction experiment: Daniel Scherer.

Enzymatic protein-amino acids extraction experiment: Sahar Akaberi.

Critical revision of the article: Ursel Hornung, Wolfgang Frey, Nicolaus Dahmen.

3. Hydrothermal liquefaction of residual microalgae biomass after pulsed electric field-assisted valuables extraction

3.1. Introduction

As stated in chapter 1, in recent years, microalgae have gained plenty of attention as biofuel feedstock (Chisti 2007, Brennan and Owende 2010, Guo, Liu et al. 2013). However, after spending several years of research on microalgae biofuels, their high production cost still remains the ultimate bottleneck for industrialization. The traditional transesterification of algal lipids requires lipid-rich strains and biomass costs should not exceed \$0.25 kg^{-1} for the process to be economically viable (Wagner, Perin et al. 2017). However, the price of algae biodiesel (\$2.76 kg^{-1}) is still much higher than fossil diesel (\$0.95 kg^{-1}) (Chen, Qiu et al. 2015). All steps of the process, such as microalgae strain selection, cultivation, harvesting, dewatering, extraction, and oil production, are costly and energy-intensive, making it less commercially competitive compared to traditional fuels and other types of biofuels.

Different processing routes promise higher added value (López Barreiro, Samorì et al. 2014), for example, by extraction of high-value compounds for applications, such as fertilizers, feed and food, pharmaceuticals, and subsequent use of the residue fractions to produce fuel (Chiaramonti, Prussi et al. 2017). From the technical point of view, the "dry" extraction route to obtain natural products is more mature and commonly applied. For water-based biomass like microalgae, however, wet extraction appears to be more suitable and economical. As a necessary pre-treatment step, as explained in chapter 1, section 1.2.4, a cell disruption process is conducted to perforate or even break the cell membranes, because most of the valuable products from the microalgae are intracellular, protected by the cell wall membranes (Carullo, Abera et al. 2018). Traditional disruption methods, such as high-pressure homogenization (Onumaegbu, Alaswad et al. 2018), bead milling (Postma, Miron et al. 2015), stream treatment, and chemical or biological treatment, however, usually are energy-intensive or harmful to target products (Alhattab, Kermanshahi-Pour et al. 2018), increasing costs and lowering the revenue of the process.

Compared to the above cell disruption methods, the pulsed electric field (PEF) treatment appears to be a promising alternative for microalgae valuables wet extraction

(Goettel, Eing et al. 2013, Zbinden, Sturm et al. 2013, Silve, Papachristou et al. 2018). Theoretically, permeabilization of the algal cell membranes can be induced when the cell is exposed to high-intensity electric field pulses and, consequently, the membrane loses its barrier function and becomes permeable (Goettel, Eing et al. 2013), thus enhancing the spontaneous release of intracellular, small, water-soluble components. Most importantly, PEF treatment does not require drying of the biomass and it entails several technical advantages for microalgae processing (Goettel, Eing et al. 2013, Postma, Pataro et al. 2016, Lam, Postma et al. 2017). For example, the relatively mild operation conditions (electric pulse duration of 1-100 µs at an electric field intensity ranging from 0.1–40 kV cm^{-1}, resulting in enhanced trans-membrane transport through the membrane pores (Arianna Ricci 2018) rather than a complete breakdown of the whole cell structure that is caused by traditional mechanical cell disruption techniques) and low temperature increase (around 15 °C (Scherer, Krust et al. 2019)) prevent undesired changes of the target product and any cell debris. Additionally, the continuous processing mode facilitates upscaling to industrial production. Moreover, PEF treatment shows high energy efficiency: with only 1 MJ kg^{-1}_{DW} of energy input, sufficient cell permeabilization can be induced. Further reduction of the energy demand is possible with increasing algae concentration (Goettel, Eing et al. 2013). In the case of PEF-assisted lipid extraction, energy consumption can even be reduced down to 0.25 MJ kg^{-1}_{DW} only (Silve, Kian et al. 2018). For the extraction of valuables from microalgae, KIT is currently operating a demonstration facility with a mass flow of up to 1000 L of concentrated microalgae slurry (100 $g_{dry\ mass}\ L^{-1}$) per hour. PEF has reached a technology readiness level of 7/8. However, most microalgae studies regarding PEF treatment focus on the extraction of valuables alone, only few of them consider utilization of the residual biomass after extraction, which obviously also plays a significant role in the overall biorefineries' economics.

When it comes to microalgae conversion, HTL to convert all the biomass components into biofuel (Elliott, Biller et al. 2015) received a lot of attention recently. HTL has been considered a promising post-treatment technology in an algae biorefinery after wet extraction of valuables (Garcia Alba, Torri et al. 2011). Hence, HTL could also have the potential to be combined with a PEF treatment, since both steps aim at avoiding the costly biomass dewatering process and, by this, intend to improve the economic performance of the overall microalgae value chain.

This chapter reports a first systematic investigation of the combination of PEF treatment, valuables extraction, and HTL to the best of our knowledge. PEF is applied as a pre-treatment to fresh microalgae slurry for facilitating extraction of either lipids, proteins, or amino acids after enzymatic hydrolysis. The raw, the PEF-treated, and the extracted valuable microalgae materials are converted into biocrude by HTL and compared in terms of chemical composition and some fuel characteristics. Different options of combining these two well-established microalgae processing techniques are analyzed in order to provide guidelines for more economical microalgae biorefinery concepts.

3.2. Material and methods

3.2.1. Algae strain selection, cultivation, and harvesting

Three microalgae strains, *Auxenochlorella protothecoides* (*A. protothecoides*), *Chlorella vulgaris* (*C. vulgaris*), and *Scenedesmus almeriensis* (*S. almeriensis*), are selected for this work. *A. protothecoides* microalgae are a well-known lipid-rich strain that is rather promising for biofuel production (Heredia-Arroyo, Wei et al. 2010). *C. vulgaris* microalgae is one of the fastest-growing and most studied strains (Chen, Li et al. 2019), containing a high amount of good quality protein (Enyidi 2017), making it suitable for food and feed application. *S. almeriensis* microalgae also produce high protein contents under nitrogen sufficient cultivation. After enzymatic protein hydrolysis, the amino acids produced can be used as biofertilizer. Therefore, *A. protothecoides*, *C. vulgaris* and *S. almeriensis* microalgae have been used for lipid extraction, protein extraction and amino acid extraction after enzymatic hydrolysis, respectively.

Fresh algae are required for an efficient cell membrane permeabilization, therefore all the algae strains were cultivated in this study. *A. protothecoides* microalgae (strain number 211-7a), were obtained from SAG, Culture Collection of algae, Göttingen, Germany. It was first mixotrophically cultivated in 1 L conical flasks (VWR International, Bruchsal, Germany) in a modified Wu medium (170 mM glucose, 5 mM KH_2PO_4, 1.7 mM K_2HPO_4, 1.2 mM $MgSO_4$, 10 µM $FeSO_4$, 1 mM glycin and 4 g L^{-1} of yeast extract) for 5 days. Afterward this culture was used to inoculate in a 25 L photo-bioreactor (PBR), with TP medium (0.02 M TRIS, 0.001 potassium phosphate buffer, 1x TAP salts (Gorman, Levine 1965), 1x Hutner's trace elements (Hutner et al. 1950), pH 7.0) for about 16 days. The detailed cultivation information can be found in Silve et al (Silve, Papachristou et al. 2018). *C. vulgaris*

microalgae (strain number 211-12) were also obtained from SAG. TP medium was used. The PBR was LED (WU-M-500-840, 4000K, Panasonic)-illuminated at 100 µmol m^{-2} s^{-1} at 25 ℃. Aeration of the culture was adjusted at 1000 cm^3 min^{-1}, and the CO_2 flow rate was set to 30 cm^3 min^{-1}. *S. almeriensis* microalgae (strain number 276-24) were kindly provided by F.G. Acien Fernandez, University of Almeria, Spain. Cultivation was performed in Arnon medium (Arnon, McSwain, et al. 1974) with the PBR illuminated at 250 µmol m^{-2} s^{-1}at 25 ℃. Aeration of the culture was adjusted to 5000 cm^3 min^{-1} and CO_2 flow rate to 25 cm^3 min^{-1}. The elemental and bio-component analysis of raw microalgae feedstock in wt.% are given in Table 3.1.

Table 3.1 Elemental and bio-component analysis in wt.% of raw microalgae feedstock.

Strain	C	H	N	S	O*	Protein	Lipid	Carbohydrates	Ash
A.protothecoides	57.8	9.8	2.2	0.6	29.6	27*	39.8	30.9	2.3
C.vulgaris.	46.1	8.4	7.9	0.9	36.5	42	28.8	25.4	5.1
S.almeriensis	48	7.6	6.9	0.5	37	41.5	24	13.7	4.9

* by difference (O=100-C-H-N-S; Protein=100-Lipid-Carbohydrates-Ash)

During the harvesting procedure, microalgae slurries were concentrated to about 100 g kg^{-1} for *A. protothecoides* microalgae, 45 g kg^{-1} for *C. vulgaris* microalgae and 40 g kg^{-1} for *S. almeriensis* microalgae by centrifugation in a swinging bucket rotor (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) at 3200 g for 10 min. In the case of *C. vulgaris* and *S. almeriensis* microalgae, the concentrated slurry was eventually slightly washed with distilled water to reach an electrical conductivity between 1 and 1.5 mS cm^{-1}, in order to achieve the target electric field amplitude of 40 kV cm^{-1} during PEF treatment. In the end, a dry mass test of the algae slurry was performed using an oven at 105 ℃, by following equation (3.1). The obtained dry mass value was used for the calculation of the product yield.

$$Dry\ mass\ (wt.\%) = \frac{Mass\ of\ sample\ tube\ after\ 105°C - Mass\ of\ empty\ sample\ tube}{Mass\ of\ sample\ tube\ before\ 105\ ^\circ\ C - Mass\ of\ sample\ tube\ after\ 105°C} \quad (3.1)$$

3.2.2. Pulsed electric field treatment of microalgae

The fresh concentrated microalgae slurry was immediately treated with PEF. Microalgae slurry was pumped into a continuous self-developed flow treatment chamber as described in Goettel et al. (Goettel, Eing et al. 2013). The flow chamber has a volume of

about 500 µl and consists of two stainless steel electrodes with a diameter of 60 mm paired in parallel. The flow rate of algae slurry was set to 3 ml min^{-1} using a peristaltic pump (MS-4/12-100 ISMATEC, Cole-Parmer GmbH, Germany) (Scherer, Krust et al. 2019). Pulses of 1 µs duration and approximately 150 KJ L^{-1}_{sus} energy were applied to microalgae slurry, corresponding to 1.5 MJ kg^{-1}_{DW}, 3.3 MJ kg^{-1}_{DW} and 3.75 MJ kg^{-1}_{DW} for *A. protothecoides*, *C. vulgaris* and *S. almeriensis* microalgae, respectively. The temperature was increased by less than 15 ℃ and cooling was performed by submerging the sample tube into an ice bath. A detailed description of PEF operation and energy input calculation can be found in a previous study (Silve, Papachristou et al. 2018). After PEF, microalgae samples were stored in ice before valuables extraction procedure.

3.2.3. Valuables extraction and hydrothermal liquefaction sample preparation

For lipid extraction, in brief, a solvent blend of ethanol, water, and hexane (volume ratio: EtOH:H_2O:Hex=1:0.41:0.05) was applied to *A. protothecoides* microalgae for 20 hours overnight with agitation and in the dark. A reference extraction for the determination of the total lipid content was also performed by a Soxhlet device (behrotest® Kompakt-Apparatur KEX 30, Behr LaborTechnik, Germany) using n-hexane as sole solvent. The amount of extracted lipid was determined gravimetrically. The detailed experimental protocol and information about yield calculation can be found in a recent study (Silve, Papachristou et al. 2018). The residual microalgae biomass after extraction was left in the air to evaporate any solvent traces for 24 hours.

Protein extraction was conducted for food and feed applications. By avoiding the usage of organic solvent, after PEF treatment, an equal volume of demineralized water was added to the *C. vulgaris* microalgae slurry and then placed in an incubator at 30 ℃ for 24 hours. Afterward, the cells were separated via centrifugation (10000 g, 10 min) from the supernatant which was then analyzed for the protein yield using a Bio-Rad DC-Assay, a modified Lowry assay. As a comparison for more complete protein extraction, *C. vulgaris* microalgae was also processed by high-pressure homogenization (Avestin Europe GmbH, Germany) at the condition of 2 kbar pressure and 5 passes for each sample. The total protein content of the microalgae was determined by infrared spectroscopy (Direct Detect® Infrared Spectrometer, Merck Chemicals GmbH, Germany).

Enzymatic protein hydrolysis-amino acids extraction was performed on *S. almeriensis* microalgae basically according to Garcia et al (Romero Garcia, Acien Fernandez et al. 2012). Amino acids extraction was performed at 50 ℃ and a pH value of 8.0 (measured by Multi 3510 IDS from Taschenmessgerät® MultiLine, Germany). Two commercial proteases, Alcalase (Subtilisin) 2.5 L (Novozyme, Denmark), and Flavourzyme 1000 L (Novozyme, Denmark) were added at 3 % (v/v) with regard to cell dry weight of the biomass. Each hydrolysis reaction lasted for 180 min, and the rate of hydrolysis was monitored by taking samples every 60 min followed by deactivating the reaction at 80 ℃ for 10 minutes. Detailed information can be found in a recent study published by Akaberi et al (Akaberi, Gusbeth et al. 2019). The amino acids concentrates were obtained from the biomass slurry by centrifugation at 10000 g for 10 min. The content of free amino acids was measured using ortho-phthaldialdehyde IR-spectrophotometric assay using serine as standard. The hydrolysis degree is calculated by the following equation (3.2).

$$Hydrolysis\ degree\ (\%) = \frac{Number\ of\ cleaved\ peptide\ bonds}{Total\ number\ of\ peptide\ bonds} \quad (3.2)$$

In this study, lipid extraction and protein extraction independent experiments were repeated at least three times and enzymatic hydrolysis-amino acids extraction experiments were repeated two times, the mean values of extraction yield are reported. The residual biomass obtained from the various extractions was subjected to HTL. For the preparation of HTL samples, distilled water was added to all the biomass residues to their exact previous volumes they had before extraction and mixed homogeneously. The dry mass of all the extracted samples was again determined using an oven at 105 ℃, and therefore a further analysis of extraction selectivity can be investigated by following equation (3.3).

$$Selectivity\ (\%) = \frac{Dry\ mass_{target\ product}}{Dry\ mass_{extract}} = \frac{Dry\ mass_{target\ product}}{Dry\ mass_{raw\ microalgae} - Dry\ mass_{microalgae\ residue}} \quad (3.3)$$

3.2.4. Hydrothermal liquefaction, product separation, and analysis

HTL of untreated, PEF treated and the extracted microalgae was done in 10 mL micro-autoclave reactor, made of stainless steel EN 1.4571. In brief, 7 g of the feedstock slurry was injected into the reactor using a pipette (Eppendorf Research Plus 5 mL, Germany), and 2.2 MPa of nitrogen gas was preloaded into the reactor to remove the residual air and then the reactor was sealed. A GC oven (Hewlett-Packard 5890 Series II, Agilent, USA) was used for

heating throughout this study, except for HTL experiments with higher heating rates for which a metal heating block was used instead. The heating period lasts for 18 min 33 s to reach target temperature 350 ℃ (about 9 min for the metal heating block), afterward a holding time was maintained before ice-cooling. After opening the autoclave to a gas collection system, the mass of product gas was calculated using the ideal gas equation with the measured pressure inside micro-autoclave and the gas composition was determined by gas chromatography (GC) analysis. Solid, aqueous and biocrude products were separated by vacuum filtration and dichloromethane (DCM, ≥99.7% purity) was used as the separation medium. DCM was added into the autoclave to absorb the biocrude formed. By vacuum filtration, the solid residue remained was separated. The filtrate consisted of the aqueous phase and DCM phase (with biocrude dissolved), showing spontaneous phase separation. The DCM phase was extracted by a syringe and placed under N_2 flow until constant weight was attained, then this is considered as the biocrude product. The detailed description of HTL and product separation procedure can be found in a previous report (López Barreiro, Samorì et al. 2014). The calculation of the product yield is based on the biomass loaded into the reactor (raw microalgae or residues), according to equation (3.4), and the aqueous phase yield is calculated by difference in this study. All the HTL experiments were repeated at least three times and the mean values are reported with the standard deviation. The product yields were also subjected to a one-way analysis of variance (ANOVA) with regard to the PEF effect at a significance level of $\alpha = 0.05$.

$$Yield_i(wt.\%) = \frac{m_i}{m_{microalgae(dry\ mass)}} \quad (3.4)$$

$i - product\ fraction\ (biocrude\ oil\ , gas, and\ solid\ residues)$

Elemental analysis on biocrude was done with a Vario EL cube (Elementar Analysensysteme GmbH, Germany) which operated at 1150 ℃ measuring the content of *C*, *H*, *N*, and *S* directly; the amount of *O* is calculated by difference. The higher-heating-value (HHV) of the biocrude is calculated with Boie's formula (3.5) (*C*, *H*, *O*, *N*, and *S* being the mass weight fractions) and the energy recovery of biocrude is calculated using the obtained HHV as equation (3.6).

$$HHV\left[\frac{MJ}{kg}\right] = 0.3516C + 1.16225H - 0.1109O + 0.0628N + 0.10465S \quad (3.5)$$

$$Energy\ recovery(\%) = \frac{HHV_{biocrude} * m_{biocrude}}{HHV_{feedstock} * m_{feedstock}} \quad (3.6)$$

The GC analysis was conducted using Agilent 1540A and Agilent 7890A, USA; both devices use a flame ionization detector (FID) and a back thermal conductivity detector (TCD) in series and Porapak Q and Molsieve columns, respectively. Proton nuclear magnetic resonance (^{1}H-NMR) measurement for the *C. vulgaris* biocrude was performed using a Bruker AVANCE 250 instrument, USA. For this, about 20 mg of biocrude was fully dissolved into 800 uL methanol-d (99.8 atom.%) with tetramethylsilane (TMS) as internal standard and spectra were acquired at 250 MHz across 32 transients. Fourier-transform infrared spectroscopy (FT-IR) spectra on the *S. almeriensis* biocrude were measured with a FT-IR-Spectrometer (Varian 660-IR, Shimadzu, Japan). A standard KBr-pressing served as background matrix for the scans from wavenumber 400 to 4000 cm^{-1}. Gel permeation chromatography (GPC) was performed for the *S. almeriensis* biocrude using a Merck Hitachi L-6200 Intelligent pump with an LaChrom RI detector (model L-7490) for determining the molecular weight distribution. 10 mg biocrude sample was dissolved in 10 mL tetrahydrofuran (with 0.4 vol.% toluene as internal standard) for the measurement. All the analytical measurements were replicated at least two times and a mean value is reported with the standard deviation.

3.2.5. Design of the experiments

In this study, as shown in Fig 3.1, two types of experiments have been conducted to study the effect of PEF treatment on HTL:

- Route 1 an 2 (in the following section 3.3.1): for the evaluation of whether PEF treatment can directly help treated samples to produce equally good biocrude at lower HTL temperature or shorter holding time, the direct effect of PEF treatment on HTL was investigated mostly using *A. protothecoides* microalgae. For these experiments, untreated and PEF-treated microalgae samples were processed by HTL at different temperatures (250 ℃ and 350 ℃), HTL holding times (5, 10, and 15 min), and HTL heating rates (18.9 ℃ min^{-1} and 37.8 ℃ min^{-1}).
- Routes 3, 4 and 5 (in section 3.3.2): the effect of PEF treatment on extraction and subsequent HTL of the microalgae residues was examined using *A. protothecoides*, *C.*

vulgaris, and *S. almeriensis* microalgae. All these experiments were conducted at 350 ℃ (18.9 ℃ min^{-1} heating rate), 25 MPa, and 15 min holding time.

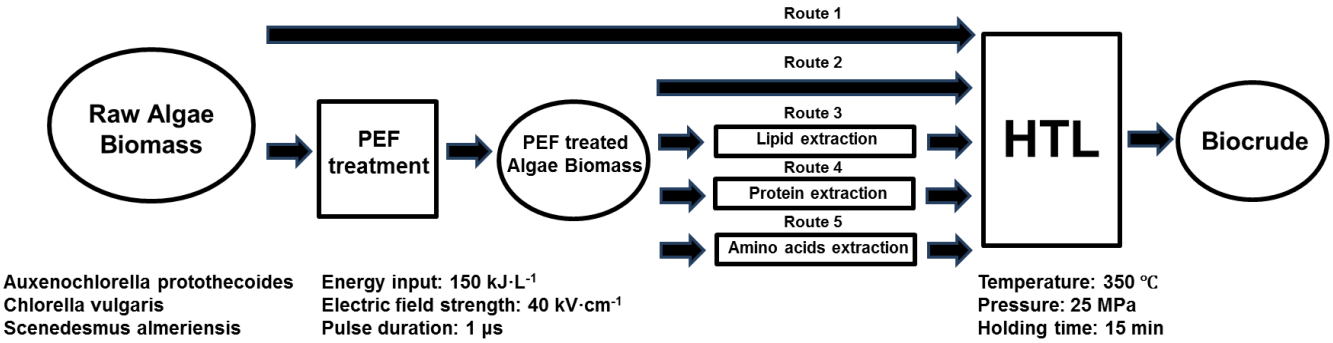

Fig 3.1 Simplified flow sheet of the PEF-HTL experimental procedure.

3.3. Results and discussion

3.3.1. Direct effect of a pulsed electric field treatment on hydrothermal liquefaction of microalgae

3.3.1.1. Hydrothermal liquefaction at different temperatures

Fig 3.2 presents the HTL product yields of untreated and PEF-treated *A. protothecoides* microalgae at 250 ℃ and 350 ℃. In both cases, about 38 wt.% of biocrude were obtained at 250 ℃, the yield of which increased up to 49 wt.% at 350 ℃. This was also observed in other studies: with higher temperature, solids (mostly unreacted microalgae) and aqueous products are increasingly converted into biocrude components, reflecting enhanced cracking reactions that lead to the formation of more non-condensable compounds as well as to the conversion of the intermediate. Increased conversion of water-soluble products into biocrude was also observed (Brilman, Drabik et al. 2017). At both temperatures, however, PEF was found to have no significant effect on the product yields. In contrast to this, the selected temperatures probably have a dominating effect on the HTL reaction.

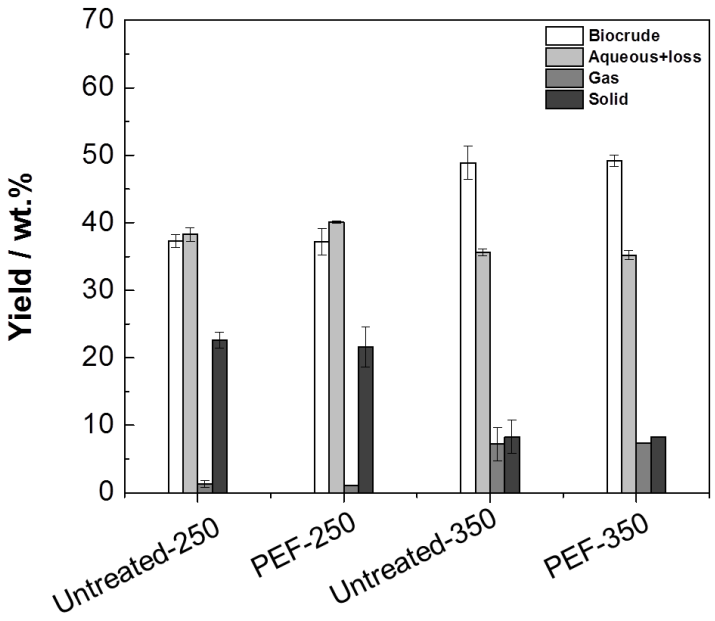

Fig 3.2 HTL product yields of untreated and PEF-treated *A. protothecoides* microalgae produced at 250 ℃ and 350 ℃, 25 MPa, and 15 min holding time. Data are shown as means of four experiments with the standard deviation. (Untreated-250 stands for samples without PEF treatment and HTL at 250 ℃; PEF-250 stands for samples with PEF treatment and HTL at 250 ℃; Untreated-350 refers to samples without PEF treatment and HTL at 350 ℃; PEF-350 denotes samples with PEF treatment and HTL at 350 ℃).

As shown in Table 3.2, the finding above was confirmed by elemental analysis of the biocrude obtained: in general, higher carbon and nitrogen contents were found in 350 ℃ biocrude, indicating a stronger degradation of proteins at 350 ℃, while no significant elemental difference was observed between untreated and PEF-treated biocrude. Another interesting phenomenon observed after PEF treatment was that microalgae suspension turned to a deeper, green color within 24 hours compared to untreated samples, which is possibly due to a total breakdown of the cell membrane. This suggested that membrane permeabilization caused by PEF was not reversible, which was necessary for the effective extraction of intracellular valuables (Goettel, Eing et al. 2013). However, this effect appeared to be irrelevant to the biocrude production in this study.

Table 3.2 Elemental analysis (wt.%) and HHV (MJ kg^{-1}) of untreated and PEF-treated *A. protothecoides* biocrude produced at 250 ℃ and 350 ℃. Data are shown as means of two repeated experiments with standard deviations. (Untreated-250 stands for samples without PEF treatment and HTL at 250 ℃; PEF-250 refers to samples with PEF treatment and HTL at 250 ℃; Untreated-350

denotes samples without PEF treatment and HTL at 350 ℃; PEF-350 stands for samples with PEF treatment and HTL at 350 ℃).

	C	H	N	S	O*	HHV
Untreated-250	73.1±0.25	11.2±0.15	0.4±0.05	0.1±0.0	15.2±0.45	37.07
PEF-250	73.4±0.2	10.7±0.2	0.9±0.15	0.1±0.0	14.9±0.2	36.66
Untreated-350	75.7±0.2	9.7±0.1	2.7±0.1	0.2±0.05	11.7±0.45	36.78
PEF-350	75.9±0.15	10±0.25	2.8±0.0	0.2±0.0	11.1±0.1	37.27

* by difference 100-C-H-N-S

3.3.1.2. Hydrothermal liquefaction at different holding times

Fig 3.3 presents the HTL product yields of untreated and PEF-treated *A. protothecoides* microalgae at holding times of 5, 10, and 15 min. It is clear that with a holding time of 5 min only, more than 60 wt.% biocrude yield can be achieved. Longer holding times promote the conversion of biocrude into gas, solids, and the aqueous phase (likely due to the degradation of cellular components (Faeth and Savage 2016)). At a short holding time, larger solid yields are obtained, which is due to either unreacted material or material formed by hydrothermal carbonization. After a minimum holding time of around 10 min, the solids yields increase slightly again, which is likely due to re-condensation of biocrude components.

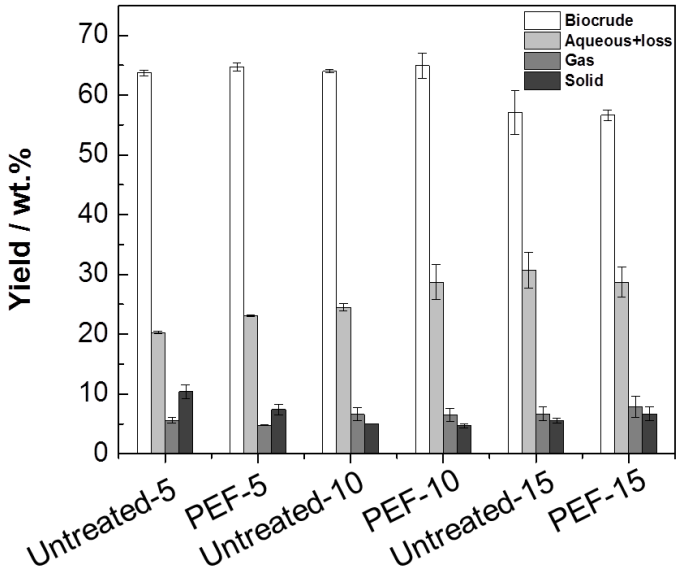

Fig 3.3 HTL product yields of untreated and PEF-treated *A. protothecoides* microalgae produced with holding times of 5, 10, and 15 min (18.9 ℃ min^{-1}, 350 ℃, 25 MPa). Data are shown as means of three experiments with standard deviations. (Untreated-5 stands for samples without PEF treatment and HTL for 5 min; PEF-5 refers samples with PEF treatment and HTL for 5 min; Untreated-10 stands for samples without PEF treatment and HTL for 10 min; PEF-10 denotes samples with PEF treatment and HTL for 10 min; Untreated-15 stands for samples without PEF treatment and HTL for 15 min; PEF-15 means samples with PEF treatment and HTL for 15 min).

It is obvious from Table 3.3 that the C content is slightly higher and the O content drops slightly with increasing holding time. For all samples, however, the C, H, N, S, and O concentrations are relatively constant with averages of 73.8±0.6 wt.%; 11.2±0.1 wt.%; 1.6±0.2 wt.%; 0.45±0.05 wt.%, and 12.75±1.1 wt.%, respectively. This means that the biocrude quality remains practically the same for all conditions investigated. In terms of biocrude yield or elemental composition, no significant difference was observed between untreated and PEF-treated samples.

Table 3.3 Elemental analysis (wt.%) and HHV (MJ kg^{-1}) of *A. protothecoides* biocrude produced (18.9 ℃ min^{-1}, 350 ℃, 25 MPa) with holding times of 5, 10, and 15 min. Data are shown as means of two repeated experiments with standard deviations. (Untreated-5 stands for samples without PEF treatment and HTL for 5 min; PEF-5 denotes samples with PEF treatment and HTL for 5 min; Untreated-10 refers to samples without PEF treatment and HTL for 10 min; PEF-10 means samples with PEF treatment and HTL for 10 min; Untreated-15 stands for samples without PEF treatment and HTL for 15 min; PEF-15 refers to samples with PEF treatment and HTL for 15 min).

	C	**H**	**N**	**S**	**O***	**HHV**
Untreated-5	73.4±0.15	11.2±0.3	1.3±0.1	0.5±0.1	13.6±0.5	37.45
PEF-5	73.3±0.2	11.4±0.1	1.5±0.1	0.4±0.0	13.4±0.2	37.67
Untreated-10	73.3±0.1	11±0.0	1.8±0.05	0.5±0.0	13.4±0.05	37.24
PEF-10	73.8±0.15	11±0.15	1.9±0.0	0.5±0.0	12.8±0.0	37.48
Untreated-15	75.1±0.05	11.3±0.0	1.7±0.05	0.4±0.0	11.5±0.1	38.41
PEF-15	74.3±0.25	11.3±0.3	1.8±0.1	0.4±0.0	12.2±0.15	38.06

* by difference 100-C-H-N-S

3.3.1.3. Hydrothermal liquefaction at higher heating rate

As mentioned in section 3.2.4, the heating period of 18.5 min is relatively long compared to the holding period. To study the influence of the heating period, experiments with a higher heating rate of 37.8 ℃·min^{-1} (in order to shorten the heating period) were conducted.

Fig 3.4 and Table 3.4 show the product yields of HTL at a higher heating rate (37.8 ℃·min^{-1}) for different holding times as well as the results of the elemental analysis of biocrude. At the higher heating rate, the holding time still was not found to have any marked influence on the biocrude quality. Compared to HTL at a lower heating rate, however, the trend to higher nitrogen and sulfur contents implies that an increased heating rate promotes protein degradation and the formation of nitrogen-containing compounds. Similar biocrude yields of around 60 wt.% and elemental compositions for all holding times investigated suggest that the higher heating rate reduces the influence of the holding time. A difference is found for the solids content, which starts at very small amounts. This might be explained by the fact that the faster heating rate decreases the formation of solids by hydrothermal carbonization. Again, hardly any significant difference was observed between untreated and PEF-treated samples at different holding times.

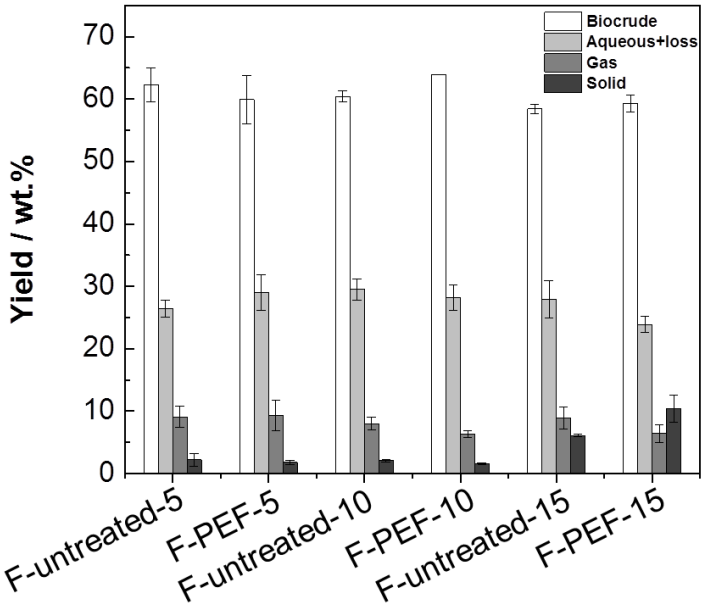

Fig 3.4 Product yields of HTL at a higher heating rate (37.8 ℃·min^{-1}) of *A. protothecoides* with different holding times of 5, 10, and 15 min (at 350 ℃, 25 MPa). Data are shown as means of three experiments with standard deviations. (F-untreated-5 stands for samples without PEF treatment, but

subjected to fast HTL for 5 min; F-PEF-5 refers to samples with PEF treatment and fast HTL for 5 min; F-untreated-10 denotes samples without PEF treatment, but with fast HTL for 10 min; F-PEF-10 means samples with PEF treatment and fast HTL for10 min; F-untreated-15 stands for samples without PEF treatment, but with fast HTL for 15 min; F-PEF-15 refers to samples with PEF treatment and fast HTL for 15 min).

Table 3.4 Elemental analysis (wt.%) and HHV (MJ kg^{-1}) of *A. protothecoides* biocrude produced with holding times of 5,10, and 15 min (37.8 °C min^{-1}, 350 °C, 25 MPa). Data are shown as means of two experiments with standard deviations. For the explanations of sample designations, see caption of Fig 3.4.

	C	H	N	S	O*	HHV
F-untreated-5	76.9±0.25	10.3±0.2	3.2±0.05	0.6±0.0	9±0.0	38.37
F-PEF-5	76.9±0.0	9.8±0.15	3.1±0.0	0.6±0.0	9.5±0.15	37.69
F-untreated-10	76.1±0.35	10.2±0.25	3.2±0.1	0.6±0.0	9.8±0.7	37.78
F-PEF-10	77±0.3	10.3±0.3	3.1±0.1	0.5±0.0	9.1±0.7	38.35
F-untreated-15	77.3±0.1	10.4±0.35	3±0.0	0.55±0.05	8.7±0.5	38.61
F-PEF-15	77.7±0.05	10.4±0.15	2.9±0.05	0.5±0.0	8.4±0.15	38.73

* by difference 100-C-H-N-S

It can be concluded that PEF has no direct influence on the HTL of microalgae, whereas the harsh HTL conditions (temperature and holding time) most probably have a dominating effect on the product yields and biocrude quality.

3.3.2. Effect of pulsed electric field treatment on valuables extraction and hydrothermal liquefaction of residues

3.3.2.1. Lipid extraction

To study the effect of the PEF-assisted extraction of lipids on HTL (Route 3), experiments were conducted on the five following samples: fresh, untreated microalgae, PEF-treated ones, lipid-extracted samples with and without PEF, and Soxhlet-extracted "lipid-free" microalgae. The total lipid content of *A. protothecoides* microalgae was determined to be 39.8±1.5 wt.% of the total biomass. After PEF treatment, about 33.9 wt.% were extracted, while only 4.3 wt.% could be extracted from the untreated sample. This means that PEF increased the lipid extraction efficiency from less than 10% to over 85%.

Fig 3.5 shows HTL product yields of *A. protothecoides* microalgae and residual biomass after lipid extraction. The biocrude yield drops from about 58 wt.% for untreated and PEF-treated microalgae to 51.6 wt.% for untreated lipid-extracted samples and significantly decreases to about 43 wt.% for PEF-assisted lipid-extracted samples. Lipids contribute most to biocrude formation compared to protein and carbohydrates. The reduced lipids proportion in the residue leads to a lower biocrude yield. However, the interaction of protein and carbohydrates, e.g. by the Maillard reaction (reactions between amines in proteins with a carbonyl group in carbohydrates that produce fission and large polymer materials, named melanoidins, in biocrude phase (Peterson, Lachance et al. 2010, Guo, Walter et al. 2019)), was enhanced when lipids were extracted (Teri, Luo et al. 2014). Hence, the residue still produced a relatively good yield of biocrude. At the same time, the solid residue yield was found to increase when more lipid was extracted, which had been reported before (Biller and Ross 2011). A similar pattern was found for the lipid-free sample, which implies that the PEF extraction method had a good selectivity for lipids and PEF treatment did not alter lipid or residue properties.

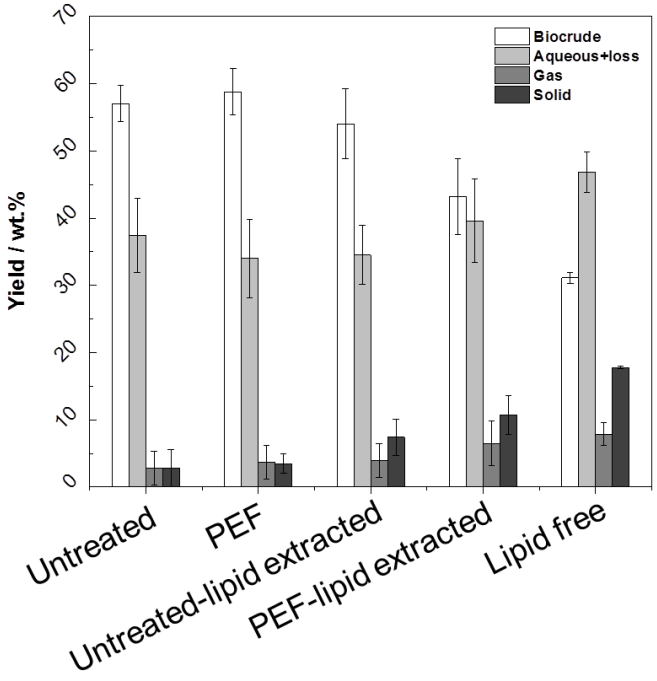

Fig 3.5 HTL product yields of *A. protothecoides* microalgae and lipid-extracted residues (18.9 ℃ min^{-1}, 350 ℃, 25 MPa for 15 min holding time). Data are shown as means of three experiments with standard deviations. (Untreated stands for samples without PEF treatment; PEF denotes samples with

PEF treatment; Untreated-lipid extracted refers to samples without PEF treatment, but with lipid extraction; PEF-lipid extracted stands for samples with PEF treatment and lipid extraction; Lipid-free means samples with completed lipid extraction by a Soxhlet process).

Table 3.5 presents the elemental compositions of the *A. protothecoides* microalgae feedstocks and the biocrudes obtained. With more lipid extracted, the algae residue contains lower carbon and hydrogen concentrations and has a lower HHV, resulting in less carbon and higher nitrogen contents in the biocrude. The energy recovery drops from 77.8% in the untreated algae to 69.1% in the PEF-lipid extracted residue. However, the PEF-lipid extracted residues still exhibit a good HTL ability, with the biocrude obtained having a relatively high HHV of 36.65 MJ kg^{-1}.

Table 3.5 Elemental analysis (wt.%), HHV (MJ kg^{-1}), and energy recovery (%) of untreated, PEF-treated, untreated and PEF-lipid extracted, lipid-free *A. protothecoides* feedstocks, and the corresponding biocrude samples. Data are shown as means of two experiments with standard deviations. (Untreated stands for samples without PEF treatment; PEF means samples with PEF treatment; Untreated-extracted denotes samples without PEF treatment, but with lipid extraction; PEF-extracted refers to samples with PEF treatment and lipid extraction; Lipid-free stands for samples with completed lipid extraction by the Soxhlet method).

	C	**H**	**N**	**S**	**O***	**HHV**	**Energy recovery**
Untreated microalgae	57.8 ±0.25	9.8 ±0.1	2.2 ±0.0	0.6 ±0.05	29.6 ±0.3	28.63	-
PEF treated microalgae	59.7 ±0.45	10 ±0.0	2 ±0.25	0.3 ±0.0	27.9 ±0.7	29.60	-
Untreated-extracted residue	56.9 ±0.35	9.6 ±0.0	2 ±0.0	0.3 ±0.0	31.2 ±0.35	27.86	-
PEF-extracted residue	49.6 ±0.1	8.2 ±0.2	3 ±0.05	0.3 ±0.0	38.9 ±0.35	22.88	-
Lipid-free residue	45.4 ±0.2	7.7 ±0.05	3.4 ±0.0	0.3 ±0.05	43.1 ±0.3	20.37	-
Untreated biocrude	75.4 ±0.2	11.7 ±0.0	1.8 ±0.15	0.5 ±0.05	10.6 ±0.0	39.10	77.8
PEF-treated biocrude	75 ±0.0	11.7 ±0.2	1.7 ±0.0	0.3 ±0.0	11.3 ±0.2	38.85	77.1
Untreated-extracted biocrude	74.6 ±0.45	11.5 ±0.2	1.8 ±0.0	0.3 ±0.1	11.8 ±0.55	38.43	74.5

PEF-extracted biocrude	74.1 ±0.35	10.1 ±0.35	3 ±0.05	0.4 ±0.05	12.4 ±0.8	36.65	69.1
Lipid-free biocrude	72.4 ±1.85	8.9 ±0.25	4 ±0.0	0.6 ±0.1	14.1 ±2.1	34.55	52.8

* by difference 100-C-H-N-S

3.3.2.2. Protein extraction

Four different experiments were performed for investigating PEF-protein extraction-HTL (Route 4) using fresh, untreated microalgae, PEF-treated ones, protein-extracted ones with PEF, and "protein-free" microalgae after extraction by high-pressure homogenization, respectively. The result obtained for protein-free samples shows that *C. vulgaris* microalgae have a total protein content of about 42 wt.% of the total dry biomass. About 17.5 wt.% (related to over 41.6 wt.% of the total protein, which is close to the PEF-protein extraction value recently reported in microalgae *A. platensis* (Jaeschke, Mercali et al. 2019)) could be extracted after PEF treatment, while no protein was extracted from untreated samples. This suggests that PEF is a decisive factor for protein extraction from *C. vulgaris* microalgae. Technically speaking, the traditional protein extraction methods already are quite efficient: up to 76 wt.% of protein recovery after precipitation can be achieved after a corresponding mechanical and chemical pre-treatment. Yet, it requires extreme conditions, such as high pressure of 270 MPa and a pH 12 environment (Ursu, Marcati et al. 2014). These conditions either are too harsh or lower the products' functional properties. PEF protein extraction as applied in this study, by contrast, has the advantage of mild operation conditions suitable for food and feed production as well as a higher protein extraction yield than that of other PEF protein extraction studies (Ursu, Marcati et al. 2014, Postma, Pataro et al. 2016). This is probably due to a higher concentration effect (Safi, Cabas Rodriguez et al. 2017) during extraction.

As shown in Fig 3.6, PEF has no direct influence on the HTL of *C. vulgaris* microalgae. The biocrude yield was increased by around 2 wt.% in the PEF-protein extracted residue. This can be attributed to the fact that after protein extraction, the residual biomass contained a higher proportion of lipid that contributed more to the biocrude phase. Similar results were reported elsewhere (Eboibi, Lewis et al. 2015): compared to untreated algae, the biocrude yield was improved by more than 50% when algae were pretreated by protein extraction. However, due to the low selectivity (35.7 % of proteins in the extract) of the extraction

method in this study, the 2 wt.% improvement was not too significant, as we did not use an elevated temperature from 130 ℃ to 200 ℃ and a high-pressure reactor for the pretreatment.

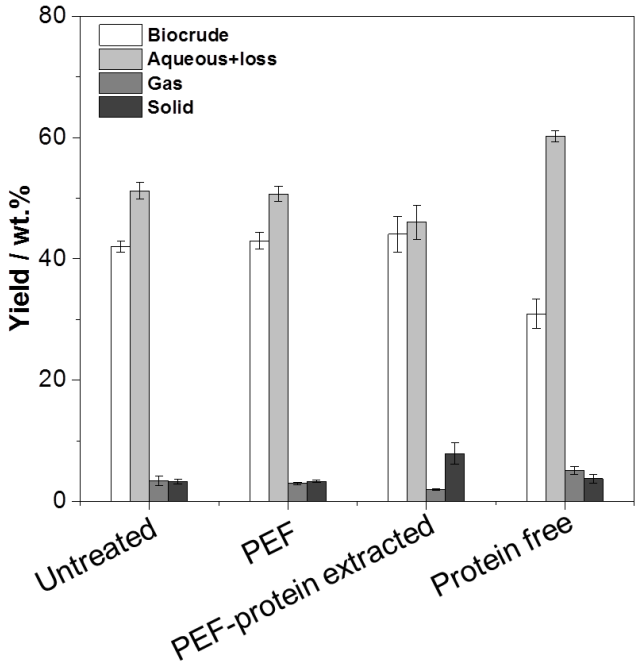

Fig 3.6 HTL product yields of *C. vulgaris* microalgae and protein-extracted residue (18.9 ℃ min^{-1}, 350 ℃, 25 MPa for 15 min holding time). Data are shown as means of four experiments with standard deviations. (Untreated stands for samples without PEF treatment; PEF denotes samples with PEF treatment; PEF-protein extracted refers to samples with PEF treatment and protein extraction; Protein-free stands for samples with completed protein extraction by high-pressure homogenization).

The results of the elemental analysis of the feedstock and relevant biocrude are shown in Table 3.6. Interestingly, the nitrogen content in the feedstock increased after PEF-assisted protein extraction, which might be caused by the low extraction selectivity. Compared to the result obtained for untreated *C. vulgaris* biocrude, the carbon content was slightly increased and the nitrogen content dropped from 6% to 5.5% after PEF protein extraction, resulting in a higher HHV of biocrude (from 37.15 MJ kg^{-1} to nearly 37.66 MJ kg^{-1}). However, energy recovery decreased after PEF extraction, probably due to the unsatisfactory extraction selectivity. An improvement of biocrude quality is also shown by the results of the ^{1}H-NMR measurement.

Table 3.6 Elemental analysis (wt.%), HHV (MJ kg^{-1}), and energy recovery (%) of untreated, PEF-treated, PEF protein-extracted *C. vulgaris* feedstocks and the corresponding biocrudes. Data are shown as means of two experiments with standard deviations. (Untreated stands for samples without PEF

treatment; PEF denotes samples with PEF treatment; PEF-extracted refers to samples with PEF treatment and protein extraction).

	C	H	N	S	O*	HHV	Energy recovery
Untreated microalgae	46.1 ±0.35	8.4 ±0.05	7.9 ±0.15	0.9 ±0.0	36.55 ±0.15	22.49	-
PEF-treated microalgae	47.8 ±0.3	8.6 ±0.0	8.1 ±0.0	0.85 ±0.05	34.6 ±0.25	23.57	-
PEF-extracted residue	52.3 ±0.3	8.8 ±0.1	8.4 ±0.15	0.6 ±0.0	29.9 ±0.55	25.89	-
Untreated biocrude	71.7 ±0.4	10.9 ±0.05	6 ±0.1	1 ±0.05	10.4 ±0.3	37.15	69.3
PEF-treated biocrude	71.6 ±0.35	10.7 ±0.15	5.8 ±0.0	0.9 ±0.15	10.9 ±0.35	36.92	65.8
PEF-extracted biocrude	72.6 ±0.35	11 ±0.0	5.5 ±0.05	0.8 ±0.05	9.9 ±0.35	37.66	64

* by difference 100-C-H-N-S

As shown in Fig 3.7, information about H-atom types in the biocrude was obtained by ^{1}H-NMR. Generally, signal peak areas can be classified into 4 groups: 0 ppm represents the internal standard TMS; 0.5-1.5 ppm reflects the H-atoms in alkanes or fatty acids (Xu, Guo et al. 2018), 1.5-3.0 ppm reflects the presence of H-atoms bonded to the heteroatomic functionalities (Gai, Zhang et al. 2014), and 5.3-5.5 ppm reveals the H-atom in aromatic compounds (Vardon, Sharma et al. 2011).

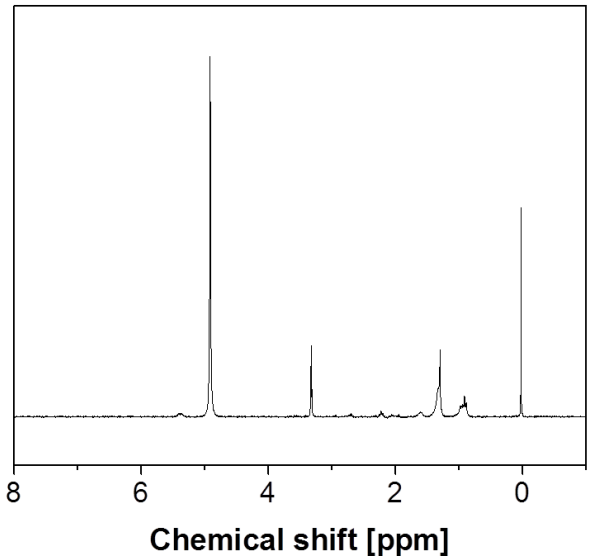

Fig 3.7 A typical ^{1}H-NMR spectrum of the *C. vulgaris* biocrude sample.

The peak areas were integrated and the corresponding relative area percentages are given in Table 3.7. Compared to untreated and PEF-treated biocrude, an increase of about 10% in the area of alkanes and a decrease in the area of heteroatomic functionalities and aromatic groups can be observed for PEF protein- extracted biocrude. This confirms that PEF protein-extracted residue reaches a better feedstock performance in HTL. In order to improve the biocrude yield and quality, the future investigation should be focused on the improvement of extraction selectivity.

Table 3.7 Relative peak area percentage (%) of different H-type groups in *C. vulgaris* biocrude. Data are shown as means of two experiments. (Untreated stands for samples without PEF treatment; PEF denotes samples with PEF treatment; PEF-extracted refers to samples with PEF treatment and protein extraction).

	Alkanes	**Heteroatomic functionalities**	**Aromatics**
Untreated biocrude	66.6 ±7.1	26.3 ±6.03	7.1 ±1
PEF biocrude	68.7 ±7.56	27.1 ±7.4	4.2 ±0.16
PEF-extracted biocrude	77.1 ±3.13	19.5 ±3.64	3.4 ±0.5

3.3.2.3. Amino acids extraction

Four groups of experiments were carried out for investigating the extraction of amino acids from *S. almeriensis* microalgae and further HTL (Route 5): experiments using untreated and PEF-treated microalgae as well as experiments using microalgae with amino acids extraction with and without PEF. The PEF treatment was observed to clearly improve the yield of extracted amino acids from 48.85 wt.% in untreated samples to 60.37 wt.% in PEF-treated samples. A similar value was also reported in (Sari, Bruins et al. 2013). Compared to other methods to extract amino acids from microalgae, such as alkaline or acidic extraction, the enzymatic hydrolysis method represents a mild and relatively environmentally friendly alternative.

Fig 3.8 presents the HTL product yields of untreated *S. almeriensis*, PEF-treated *S. almeriensis,* and *S. almeriensis* residues after the extraction of amino acids. Again, no direct effect of PEF was found on the HTL *of S. almeriensis* microalgae. Compared to untreated and PEF samples, an average increase in biocrude yield by about 4 wt.% and 6 wt.% and decrease in the aqueous phase yield by 8 wt.% and 7 wt.%, respectively were observed in untreated samples after the extraction of amino acids and PEF-treated samples after the extraction of amino acids. As for *C. vulgaris* protein extraction, this was explained by a higher proportion of lipids remaining in the biomass after extraction.

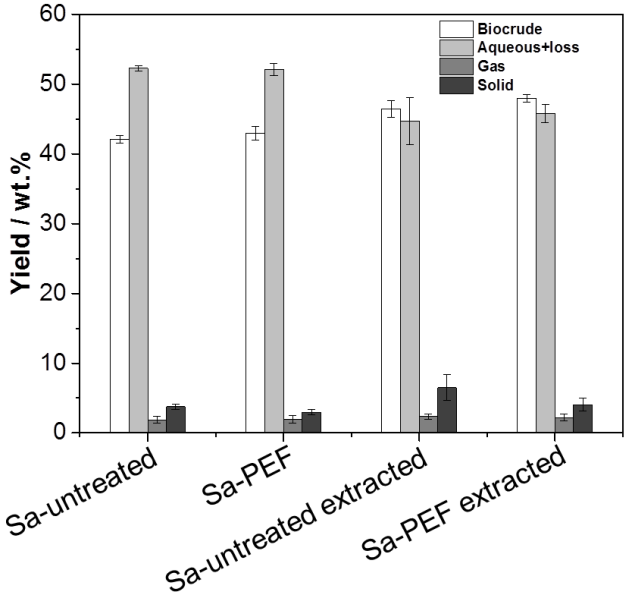

Fig 3.8 HTL product yields of *S. almeriensis* microalgae (18.9 ℃ min^{-1}, 350 ℃, 25 MPa for 15 min holding time, extraction for 180 min). Data are shown as means of four experiments with standard deviations. (Untreated stands for samples without PEF treatment; PEF denotes samples with PEF treatment; Untreated extracted refers to samples without PEF treatment, but with amino acids extraction; PEF extracted stands for samples with PEF treatment and amino acids extraction).

It is evident from Table 3.8 that after amino acids extraction, the nitrogen content decreased from 5.7 wt.% in untreated *S. almeriensis* biocrude to 4 wt.% in PEF-extracted biocrude and the oxygen content showed a slight decrease as well. Energy recovery improved by around 7%, revealing that the residue after PEF treatment and extraction of amino acids was better suited for HTL.

Table 3.8 Elemental analysis (wt.%), HHV (MJ kg^{-1}), and energy recovery (%) of untreated and PEF-treated *S. almeriensis*, *S. almeriensis* subjected to PEF treatment and amino acids extraction, and of the corresponding biocrude. Data of the feedstocks are shown as means of two experiments with standard deviations. Data of the biocrude are shown as means of three experiments with standard deviations. (Untreated stands for samples without PEF treatment; Untreated extracted denotes samples without PEF treatment, but with the extraction of amino acids; PEF extracted refers to samples with PEF treatment and extraction of amino acids).

	C	**H**	**N**	**S**	**O***	**HHV**	**Energy recovery**
Untreated microalgae	48 ±0.15	7.6 ±0.25	6.9 ±0.05	0.5 ±0.0	37 ±0.45	22.08	-
Untreated-extracted residue	48.7 ±0.0	7.8 ±0.0	6.6 ±0.2	0.5 ±0.05	36.45 ±0.15	22.61	-
PEF-extracted residue	50.4 ±0.2	8 ±0.0	5.3 ±0.1	0.4 ±0.0	35.9 ±0.3	23.41	-
Untreated biocrude	73.5 ±0.6	11 ±0.2	5.7 ±0.1	0.5 ±0.0	9.3 ±0.7	38.01	72.3
Untreated-extracted biocrude	73.6 ±1.9	11.1 ±0.5	4.4 ±0.3	0.3 ±0.04	10.6 ±2.5	37.92	77.1
PEF-extracted biocrude	74.8 ±1.3	11.4 ±0.2	4 ±0.2	0.4 ±0.04	9.4 ±1.5	38.79	79.5

*by difference 100-C-H-N-S

The FTIR results of *S. almeriensis* biocrude in Fig 3.9 show that the peak between 2800 cm^{-1} and 3000 cm^{-1}, which is attributed to alkanes (Guo, Zhou et al. 2015), becomes sharper in the samples subjected to amino acids extraction compared to samples that were not subjected to extraction. This indicates an increase in the proportion of long-chain fatty acids in the biocrude. The peak around 1716 cm^{-1} (S. Syngellakis 2014), which belongs to the carboxyl acid, becomes more visible after extraction, and the peak at 1690–1675 cm^{-1}, which is related to compounds with C=O double bonds possibly contained in ketones or aldehydes (Coates), is eliminated after the extraction process. All changes observed by FTIR suggest an improvement of biocrude quality after PEF-assisted amino acids extraction.

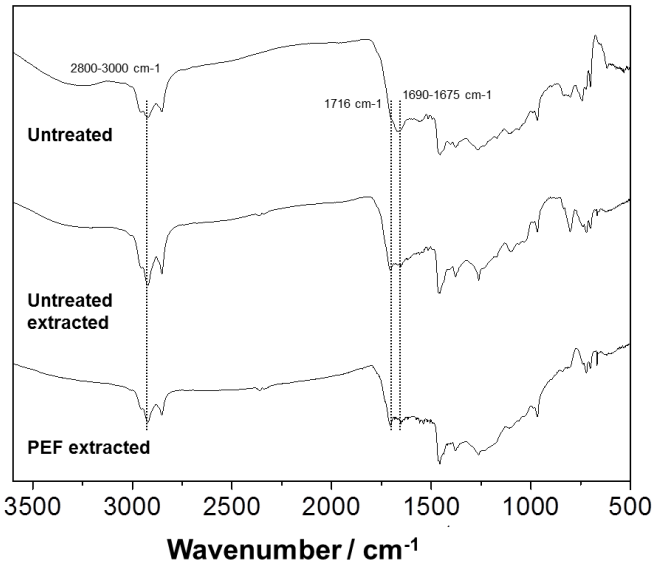

Fig 3.9 FTIR spectrometry of untreated, untreated-extracted, and PEF-extracted *S. almeriensis* biocrude. (Untreated stands for *S. almeriensis* sample without PEF treatment; Untreated extracted denotes an *S. almeriensis* sample without PEF treatment, but with the extraction of amino acids; PEF extracted refers to an *S. almeriensis* sample subjected to both PEF treatment and amino acids extraction).

Fig 3.10 reveals the distribution of molar mass determined by GPC measurement in the *S. almeriensis* biocrude. It is clearly visible that the molar mass distribution in the PEF-extracted samples is much more compact than that of untreated-extracted and untreated samples, which suggests a lower dispersity in the biocrude components. The average molar masses of three samples range between 423 and 538 g·mol^{-1}, which is in line with an average molar mass distribution from 300 to 600 g·mol^{-1} for *S. almeriensis* microalgae-biocrude reported elsewhere (López Barreiro, Riede et al. 2015). Therefore, it can be concluded that

higher yields and better qualities of biocrude can be achieved from the residue after both PEF treatment and extraction of amino acids.

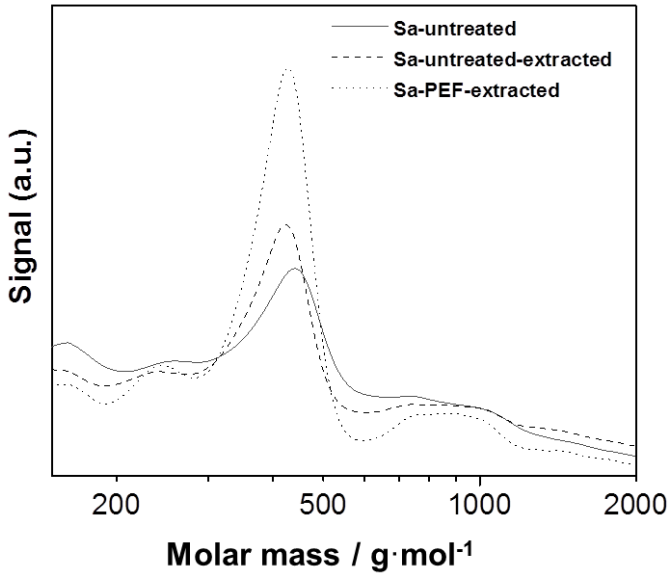

Fig 3.10 GPC results of untreated, untreated-extracted, and PEF-extracted *S. almeriensis* biocrude. (Untreated stands for a sample without PEF treatment; Untreated extracted denotes a sample without PEF treatment but subjected to amino acids extraction; PEF extracted refers to a sample subjected to both PEF treatment and amino acids extraction).

Another interesting phenomenon was observed for the hydrolysis rate during extraction. As shown in Fig 3.11, the correlation of hydrolysis degree and incubation time was studied over the whole extraction period. It was found that the hydrolysis rate during the first 60 min was highest and slowed down significantly afterward. When assisted by the PEF treatment, the hydrolysis rate increased further, with the extraction yield being about 150% higher than that of the untreated samples in the first 60 min. Over a sufficiently long period of time, however, the difference of hydrolysis degrees of untreated and PEF-treated samples is assumed to eventually disappear (drop to about 123% after 180 min of enzymatic hydrolysis). It can be said that PEF accelerates hydrolysis and amino acids extraction during the first 60 min of extraction, while this effect tends to fade out with longer time. Time dependence of extraction by PEF was noticed especially in the first 60 min (Parniakov, Barba et al. 2015, Carullo, Abera et al. 2018). This phenomenon was investigated further by Akaberi et al (Akaberi, Gusbeth et al. 2019).

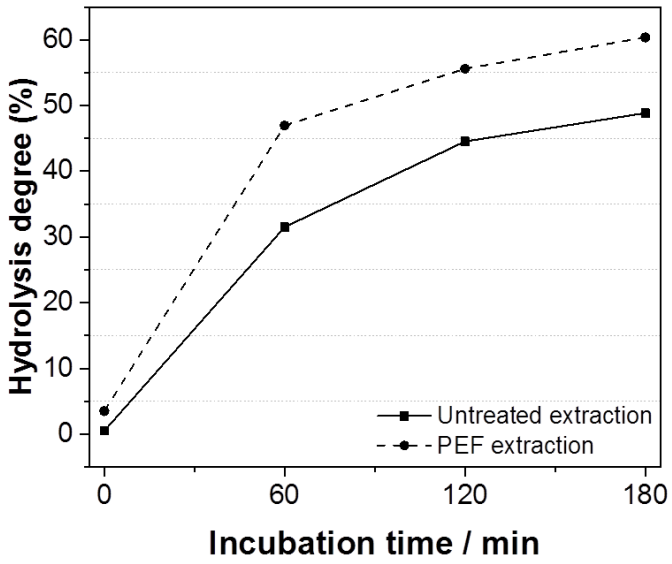

Fig 3.11. Hydrolysis degree of untreated and PEF-treated *S. almeriensis* microalgae during incubation. (Untreated extraction stands for samples without PEF treatment; PEF extraction stands for samples with PEF treatment).

Another group of HTL experiments was carried out to better understand this accelerating effect on HTL. For these experiments, the following samples were used: untreated-extracted for 60 min, untreated-extracted for 180 min, PEF-extracted for 60 min, and PEF-extracted for 180 min. These samples were subjected to HTL under the standard conditions chosen in this study.

Fig 3.12 shows the product yields of these samples. When increasing the extraction time from 60 min to 120 min, the biocrude yield increased by about 2 wt.% after HTL of the untreated-extracted samples and by approximately 3 wt.% in the PEF-extracted samples. Although more amino acids were extracted from the PEF-treated after 60 min, however, the biocrude yield did not increase. This was explained by the fact that not only extraction was facilitated, but also the release of other components during extraction. The composition of the extract was analyzed to verify this explanation.

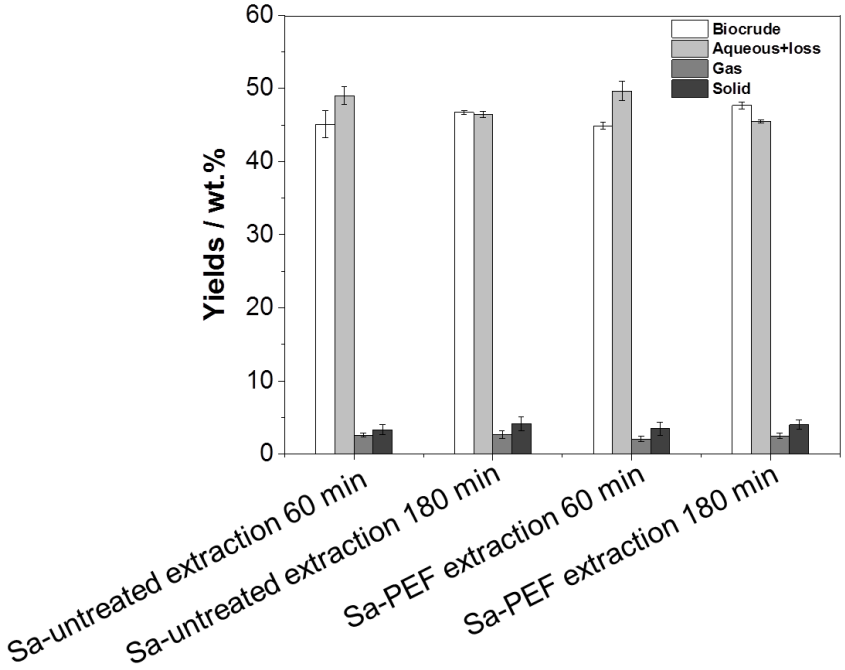

Fig 3.12. HTL product yields of *S. almeriensis* for different extraction times (18.9 ℃ min^{-1}, 350 ℃, 25 MPa for 15 min holding time). Data are shown as means of four identical experiments with standard deviations. (Untreated extracted 60 min stands for samples without PEF treatment, but with amino acids extraction for 60 min; Untreated extracted 180 min refers to samples without PEF treatment, but with amino acids extraction for 180 min; PEF extracted 60 min denotes samples subjected to both PEF treatment and amino acids extraction for 60 min; PEF extracted 180 min stands for samples subjected to PEF treatment and amino acids extraction for 180 min).

As shown in Fig 3.13 for untreated-extracted 60 min samples, the protein in the extract accounts for 60% of the total mass. When the extraction process is assisted by the PEF treatment, the apparent yield of amino acids is increased, as is the release of other components. The selectivity of amino acid decreases to 45 %. After 180 min, the PEF treatment does not have any major influence on the release of other components and another 120 min of extraction seem to increase the amino acid yield slightly only. PEF treatment can accelerate both protein hydrolysis and release of other components into water during the first 60 min. This, however, will reduce biocrude production. Since extraction was mostly completed within the first 60 min and 3% difference in biocrude yield only is unlikely to outweigh 120 min of extraction time, it is recommended to apply algae residues after PEF treatment and extraction for 60 min for HTL. However, this remains to be confirmed by further analysis.

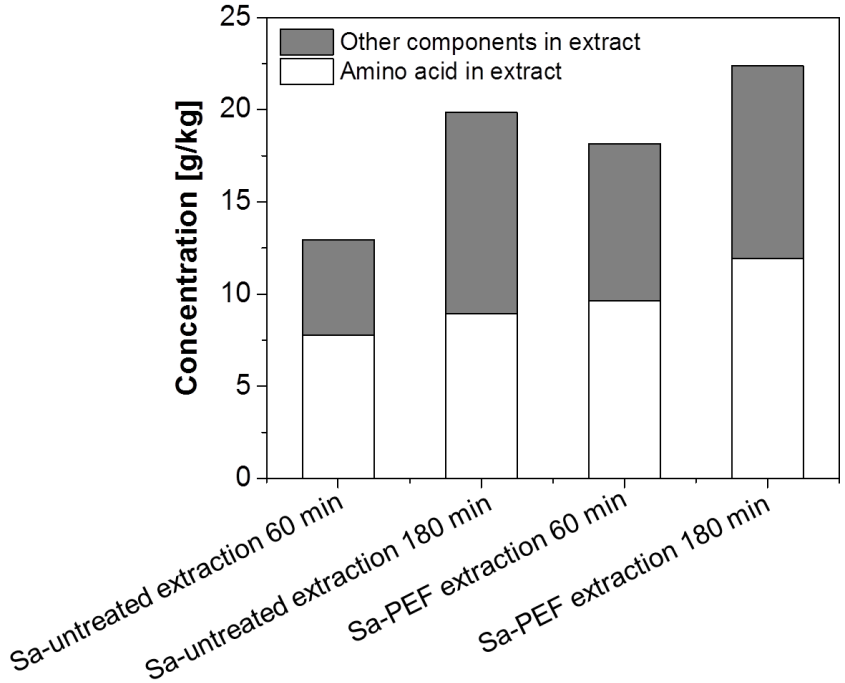

Fig 3.13. Compositions of extract phases of the *S. almeriensis* amino acids after different extraction times. Data are shown as means of two experiments with standard deviations. (Sa-untreated extraction 60 min stands for samples without PEF treatment, but with an amino acids extraction for 60 min; Sa-untreated extraction 180 min stands for samples without PEF treatment, but with an amino acids extraction for 180 min; Sa-PEF extraction 60 min refers to samples subjected to both PEF treatment and amino acids extraction for 60 min; Sa-PEF extraction 180 min are samples subjected to both PEF treatment and amino acids extraction for 180 min).

3.3.2.4. Overall mass balance of pulsed electric field-assisted extraction of valuables and hydrothermal liquefaction

The overall mass balance as a function of the original algae biomass is shown in Fig 3.14. After PEF-assisted valuables extraction, the biocrude yield decreased from 57 wt.% to 23.4 wt.%, 42.05 wt.% to 22.46 wt.%, and 42.05 wt.% to 18. 54 wt.% for *A. protothecoides*, *C. vulgaris*, and *S. almeriensis* algae, respectively. The respective aqueous yields were reduced from 37.4 wt.% to 21.5 wt.%, 51.23 wt.% to 23.46 wt.%, and 52.29 wt.% to 23.06 wt. %. The extraction yields and selectivities were determined for each processing route. PEF-assisted extraction of lipid, protein, or amino acids were found to have the highest impact on the biocrude phase and aqueous phase in the following HTL of the algae investigated in this study. However, this phenomenon may be algae strain-dependent, as was reported before (López Barreiro, Samorì et al. 2014). Extracting proteins prior to HTL could be a more

interesting route to improve HTL economics and biocrude quality (López Barreiro, Samorì et al. 2014). Amino acids extraction from Sa microalgae by enzymatic hydrolysis produces a higher extraction yield and target selectivity than protein extraction from *C. vulgaris* microalgae. Therefore, the PEF-amino acids extraction-HTL process (Route 5) appears to be the most promising. This still remains to be confirmed by an application assessment of the extracted product.

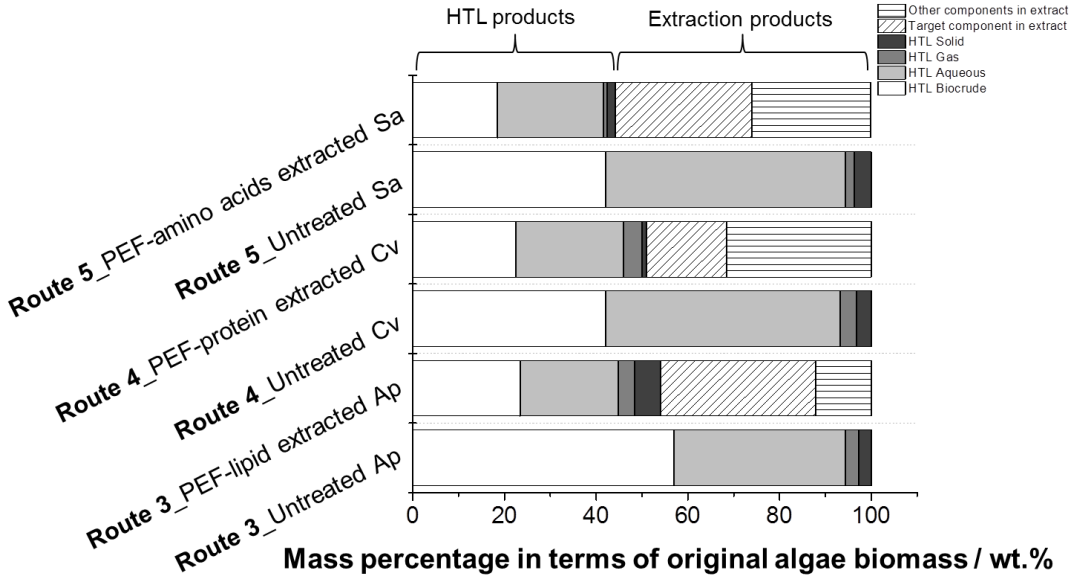

Fig 3.14. Overall mass balance of PEF-assisted valuables extraction and HTL products (based on the original algae biomass) compared to untreated microalgae for all investigated strains of *A. protothecoides (Ap), C. vulgaris (Cv),* and *S. almeriensis (Sa).*

3.4. Conclusions

The results of this chapter show that PEF treatment alone does not have any direct influence on the HTL of all three microalgae investigated in this study. Neither product yields nor biocrude qualities were significantly changed after the PEF treatment.On the other hand, PEF treatment has a significant impact on extraction and, consequently, on HTL of extracted residues. When using *A. protothecoides* microalgae, PEF treatment enhances the lipid extraction yield from 4 wt.% to 33 wt.% (more than 80% of the total lipids), which decreases the biocrude yields from 58 wt.% to 43 wt.%. In spite of the higher nitrogen content, the biocrude produced still exhibits a relatively high HHV of 36.7 MJ·kg^{-1}. The overall economics of the PEF treatment is further improved by the low energy consumption (could be

as low as 0.25 MJ ₭g^{-1}_{DW} (Silve, Kian et al. 2018)). Further research will address the reduction of the extraction solvent amount and optimization of PEF parameters.

PEF treatment was found to be a decisive factor for protein extraction from *C. vulgaris* microalgae. The biocrude obtained has a lower nitrogen content (from 6 wt.% to 5.5 wt.%). As only demineralized water is used, the process is environmentally friendly and the product is expected to better suit for later food and feed application. However, the selectivity of this method still needs to be optimized to improve the yields.

The most promising route of combining PEF treatment and HTL application seems to be the enzymatic protein hydrolysis and extraction of amino acids from *S. almeriensis* microalgae. After extraction of amino acids, the algae residues are well suited for HTL, with the biocrude yield being 6 wt.% higher and the nitrogen content reduced. PEF treatment accelerates amino acids extraction and improves the extraction efficiency by up to 150% in the first 60 min. Considering the decreasing hydrolysis rate and time consumption, extraction for 60 min is recommended. The enzymatic hydrolysis method represents a mild and relatively environmentally friendly alternative for amino acids extraction from microalgae.

Chapter 4. Aqueous product recycle from continuous hydrothermal liquefaction of Chlorella vulgaris for algae cultivation

Chapter redrafted after:

Guo, B., Yang, B., Weil, P., Hornung, U., Dahmen, N. (2019) *(Under preparation)*.

Declaration of contributions

cHTL and separation experiments: Bingfeng Guo and Boda Yang.

Analysis, interpretation of the data and draft of manuscript: Bingfeng Guo.

Algae cultivation experiment: Peter Weil.

Critical revision of the article: Ursel Hornung, Nicolaus Dahmen.

4. Aqueous product recycle from continuous hydrothermal liquefaction of Chlorella vulgaris for algae cultivation

4.1. Introduction

Using side product from hydrothermal conversion as alternative nutrient source is becoming a focus in sustainable source management (Belete, Leu et al. 2019). As discussed in chapter 1, section 1.2.7, recycle of water and nutrient from HTL aqueous product (HTL-AP) for algae cultivation could improve the economics of a microalgae biorefineries. The product separation step plays a significant role in the properties of the biocrude as well as the composition of AP. Dichloromethane (DCM) is a commonly used organic solvent for product separation in laboratory, especially for microalgae biofuel research. However, it seems to be inapplicable in industrial production, due to environmental and health problem (cause damage to brain and central nervous system, classified by Environmental Protection Agency (EPA) as a probable human carcinogen). Moreover, it has been demonstrated that an overestimation of biocrude yield can be obtained due to the usage of DCM in batch HTL (López Barreiro, Riede et al. 2015). Recently, the study of microalgae HTL in a continuous mode (cHTL) has been receiving increasing interest (Elliott, Hart et al. 2013, Elliott, Biller et al. 2015, Castello, Pedersen et al. 2018), since this is the prerequisites for the microalgae biofuel industrial production. This technology performs at hydrothermal condition with different types of continuous reactor, such as plug flow reactor (Patel and Hellgardt 2015, Lababpour 2018), pilot plant (Jazrawi, Biller et al. 2013, Anastasakis, Biller et al. 2018) or semi-continuous system (Prapaiwatcharapan, Sunphorka et al. 2015). Therefore the understanding of DCM impact in the product separation of a cHTL process could help to provide more realistic data for biocrude productivity and AP recovery, as well as establishing a microalgae biorefinery.

In this chapter, the impact of separation solvent DCM on HTL products, specifically at a concept of continuous production mode and the follow-up AP recovery for algae cultivation are investigated. The yield and quality of produced biocrude and AP have been examined along with their HTL temperature dependency (from 300 ℃ to 400 ℃). After purification treatment, the obtained cHTL-AP is examined and used for the algae cultivation.

4.2. Materials and methods

4.2.1. Feedstock preparation

Microalgae Chlorella vulgaris (*C. vulgaris*) was selected for this study and obtained in a dry powder state from Roquette Klötze GmbH (Germany). The preparation of algae slurry was performed with distilled water, for each cHTL run, around 3.2 L of 5 wt.% biomass slurry was homogenized with motor-stirring for 30 min in a 5 L beaker. The elemental and bio-component characterization of the feedstock is given in Table 4.1.

Table 4.1 Feedstock characterization of *C. vulgaris* by elemental analysis (wt.%), biochemical composition (wt.%), ash (wt.%), moisture content (wt.%) and higher heating value (HHV) (MJ kg^{-1}).

Elemental analysis					**Bio-component analysis**					
C	**H**	**N**	**S**	**O***	**Protein**	**Lipid**	**Carbohydrates**	**Ash**	**Moisture**	**HHV**
47.7	7.5	8.4	0.5	35.9	51.9	23.6	9.2	6.8	3.6	22.03

*by difference (100-C-H-N-S)

4.2.2. Continuous hydrothermal liquefaction

Microalgae cHTL experiments were performed in a continuous stirred tank reactor (CSTR). The CSTR schematic setups are shown in Fig 4.1. There are 8 electric heating cartridges placed around the tank and kept heating the reactor to maintain the target temperature during the whole experiment. Detailed information about the reactor can be also found in a previous study (Barreiro, Gómez et al. 2015). In brief, approximately 2 hours preheating using water was firstly conducted for the reactor to reach target temperature (300, 325, 350, 375 and 400 ℃) and then algae slurry was started to feed into the reactor with a double press pump. Then another 90 min (about 6 times the HTL residence time) was maintained to reach a steady HTL reaction state, and all the samples were collected during this state. For the product yield calculation, in total 18 samples were taken respectively during the whole cHTL operation period (about 100 min, samples were taken every 20 min) in each temperature investigation, and the mean values with standard deviation are reported. Product gas was lost in this work, while biocrude, aqueous phase, and solid residues were collected into a pre-weighted tube for further separation.

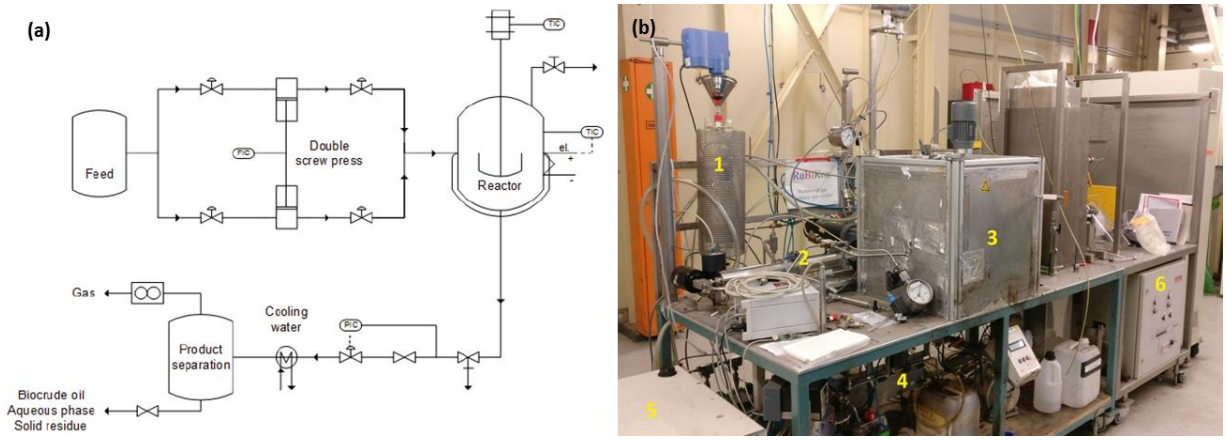

Fig 4.1 (a) Setup of the continuous stirring tank reactor (Barreiro, Gómez et al. 2015) (b) cHTL reactor –1 feeding system –2 double screw press –3 insulated reactor covering –4 downstream –5 cooling system –6 flow rate regulator.

4.2.3. Product separation

In order to investigate the effect of DCM on cHTL products, two different separation methods were applied in this study. For the non-DCM method, the biocrude, solid and aqueous product in the sample tube was first transferred into a falcon tube. Then the biocrude with the solid product was separated from aqueous phase using 8000 rpm centrifugation for 5 mins. Afterward, the biocrude and solid product were forced into one phase attached on the preweighted centrifugation tube, then the aqueous phase was removed and placed into a preweighted tube for a direct dry mass content measurement. The dry mass content was directly measured using 60 ℃ oven described in a previous study (López Barreiro, Samorì et al. 2014), following Equation (4.1). Moreover, the yield of aqueous product is also calculated by dry mass difference and compared with the results of direct measurement. As all the samples were collected in the stationary HTL period, during which the output of mass was assumed to the same as the input to the reactor. Therefore the calculation of yield is as in Equation (4.2).

$$Dry\ mass\ content_{aqueous} = \frac{Mass_{tube+AP\ after\ 60°C\ oven} - Mass_{empty\ tube}}{Mass_{tube+AP\ before\ 60°C\ oven} - Mass_{empty\ tube}} \quad (4.1)$$

$$Yield_{aqueous} = \frac{Dry\ content_{aqueous} * Mass_{aqueous}}{Dry\ content_{algae} * Mass_{product\ in\ 2\ min}} \quad (4.2)$$

The DCM separation was performed as follows: 9 ml DCM (≥99.7% purity) was added into the sample tube and agitated for a complete contact among different products, and after a

stationary step, the phase separation spontaneously occurred. Afterward, the removal of the upper aqueous phase was done carefully with a pipette. The DCM phase left in the tube, which consisted of biocrude and solid, was placed under nitrogen flow for 24 hours. After evaporation, the biocrude and solid were determined together by weight. Therefore the biocrude and solid-phase together are reported in this study. The aqueous phase yield is calculated in the same way aforementioned for the comparison.

4.2.4. Purification of HTL-AP

Due to the potentially toxic substances evolved from HTL, the growth of algae could be inhibited (López Barreiro, Bauer et al. 2015), therefore a pretreatment to remove or reduce the toxic compounds in AP is performed (Leng, Li et al. 2018). Purification methods such as precipitation, membrane filtration, air stripping et al. were generally reported but for their application in microalgae HTL-AP are still limited. Supercritical water gasification (SCWG) has been reported to effectively remove the harmful compounds in the HTL-AP for re-cultivation (López Barreiro, Bauer et al. 2015). Another commonly used purification method is adsorption. Adsorption is a common process for the removal of pollutants from water body, especially using activated carbon absorption (ACA) treatment because it is cost-effective, easily adaptable and environmentally compatible. The enhancement of nutrient recovery and bio-oil from microalgae HTL by using activated carbon has been reported (Fushimi, Kakimura et al. 2016). Therefore, SCWG and ACA treatment were tested for purification of the cHTL-AP in this study.

The SCWG treatment was performed using 24.5 ml autoclaves, made of stainless steel EN 1.4980. A pre-calculated quantity of 2.5 mL AP was injected into the autoclave and about 0.5 MPa nitrogen was preloaded into the autoclave, making the water turn into a supercritical state (450 ℃, 23.9 MPa) when heating up to 450 ℃ by a metal block heater. The heating block was preheated to 450 ℃ and then the autoclave was placed inside as residence time of 30 min. The ACA purification was carried out using activated carbon from VWR Chemicals (CAS: 7440-44-0), with maximum particle size of 80 μm. 100 g of activated carbon was added into 1 L continuous HTL-AP and magnet-stirred for 30 min. Afterward, a vacuum filtration was done with LLG filter paper (Lab Logistics Group GmbH, diameter 125 mm and pore size 5-8 μm) to separate the carbon and AP. For each treated sample, the purification was

repeated 2 times. A comparison of samples before and after ACA purification is shown in Fig 4.2.

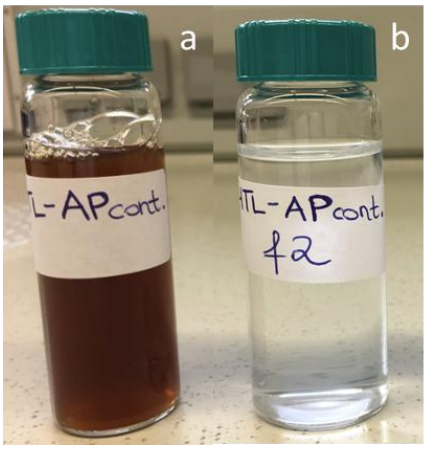

Fig 4.2 cHTL-AP with ACA purification before (a) after (b).

4.2.5. Algae cultivation with recovered AP

ACA-treated AP was selected to perform further algae cultivation, due to the limited available amount of SCWG-AP. The treated AP was first diluted with distilled water as a factor of 15 and then some additional nutrients were added into diluted AP to reach a comparable nutrient level to the standard medium. A standard medium was also prepared for the comparison, the composition of a standard medium is shown in Table 4.2.

Table 4.2 Chemical composition of standard medium (in 1000 mL solution).

TAP salt	NH_4Cl	15 g
	$MgSO_4\ 7H_2O$	4 g
	$CaCl_2\ 2H_2O$	2 g
Phosphor solution	K_2HPO_4	28.8 g
	KH_2PO_4	14.4 g
	$Na_2EDTA\ H_2O$	2.5 g
	$ZnSO_4\ 7H_2O$	1.1 g

Hutner ́s trace elements	H_3BO_3	570 mg
	$MnCl_2$ $4H_2O$	255 mg
	$FeSO_4$ $7H_2O$	250 mg
	$CoCl_2$ $6H_2O$	80 mg
	$CuSO_4$ $5H_2O$	80 mg
	$(NH_4)_6Mo_7O_{24}$ $4H_2O$	55 mg

The cultivation was carried out in a 200 mL flask on a vibration plate (100 rpm) at a temperature of 25 ℃. The light intensity during the cultivation was adjusted to 125 µE/m^2s (LI-250 Light Meter, Li-Cor, USA), about 5 % CO_2 was continuously input at a rate of 25 ml·min^{-1} for maintaining pH value around 7.3. The overall cultivation investigation lasted for 8 days. Cultivations with standard medium, with untreated AP and with ACA treated AP were investigated and two flasks were performed for each cultivation condition.

4.2.6. Analysis of cHTL product, purified AP and algae cultivation

The elemental analysis (EA) was performed on biocrude+solid sample using a Vario EL Cube Analyser, the content of O is calculated by difference (100-C-H-N-S), and Boie's formal, as following equation (4.3), is applied for the HHV calculation.

$$HHV\ [MJ/kg] = 0.3516 * C + 1.16225 * H - 0.1109 * O + 0.0628 * N + 0.10465 * S \quad (4.3)$$

The pH value of the cHTL-AP was measured using pH indicator Multi 3510 IDS from Taschenmessgerät® MultiLine. The total inorganic carbon (TIC), total carbon (TC) and total nitrogen bound (TN_b) analysis were performed onto the cHTL-AP and purified AP by a DIMATOC 2100 instrument, the total organic carbon (TOC) was calculated by difference, TOC= TC-TIC. Inductively coupled plasma optical emission spectroscopy (ICP-OES) on a 725-ES emission spectrometer using argon as plasma gas from Agilent Technologies was used to determine the inorganic matter. High-performance liquid chromatography (HPLC) was used to examine the organic acids and alcohols in the AP. The recovery of TIC, TC, TOC, TNb, partial ions have been calculated as below equation (4.4)

$$Recovery\ (\%) = \frac{Concentration\ of\ target\ component_{aqueous\ phase} * Volumn_{aqueous\ phase}}{Concentration\ of\ target\ component_{algae\ slurry} * Volumn_{algae\ slurry}} \quad (4.4)$$

The cultivation of microalgae was monitored by optical density test (OD) at 750 nm, and dry biomass was determined at harvest time. A correlation between OD and dry mass was made as to the basis for the growth curve, R-value in all groups equal to more than 0.99. The mean value for each cultivation condition was reported with the standard deviation.

4.3. Results and discussion

4.3.1. Continuous HTL experiments

4.3.1.1. Product yields

Fig 4.3 presents the average biocrude+solid yields at different HTL temperatures with/ without DCM separation medium. It can be seen that the yield increases with temperature and reach the maximum at 350 ℃ (about 38.9 ±1.4 wt.% for non-DCM method and 47.5 ±2.5 wt.% for DCM method), and then drop down with higher temperature. 350 ℃ seems to be the optimal temperature for high biocrude yields in cHTL, as suggested by many studies in batch system (Brown, Duan et al. 2010, Jena, Das et al. 2011, López Barreiro, Prins et al. 2013): the increasing temperature promotes degradation of proteins and converts to biocrude phase, when surpassing water critical point, radical-induced cracking of biocrude, repolymerization and recomposition of intermediate products were enhanced to form lighter compounds, leading to a decrease of biocrude yield (López Barreiro, Prins et al. 2013, Xue, Chen et al. 2016). The biocrude+solid product generated at 300 ℃ cHTL has a clear dark green color, implying the majority of the product was unreacted biomass, this indicates 300 ℃ was too mild to trigger significant reaction. Besides, the yields present a relatively lower fluctuation at higher temperature (375 and 400 ℃), this indicates the performance of cHTL reactor is more stable when applying higher temperature. Besides, by using DCM, a mean of 9 wt.% higher yield can be achieved compared to that of non-DCM method, this value is quite close to a similar study using batch reactor, which was reported about 8.4 wt.% of the total biocrude will be lost if the water-soluble portion was not recovered (Xu and Savage 2014). An average biocrude yield estimation can be proposed: $Yield_{non\text{-}DCM} \approx 81.8\% * Yield_{DCM}$. 300 ℃ samples exhibit lowest yield in DCM method while the highest in non-DCM method, which confirms 300 ℃ is not intensive enough to convert all the biomass and great amount of unreacted algae remained as solid residues.

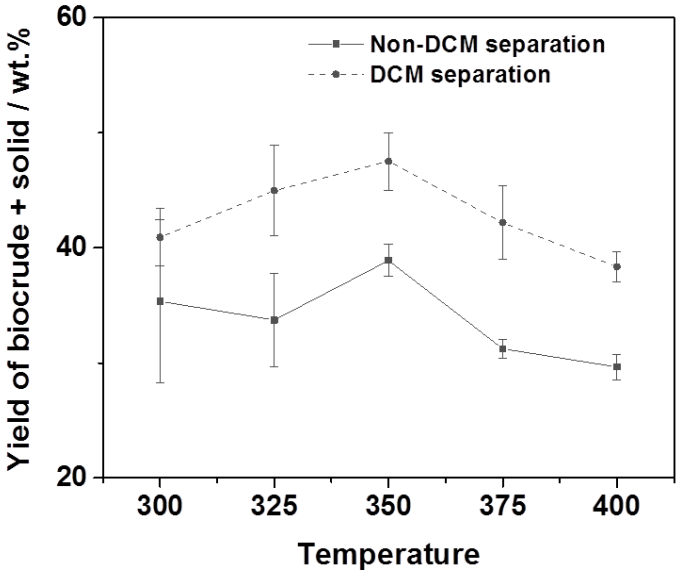

Fig 4.3 Biocrude+solid product yield at different cHTL temperatures (℃) with/without DCM separation. Data are shown as the mean of 18 measurements for each temperature during 100 min of cHTL running with the standard deviation.

When it comes to the yield of cHTL-AP as presented in Fig 4.4, the calculation by difference and a direct measurement show quite different trends over temperatures: in general, decreasing aqueous yield was observed along with the increasing temperature in direct measurement, revealing that higher temperatures promoted the conversion of intermediate water-soluble products. More light organics were generated in AP higher than 350 ℃, which was also easier to lose during the direct measurement. This explains the bigger gap between two methods higher than 350 ℃. At 350 ℃, the yield values in two methods reach the closest, indicating 350 ℃ converts the least amount of light organics into AP, possibly more to biocrude phase, this could be the evidence supporting 350 ℃ is the optimal temperature for running cHTL of *Chlorella vulgaris*.

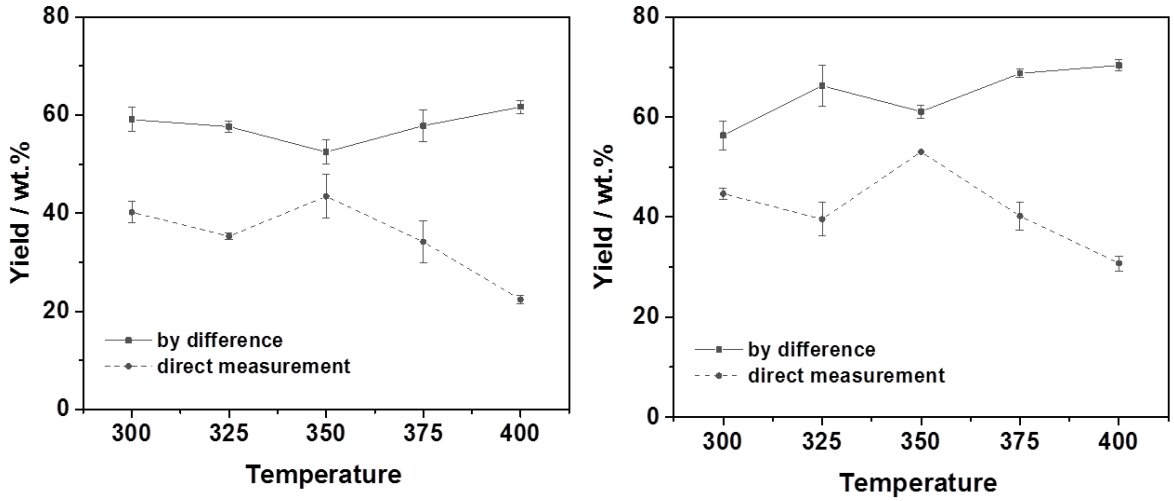

Fig 4.4 AP yield under different cHTL temperature using DCM (a) and non-DCM method (b). Data are shown as the mean of 18 measurements for each temperature with the standard deviation.

4.3.1.2. Elemental analysis

Table 4.3 shows the elemental content of biocrude+solid produced at different HTL temperatures. The obtained biocrude contains a range of HHV from 24.8 to 34 MJ kg^{-1}, which was substantially higher than the microalgae feedstock (22.03 MJ kg^{-1}). Generally, the higher temperature increases C and H content in the biocrude, at the same time lowering O content, resulting in a higher HHV. However, it can be noted that in cHTL, this pattern is not that significant after 350 ℃, indicating the effect of temperature on the biocrude chemical composition is less influential when surpassing the critical point of water.

Table 4.3 Elemental analysis in wt.% of biocrude+solid produced at different HTL temperatures and HHV (MJ kg^{-1}). Data are shown as the mean of two measurements with the standard deviation.

Temperature		C	H	N	S	O*	HHV
300 ℃	Non-DCM separation	54.6±0.2	7.35±0.05	7.15±0.05	0.5	30.4	24.87
	DCM separation	56.6±2.7	7.6±0.1	8.3±1.1	0.56±0.04	26.94	26.33
325 ℃	Non-DCM separation	64.7±1.14	8.07±0.12	5.7±0.37	0.33±0.05	21.2	30.17
	DCM separation	66.9±1.25	8.25±0.15	6.6	0.4	17.8	31.61

350 ℃	Non-DCM separation	65.7±2.2	8.8±0.2	5.8±0.3	0.6±0.05	19.1	31.64
	DCM separation	67.2±2.3	8.8±0.2	6.8±0.3	0.6±0.04	16.6	32.5
375 ℃	Non-DCM separation	70.9±0.66	8.67±0.12	5.67±0.05	0.37±0.05	14.42	33.79
	DCM separation	69.3±2	8.47±0.21	6.53±0.24	0.57±0.17	15.13	33
400 ℃	Non-DCM separation	70.5±0.45	8.95±0.05	5.55±0.05	0.7	14.3	34.03
	DCM separation	69.2±1	9.1±0.2	6.6±0.08	0.75±0.04	14.35	33.81

*by difference 100-C-H-N-S

Generally, within the temperature range investigated in this study, about 9 wt.% higher yield and 1 wt.% higher nitrogen content were achieved in biocrude+solid phase when separating the products by DCM in cHTL. And by using DCM, higher carbon and nitrogen content were obtained in biocrude+solid phase, especially at 300-350 ℃, the use of the solvent increases the biocrude yield but decreases its quality (Xu and Savage 2014), however this effect on biocrude quality tends to be alleviated when HTL temperature higher than 350 ℃, the carbon and hydrogen content seems to be stable regardless of DCM applied or not. This knowledge could be helpful to scale up a continuous process based on data achieved by means of DCM separation.

4.3.2. Analysis of aqueous product

4.3.2.1. Composition of continuous HTL aqueous product

A detailed examination regarding pH value, total carbon (organic and inorganic), total nitrogen, ions and organic acids composition of the cHTL-AP was performed, as shown in Table 4.4. With the increasing cHTL temperature, the pH values of AP rise accordingly from 6.8 to over 8, which is in the range of the reported values (Leng, Li et al. 2018), probably due to the decarboxylation of organic acids and deamination of protein (Yu, Zhang et al. 2011). Besides, more inorganic carbon was generated with higher temperature, while using DCM, less carbon was converted to AP, which on the other hand, is believed to transfer into the

biocrude phase. Total nitrogen content maintained around 3200 mg Ł$^{-1}$among all the samples, while more NH^{4+} have been found in AP produced at a higher temperature, which also is in line with the stronger deamination of protein over temperature, while in this case there is no significant difference between non-DCM and DCM samples. In terms of ion recovery, Mg, P, S, Fe, Ca present a decreasing pattern over the cHTL temperature, while DCM seems to play no role. While K and Na show a stable recovery, regardless of the cHTL temperature or the usage of solvent.

Table 4.4 pH value, composition and recovery percentage of several ions in the AP from original algae feedstock at different cHTL temperatures. Data are shown as the mean of two measurements.

	300 ℃		325 ℃		350 ℃		375 ℃		400 ℃	
	Non-DCM separation	DCM separation	Non-DCM separation	DCM separation	Non-DCM separation	DCM separation	Non-DCM separation	DCM separation	Non-DCM separation	DCM separation
pH	6.85	7.15	7.56	7.68	7.85	7.89	8.01	8.03	8.58	8.09
TC (mg Ł$^{-1}$)	14588	13368	13594	12641	16970	14000	12917	10343	13413	8556
TIC (mg Ł$^{-1}$)	0	0	86	90	191	224	543	548	607	604
TOC (mg Ł$^{-1}$)	14588	13368	13509	12554	16778	13775	12374	9794	12806	8285
TOC/C	55.9%	48.6%	50.1%	43.9%	68.4%	53.6%	55.2%	42.8%	47.5%	29.6%
TNb (mg Ł$^{-1}$)	3424	3082	3482	3270	4299	3642	3402	3164	3634	2941
TNb/N	74.5%	63.6%	73.3%	62.7%	94.5%	80.1%	86.2%	78.6%	76.6%	59.7%
NH_4^+(mg Ł$^{-1}$)	1058	1159	1573	1488	1943	2126	2509	2166	2379	2263
NH_4^+/N	23%	23.9%	33.1%	28.5%	42.7%	46.7%	63.6%	53.8%	50.2%	45.9%
Fe (mg Ł$^{-1}$)	3.3	3.3	2.3	3	2.2	1.8	1	1	1	1
Recovery	4%	3.8%	2.7%	3.3%	2.8%	2.2%	1.4%	1.4%	1.2%	1.1%
K (µg ml^{-1})	971	951	974	951	1016	968	1003	964	973	963
Recovery	88.7%	82.5%	86.1%	79.3%	98.8%	89.4%	106.8%	147.9%	86.1%	82.1%
Na (µg ml^{-1})	10	11	9.4	8.7	11	10	9.8	8.3	8.7	8.6
Recovery	6.7%	7.1%	6.1%	5.4%	7.9%	6.8%	7.7%	6.4%	5.7%	5.4%
Ca (µg ml^{-1})	28.1	27.3	14.1	17.1	11.9	20.7	4.6	5.3	5	7.5
Recovery	8.4%	7.7%	4.1%	4.7%	3.8%	6.3%	1.6%	1.8%	1.4%	2.1%

Mg (μg ml^{-1})	37.2	36.1	33.5	15.8	7.8	6	4.6	4.6	2	1
Recovery	25.2%	23.2%	21.9%	9.8%	5.6%	4.1%	3.6%	3.5%	1.3%	0.6%
P (μg ml^{-1})	511	509	517	492	532	535	498	513	487	488
Recovery	68.7%	54.9%	66.2%	60.4%	76.1%	72.7%	77.9%	78.7%	63.4%	61.2%
S (μg ml^{-1})	203	163	224	160	231	179	206	145	191	133
Recovery	74.2%	56.5%	79.2%	53.4%	89.8%	66.1%	87.7%	60.5%	67.6%	45.4%
PO_4^{3-} (mg L^{-1})	1179	1130	1277	1226	1370	1280	1269	1266	1219	1224
Cl^- (mg L^{-1})	42.6	41.5	44.3	37	39	41	41	42.6	43	45
Acetic acid (mg L^{-1})	571	535	742	165	1007	965	1116	1130	1264	1222
EtOH (mg L^{-1})	2793	2123	3313	1408	2973	2013	2962	2862	2182	2066
Formic acid (mg L^{-1})	203	177	150	NF	152	136	155	NF	NF	NF

4.3.2.2. Composition of purified aqueous product

The obtained AP from non-DCM applied sample produced at 350 ℃ was selected for further SCWG and ACA purification treatment. The selection of the samples at this condition is based on the consideration that the usage of organic solvent in the real industrial application is unlikely and 350 ℃ was proved to be the optimal temperature for cHTL, which has been discussed in section 4.3.1.1.

Table 4.5 presents the composition and recovery of AP after two purification treatments. With both treatments, the TOC and TNb content were reduced, however, compared to SCWG treatment, the ACA treatment has a stronger reduction, indicating the absorption might remove a greater proportion of carbon source. While most of NH^{4+} remained after both treatments. After SCWG treatment, the content of K reduced from about 1000 μg ml^{-1} to only 600 μg ml^{-1}, while surprisingly it increased up to more than 2300 μg ml^{-1} after ACA treatment. A similar phenomenon also occurred in the case of Na and Mg, probably due to some of these ions dissolved from the activated carbon into the cHTL-AP. For such ions, this is not considered as a recovery. The other ions remained around the same level as before the treatments. Most of the organic acids were below the detection limitation after purification.

Table 4.5 Composition and recovery percentage of AP with SCWG or ACA purification treatment. NF means not found.

	350 ℃ SCWG	350 ℃ ACA
TC (mg Ł$^{-1}$)	8599	3230
TIC (mg Ł$^{-1}$)	1628	1095
TOC (mg Ł$^{-1}$)	6970	2136
TOC/C	27.7%	8.7%
TNb (mg Ł$^{-1}$)	3534	1361
Recovery	81.8%	31.5%
NH_4^+ (mg Ł$^{-1}$)	2064	1981
Recovery	47.8%	45.8%
Fe (mg Ł$^{-1}$)	5	0.25
Recovery	6.48%	0.23%
PO_4^{3-} (mg Ł$^{-1}$)	172	21.38
Cl^- (mg Ł$^{-1}$)	54	97.7
SO_4^{2-} (mg Ł$^{-1}$)	300	609
K (µg ml^{-1})	595	2345
Recovery	57.8%	228%
Na (µg ml^{-1})	2.3	158
Recovery	1.7%	113.8%
Ca (µg ml^{-1})	NF	15.45
Mg (µg ml^{-1})	2	116.3
Recovery	1.4%	83.76%

P (µg ml^{-1})	90	90
Recovery	12.9%	12.9%
S (µg ml^{-1})	160	160
Recovery	62.2%	62.2%
Mo (µg ml^{-1})	4	0.21
Ni (µg ml^{-1})	NF	<0.2
Zn (µg ml^{-1})	NF	0.04
Al (µg ml^{-1})	<10	1.5
Glycols (mg L^{-1})	NF	NF
Acetic acid (mg L^{-1})	1788	1788
MeOH (mg L^{-1})	NF	NF
EtOH (mg L^{-1})	NF	NF
Formic acid (mg L^{-1})	NF	NF

4.3.3. Algae cultivation with recovered aqueous product

As presented in Fig 4.5 and Fig 4.6, without ACA treatment, the algae growth seems to be inhibited since cultivation Day 2. While the ACA treated AP exhibits a better growing behavior than the ones with standard culture, which proves that ACA treatment not only can remove the undesired compounds (phenolics, cyclic nitrogenous compounds or heavy metals) effectively but also promote the further growth, probably due to the dissolved K and Na. The cultivation of *C. vulgaris* algae was successfully performed. It can be noted that from Day 2, the growth with ACA-treated AP exhibits a rapid increase over the cultivation time. While the growth with untreated AP seems to be stopped from Day 3. This shows that the recycling of the cHTL-AP with ACA purification treatment is technically feasible, and to use the biochar generated from microalgae thermal conversion could also be one alternative for replacing the

commercial activated carbon, in order to achieve full utilization of microalgae feedstock and cost reduction of the purification treatment.

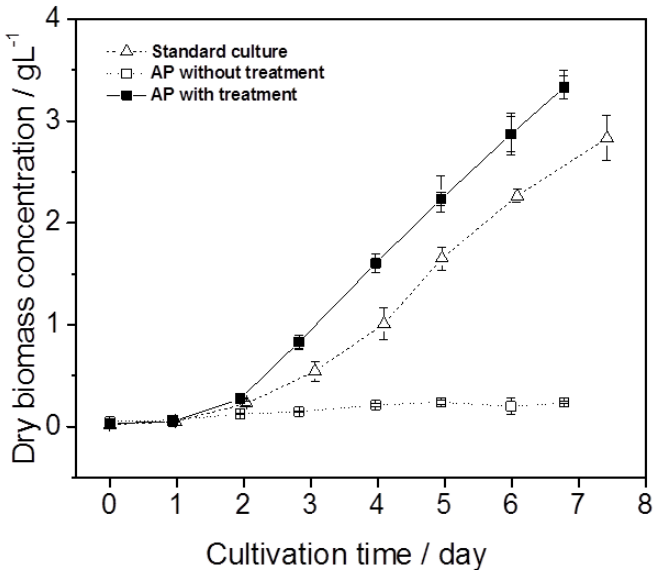

Fig 4.5 Algae growth curve (dry biomass concentration with cultivation days). Data are shown as mean of two cultivations in each condition with the standard deviation.

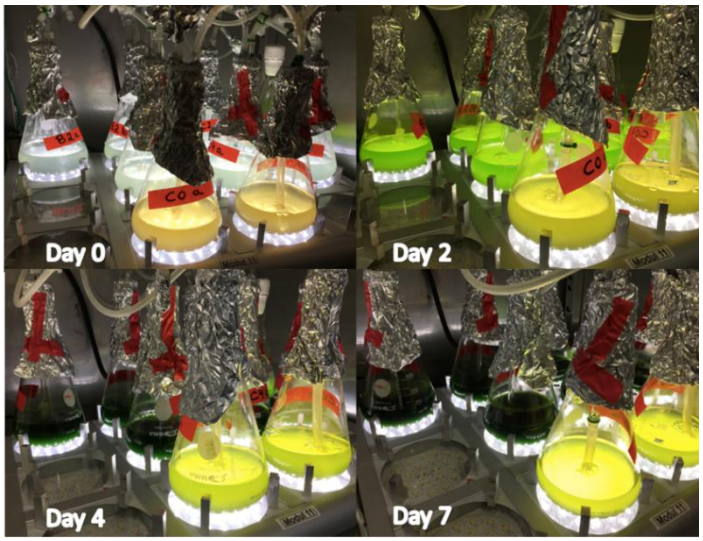

Fig 4.6 Algae cultivation with recovered ACA treated HTL-AP (front right two flasks present cultivation with no treated AP; back left two flasks present cultivation with treated AP; the middle two flasks present cultivation with the standard medium).

4.4. Conclusions

In this study, microalgae *Chlorella vulgaris* was processed via HTL in a continuous stirred tank reactor at different temperatures. The HTL products were separated with and

without DCM. A temperature 350 ℃ was proved as the optimal temperature for cHTL as suggested by batch experiments, obtaining the highest biocrude+solid yield and increasing temperature after 350 ℃ seems to have less influence on biocrude elemental content. The addition of DCM induced about 9 wt.% higher of biocrude yields at the expense of more nitrogen into the biocrude in microalgae cHTL, while this effect on biocrude quality tends to be alleviated at higher cHTL temperature. An average biocrude yield estimation can be proposed by $Yield_{non\text{-}DCM} \approx 81.8\% * Yield_{DCM}$.

After purification, the ACA treatment was proved to remove the toxic components effectively. Probably due to more K and Ca ion content dissolved from the activated carbon into the AP, the algae showed a better growth with treated AP than with the standard culture medium. Algae grew faster and obtained higher biomass content with treated AP. The results proved the feasibility of using the Ap from cHTL to improve the overall nutrient efficiency and potential to reduce the overall cost for a microalgae biorefinery.

Chapter 5. Hydrothermal liquefaction of Chlorella vulgaris and Nannochloropsis gaditana in a continuous stirred tank reactor and hydrotreating of biocrude by nickel catalysts

Chapter redrafted after:

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Declaration of contributions

HTL and upgrading experiments: Bingfeng Guo and Vincent Walter.

Analysis, interpretation of the data: Bingfeng Guo and Vincent Walter.

Draft of manuscript: Bingfeng Guo.

Critical revision of the article: Ursel Hornung, Nicolaus Dahmen.

5. Hydrothermal liquefaction of Chlorella vulgaris and Nannochloropsis gaditana in a continuous stirred tank reactor and hydrotreating of biocrude by nickel catalysts

5.1. Introduction

For improving the commercial competitiveness and usability of microalgae biocrude, as discussed in Chapter 1, section 2.6, the catalytic upgrading of raw HTL biocrude is another significant point. However, most of the related studies were focused on upgrading step alone, while the combination study of HTL and upgrading is of utmost importance for the technical and economic evaluations of the whole microalgae biofuel application (Castello, Pedersen et al. 2018, Hu, Gong et al. 2019). Based on the work from chapter 4, further work is designed to combine the continuous HTL and catalytic upgrading of the obtained biocrude.

In this chapter, a continuous processing of microalgae by HTL and additional catalytic upgrading experiments are reported. Two microalgae strains are hydrothermally liquefied in a continuous stirred tank reactor (CSTR). After product separation, the obtained biocrudes are catalytically upgraded in a micro autoclave under pressurized hydrogen. The aim is to contribute a further viewing into the microalgae cHTL and followed-up catalytic upgrading opportunities, in the view of proposing recommendations for algae biofuel further process development.

5.2. Materials and methods

5.2.1. Microalgae feedstock preparation

Chlorella vulgaris (*C. vulgaris*), as the most studied algae strains (Chen, Li et al. 2019), it contains high-quality protein for food and feeds application. *C. vulgaris* algae in a dry powder state was obtained from Roquette Klötze GmbH (Germany). *Nannochloropis gaditana* (*N. gaditana*), is found to contain high amounts of linolenic acid which is important for biodiesel quality. Fine dry *N. gaditana* algae powder was obtained from Astaxa GmbH (Germany). A detailed elemental and bio-component characterization of the feedstock is given in Table 5.1.

Table 5.1 Feedstock characterization for microalgae strains *C. vulgaris* and *N. gaditana* by elemental analysis (wt.%), biochemical composition (wt.%), ash (wt.%), moisture content (wt.%) and HHV (MJ ₭g^{-1}).

Strain	C	H	N	S	O*	Protein	Lipid	Carbohydrates	Ash	Moisture	HHV
C. vulgaris	47.7	7.5	8.4	0.5	35.9	51.9	23.6	9.2	6.8	3.6	22.03
N. gaditana	46.8	7.8	7.4	0.7	37.3	43.8	18	25.8	11.8	3.5	21.84

*by difference (100-C-H-N-S)

The feedstock and its preparation are shown in Fig 5.1. Suspensions with 5 wt.% of microalgae mass were prepared with distilled water in a 5 L beaker and motor-stirred at 100 rpm for 30 min to avoid agglomeration and make the suspension homogenous, facilitating later feeding into the continuous reactor.

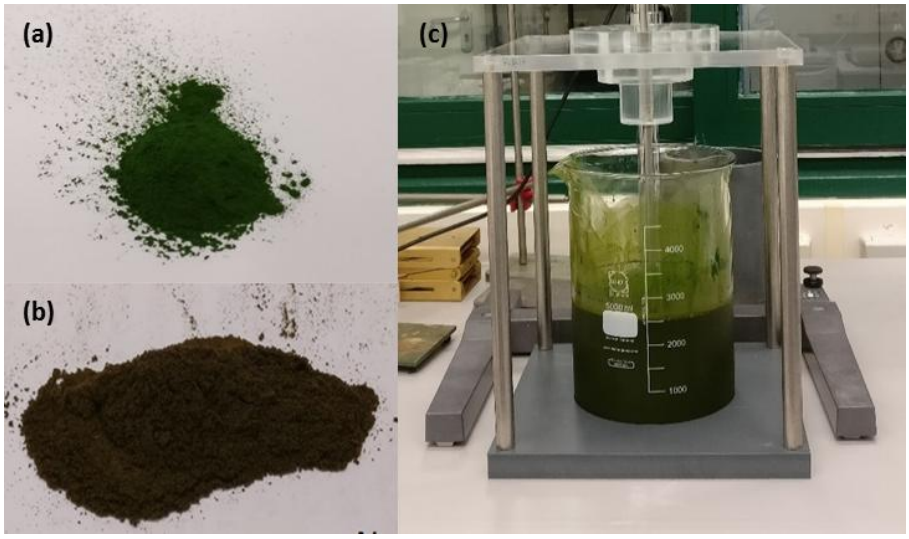


Fig 5.1 Microalgae strains (a) *C. vulgaris* (b) *N. gaditana* and (c) preparation of algae slurry.

5.2.2. Continuous hydrothermal liquefaction and products separation

Detailed information about the reactor can be found in chapter 4, section 4.2.2. According to previous studies on continuous HTL, as presented in chapter 1, table 1.4, consistent findings are the application of short residence times from 0.5 min to 15 min at temperatures from 250 ℃ to 380 ℃, depending on the different strains and reaction modes. In this study, a double screw press was used to feed the algae slurry continuously into the reactor, with the flowing rate adjusted to a residence time of 15 min, which was proved to

obtain high biocrude yield (Barreiro, Gómez et al. 2015) and cHTL experiments were carried out at a temperature of 350 ℃ and pressure of 24 MPa, this is proved to be the most optimized condition for cHTL in chapter 4. After about 2 h preheating period of the whole system with pure water, the temperature inside the reactor was heated to the target temperature 350 ℃, then the algae slurry was started to feed into the reactor, and another 90 min (about 6 times the CSTR residence time) was maintained to ensure a stationary HTL state for sample collection. The product stream was depressurizated, cooled and released rapidly from a pipe outlet, stabilizing the pressure in the cHTL system around 24±1 MPa. The gas sample was lost during the process and the mixture of biocrude, aqueous and solid product was released together and collected in a pre-weighed bottle. After reaching the steady HTL state, sample collection for yield calculation was performed with an exact time of 3 min sampling duration and taken every 20 min. At least, three samples were taken at each time point for yield calculation. Afterward, the reactor was given enough time to convert all the remaining algae slurry to produce sufficient amount of biocrude for further upgrading experiments.

Batch experiments of two algae strains were also performed using the autoclaves at similar conditions (350 ℃, 25 MPa and 15 min holding time). In brief, 7 g of 5 wt.% algae slurry was injected into 10 mL micro autoclave (made of stainless steel EN 1.4571). After 2.2 MPa N_2 pressurized, autoclave was sealed and placed into a heating oven for HTL reaction. The detailed experimental procedure is followed the same way as (López Barreiro, Samorì et al. 2014), which was described in chapter 3, section 3.2.4 as well.

The aqueous phase, biocrude and solid residue from cHTL and batch were separated using vacuum filtration with a Whatman nylon membrane filter (47 mm, 0.45 μm pore size). 35 mL of dichloromethane (DCM, ⩾99.7% purity) was stepwise added into the sample bottle to dissolve the biocrude attached inside and then filtered. The solid residue remaining on the filter material was further dried at 105 ℃ in an oven overnight and measured in weight as the value of cHTL solid product. The filtrate was a mixture of aqueous phase and DCM phase (with biocrude dissolved); after the phases separated spontaneously, the aqueous phase was recovered with a syringe. The DCM phase was evaporated by nitrogen flushing until constant weight was reached. This then was considered as the biocrude product. Two 5 mL of the aqueous phase was placed at 105 ℃ in an oven for determining the dry mass content. The

aqueous product yield is calculated using the dry mass content and the weight of aqueous phase.

The biocrude for the upgrading experiment (larger sample) was separated in a similar way: for the purpose of reduction of separation time and solvent amount, after overnight sedimentation, most of the aqueous product remaining in the upper phase of the bottle was removed with a pipette. The remaining liquid separation was carried out with DCM along with the same procedure aforementioned. It should be noted that this procedure difference might affect the biocrude properties, and result in a lower biocrude yield since DCM can convert organics components from aqueous phase into biocrude (López Barreiro, Riede et al. 2015). With assist of a 50 ℃ heating plate and N_2 flushing, the completed DCM evaporation of the larger sample took around one week until a constant weight was reached.

5.2.3. Heterogeneous catalytic upgrading of biocrude

The catalytic upgrading of algal biocrude was conducted using 10 mL microautoclave, made of EN 1.5471 stainless steel. The hydrotreating reaction conditions were selected as 250 ℃ and 400 ℃ and a holding time of 4 h.

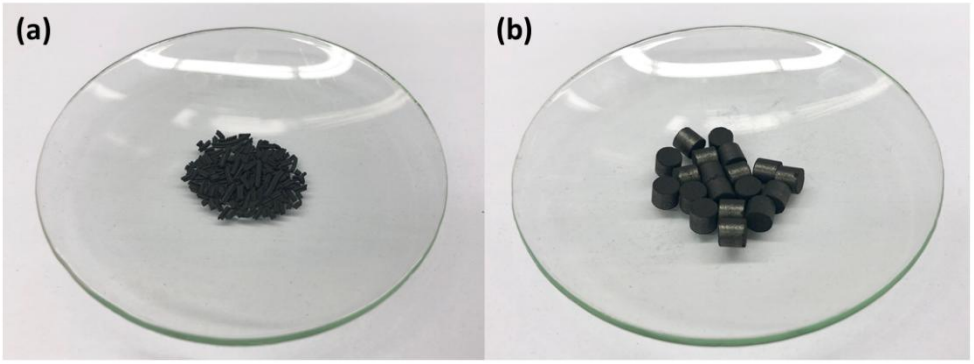


Fig 5.2 $NiMo/Al_2O_3$ catalyst (a) and NiW/ Al_2O_3 catalyst (b).

As shown in Fig 5.2, commercial presulphided $NiMo/Al_2O_3$ (pellets size: 1.3-2.5 mm) and NiW/Al_2O_3 (pellets size around 5 mm, pretreated into smaller size before use to increase the contact with biocrude) catalyst in the form of extrudates were used in this study and they were obtained from Süd-Chemie AG, München. A loading of 20 wt.% catalyst (around 0.2 g) was applied to 1 g raw biocrude and mix them carefully and homogenously. Although it has been reported (Duan, Bai et al. 2013), 5 wt.% loading could be the ideal amount for limiting

coke formation, since high nitrogen content was found in *C. vulgaris* and *N. gaditana* raw biocrude, which appears to reduce with the increasing catalyst loading, a loading of 20 wt.% is expected for the highest energy recoveries. After nitrogen flushing to remove the air from inside the autoclave, 6 MPa of H_2 was preloaded into the micro autoclave at room temperature, during the upgrading, a pre-calculated pressure of 10.7 and 13.8 MPa was reached at 250 ℃ and 400 ℃, respectively. The micro autoclaves were then placed in a GC oven, about 13 and 25 min were required for heating up to the target temperature 250 ℃ and 400 ℃, respectively. After the preheating period, another 4 hour holding time was maintained before quenching in an ice bucket. Autoclaves were then air-dried and opened in a gas collection system, which allowed for measuring the gas amount and to collect samples for gas chromatography measurement in order to determine gas product mass and product distribution. The biocrude recovery was carried out by a similar DCM solvent extraction procedure aforementioned in section 5.2.2. Due to the limited amount of generated water, DCM was used for removing upgraded products from the reactor and separating the bicorude from solids (spent catalysts+solid product). To investigate the effect of catalyst on biocrude upgrading, a blank sample without any catalyst for comparison was also prepared. The different material groupings along with their abbreviations used in the following are presented in Fig 5.3. All experiments within a group were repeated at least threefold.

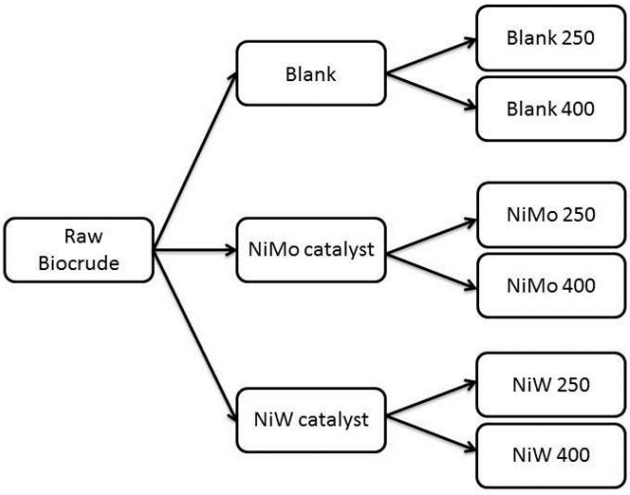


Fig 5.3 Upgrading groupings and abbreviations.

5.2.4. Determination of cHTL and upgrading yields

The yields of cHTL products are examined on the dry mass basis, as the ratio of the different fractions recovered mass and the mass of algae feedstock delivered during the 3 min sampling time, according to the following equation (5.1):

$$\text{Product yield [wt.\%]} = \frac{\text{Product mass that released from the reactor}_{\text{[g,dry]}}}{\text{Microalgae feedstock mass that flowed into the reactor}_{\text{[g,dry]}}} * 100\% \quad (5.1)$$

The upgraded products in the autoclave consisted of four fractions: upgraded oil, gas product, solid residue and water phase. However, the generated water phase was mainly spread as drops around the autoclave cap and got lost during the solvent evaporation. Therefore only the other three phases were managed to be measured quantitatively, the value of the solid product is the mass remained on the filter minus the amount of catalyst applied. The gas product amount and distribution was calculated with gas chromatography results and pressure inside the autoclave, by ideal gas law.

The yields of upgraded products are determined by the following equation (5.2):

$$\text{Product yield [wt.\%]} = \frac{\text{Product mass}_{\text{[g,dry]}}}{\text{Raw biocrude mass}_{\text{[g,dry]}}} * 100\% \quad (5.2)$$

5.2.5. Analysis of cHTL and upgraded products

The elemental analysis (EA) of feedstock, raw and upgraded biocrude was measured by a Vario EL Cube Analyser, the oxygen content is determined by difference (100-C-H-N-S), and Boie's formula, as shown below (5.3), is used to calculate the higher heating value (HHV). Direct measurement of HHV (MJ ₭g^{-1})was done on partial samples to compare with the calculated value.

$$HHV = 0.3516 * w(C) + 1.16225 * w(H) - 0.1109 * w(O) + 0.0628 * w(N) + 0.10465 * w(S) \quad (5.3)$$

With w being the mass fractions of the different elements.

The total organic carbon (TOC), total inorganic carbon (TIC), total carbon (TC) and total nitrogen bound (TN_b) analysis in the cHTL aqueous product were carried out using a DIMATOC 2100. 50 µL of aqueous product is diluted with distilled water by 1:100. The gas product composition from the upgrading experiments was double-checked with two gas chromatographs (Agilent 1540A and 7890A) with a front flame ion detector and a back

thermal conductivity detector (GC-FID/TCD) using porapak Q and molsieve 5 Å column. The carrier gas is helium with a flow of 18 mL min^{-1}, and detector temperature is 250 ℃. Gel permeation chromatography (GPC) from Merck Hitachi with LaChrom RI detector was applied to raw and upgraded biocrude samples to determine the molecular weight. 10 mg sample was dissolved in 10 mL tetrahydrofuran and drops of toluol for the measurement. Thermogravimetric analysis (TGA) was performed to investigate the fraction of raw and upgraded biocrude; about 10 mg of biocrude was measured by a Mettler toledo DSC 822 under N_2-flow with a heating rate of 10 ℃ min^{-1}, from 20 ℃ to 800 ℃. ^{1}H-NMR was done with Bruker Avance 250. For each analysis, about 20 mg of biocrude was well dissolved into 800 uL deuterated chloroform ($CDCL_3$, 99.8% purity) in a 5mm diameter NMR tube and spectra were acquired at 250 MHz across 32 transients. All measurements are repeated at least in duplicates from different independent experiments and a mean value is reported.

5.3. Results and discussion

5.3.1. cHTL experiments

5.3.1.1. Product yields

Fig 5.4 presents the product yields during the stationary period in cHTL. An average of 36.2 wt.% and 31.5 wt.% biocrude yield for *C. vulgaris* and *N. gaditana* algae have been obtained, respectively. A difference of about 5 wt.% biocrude yield between two algae could be caused by a lower lipid content in *N. gaditana* algae. Besides, compared to a previous study performed at the same HTL condition in (Barreiro, Gómez et al. 2015), *N. gaditana* algae presented 54.8 wt.% and 50.8 wt.% biocrude yield at 18.2 wt.% and 9.1 wt.% feeding concentration, respectively, thus it becomes clear that a lower feeding concentration leads to a lower biocrude yield. That means, in return, that aqueous organics become the dominating products at low feeding condition, which is probably caused by more oxygenated compounds converted to water at low feeding concentration (Castello, Pedersen et al. 2018). Similar results were reported in (Jena, Das et al. 2011) as well. Besides, more gas product was produced from *N. gaditana* than *C. vulgaris*, which is possibly due to the high carbohydrate content in *N. gaditana* feedstock, because carbohydrates are easier to decompose to gas products via HTL at the applied reaction conditions (Teri, Luo et al. 2014). Technically, another interesting phenomenon was noticed, instead of a later sharp drop in biocrude yield (probably due to pump blockage) which was previously reported (Barreiro, Gómez et al.

2015), a slight descension in biocrude yield was noticed all over the whole processing time. This could be caused by the lower feeding concentration, leading to less solid products and therefore pump blockage was prevented. In general, a much smoother cHTL operation with low feeding was experienced than before (Barreiro, Gómez et al. 2015). Lower feedstock loading is more suggested in running cHTL when it comes to the technical feasibility, while high feedstock loading (normally higher than 15 wt.%) certainly brings more economical benefits and prompts a great increase in biocrude yield (nearly 20 wt.% higher in (Barreiro, Gómez et al. 2015)).

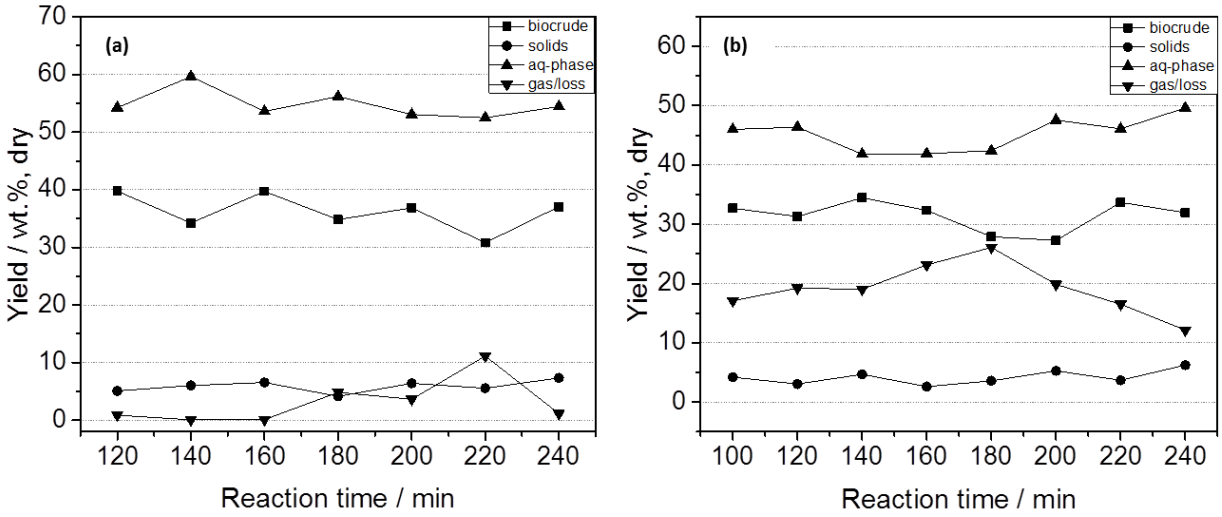


Fig 5.4 Product yields of (a) *C. vulgaris* (b) *N. gaditana* algae in cHTL (350 ℃, 24 MPa and 15 min residence time).

The average yields of cHTL and batch experiments are shown in Table 5.2. Generally, compared to batch results, cHTL of both algae shows lower biocrude yields, probably because of the different heating rate and residence time: for example, in cHTL, 15 min of residence time was shorter compared to that (about 30 min including heating-up and cooling) of batch experiment. Besides, due to the intensive back mixing in CSTR, an "active hydrogen" effect was prompted (Kruse and Faquir 2007). Fresh feedstock and reaction products were mixed, the intermediate products were then more formed and high order reactions were suppressed, i.e. repolymerization, thus reducing the biocrude yield. It is noteworthy that *N. gaditana* algae have a much higher biocrude yield than that of *C. vulgaris* in batch reactor, which is the opposite during cHTL. This indicates that when given more residence time, more completed hydrolysis of protein might occur and interacts with its carbohydrates, leading to a higher

biocrude yield. Therefore, longer residence time in cHTL is suggested for algae with high protein and carbohydrates content.

Table 5.2 Average product yields of *C. vulgaris* and *N. gaditana* algae in continuous and batch HTL (350 ℃, 25 MPa and 15 min residence time).

	Biocrude	**Aqueous phase**	**Solid**	**Gas (Difference+loss)**
C. vulgaris cHTL	36.2±2.9	55.4±2.3	5.9±0.9	2.5±1.3
C. vulgaris batch	42.5±1.1	47.9±0.2	6±0.2	6.8±1.8
N. gaditana cHTL	31.5±2.4	45.2±2.7	4.2±1.1	19.1±3.4
N. gaditana batch	47.9±4.6	40.4±4.7	5.34±0.1	6.3±0.1

5.3.1.2. Analysis of cHTL biocrude and aqueous product

As presented in Table 5.3, the elemental analysis of raw biocrude is compared for cHTL evaluation. It is noticeable that *N. gaditana* biocrude has a higher carbon and hydrogen content than that of *C. vulgaris* biocrude, possibly caused by a higher amount of carbohydrates in *N. gaditana*, which interacts more intense with amino acids from proteins in the biomass by Maillard reactions (numerous reactions of amine group in proteins with carbonyl group in carbohydrates, producing fission and large polymeric materials, called melanoidins (Peterson, Lachance et al. 2010)), which are believed to elevate low-lipid algae biocrude quality (Zhang, Tang et al. 2016). The oxygen content was significantly reduced via cHTL from about 36 wt.% in the feedstock to 14.6 and 10.3 wt.%, respectively, in *C. vulgaris* and *N. gaditana* biocrude. A reduction in nitrogen content was also observed by around 18% and 23% via cHTL, suggesting a strong denitrogenation reaction taking place during cHTL. The elemental composition of *N. gaditana* biocrude is similar to earlier results (Barreiro, Gómez et al. 2015), indicating that conditions applied have more impact on biocrude composition than feedstock concentration in cHTL. Apart from this, the calculated HHV was prompted from 22 to around 35 MJ·kg^{-1}; the measured HHV was found to differ with less than 5%, proving that the Boie equation applied here is suitable for cHTL biocrude. In terms

of biocrude elemental composition and HHV, *N. gaditana* algae generally exhibit a better feedstock cHTL performance than *C. vulgaris* algae. However, around 6 wt.% of nitrogen and 0.8 wt.% of sulfur in both biocrudes are still unacceptably high for fuel applications.

Table 5.3 Elemental analysis (wt.%) and HHV(MJ ŀkg^{-1}) of raw biocrude produced in cHTL (350 ℃, 24 MPa and 15 min residence time).

	C	**H**	**N**	**S**	**O***	**HHV measured**	**HHV calculated**
C. vulgaris biocrude	68.4	9.3	6.9	0.8	14.6	35.12	33.76
N.gaditana biocrude	73.1	10.2	5.7	0.7	10.3	36.23	36.85

*by difference (100-C-H-N-S)

The results of TOC, TIC, TC, and TN_b in aqueous product are presented in Table 5.4. Most of the carbon in aqueous phase is in organic form. After HTL, more carbon and nitrogen of *C. vulgaris* algae was recovered in the aqueous phase than that of *N. gaditana*, this was in line with the assumption that higher carbohydrate contents in *N. gaditana* enhance the Maillard reaction, forming more biocrude components during cHTL. It is also consistent with the lower carbon content in *C. vulgaris* biocrude, indicating that *N. gaditana* is a better strain than *C. vulgaris* in terms of carbon conversion into biocrude.

Table 5.4 TOC (mg ŀL^{-1}), TIC (mg ŀL^{-1}), TC (mg ŀL^{-1}), and TNb (mg ŀL^{-1}) analysis of cHTL aqueous product.

Strain	**TOC**	**TIC**	**TC**	**TN_b**
C. vulgaris aqueous product	13294±1466	<1000	13501±1489	4303±491
N. gaditana aqueous product	9863±1029	<1000	10048±998	2834±244

5.3.2. Upgrading experiments

5.3.2.1. Product yields

The overall yields of the upgrading experiments are shown in Fig 5.5. Clearly, the upgrading temperature makes a significant influence on upgraded biocrude yields. For *C. vulgaris* raw biocrude, in general, over 90 wt.% of the biocrude remained at 250 ℃, while at 400 ℃ the yield shrinks to around 54 wt.% by catalytic conversion, and the yield of other products increase correspondingly. This is evidence that a stronger cracking reaction occurred at 400 ℃ and more volatiles are generated. The similar phenomenon was observed before (Elliott, Hart et al. 2009), increasing temperature leads to decreased wood-based fast pyrolysis bio-oil yield, especially at high-pressure condition (Schmitt, Raffelt et al. 2018). Besides, the mass balance closes to almost 100 % at 250 ℃, while about 25-30 wt.% mass loss is observed for *C. vulgaris* at 400 ℃. This is possibly due to a stronger hydrodeoxygenation reaction occurred in *C. vulgaris* than *N. gaditana* at 400 ℃, leading to loss by water, since more water drops from *C. vulgaris* biocrude were found left on the cap and inner wall of the autoclaves but which could not be quantified. The molecular weight of two biocrudes was measured by GPC analysis, *C. vulgaris* raw biocrude has an average molecular weight of 479.5 g $\cdot$mol^{-1} while *N. gaditana* raw biocrude has a higher molecular weight of 642.1 g $\cdot$mol^{-1}. The lower average molecular weight of *C. vulgaris* suggests more light organics might exist in *C. vulgaris* raw biocrude, which is easier to lose during the evaporation process, leading to a bigger mass loss. Its higher gas yield also indicates a much stronger cracking reaction at 400 ℃ than that of *N. gaditana*. In terms of *N. gaditana* raw biocrude upgrading, a similar pattern can be found in product distribution, however, less water and higher molecular weight were obtained in the upgrading process, leading to a better mass balance closure of 97 % for 250 ℃ and 87 % for 400 ℃. Generally, the temperature remains as key factor in upgrading yields determination; while 250 ℃ seems to be too mild for cracking reaction to take place, 400 ℃ is more suitable for prompting hydrodeoxygenation and other upgrading reactions.

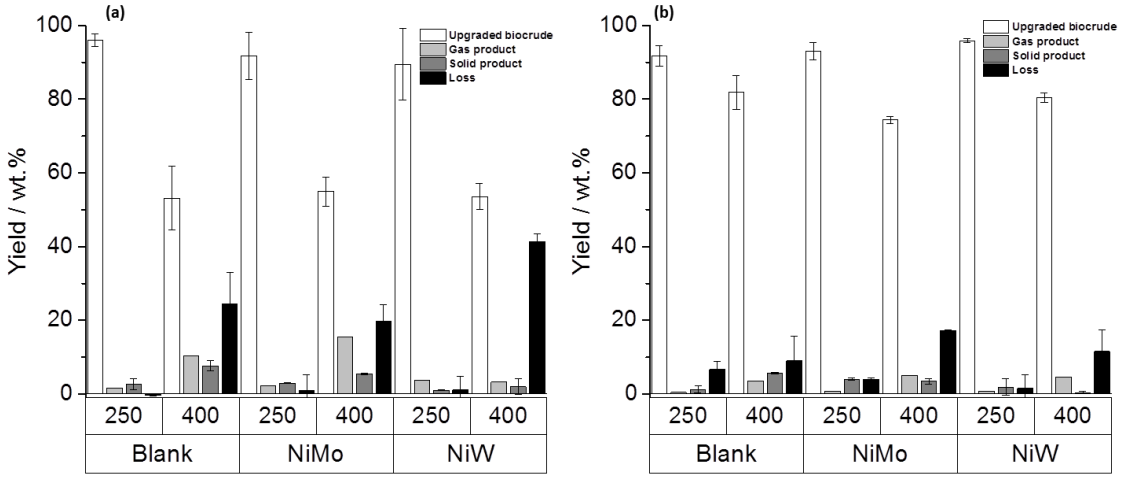


Fig 5.5 Product yields of (a) *C. vulgaris* (b) *N. gaditana* biocrude in upgrading experiments (250 ℃, 10.7 MPa and 400 ℃, 13.9 MPa under a hydrogen atmosphere for a holding time of 4 hour).

5.3.2.2. Gas product

Gas chromatography was used to analyze the gas product of upgrading experiments. Fig 5.6 displays the gas composition for the different groups of experiments. In both algae biocrudes, excluding the remaining preloaded H_2, the gaseous product at 250 ℃ mainly composes carbon dioxide, carbon monoxide and little amount of methane. Much more methane, ethane, ethene, and alkane are generated at 400 ℃, which could be evidence of intensive cracking reaction. Furthermore, on average about 5.13 times the mass of the total gas was generated in the reactor at 400 ℃ samples than that of 250 ℃ samples. This reveals a low reactivity at 250 ℃ reaction, mainly decarbonylation and decarboxylation. In contrast, stronger reactions took place at 400 ℃ such as hydrogenation and cracking.

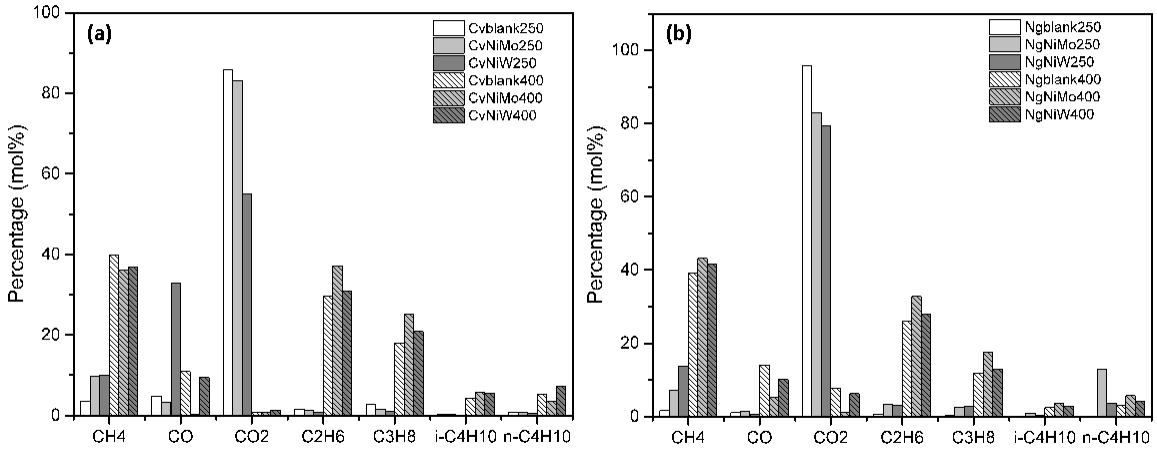


Fig 5.6 Gas product distribution of (a) *C. vulgaris* (b) *N. gaditana* biocrude in upgrading experiments.

With the result from GC analysis and pressure measured in the reactor, the H_2 consumption during each upgrading group is calculated, as presented in Table 5.5. It can be noted that during 250 ℃ upgrading, there was almost no H_2 consumed (in the case of *C. vulgaris* biocrude, there are even negative values, indicating more H_2 released from the biocrude than consumed), which is in line with the general low reactivity at 250 ℃. While 400 ℃ promotes stronger hydrogenation reaction, evidenced by the high H_2 consumption in both biocrude upgrading.

Table 5.5 Average hydrogen consumption ($kg_{H2}/kg_{raw\ biocrude}$) in each upgrading group.

Groups	***C. vulgaris* upgrading**	***N. gaditana* upgrading**
Blank 250	-0.011261	0.001418
Blank 400	0.011883	0.016956
NiMo 250	-0.009576	-0.000163
NiMo 400	0.016656	0.022377
NiW 250	-0.005708	0.001283
NiW 400	0.019783	0.022895

5.3.2.3. Elemental analysis and Van Krevelen diagrams

The elemental results of upgraded biocrude together with their calculated HHV are presented in Table 5.6. After upgrading, the carbon and hydrogen content of the *C. vulgaris* biocrude are markedly increased, especially at 400 ℃. The removal of heteroatom is significant: the oxygen content drops from 14.6 wt.% to only 1 wt.% in the best case of NiMo catalyst at 400 ℃. Similar results were reported in (Haider, Castello et al. 2018), which found temperature is the only significant factor influencing the oxygen removal. Meanwhile its nitrogen content reduces to its original 52% and its sulphur content turns to 25%, which also leads to a great improvement of the HHV, increasing from 33.76 to 42 MJ ₭g^{-1}. The same pattern can be found in *N. gaditana* biocrude as well, its highest HHV can be increased up to 43.74 MJ ₭g^{-1}. In general, NiMo catalyst presents good performance on the deoxygenation and denitrogenation while NiW catalyst promotes stronger desulphurization reaction. Both catalysts exhibit a higher reactivity for *N. gaditana* biocrude.

Table 5.6 Elemental analysis (wt. %) of cHTL raw biocrude, upgraded biocrude and calculated HHV (MJ ₭g^{-1}).

Groups	C	H	N	S	O*	HHV
C. vulgaris raw biocrude	68.4	9.3	6.9	0.8	14.6	33.76
C. vulgaris blank 250	73.6	10.4	5.5	0.7	9.7	37.31
C. vulgaris NiMo 250	74.0	10.5	5.6	0.4	9.3	37.66
C. vulgaris NiW 250	74.2	10.4	5.4	0.2	9.7	37.48
C. vulgaris blank 400	82.9	11.4	4	0.3	1.3	42.59
C. vulgaris NiMo 400	83.7	11.4	3.6	0.2	1	42.88
C. vulgaris NiW 400	83	11.3	4.1	0.1	1.4	42.43
N. gaditana raw biocrude	73.1	10.2	5.7	0.7	10.3	36.85
N. gaditana blank 250	75.7	10.8	4.8	0.5	8.2	38.61
N. gaditana NiMo 250	75.2	11.2	4.8	0.4	8.4	38.87

N. gaditana NiW 250	74.9	11.1	4.8	0.2	9	38.56
N. gaditana blank 400	78.8	11.2	4.7	0.3	5	40.50
N. gaditana NiMo 400	82.3	12.7	3.2	0.2	1.6	43.74
N. gaditana NiW 400	80.9	11.9	4.4	0.2	2.6	42.28

*****calculated by difference (100-C-H-N-S)

This phenomenon can be clearly seen by Van Krevelen diagrams, which are made to categorize biofuels by their elemental C, H, N and O ratio for comparison with diesel fuels. As can be seen in Fig 5.7, the upgrading of *C. vulgaris* biocrude seems to be only temperature dependent, while NiMo catalyst removes more nitrogen and oxygen from *N. gaditana* biocrude, especially that of 400 ℃. The different catalytic behaviors of two algae biocrude could be explained as all molecules in *C. vulgaris* biocrude, which can react with the catalyst, are not any more present in the biocrude, but in the aqueous phase, evidenced by the high TOC value in its aqueous phase and a relatively low molecular weight of biocrude. While in *N. gaditana* biocrude, due to a stronger Maillard reaction, more "catalytic active" components generated in the biocrude phase, resulting in a higher catalyst reactivity. In terms of O/C, the 400 ℃ groups are even close to the range of diesel fuel, representing the catalytic upgrading processing and improvement of biocrude quality were successfully carried out.

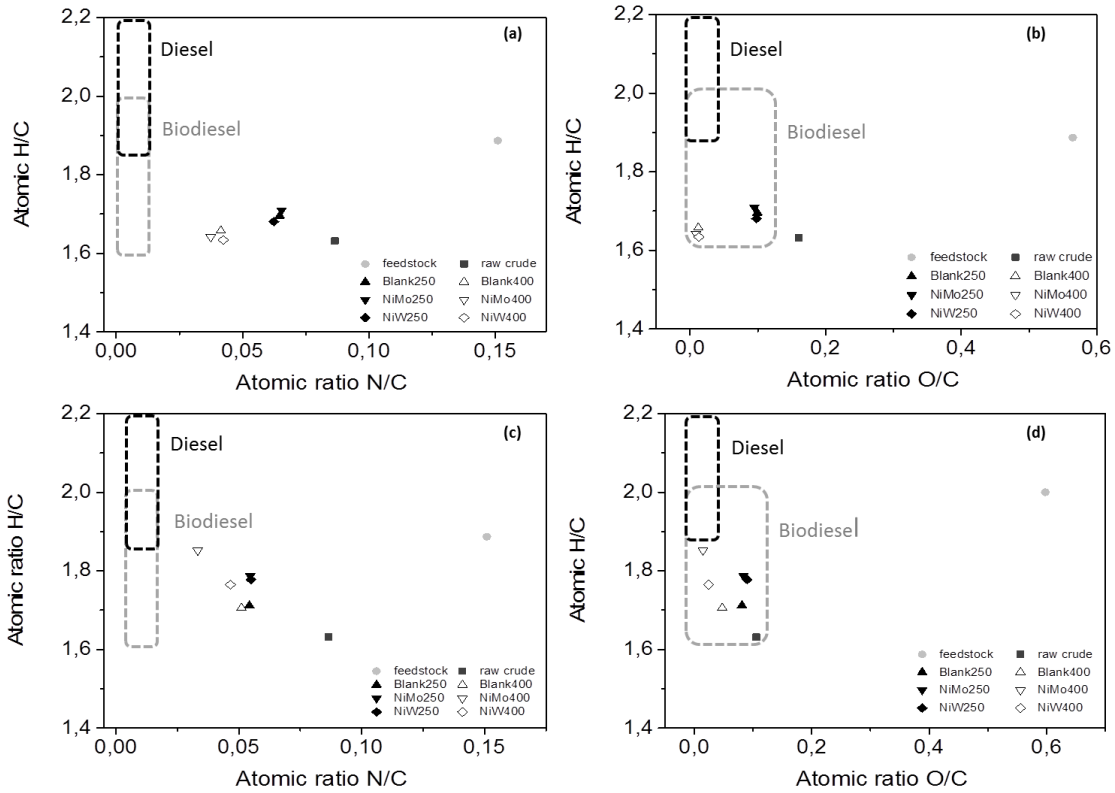


Fig 5.7 Van Krevelen diagrams of algae feedstock, raw biocrude, and upgraded biocrude. (a) and (b) *C. vulgaris*; (c) and (d) *N. gaditana*.

5.3.2.4. Biocrude distillation and fuel fraction type distribution

In order to quantify the fuel improvement, by TGA, a distillation of the biocrude from 25 ℃ to 800 ℃ was performed and the different oil fractions from raw and upgraded biocrude were determined according to (Gai, Zhang et al. 2014).

As presented in Table 5.7, both algae biocrudes exhibit a similar pattern: the C14-C20 (diesel oils), C20-C50 (lubricating oils) and >C70 (residues) fractions contain the most part of the biocrude mass: a slight reduction is observed in diesel oils range after 250 ℃ upgrading, while lubricating oils fraction remains around 50 wt.%. The residue has an average increase of 8 wt.%, which may be due to more repolymerization reactions at 250 ℃. When it comes to 400 ℃ upgrading, more fractions are formed in diesel oils and kerosene, besides a significant decrease in lubricating oils has been seen, which indicates lower molecular weight of the biocrude and a lower viscosity consequently. The results from GPC measurement confirm this assumption: the molecular weight of upgraded biocrude seems to be temperature dependent, where upgraded *C. vulgaris* biocrude has the average value of 693.3 g mol^{-1} and

595.3 g·mol^{-1} at 250 ℃ and 400 ℃ upgrading, and *N. gaditana* biocrude has the average value of 850.4 g·mol^{-1} and 512.6 g·mol^{-1} at 250 ℃ and 400 ℃ upgrading, respectively. Improved lower viscosity and better flowability of upgraded samples are also observed, however, due to the limited amount, a direct viscosity measurement was not done. In general, the upgraded oil presents better physical properties for transportation fuels, especially after 400 ℃ upgrading: gasoline, kerosene and diesel oils increase from about 18 wt.% to more than 30 wt.% of biocrude after catalytic upgrading.

Table 5.7 Oil type distribution of raw and upgraded biocrude.

Carbon range **Distillation range** **Oil type (wt.%)**	**C1-C9** **(< 70 ℃)** **Gas/Naptha**	**C5-C10** **(70-120 ℃)** **Gasoline**	**C10-C16** **(120-170 ℃)** **Kerosene**	**C14-C20** **(170-250 ℃)** **Diesel oils**	**C20-C50** **(250-500 ℃)** **Lubricating oils**	**C20-C70** **(500-600 ℃)** **Fuel Oils**	**>C70** **(> 600 ℃)** **Residue**
C. vulgaris raw biocrude	0	0.7	3.2	14.2	50.6	6.8	24.6
C. vulgaris Blank 250	0.09	0.37	2.06	11.25	49.6	3.6	33.03
C. vulgaris Blank 400	0.08	0.72	3.9	22.8	39.3	3.6	29.6
C. vulgaris NiMo 250	0.16	1.28	3.06	11.7	51.99	3.42	28.39
C. vulgaris NiMo 400	0.06	0.79	4.75	24.4	39.9	2.85	27.25
C. vulgaris NiW 250	0.02	0.48	2.27	9.93	51.7	2.5	33.1
C. vulgaris NiW 400	0.03	0.47	3.3	22	41.7	3	29.5
N. gaditana raw biocrude	0.22	0.83	2.85	15.2	49.2	3.2	28.5
N. gaditana Blank 250	0.04	0.26	1.49	9.36	53.88	2.86	32.11
N. gaditana Blank 400	0.74	2.33	7.31	21.4	30.47	2.82	34.93
N. gaditana NiMo 250	0.08	0.31	1.41	9.59	55.49	2.62	30.5
N. gaditana NiMo 400	1.35	2.58	7.55	21.49	32.44	2.91	31.68
N. gaditana NiW 250	0.15	0.36	1.32	9.37	53.05	2.93	32.82
N. gaditana NiW 400	1.85	3.15	8.85	21.15	28.05	2.84	34.14

5.3.2.5. ^{1}H-NMR analysis

The ^{1}H-NMR analysis was applied on biocrude samples for revealing the distribution of different types of functional groups in the biocrude. The spectrum looks similar regardless of which biocrude and catalyst were used; a typical spectrum is shown in Fig 5.8 as an example.

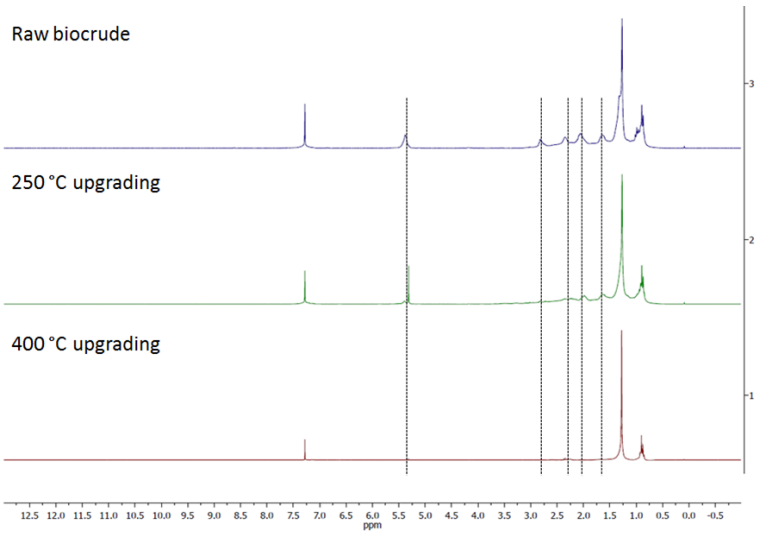


Fig 5.8 A typical ^{1}H-NMR spectrometry of raw and upgraded biocrude.

The most significant changes appear in the chemical shift region from 0.5 ppm to 3 ppm. The peaks at 0.5-1.5 ppm represent terminal hydrogen atoms in alkanes (Xu, Guo et al. 2018), the high alkane content attributed to the fatty acids, derived from the decomposition of triglycerides during HTL (Gai, Zhang et al. 2014). It is obvious that after upgrading this area becomes more narrow and sharper, which indicates more alkanes and aliphatic functional groups were generated. The peaks around 1.5-1.6 and 1.7-2.8 ppm represent protons in β-position and α-position in the heteroatomic functionalities, mainly due to the nitrogenous and oxygenated compounds originated from decomposition of protein (Gai, Zhang et al. 2014). The peak at 5.4 ppm represents phenolic –OH (Mullen and Boateng 2011) and aromatics (Vardon, Sharma et al. 2011). The reduction of these peaks after upgrading shows the fade of aromatic and unsaturated functional components and relatively higher aliphatic compounds in the biocrude phase. The NMR results confirm that the biocrude has been upgraded into a less nitrogen and closer to fuel standard material.

Further integration of different peak areas is listed in Table 5.8 and supports the above-discussed observations from the ^{1}H-NMR spectra. It is clearly shown that both catalysts have an effect on 250 ℃ upgrading, proved by the increase of alkane portion (0.5-1.4), while at 400 ℃, the relative effect of catalyst becomes less influential.

Table 5.8 Integration of different peak area distribution (%) in raw and upgraded biocrude.

Biocrude (Chemical shift)	**Portion of (0.5-1.4)**	**Portion of (1.7-2.3)**	**Portion of (2.5-3)**	**Portion of (5.3-5.5)**
C. vulgaris raw biocrude	49	11	3.8	6.8
C. vulgaris Blank250	52	7.5	0.8	5.3
C. vulgaris Blank400	76.2	0.3	0	0.1
C. vulgaris NiMo250	65	5	0.5	2.7
C. vulgaris NiMo400	71.1	0.9	0	0
C. vulgaris NiW250	65	4	0	4.3
C. vulgaris NiW400	74	1.7	0	0.1
N. gaditana raw biocrude	48.4	8.6	3.6	7.3
N. gaditana Blank250	59.3	5.9	0.3	4.9
N. gaditana Blank400	73.7	1.1	0	2
N. gaditana NiMo250	61.1	7.1	0	3.2
N. gaditana NiMo400	74.8	0.6	0	1.7
N. gaditana NiW250	66.7	4.8	0	1.6
N. gaditana NiW400	70.9	0.2	0	6

5.4. Conclusions

cHTL of two microalgae species was successfully performed at 350 ℃, 24 MPa and 15 min residence time. cHTL of *C. vulgaris* algae results in a higher biocrude yield than that of *N. gaditana* algae, while *N. gaditana* biocrude presents better elemental composition as well as HHV. While for both algae strains, lower yields were obtained in cHTL compared to batch experiments, so it can be recommended to expect lower yields in cHTL. Besides, the longer residence time is more suggested for algae with high protein and carbohydrates content in

cHTL for a higher biocrude yield. When upgrading of cHTL biocrude, *C. vulgaris* biocrude seems to be more temperature dependent, and has a better quality improvement than *N. gaditana* biocrude. While *N. gaditana* biocrude shows a higher reactivity with both catalysts, possibly due to more Malliard reaction products in biocrude, the NiMo catalyst shows a better performance in removing oxygen and nitrogen (more than 93% and nearly 50%, respectively) and leads to a maximum HHV of 43.7 MJ kg^{-1}. Therefore the use of conventional deoxygenation catalysts can be recommended, even though HTL biocrude is already lower in oxygen than bio-oil e.g. from fast pyrolysis. The highest sulphur removal was achieved by NiW catalyst, with a maximum value of 80%. Even though temperature plays a major role in upgrading, the reactivity of catalyst is strain-dependent, the selection of specific catalyst for different algae biocrude is worthy of further investigation.

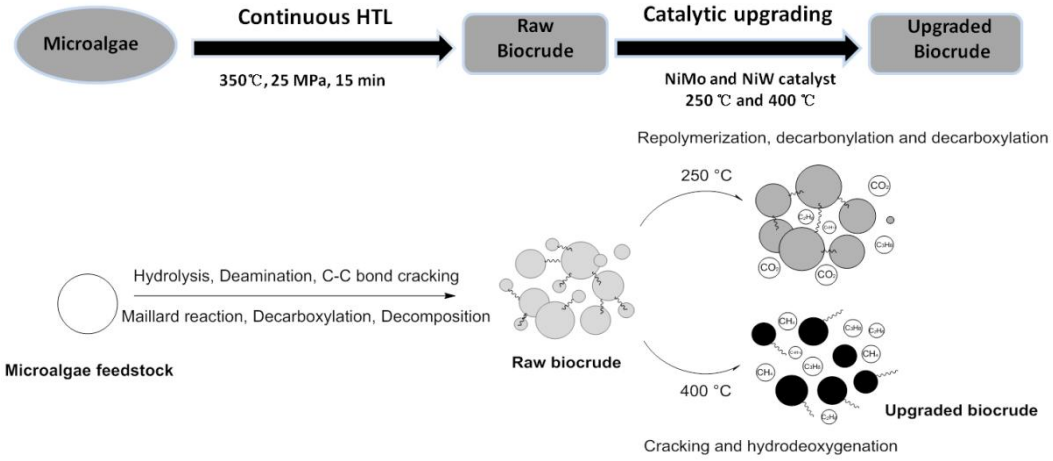


Fig 5.9 Simplified flowsheet of microalgae cHTL and biocrude upgrading.

A simplified flowsheet of this study is given in Fig 5.9. A deeper color of the biocrude molecule indicates a higher energy density and higher molecular weight of biocrude components. Possible reactions are proposed in this study: a variety of reactions are expected during cHTL, such as hydrolysis of proteins, deamination, decarboxylation, Maillard reactions, C-C bond cracking. For upgrading, 250 ℃ appears too mild to trigger hydrogenation reactions on cHTL biocrude and mainly supports repolymerization, decarboxylation, and decarbonylation. A higher temperature of 400 ℃ generates more volatile components, leading to a lower biocrude recovery but it promotes a variety of reactions and improves biocrude quality significantly. Gasoline, kerosene and diesel oils

components increase from 18 wt.% to more than 30 wt.% of the biocrude with catalytic upgrading.

Chapter 6. Techno-economic assessment of a microalgae biorefinery

6. Techno-economic assessment of a microalgae biorefinery

6.1. Introduction

The development of microalgae biofuel via HTL is challenged by the financial viability of scale-up of the various production steps. As proposed in Fig 2.1 and investigated in chapter 3, the PEF treatment and fractionation step are supposed to improve the economics of the process. HTL of the residues after PEF assisted amino acids extraction is found to be a promising route for the combination of PEF and HTL technology since higher yield of biocrude with better quality could be obtained (Guo, Yang et al. 2019). However, the extent of economic benefits provided by the utilization of PEF is still not clear. Therefore in this chapter, a techno-economic evaluation of a microalgae biorefinery was conducted using the data obtained from previous chapters and being based on a previous microalgae biorefinery model (Barreiro. 2015) (a pilot plant with an output of 0.5 MW in the form of biocrude produced via HTL).

6.2. Materials and methods

6.2.1. Assumptions of the process

The flowchart of a potential plant is shown in Fig 6.1, adapted from the previous study (Barreiro. 2015). The model system, location and main assumptions of the plant are taken from the previous study, too. In brief, the plant is designed to be operated annually for 7500 h at a lifetime of 20 years. A flat panel airlift (FPA) technology (Subitech) is applied for algae cultivation using saltwater. The water streams consist of water recovered from harvesting and aqueous phase as well as fresh seawater. A stepwise combination of sedimentation and centrifugation (Evodos 25 spiral plate technology) is designed for concentrating the algae slurry to 150 g kg^{-1}(dry mass). Annually, about 818 ton microalgae biomass is required. The fractionation step is based on the technology developed by the University of Almería (Romero Garcia, Acien Fernandez et al. 2012). This process includes pre-treatment for cell disruption, enzymatic hydrolysis (using commercial enzymes Alcalse and Flavourzyme) and final separation by centrifugation. Microalgae *(S. almeriensis* is selected in this study because it is a suitable strain in southern Spain where the plant is designed for. More importantly, the biocrude from residues of PEF assisted amino acids extraction is considered the most

promising route as stated in chapter 3. The conversion of microalgae is assumed to be done in a continuously operated tubular reactor with residence time of 15 min at 350 ℃, 24 MPa, which is in the same range of the continuous HTL study described in chapters 4 and 5. The enzymatic hydrolysis of proteins and amino acid extraction are performed at 50 ℃ for 180 min. The extraction yields and HTL product yields of the process are being based on the results from chapter 3. Besides, the overestimation by the usage of DCM as separation solvent (results from chapter 4) is also considered ($Yield_{non\text{-}DCM} \approx 81.8\% * Yield_{DCM}$). Moreover, a lower biocrude yield is expected in a continuous mode (results from chapter 5, 65% to 96% of the batch-biocrude yield (Barreiro, Gómez et al. 2015, Guo, Walter et al. 2019)), and therefore averagely 82% of the batch-biocrude yield is taken for transferring the data from batch to continuous operation mode in this study ($Yield_{continuous} \approx 82\% * Yield_{batch}$). However, it should be notable that the difference of microalgae strains in these studies could also have an impact on these processing parameters. A flash separation unit is used to split up the gas-aqueous-biocrude mixture. For separating the biocrude from aqueous phase, an oil skimmer is used.

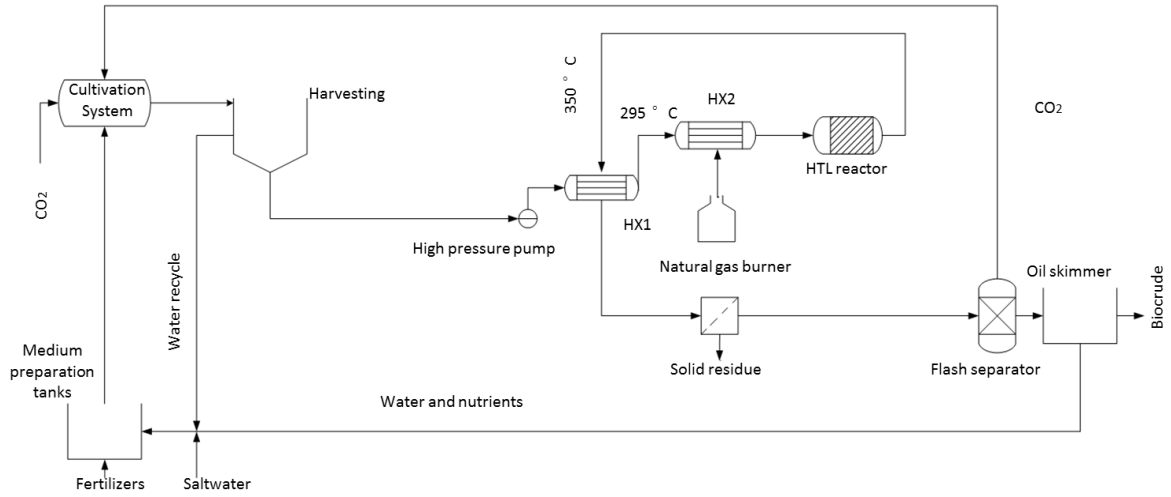


Fig 6.1 Flowchart of the microalgae biorefinery plant.

The biocrude is assumed to be sold at price of 0.37 € kg^{-1}, as this is the value of the crude oil price at September 2019, which would be expected to compete with biocrude. According to similar products already available on the market, the biofertilizer (made of 60 wt.% amino acids) is supposed to be sold between 7.35 € L^{-1} (Aminosol-PS, Lebosol Dünger GmbH, derived from meat residues, therefore is seen as the lowest benchmark) and 10 € L^{-1} (Algafert, Biorizon biotech, Spain). The local electricity price is assumed to be about 0.065 € kWh^{-1}.

6.2.2. Assumptions of costs

The pump ability and harvesting behavior of microalgae *S. almeriensis* are considered to be similar to the microalgae *N. gaditana* of the previous study (Barreiro. 2015). The fixed costs and variable costs are assumed to be the same as the previous work, as presented in Table 6.1 and 6.2 in € a^{-1}. The annual costs is the sum of the fixed costs and variable costs, which is about 2.75 M€ a^{-1}. It is noteworthy that the cultivation of microalgae *S. almeriensis* would require more Mg and Ca species than *N. gaditana*, however, the cost of the fertilizer contributes less than 5 % of the total costs, which can be considered as a minor factor.

Table 6.1 Fixed costs (FC) (in €·a^{-1}).

Item	Cost	% of FC
Depreciation		
Major equipment	795630	36.8
Piping	159126	7.4
Instrumentation	119344	5.5
TOTAL	1074100	49.7
Engineering	322230	14.9
Labor	675000	31.3
Insurance	6445	0.3
Land	1945	0.1
Contingency	79563	3.7
Total FC	2159294	

Table 6.2 Variable costs (VC) (in €·a^{-1}).

Item	Cost	% of VC
Process materials		
Fertilizers	137162	23.0
Natural gas	29228	4.9
Electricity	397313	66.7
TOTAL	563703	94.7
Maintenance and repair	31825	5.3

TOTAL VC	595528

The cost for PEF treatment is assumed to contain three contributions: PEF equipment cost, labor cost and electricity cost, as shown in Table 6.3. The electricity efficiency of PEF treatment is about 75 % and 40 % of the operating time is assumed to be supervised by man with an average labor cost of 30 € h^{-1}.

Table 6.3 PEF treatment costs (PC) (in € a^{-1}).

Item	Cost	% of PC
Depreciation		
PEF equipment	10000	8.5
Labour	90000	75
Electricity	19696	16.5
TOTAL PEF	119696	

The cost for the fractionation step is assumed to be 1 € L^{-1} amino acids concentrate according to the data from the University of Almería in this chapter. However, this is one of the best secrets kept in the biofertilizer company, a cost calculation on the laboratory level comes to 58.34 M€ a^{-1} when scaling up to the scope of this study, thus deeply requires further consideration.

The economic key numbers such as income, cost, return and minimum fuel selling price (MFSP, the price for compensating the production costs) are calculated using following equations (6.1, 6.2, 6.3 and 6.4):

$$\text{Income} = \textit{yearly productivity of biocrude} * \text{unit price of biocrude} + \text{yearly productivity of high value product} * \text{unit price of high value product} \quad (6.1)$$

$$\text{Cost} = \textit{Fixed costs} + \textit{Variable costs} + \textit{PEF costs} + \textit{Fractionation costs} \quad (6.2)$$

$$\text{Return} = \textit{Income} - \textit{Cost} \quad (6.3)$$

$$\text{MFSP} = \frac{(\textit{Fixed costs} + \textit{Variable costs} + \textit{PEF costs} + \textit{Fractionation costs} - (\text{yearly productivity of high value product} * \text{unit price of high value product}))}{\textit{yearly productivity of biocrude}} \quad (6.4)$$

6.2.3. Value chain scenarios

In order to study the economic improvement by single fractionation and PEF assisted fractionation, three scenarios have been assessed, as presented in Fig 6.2: Scenario 1, as the base case, microalgae biomass are directly converted to biocrude via HTL as the core technology; Scenario 2, microalgae first undergo a fractionation procedure for the extraction of amino acids concentrates, and then the residual biomass is converted to biocrude via HTL; Scenario 3, based on scenario 2, PEF treatment is introduced for assisting the extraction of amino acids concentrate.

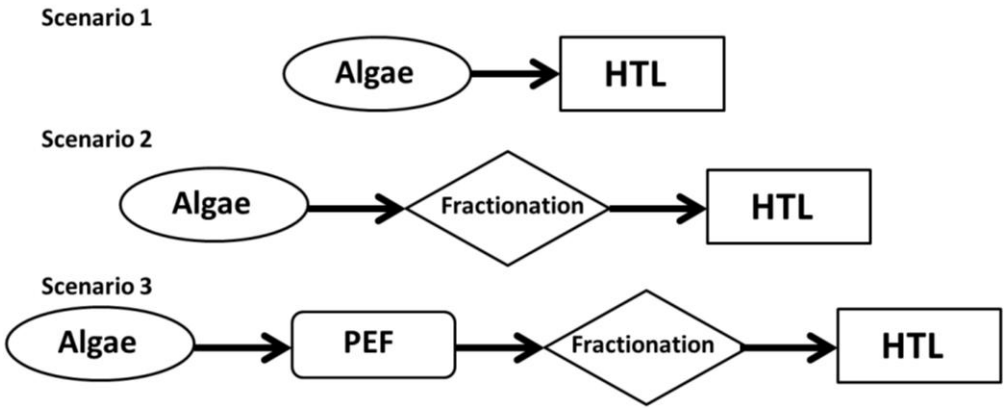


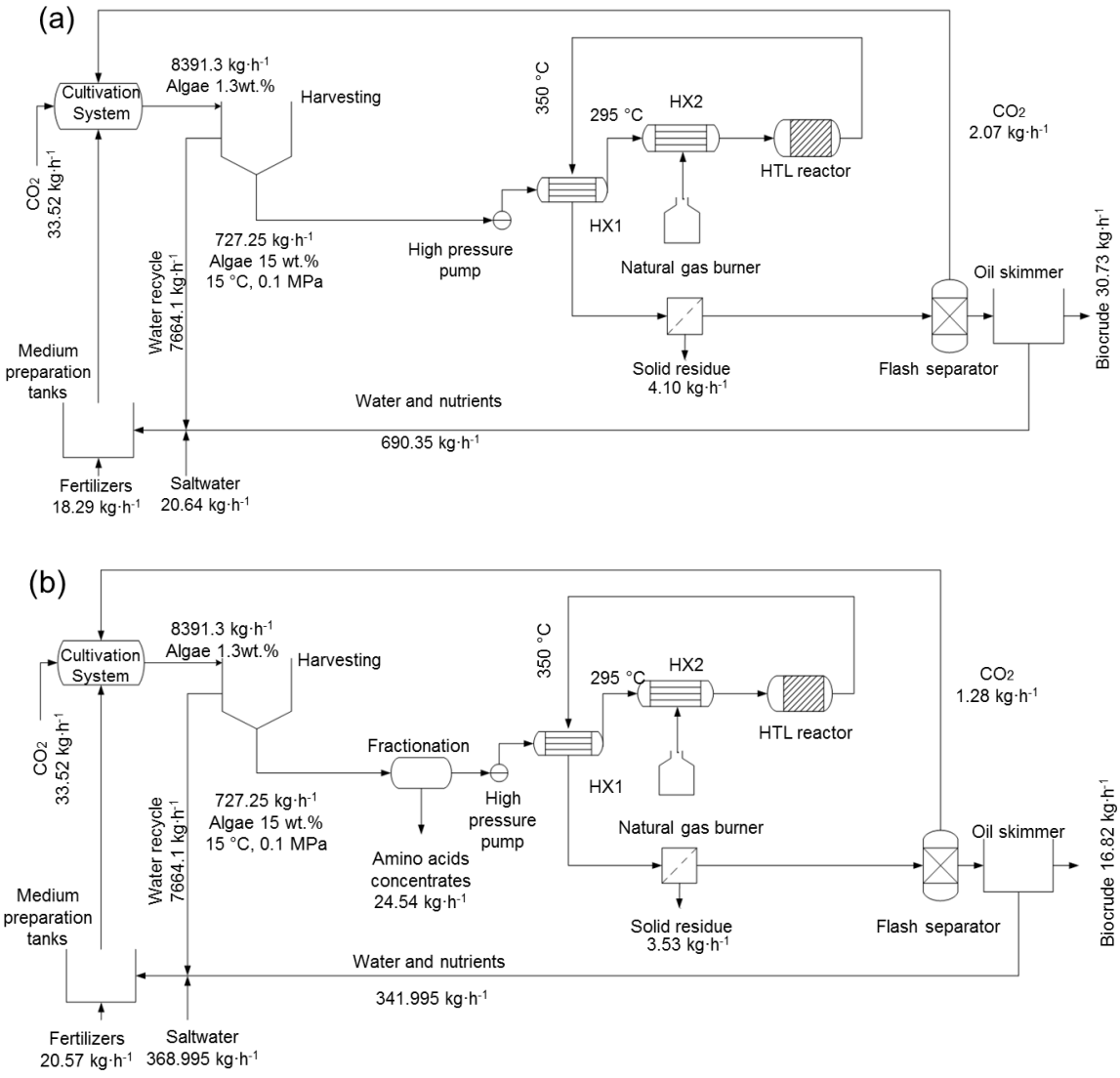
Fig 6.2 Three scenarios investigated in this work.

6.3. Results and discussion

6.3.1. Mass flow of three scenarios

Fig 6.3 presents the mass flow of each scenario in the proposed plant. After harvesting and concentration, about 727.25 kg $ħ^{-1}$ microalgae slurry (15 wt.%) is supposed to be transferred into the production steps. In scenario 1, around 30.73 kg $ħ^{-1}$ biocrude oil can be produced (calculation based on the results from chapter 3). While in scenario 2, the biocrude productivity is reduced to 16.82 kg $ħ^{-1}$, since only about 50 wt.% of the microalgae slurry flows to the HTL step. Meanwhile, the other 50 wt.% of the microalgae slurry generates 24.54 kg $ħ^{-1}$ amino acids concentrate. It is noteworthy that there are also other components in the extract, which is not the amino acids concentrates (not shown in the chart). In this case, they are not calculated in terms of incomes in this study. In scenario 3, 32.5 kg $ħ^{-1}$ amino acids concentrate is supposed to be produced with the assist of PEF treatment, leading to a reduced production of 13.53 kg $ħ^{-1}$ biocrude. It is noteworthy that the required amount of fertilizers is higher in scenario 2 and 3, this is due to the lower HTL fraction. However, since most of the

nutrients were recovered with the water in the harvesting step, the increase of the fertilizer is less than 2 kg h^{-1}, which is negligible in this study (no more than 3% of the variable cost (0.6% of the total cost)).



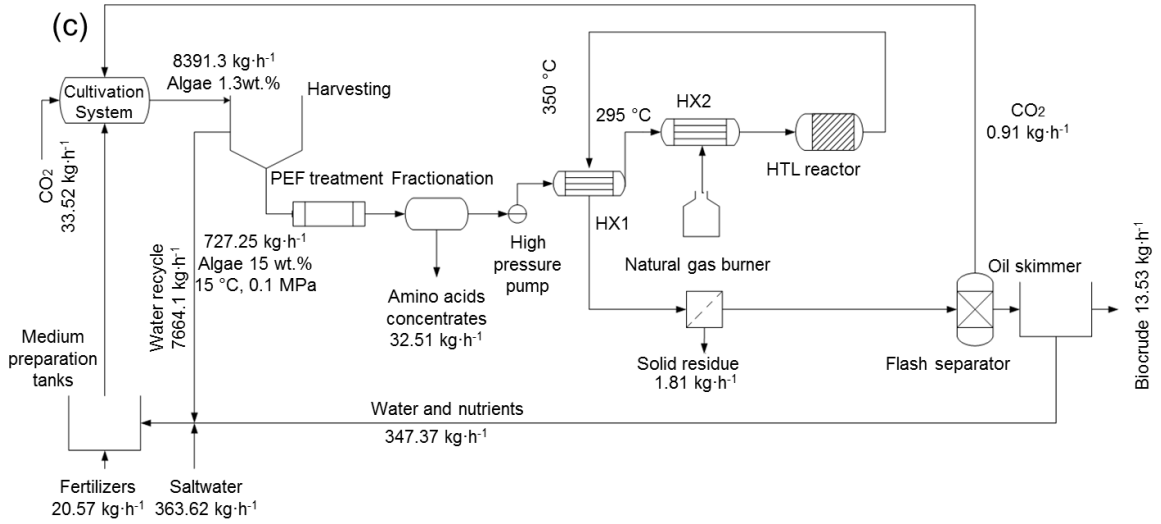


Fig 6.3 Mass flow of proposed microalgae biorefinery plant. (a) scenario 1 (b) scenario 2 (c) scenario 3.

6.3.2. Economics of the three scenarios

As shown in Table 6.4, data of scenario 1 indicates that if the plant is designed only for the microalgae biofuels production, this will lead to a net loss of 2.615 M€ a^{-1}. The MFSP of 11.72 € kg^{-1} is much higher than the fossil crude oil price (0.37 € kg^{-1}), which makes the competitiveness of microalgae biofuels quite weak. This is even higher than the previous estimated price of 4.6-6.5 € kg^{-1} (Barreiro. 2015). The main reasons for such a high value are the low HTL biocrude yield of microalgae *S. almeriensis* itself and the modifications of an expected lower biocrude yield in continuous HTL. This provides a more realistic estimation of the capital return. Nevertheless, when high value product is generated (scenario 2a), the MFSP is reduced by over 50%, this encourages further studies on valuables extraction prior to HTL conversion. As for scenario 3a, due to the implementation of PEF, more amino acids concentrates are produced as well as better economics return. The MFSP of 0.78 € kg^{-1} brings microalgae biofuel to a competitive level against fossil crude oil. When the biofertilizer is supposed to be sold at 10 € L^{-1}, net revenues have been already obtained in both scenarios. It can be noted that the market price for high-value product plays a sensitive role in final capital, if selling biocrude at the same price of current crude oil (0.37 € L^{-1}), the biofertilizer price is supposed to be at least 7.43 € L^{-1} for a positive economic return.

Table 6.4 Economic key numbers for three scenarios, and MSFP. (a denotes the case when biofertilizer is sold at 7.35 € Ł$^{-1}$, b denotes the case when biofertilizer is sold at 10 € Ł$^{-1}$.)

Scenario	Incomes (M€ a^{-1})	Costs (M€ a^{-1})	Return (M€ a^{-1})	MFSP (€ kg^{-1})
1	0.085	2.7	-2.615	11.72
2a	2.30	2.884	-0.58	4.98
2b	3.13	2.884	0.246	-
3a	3.02	3.06	-0.04	0.78
3b	4.11	3.06	1.05	-

As shown in Fig 6.4, with the data presented under the proposed scale, the PEF treatment and further fractionation are believed to play a significant role in promoting the economics of the whole process.

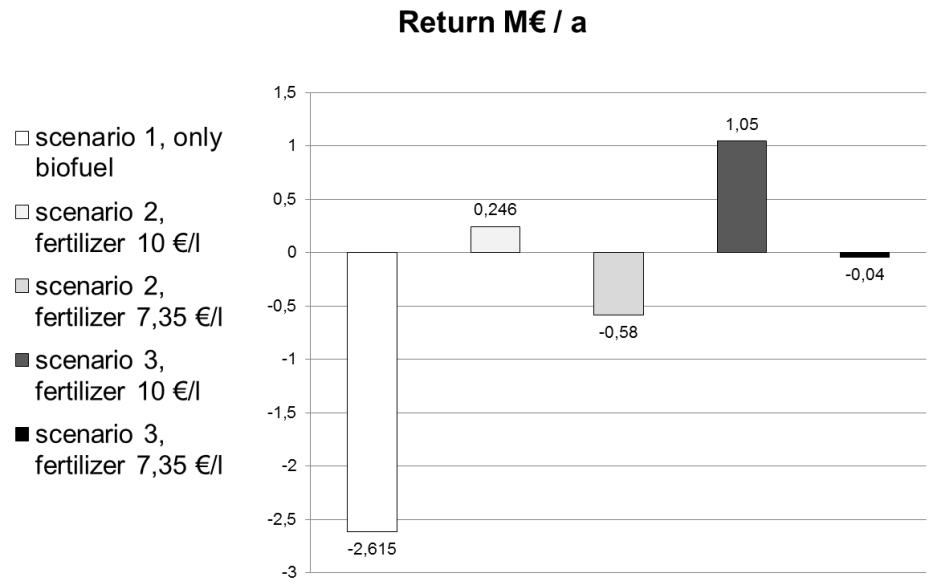


Fig 6.4 Return of capital in different scenarios.

6.4. Conclusions

An earlier techno-economic evaluation of a microalgae biorefinery plant has been updated considering the results from previous chapters. The economic benefit of

implementing PEF treatment and fractionation has been confirmed. It can be confirmed that production of microalgae biofuel alone is still far from commercial application, leading to an annual loss of 2.615 M€. It can be concluded that a fractionation step to extract high-value products promises to reduce the overall capital costs significantly; even considering the lowest benchmark price of the value added amino-acid based product, PEF treatment could improve this effect bringing the by-produced microalgae biofuel to a competitive level (0.78 $€\cdot kg^{-1}$) compared to that of current fossil crude oil (0.37 $€\cdot kg^{-1}$). The market price for high-value product plays an essential role in the final costs. Given that selling microalgae biofuel should occur at the same price of the crude oil, the biofertilizer minimum price is supposed to be 7.43 $€\cdot L^{-1}$ for a positive capital return.

Chapter 7. Conclusions and outlook

7. Conclusions and outlook

Microalgae biofuel production via HTL within a biorefinery has the potential to significantly contribute to renewable energy. As worked out in the introduction of this thesis, there is still a lack of fundamental knowledge and limited upscaling trials towards its industrial application, from which research questions were raised. In this thesis, as presented in Fig 7.1 (same as Fig 2.1), some key down-stream processing steps using HTL as the core conversion technology are examined within a microalgae biorefinery concept, with the aim to obtain deeper insight to further improve microalgae biofuel economics and scale-up possibility.

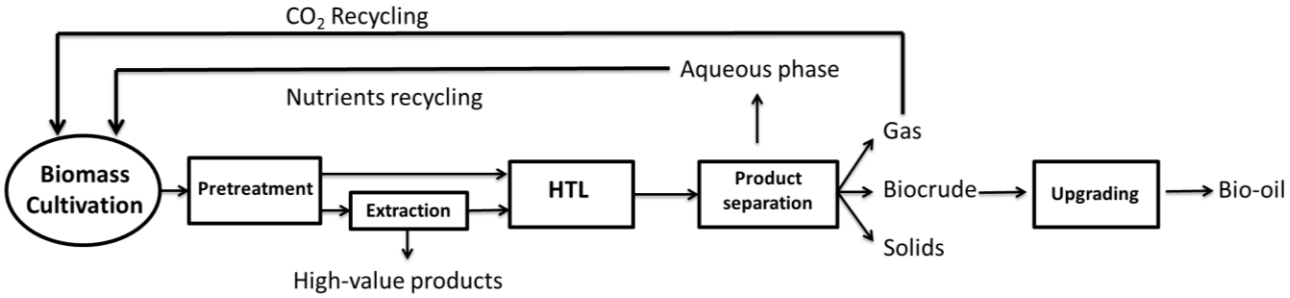


Fig 7.1 Simplified flowchart of the processing steps investigated in this thesis.

Herein, the research questions of this thesis can be answered:

- Combining pre-treatment and HTL conversion. How can pre-treatment for valuables extraction and HTL for biofuel production be combined efficiently? How to extract valuables from wet microalgae biomass energy-efficiently? How is the HTL behavior of the residual biomass?

Applying microalgae only for the biofuel production is not economically viable, additional high-value products need to be generated. PEF treatment alone does not have any direct influence on HTL of all three microalgae investigated. Neither product yields nor biocrude qualities were significantly changed after PEF treatment. However, using PEF treatment has been proved to be an effective method to assist value-added products: when using *A. protothecoides* microalgae, PEF treatment enhances the lipid extraction yield from 4 wt.% to 33 wt.% (more than 80% of the total lipids), which decreases the biocrude yields

from 58 wt.% to 43 wt.%. In spite of the higher, for fuel applications non-desired nitrogen content, the biocrude produced exhibits a relatively high HHV of 36.7 MJ kg^{-1}. PEF treatment was found to be a decisive factor for protein extraction from *C. vulgaris* microalgae. The biocrude obtained has a lower, but still unfavorable nitrogen content (from 6 wt.% to 5.5 wt.%). The most promising route of combining PEF treatment and HTL application seems to be the enzymatic protein hydrolysis and subsequent extraction of amino acids from *S. almeriensis* microalgae. Due to a lower fraction of protein, 6 wt.% higher yield and better quality of biocrude can be produced from the residual biomass via HTL. PEF treatment accelerates amino acids extraction and improves the extraction efficiency by up to 150% in the first 60 min. As a next steps scale-up of the PEF and HTL processes and market research of these high-value products need to be done.

- Continuous HTL and product separation step. How is the HTL behavior of microalgae in a continuous operation mode? How to reuse the aqueous phase for algae cultivation? What is the impact of DCM solvent on a continuous HTL product separation?

A temperature of 350 ℃ has been proved to be the optimized temperature for microalgae *C. vulgaris* conversion in continuous HTL, obtaining the highest biocrude+solid yield (about 38.9 ±1.4 wt.% for non-DCM method and 47.5 ±2.5 wt.% for DCM method). In terms of the "overestimation effect" by DCM, similar to the batch experiments, a biocrude+solid yield increment (9 wt.%, averagely) was achieved in continuous mode in the temperature range investigated. A general biocrude yield correlation can be proposed: $Yield_{non\text{-}DCM} \approx 81.8\% * Yield_{DCM}$. By this data, better comparison can be made in terms of the reported data between different systems when using DCM as the solvent. Besides, the recovery of AP from continuous HTL was proved to be feasible when activated carbon was used to remove the toxic component. Further studies on the purification method are recommended in terms of the processing cost. Possible alternatives could be the use of biochar as the feedstock for activation carbon, a very interesting route could be utilization of the microalgae HTL solid product, since it is rich in carbon.

- Biocrude upgrading step. How is the cHTL and upgrading behavior when using different microalgae strains? What are the possibilities to upgrade cHTL biocrude? Which types of reactions take place during catalytic upgrading?

Lower yields are reasonable to be expected in cHTL, compared to batch. Besides, longer residence time is more suggested for algae with high protein and carbohydrate content in cHTL to obtain higher biocrude yields. Upgrading of cHTL biocrude derived from *C. vulgaris* seems to be more temperature dependent and leads to a better quality improvement than upgrading of *N. gaditana* biocrude. On the other hand *N. gaditana* biocrude shows a higher reactivity with the catalysts used. The NiMo catalyst shows a better performance in removing oxygen and nitrogen (more than 93% and nearly 50%, respectively) and leads to a maximum HHV of 43.7 MJ łkg^{-1}. Therefore the use of conventional deoxygenation catalysts can be recommended. The NiW catalyst is found to remove the sulfur content more effectively. Temperature is the main factor for upgrading by hydrotreatment; 250 ℃ appears as too mild to trigger hydrogenation reactions in cHTL biocrude and mainly supports repolymerization, decarboxylation, and decarbonylation. At higher temperature of 400 ℃ more volatile components are generated, consequently leading to lower biocrude recovery but it promotes a variety of hydrogenation reactions and therefore improves biocrude quality significantly.

- Techno-economic assessment: Is it economically viable to realize such a processing chain? What is the recommendation for commercialization of microalgae biofuel production?

Based on the results obtained in the here performed studies, a techno-economic assessment is updated on a microalgae biorefinery pilot plant with an output of 0.5 MW related to biocrude thermal fuel capacity. Three scenarios regarding the valuables extraction and the effect of PEF treatment have been investigated in terms of mass flows and economics analysis. When biocrude is the only product, the proposed biorefinery plant leads to approximately 2.615 M€ loss yearly, which confirms that to use microalgae for biofuel production exclusively is not economically feasible today. In contrast, the implementation of PEF and amino acids extraction bring microalgae biofuel co-production to a competitive level (0.78 €·kg^{-1}) compared to current fossil crude oil (0.37 €·kg^{-1}). The market price for high-value product plays the determining role in the final cost structure: the minimum biofertilizer selling price is supposed to be 7.43 €·L^{-1}, to be able to sell the microalgae biofuel at the same price as crude oil. This underlines the usefulness of research towards PEF assisted extraction of high value products along with biofuel production as a promising way for the commercialization of microalgae biofuel.

References

Akaberi, S., C. Gusbeth, A. Silve, D. S. Senthilnathan, E. Navarro-López, E. Molina-Grima, G. Müller and W. Frey (2019). "Effect of pulsed electric field treatment on enzymatic hydrolysis of proteins of Scenedesmus almeriensis." Algal Research **43**.

Alhattab, M., A. Kermanshahi-Pour and M. S.-L. Brooks (2018). "Microalgae disruption techniques for product recovery: influence of cell wall composition." Journal of Applied Phycology **31**(1): 61-88.

Anastas, P. T. and J. B. Zimmerman (2003). Peer reviewed: design through the 12 principles of green engineering, ACS Publications.

Anastasakis, K., P. Biller, R. Madsen, M. Glasius and I. Johannsen (2018). "Continuous Hydrothermal Liquefaction of Biomass in a Novel Pilot Plant with Heat Recovery and Hydraulic Oscillation." Energies **11**(10).

Arianna Ricci, G. P. P., Andrea Versari (2018). "Recent Advances and Applications of Pulsed Electric Fields (PEF) to Improve Polyphenol Extraction and Color Release during Red Winemaking." Beverages **4**(1).

Arvindnarayan, S., K. K. Sivagnana Prabhu, S. Shobana, G. Kumar and J. Dharmaraja (2017). "Upgrading of micro algal derived bio-fuels in thermochemical liquefaction path and its perspectives: A review." International Biodeterioration & Biodegradation **119**: 260-272.

Bahadar, A. and M. Bilal Khan (2013). "Progress in energy from microalgae: A review." Renewable and Sustainable Energy Reviews **27**: 128-148.

Bai, X., P. Duan, Y. Xu, A. Zhang and P. E. Savage (2014). "Hydrothermal catalytic processing of pretreated algal oil: A catalyst screening study." Fuel **120**: 141-149.

Barreiro, D. L., "Hydrothermal liquefaction of microalgae". Ph.D thesis.

Barreiro, D. L., B. R. Gómez, U. Hornung, A. Kruse and W. Prins (2015). "Hydrothermal Liquefaction of Microalgae in a Continuous Stirred-Tank Reactor." Energy & Fuels **29**(10): 6422-6432.

Barros, A. I., A. L. Gonçalves, M. Simões and J. C. M. Pires (2015). "Harvesting techniques applied to microalgae: A review." Renewable and Sustainable Energy Reviews **41**: 1489-1500.

Belete, Y. Z., S. Leu, S. Boussiba, B. Zorin, C. Posten, L. Thomsen, S. Wang, A. Gross and R. Bernstein (2019). "Characterization and utilization of hydrothermal carbonization aqueous phase as nutrient source for microalgal growth." Bioresour Technol **290**: 121758.

Bian, J., Q. Zhang, P. Zhang, L. Feng and C. Li (2017). "Supported Fe_2O_3 nanoparticles for

catalytic upgrading of microalgae hydrothermal liquefaction derived bio-oil." Catalysis Today **293-294**: 159-166.

Biller, P. and A. B. Ross (2011). "Potential yields and properties of oil from the hydrothermal liquefaction of microalgae with different biochemical content." Bioresour Technol **102**(1): 215-225.

Biller, P., A. B. Ross, S. C. Skill, A. Lea-Langton, B. Balasundaram, C. Hall, R. Riley and C. A. Llewellyn (2012). "Nutrient recycling of aqueous phase for microalgae cultivation from the hydrothermal liquefaction process." Algal Research **1**(1): 70-76.

Biller, P., B. K. Sharma, B. Kunwar and A. B. Ross (2015). "Hydroprocessing of bio-crude from continuous hydrothermal liquefaction of microalgae." Fuel **159**: 197-205.

Brennan, L. and P. Owende (2010). "Biofuels from microalgae—A review of technologies for production, processing, and extractions of biofuels and co-products." Renewable and Sustainable Energy Reviews **14**(2): 557-577.

Brilman, D. W. F., N. Drabik and M. Wadrzyk (2017). "Hydrothermal co-liquefaction of microalgae, wood, and sugar beet pulp." Biomass Conversion and Biorefinery **7**(4): 445-454.

Brown, T. M., P. Duan and P. E. Savage (2010). "Hydrothermal Liquefaction and Gasification of Nannochloropsis sp." Energy & Fuels **24**(6): 3639-3646.

Caporgno, M. P., E. Clavero, C. Torras, J. Salvadó, O. Lepine, J. Pruvost, J. Legrand, J. Giralt and C. Bengoa (2016). "Energy and Nutrients Recovery from Lipid-Extracted Nannochloropsis via Anaerobic Digestion and Hydrothermal Liquefaction." ACS Sustainable Chemistry & Engineering **4**(6): 3133-3139.

Carullo, D., B. D. Abera, A. A. Casazza, F. Donsì, P. Perego, G. Ferrari and G. Pataro (2018). "Effect of pulsed electric fields and high pressure homogenization on the aqueous extraction of intracellular compounds from the microalgae Chlorella vulgaris." Algal Research **31**: 60-69.

Castello, D., T. Pedersen and L. Rosendahl (2018). "Continuous Hydrothermal Liquefaction of Biomass: A Critical Review." Energies **11**(11).

Chen, C. Y., K. L. Yeh, R. Aisyah, D. J. Lee and J. S. Chang (2011). "Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review." Bioresour Technol **102**(1): 71-81.

Chen, H., T. Li and Q. Wang (2019). "Ten years of algal biofuel and bioproducts: gains and pains." Planta.

Chen, H., T. Qiu, J. Rong, C. He and Q. Wang (2015). "Microalgal biofuel revisited: An

informatics-based analysis of developments to date and future prospects." Applied Energy **155**: 585-598.

Chen, L., T. Zhu, J. S. M. Fernandez, S. Chen and D. Li (2017). "Recycling nutrients from a sequential hydrothermal liquefaction process for microalgae culture." Algal Research **27**: 311-317.

Chiaramonti, D., M. Prussi, M. Buffi, A. M. Rizzo and L. Pari (2017). "Review and experimental study on pyrolysis and hydrothermal liquefaction of microalgae for biofuel production." Applied Energy **185**: 963-972.

Chisti, Y. (2007). "Biodiesel from microalgae." Biotechnol Adv **25**(3): 294-306.

Coates, J. "Interpretation of infrared spectra, a practical approach." Encyclopedia of analytical chemistry: 10815-10837.

Delrue, F., Y. Li-Beisson, P. A. Setier, C. Sahut, A. Roubaud, A. K. Froment and G. Peltier (2013). "Comparison of various microalgae liquid biofuel production pathways based on energetic, economic and environmental criteria." Bioresour Technol **136**: 205-212.

Demirbas, A. (2010). "Use of algae as biofuel sources." Energy Conversion and Management **51**(12): 2738-2749.

Du, Z., B. Hu, A. Shi, X. Ma, Y. Cheng, P. Chen, Y. Liu, X. Lin and R. Ruan (2012). "Cultivation of a microalga Chlorella vulgaris using recycled aqueous phase nutrients from hydrothermal carbonization process." Bioresour Technol **126**: 354-357.

Duan, P., X. Bai, Y. Xu, A. Zhang, F. Wang, L. Zhang and J. Miao (2013). "Catalytic upgrading of crude algal oil using platinum/gamma alumina in supercritical water." Fuel **109**: 225-233.

Duan, P., B. Wang and Y. Xu (2015). "Catalytic hydrothermal upgrading of crude bio-oils produced from different thermo-chemical conversion routes of microalgae." Bioresour Technol **186**: 58-66.

Duong, V. T., Y. Li, E. Nowak and P. M. Schenk (2012). "Microalgae Isolation and Selection for Prospective Biodiesel Production." Energies **5**(6): 1835-1849.

Eboibi, B. E., D. M. Lewis, P. J. Ashman and S. Chinnasamy (2015). "Influence of process conditions on pretreatment of microalgae for protein extraction and production of biocrude during hydrothermal liquefaction of pretreated Tetraselmis sp." RSC Advances **5**(26): 20193-20207.

Ehimen, E. A., Z. F. Sun and C. G. Carrington (2010). "Variables affecting the in situ

transesterification of microalgae lipids." Fuel **89**(3): 677-684.

Elliott, D. C., P. Biller, A. B. Ross, A. J. Schmidt and S. B. Jones (2015). "Hydrothermal liquefaction of biomass: developments from batch to continuous process." Bioresour Technol **178**: 147-156.

Elliott, D. C., T. R. Hart, G. G. Neuenschwander, L. J. Rotness and A. H. Zacher (2009). "Catalytic hydroprocessing of biomass fast pyrolysis bio-oil to produce hydrocarbon products." Environmental Progress & Sustainable Energy **28**(3): 441-449.

Elliott, D. C., T. R. Hart, A. J. Schmidt, G. G. Neuenschwander, L. J. Rotness, M. V. Olarte, A. H. Zacher, K. O. Albrecht, R. T. Hallen and J. E. Holladay (2013). "Process development for hydrothermal liquefaction of algae feedstocks in a continuous-flow reactor." Algal Research **2**(4): 445-454.

Enyidi, U. (2017). "Chlorella vulgaris as Protein Source in the Diets of African Catfish Clarias gariepinus." Fishes **2**(4).

Faeth, J. L. and P. E. Savage (2016). "Effects of processing conditions on biocrude yields from fast hydrothermal liquefaction of microalgae." Bioresour Technol **206**: 290-293.

Fang, M., S. Ma, T. Wang, Z. Xia, W. Tang, L. Xia and Z. Luo (2017). "Hydrotreatment of model compounds with catalysts of NiW/Al_2O_3 and NiWP/Al_2O_3 to simulate low temperature coal tar oil." RSC Advances **7**(86): 54512-54521.

Fushimi, C., M. Kakimura, R. Tomita, A. Umeda and T. Tanaka (2016). "Enhancement of nutrient recovery from microalgae in hydrothermal liquefaction using activated carbon." Fuel Processing Technology **148**: 282-288.

Gai, C., Y. Zhang, W.-T. Chen, P. Zhang and Y. Dong (2014). "Energy and nutrient recovery efficiencies in biocrude oil produced via hydrothermal liquefaction of Chlorella pyrenoidosa." RSC Advances **4**(33).

Gai, C., Y. Zhang, W. T. Chen, Y. Zhou, L. Schideman, P. Zhang, G. Tommaso, C. T. Kuo and Y. Dong (2015). "Characterization of aqueous phase from the hydrothermal liquefaction of Chlorella pyrenoidosa." Bioresour Technol **184**: 328-335.

Garcia Alba, L., C. Torri, D. Fabbri, S. R. A. Kersten and D. W. F. Brilman (2013). "Microalgae growth on the aqueous phase from Hydrothermal Liquefaction of the same microalgae." Chemical Engineering Journal **228**: 214-223.

Garcia Alba, L., C. Torri, C. Samor ̧ J. van der Spek, D. Fabbri, S. R. A. Kersten and D. W. F. Brilman (2011). "Hydrothermal Treatment (HTT) of Microalgae: Evaluation of the Process As

Conversion Method in an Algae Biorefinery Concept." Energy & Fuels **26**(1): 642-657.

Gille, A., A. Trautmann, M. R. Gomez, S. C. Bischoff, C. Posten and K. Briviba (2019). "Photoautotrophically Grown Chlorella vulgaris Shows Genotoxic Potential but No Apoptotic Effect in Epithelial Cells." J Agric Food Chem **67**(31): 8668-8676.

Goettel, M., C. Eing, C. Gusbeth, R. Straessner and W. Frey (2013). "Pulsed electric field assisted extraction of intracellular valuables from microalgae." Algal Research **2**(4): 401-408.

Guiry, M. D. (2012). "How Many Species of Algae Are There?" J Phycol **48**(5): 1057-1063.

Gunerken, E., E. D'Hondt, M. H. Eppink, L. Garcia-Gonzalez, K. Elst and R. H. Wijffels (2015). "Cell disruption for microalgae biorefineries." Biotechnol Adv **33**(2): 243-260.

Guo, B., V. Walter, U. Hornung and N. Dahmen (2019). "Hydrothermal liquefaction of Chlorella vulgaris and Nannochloropsis gaditana in a continuous stirred tank reactor and hydrotreating of biocrude by nickel catalysts." Fuel Processing Technology **191**: 168-180.

Guo, B., B. Yang, A. Silve, S. Akaberi, D. Scherer, I. Papachristou, W. Frey, U. Hornung and N. Dahmen (2019). "Hydrothermal liquefaction of residual microalgae biomass after pulsed electric field-assisted valuables extraction." Algal Research **43**.

Guo, B., W. Zhou, S. Liu, Y. Liu, J. Wang and R. Yin (2015). "Effect of γ-ray irradiation on the structure and electrochemical liquefaction of Shenhua coal." Fuel **143**: 236-243.

Guo, Z., Y. Liu, H. Guo, S. Yan and J. Mu (2013). "Microalgae cultivation using an aquaculture wastewater as growth medium for biomass and biofuel production." Journal of Environmental Sciences **25**: S85-S88.

Haider, M., D. Castello, K. Michalski, T. Pedersen and L. Rosendahl (2018). "Catalytic Hydrotreatment of Microalgae Biocrude from Continuous Hydrothermal Liquefaction: Heteroatom Removal and Their Distribution in Distillation Cuts." Energies **11**(12).

He, Y., X. Liang, C. Jazrawi, A. Montoya, A. Yuen, A. J. Cole, N. Neveux, N. A. Paul, R. de Nys, T. Maschmeyer and B. S. Haynes (2016). "Continuous hydrothermal liquefaction of macroalgae in the presence of organic co-solvents." Algal Research **17**: 185-195.

He, Z., D. Xu, S. Wang, H. Zhang and Z. Jing (2018). "Catalytic Upgrading of Water-Soluble Biocrude from Hydrothermal Liquefaction of Chlorella." Energy & Fuels **32**(2): 1893-1899.

Heredia-Arroyo, T., W. Wei and B. Hu (2010). "Oil accumulation via heterotrophic/mixotrophic Chlorella protothecoides." Appl Biochem Biotechnol **162**(7): 1978-1995.

Hosseinpour, M., A. Golzary, M. Saber and K. Yoshikawa (2017). "Denitrogenation of

biocrude oil from algal biomass in high temperature water and formic acid mixture over H + ZSM-5 nanocatalyst." Fuel **206**: 628-637.

Hu, Y., M. Gong, S. Feng, C. Xu and A. Bassi (2019). "A review of recent developments of pre-treatment technologies and hydrothermal liquefaction of microalgae for bio-crude oil production." Renewable and Sustainable Energy Reviews **101**: 476-492.

Huber, G. W., S. Iborra and A. Corma (2006). "Synthesis of transportation fuels from biomass: chemistry, catalysts, and engineering." Chem Rev **106**(9): 4044-4098.

Jaeschke, D. P., G. D. Mercali, L. D. F. Marczak, G. Muller, W. Frey and C. Gusbeth (2019). "Extraction of valuable compounds from Arthrospira platensis using pulsed electric field treatment." Bioresour Technol **283**: 207-212.

Jazrawi, C., P. Biller, A. B. Ross, A. Montoya, T. Maschmeyer and B. S. Haynes (2013). "Pilot plant testing of continuous hydrothermal liquefaction of microalgae." Algal Research **2**(3): 268-277.

Jena, U., K. C. Das and J. R. Kastner (2011). "Effect of operating conditions of thermochemical liquefaction on biocrude production from Spirulina platensis." Bioresour Technol **102**(10): 6221-6229.

Jin, M., Y.-K. Oh, Y. K. Chang and M. Choi (2017). "Optimum Utilization of Biochemical Components in Chlorella sp. KR1 via Subcritical Hydrothermal Liquefaction." ACS Sustainable Chemistry & Engineering **5**(8): 7240-7248.

Klassen, V., O. Blifernez-Klassen, D. Wibberg, A. Winkler, J. Kalinowski, C. Posten and O. Kruse (2017). "Highly efficient methane generation from untreated microalgae biomass." Biotechnol Biofuels **10**: 186.

Kröger, M. and F. Müller-Langer (2014). "Review on possible algal-biofuel production processes." Biofuels **3**(3): 333-349.

Kruse, A. and N. Dahmen (2018). "Hydrothermal biomass conversion: Quo vadis?" Journal of Supercritical Fluids **134**: 114-123.

Kruse, A. and M. Faquir (2007). "Hydrothermal Biomass Gasification – Effects of Salts, Backmixing and Their Interaction." Chemical Engineering & Technology **30**(6): 749-754.

Lababpour, A. (2018). "Continuous Hydrothermal Liquefaction for Biofuel and Biocrude Production from Microalgal Feedstock." ChemBioEng Reviews **5**(2): 90-103.

Lam, G. P. t., P. R. Postma, D. A. Fernandes, R. A. H. Timmermans, M. H. Vermuë, M. J. Barbosa, M. H. M. Eppink, R. H. Wijffels and G. Olivieri (2017). "Pulsed Electric Field for

protein release of the microalgae Chlorella vulgaris and Neochloris oleoabundans." Algal Research **24**: 181-187.

Larkum, A. W., I. L. Ross, O. Kruse and B. Hankamer (2012). "Selection, breeding and engineering of microalgae for bioenergy and biofuel production." Trends Biotechnol **30**(4): 198-205.

Lee, S. H., H. M. Oh, B. H. Jo, S. A. Lee, S. Y. Shin, H. S. Kim, S. H. Lee and C. Y. Ahn (2014). "Higher biomass productivity of microalgae in an attached growth system, using wastewater." J Microbiol Biotechnol **24**(11): 1566-1573.

Leng, L., J. Li, Z. Wen and W. Zhou (2018). "Use of microalgae to recycle nutrients in aqueous phase derived from hydrothermal liquefaction process." Bioresour Technol **256**: 529-542.

Li, Z. and P. E. Savage (2013). "Feedstocks for fuels and chemicals from algae: Treatment of crude bio-oil over HZSM-5." Algal Research **2**(2): 154-163.

Liu, X., B. Saydah, P. Eranki, L. M. Colosi, B. Greg Mitchell, J. Rhodes and A. F. Clarens (2013). "Pilot-scale data provide enhanced estimates of the life cycle energy and emissions profile of algae biofuels produced via hydrothermal liquefaction." Bioresour Technol **148**: 163-171.

López Barreiro, D., M. Bauer, U. Hornung, C. Posten, A. Kruse and W. Prins (2015). "Cultivation of microalgae with recovered nutrients after hydrothermal liquefaction." Algal Research **9**: 99-106.

López Barreiro, D., B. R. Gómez, F. Ronsse, U. Hornung, A. Kruse and W. Prins (2016). "Heterogeneous catalytic upgrading of biocrude oil produced by hydrothermal liquefaction of microalgae: State of the art and own experiments." Fuel Processing Technology **148**: 117-127.

López Barreiro, D., W. Prins, F. Ronsse and W. Brilman (2013). "Hydrothermal liquefaction (HTL) of microalgae for biofuel production: State of the art review and future prospects." Biomass and Bioenergy **53**: 113-127.

López Barreiro, D., S. Riede, U. Hornung, A. Kruse and W. Prins (2015). "Hydrothermal liquefaction of microalgae: Effect on the product yields of the addition of an organic solvent to separate the aqueous phase and the biocrude oil." Algal Research **12**: 206-212.

López Barreiro, D., C. Samorì, G. Terranella, U. Hornung, A. Kruse and W. Prins (2014). "Assessing microalgae biorefinery routes for the production of biofuels via hydrothermal liquefaction." Bioresource Technology **174**: 256-265.

Mata, T. M., A. A. Martins and N. S. Caetano (2010). "Microalgae for biodiesel production and other applications: A review." Renewable & Sustainable Energy Reviews **14**(1): 217-232.

Mortensen, P. M., J. D. Grunwaldt, P. A. Jensen, K. G. Knudsen and A. D. Jensen (2011). "A review of catalytic upgrading of bio-oil to engine fuels." Applied Catalysis A: General **407**(1-2): 1-19.

Mørup, A. J., J. Becker, P. S. Christensen, K. Houlberg, E. Lappa, M. Klemmer, R. B. Madsen, M. Glasius and B. B. Iversen (2015). "Construction and Commissioning of a Continuous Reactor for Hydrothermal Liquefaction." Industrial & Engineering Chemistry Research **54**(22): 5935-5947.

Mullen, C. A. and A. A. Boateng (2011). "Characterization of water insoluble solids isolated from various biomass fast pyrolysis oils." Journal of Analytical and Applied Pyrolysis **90**(2): 197-203.

Onumaegbu, C., A. Alaswad, C. Rodriguez and A. Olabi (2018). "Optimization of Pre-Treatment Process Parameters to Generate Biodiesel from Microalga." Energies **11**(4).

Parniakov, O., F. J. Barba, N. Grimi, L. Marchal, S. Jubeau, N. Lebovka and E. Vorobiev (2015). "Pulsed electric field assisted extraction of nutritionally valuable compounds from microalgae Nannochloropsis spp. using the binary mixture of organic solvents and water." Innovative Food Science & Emerging Technologies **27**: 79-85.

Patel, B., P. Arcelus-Arrillaga, A. Izadpanah and K. Hellgardt (2017). "Catalytic Hydrotreatment of algal biocrude from fast Hydrothermal Liquefaction." Renewable Energy **101**: 1094-1101.

Patel, B. and K. Hellgardt (2015). "Hydrothermal upgrading of algae paste in a continuous flow reactor." Bioresour Technol **191**: 460-468.

Peterson, A. A., R. P. Lachance and J. W. Tester (2010). "Kinetic Evidence of the Maillard Reaction in Hydrothermal Biomass Processing: Glucose−Glycine Interactions in High-Temperature, High-Pressure Water." Industrial & Engineering Chemistry Research **49**(5): 2107-2117.

Posten, C. (2009). "Design principles of photo-bioreactors for cultivation of microalgae." Engineering in Life Sciences **9**(3): 165-177.

Postma, P. R., T. L. Miron, G. Olivieri, M. J. Barbosa, R. H. Wijffels and M. H. M. Eppink (2015). "Mild disintegration of the green microalgae Chlorella vulgaris using bead milling." Bioresour Technol **184**: 297-304.

Postma, P. R., G. Pataro, M. Capitoli, M. J. Barbosa, R. H. Wijffels, M. H. Eppink, G. Olivieri and G. Ferrari (2016). "Selective extraction of intracellular components from the microalga Chlorella vulgaris by combined pulsed electric field-temperature treatment." Bioresour Technol **203**: 80-88.

Prapaiwatcharapan, K., S. Sunphorka, P. Kuchonthara, K. Kangvansaichol and N. Hinchiranan (2015). "Single- and two-step hydrothermal liquefaction of microalgae in a semi-continuous reactor: Effect of the operating parameters." Bioresour Technol **191**: 426-432.

Ramirez, J., R. Brown and T. Rainey (2015). "A Review of Hydrothermal Liquefaction Bio-Crude Properties and Prospects for Upgrading to Transportation Fuels." Energies **8**(7): 6765-6794.

Romero Garcia, J. M., F. G. Acien Fernandez and J. M. Fernandez Sevilla (2012). "Development of a process for the production of L-amino-acids concentrates from microalgae by enzymatic hydrolysis." Bioresour Technol **112**: 164-170.

Rosello Sastre, R., Z. Csogor, I. Perner-Nochta, P. Fleck-Schneider and C. Posten (2007). "Scale-down of microalgae cultivations in tubular photo-bioreactors--a conceptual approach." J Biotechnol **132**(2): 127-133.

Ruiz, J., G. Olivieri, J. de Vree, R. Bosma, P. Willems, J. H. Reith, M. H. M. Eppink, D. M. M. Kleinegris, R. H. Wijffels and M. J. Barbosa (2016). "Towards industrial products from microalgae." Energy & Environmental Science **9**(10): 3036-3043.

S. Syngellakis (2014). "Biomass to biofuels, WIT Press,."

Safi, C., L. Cabas Rodriguez, W. J. Mulder, N. Engelen-Smit, W. Spekking, L. A. M. van den Broek, G. Olivieri and L. Sijtsma (2017). "Energy consumption and water-soluble protein release by cell wall disruption of Nannochloropsis gaditana." Bioresour Technol **239**: 204-210.

Sari, Y. W., M. E. Bruins and J. P. M. Sanders (2013). "Enzyme assisted protein extraction from rapeseed, soybean, and microalgae meals." Industrial Crops and Products **43**: 78-83.

Scherer, D., D. Krust, W. Frey, G. Mueller, P. Nick and C. Gusbeth (2019). "Pulsed electric field (PEF)-assisted protein recovery from Chlorella vulgaris is mediated by an enzymatic process after cell death." Algal Research **41**.

Schmitt, C. C., K. Raffelt, A. Zimina, B. Krause, T. Otto, M. Rapp, J.-D. Grunwaldt and N. Dahmen (2018). "Hydrotreatment of Fast Pyrolysis Bio-oil Fractions Over Nickel-Based Catalyst." Topics in Catalysis **61**(15-17): 1769-1782.

Scott, S. A., M. P. Davey, J. S. Dennis, I. Horst, C. J. Howe, D. J. Lea-Smith and A. G. Smith

(2010). "Biodiesel from algae: challenges and prospects." Curr Opin Biotechnol **21**(3): 277-286.

Shanmugam, S. R., S. Adhikari and R. Shakya (2017). "Nutrient removal and energy production from aqueous phase of bio-oil generated via hydrothermal liquefaction of algae." Bioresour Technol **230**: 43-48.

Silve, A., C. B. Kian, I. Papachristou, C. Kubisch, N. Nazarova, R. Wustner, K. Leber, R. Strassner and W. Frey (2018). "Incubation time after pulsed electric field treatment of microalgae enhances the efficiency of extraction processes and enables the reduction of specific treatment energy." Bioresour Technol **269**: 179-187.

Silve, A., I. Papachristou, R. Wüstner, R. Sträßner, M. Schirmer, K. Leber, B. Guo, L. Interrante, C. Posten and W. Frey (2018). "Extraction of lipids from wet microalga Auxenochlorella protothecoides using pulsed electric field treatment and ethanol-hexane blends." Algal Research **29**: 212-222.

Singh, G. and S. K. Patidar (2018). "Microalgae harvesting techniques: A review." J Environ Manage **217**: 499-508.

Stephens, E., I. L. Ross, J. H. Mussgnug, L. D. Wagner, M. A. Borowitzka, C. Posten, O. Kruse and B. Hankamer (2010). "Future prospects of microalgal biofuel production systems." Trends Plant Sci **15**(10): 554-564.

Teri, G., L. Luo and P. E. Savage (2014). "Hydrothermal Treatment of Protein, Polysaccharide, and Lipids Alone and in Mixtures." Energy & Fuels **28**(12): 7501-7509.

Teymouri, A., S. Kumar, E. Barbera, E. Sforza, A. Bertucco and T. Morosinotto (2017). "Integration of biofuels intermediates production and nutrients recycling in the processing of a marine algae." AIChE Journal **63**(5): 1494-1502.

Tredici, M. R. (2014). "Photobiology of microalgae mass cultures: understanding the tools for the next green revolution." Biofuels **1**(1): 143-162.

Ursu, A. V., A. Marcati, T. Sayd, V. Sante-Lhoutellier, G. Djelveh and P. Michaud (2014). "Extraction, fractionation and functional properties of proteins from the microalgae Chlorella vulgaris." Bioresour Technol **157**: 134-139.

Vardon, D. R., B. K. Sharma, J. Scott, G. Yu, Z. Wang, L. Schideman, Y. Zhang and T. J. Strathmann (2011). "Chemical properties of biocrude oil from the hydrothermal liquefaction of Spirulina algae, swine manure, and digested anaerobic sludge." Bioresour Technol **102**(17): 8295-8303.

Venteris, E. R., M. S. Wigmosta, A. M. Coleman and R. L. Skaggs (2014). "Strain Selection, Biomass to Biofuel Conversion, and Resource Colocation have Strong Impacts on the Economic Performance of Algae Cultivation Sites." Frontiers in Energy Research **2**.

Wagner, I., M. Braun, K. Slenzka and C. Posten (2016). "Photobioreactors in Life Support Systems." Adv Biochem Eng Biotechnol **153**: 143-184.

Wagner, J. L., C. D. Le, V. P. Ting and C. J. Chuck (2017). "Design and operation of an inexpensive, laboratory-scale, continuous hydrothermal liquefaction reactor for the conversion of microalgae produced during wastewater treatment." Fuel Processing Technology **165**: 102-111.

Wagner, J. L., J. Perin, R. S. Coelho, V. P. Ting, C. J. Chuck and T. Teixeira Franco (2017). "Hydrothermal Conversion of Lipid-Extracted Microalgae Hydrolysate in the Presence of Isopropanol and Steel Furnace Residues." Waste and Biomass Valorization **9**(10): 1867-1879.

Wang, L., M. Min, Y. Li, P. Chen, Y. Chen, Y. Liu, Y. Wang and R. Ruan (2010). "Cultivation of green algae Chlorella sp. in different wastewaters from municipal wastewater treatment plant." Appl Biochem Biotechnol **162**(4): 1174-1186.

Wang, Z., S. Adhikari, P. Valdez, R. Shakya and C. Laird (2016). "Upgrading of hydrothermal liquefaction biocrude from algae grown in municipal wastewater." Fuel Processing Technology **142**: 147-156.

Watson, J., J. Lu, R. de Souza, B. Si, Y. Zhang and Z. Liu (2019). "Effects of the extraction solvents in hydrothermal liquefaction processes: Biocrude oil quality and energy conversion efficiency." Energy **167**: 189-197.

Wild, K. J., A. Trautmann, M. Katzenmeyer, H. Steinaß, C. Posten and M. Rodehutscord (2019). "Chemical composition and nutritional characteristics for ruminants of the microalgae Chlorella vulgaris obtained using different cultivation conditions." Algal Research **38**.

Xiu, S. and A. Shahbazi (2012). "Bio-oil production and upgrading research: A review." Renewable and Sustainable Energy Reviews **16**(7): 4406-4414.

Xu, D., S. Guo, L. Liu, H. Hua, Y. Guo, S. Wang and Z. Jing (2018). "Ni-Ru/CeO_2 Catalytic Hydrothermal Upgrading of Water-Insoluble Biocrude from Algae Hydrothermal Liquefaction." BioMed Research International **2018**: 1-9.

Xu, D. and P. E. Savage (2014). "Characterization of biocrudes recovered with and without solvent after hydrothermal liquefaction of algae." Algal Research **6**: 1-7.

Xu, D. and P. E. Savage (2018). "Supercritical water upgrading of water-insoluble and water-

soluble biocrudes from hydrothermal liquefaction of Nannochloropsis microalgae." The Journal of Supercritical Fluids **133**: 683-689.

Xue, Y., H. Chen, W. Zhao, C. Yang, P. Ma and S. Han (2016). "A review on the operating conditions of producing bio-oil from hydrothermal liquefaction of biomass." International Journal of Energy Research **40**(7): 865-877.

Yu, G., Y. Zhang, L. Schideman, T. Funk and Z. Wang (2011). "Distributions of carbon and nitrogen in the products from hydrothermal liquefaction of low-lipid microalgae." Energy & Environmental Science **4**(11).

Zbinden, M. D., B. S. Sturm, R. D. Nord, W. J. Carey, D. Moore, H. Shinogle and S. M. Stagg-Williams (2013). "Pulsed electric field (PEF) as an intensification pretreatment for greener solvent lipid extraction from microalgae." Biotechnol Bioeng **110**(6): 1605-1615.

Zhang, C., X. Tang, L. Sheng and X. Yang (2016). "Enhancing the performance of Co-hydrothermal liquefaction for mixed algae strains by the Maillard reaction." Green Chemistry **18**(8): 2542-2553.

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