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The Effect of Dichloromethane on Product Separation during Continuous Hydrothermal Liquefaction of *Chlorella vulgaris* and Aqueous Product Recycling for Algae Cultivation

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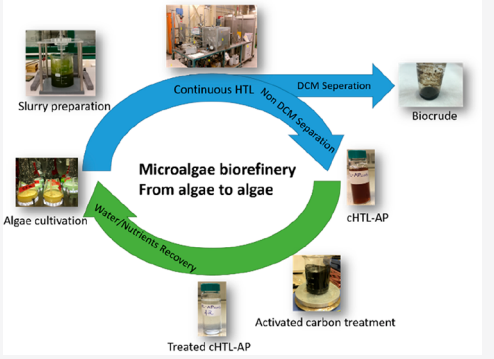
ABSTRACT: Dichloromethane (DCM) is a solvent commonly used in laboratories for microalgae hydrothermal liquefaction (HTL) product separation. The addition of DCM would lead to an "overestimation effect" of biocrude yield and diminish biocrude quality. However, it is currently not clear to what extent this overestimation effect will impact a continuous HTL process. In this study, *Chlorella vulgaris* microalgae was processed in a continuous stirred tank reactor at different temperatures (300, 325, 350, 375, and 400 °C) at 24 MPa for 15 min holding time. Two separation methods were applied to investigate the effect of using DCM in a cHTL product separation procedure in terms of product yield, biocrude elemental content, and aqueous product (AP) composition. Subsequently, the feasibility of reusing AP for algae cultivation has been evaluated. Results suggest that 350 °C is the optimal temperature for cHTL operation, leading to the highest biocrude yield, and an average increase in biocrude yield of 9 wt % was achieved when using DCM in cHTL product separation. Within the temperature range investigated, an average biocrude yield estimation can be proposed by $yield_{non\text{-}DCM} \approx 0.818 \times yield_{DCM}$. The AP has been characterized by total organic carbon and total nitrogen, high-performance liquid chromatography, and inductively coupled plasma optical emission spectroscopy. Results show that at 350−375 °C more nitrogen and other ions were directed into the AP, which could be advantageous in nutrient recovery. With the help of optical density testing, algae was shown to exhibit a better growth using AP with activated carbon absorption purification treatment as compared to the standard medium. The recovery of water and nutrients from the HTL-AP could improve the economics of a microalgae biorefinery process.



1. INTRODUCTION

In the past decade, microalgae have been regarded as a promising source for sustainable biofuel production due to their fast growth rate, higher photosynthetic efficiency compared to terrestrial lignocellulosic plants, ability to accumulate a high lipid content, high applicability to nonarable land environments, etc.[1−3] For the conversion of microalgae biomass into biofuel, hydrothermal liquefaction (HTL) is considered to be a suitable thermochemical process,[4,5] as it most importantly avoids the energy-intensive biomass drying process. Recently, the study of microalgae HTL in a continuous mode has been receiving increasing interest,[6−8] because it is the prerequisite for the industrial production of microalgae biofuel and microalgae-based value-added products. This technology performs in hydrothermal conditions, mostly in a temperature range of 200−380 °C and a pressure range of 5−28 MPa, utilizing different types of reactors in different scales, such as plug flow reactors[9,10] and pilot plants[11,12] in continuous or semicontinuous operation.[13] Chemically, by utilizing the hot, pressurized water environment, organics in the algae biomass undergo various reactions including hydrolysis, decarboxylation, dehydration, depolymerization, and repolymerization. Four main products are obtained: a biocrude product with higher carbon and energy density compared to the feedstock, which can be used as fuel after a further upgrading step; a solid residue that can be used as fertilizer; a gaseous product mainly composed of CO_2 and minor amounts of CO and light alkanes; and an aqueous product (AP) derived from HTL, containing water-soluble organics (alcohols, acids, and phenols, etc.) and dissolved nutrients with essential elements (N, P, and K[14]). The AP contains approximately 30%−60% of the feedstock carbon and more than 50% of the nitrogen.[15] It has been suggested that the AP and HTL biochar[16] could be recovered as a source of water and nutrients for algae cultivation. The effective

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Table 1. Recent Studies on the Recovery of Nutrients from Microalgae HTL Aqueous Product

groups	algae strains	HTL conditions	product separation and pretreatment of AP	main findings in nutrients recovery	main findings related to HTL or cultivation
[21]	*Chlorella pyrenoidosa* (15–35 wt % loading)	batch reactor 260–300 °C, holding time 30–90 min	filtration (no solvent)	The concentration of total nitrogen gradually increased with the increment of reaction temperature at a 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 a lower concentration of N- and O- heterocyclic compounds when higher energy recoveries were obtained at optimized operating conditions (280 °C, 60 min, 35 wt % and 300 °C, 60 min, 25 wt %).
[22]	*F. solaris* (1 wt % loading)	autoclave HTL reactor 320 °C, 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.	The enhancement of total nitrogen recovery is considered to be 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.
[23]	*Nannochloropsis oculata* (20 wt % loading)	batch reactor 300 °C, 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 of water discharge.
[24]	*Nannochloropsis* sp. (15 wt % loading)	320 °C 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 the bio-oil aqueous phase.
[20]	*C. sorokiniana*, *C. vulgaris*, and *G. sulphuraria* (10 wt % loading)	sequential HTL (bomb type reactor) 160 °C 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.
[25]	*Chlorella* sp. KR1 (10 wt % loading)	high-pressure batch-type reactor 150–300 °C holding time 1 h	DCM	The total N content gradually increased with increasing temperature. The P concentration slowly increased with HTL temperature up to 200 °C and then decreased at higher temperatures.	In the aqueous phase, carbohydrates could be extracted at mild temperatures (<200 °C), mainly as polysaccharides. At high temperatures above 200 °C, however, organic products that are toxic to subsequent bioprocesses (e.g., acetone and phenols) were generated with the consumption of carbohydrates.
[26]	*Nannochloropsis gaditana* (20.56 wt % loading)	flash hydrolysis (FH) 280 °C, holding time 9 s	centrifugation and filtration (no solvent)	The results from elemental composition and XRF spectroscopy analysis also confirmed that FH on *Nannochloropsis gaditana* efficiently extracted most macronutrients such as nitrogen and phosphorus (between 50 and 60 wt %).	The liquid hydrolysate, rich in nutrients, was tested for direct nutrient 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.

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Table 2. Feedstock Characterization of *Chlorella vulgaris* by Elemental Analysis (wt %), Biochemical Composition (wt %), Ash (wt %), Moisture Content (wt %), and Higher Heating Value (HHV; MJ/kg)

elemental analysis					biocomponent analysis					
C	H	N	S	O[a]	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

[a]By difference (100−C−H−N−S).

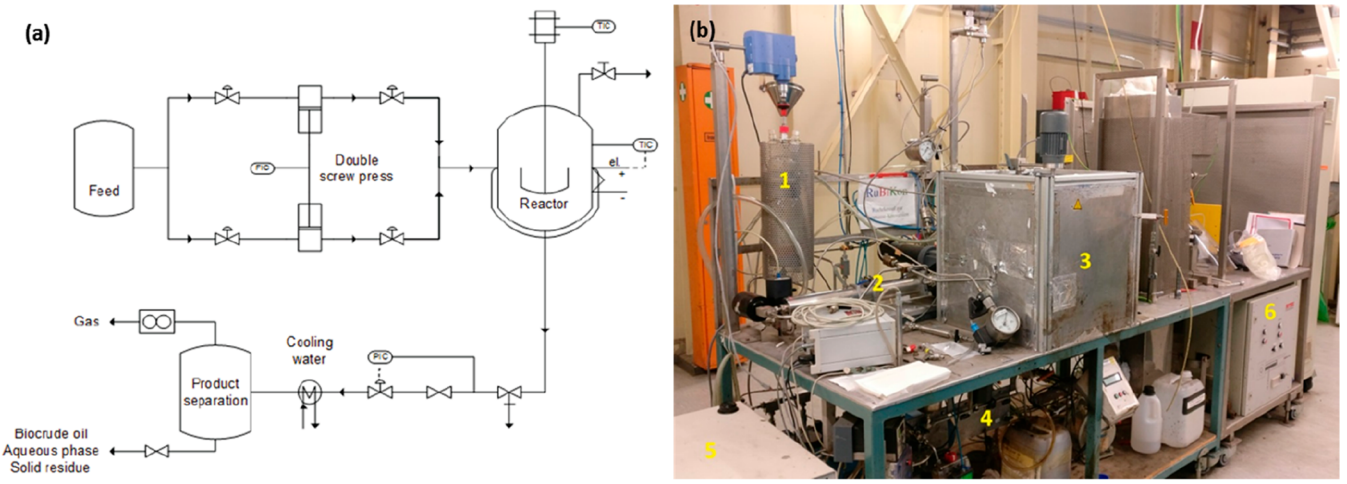

Figure 1. (a) Setup of the continuous stirring tank reactor.[33] (b) cHTL reactor: 1, feeding system; 2, double screw press; 3, insulated reactor covering; 4, downstream; 5, cooling system; 6, flow rate regulator.

utilization of the organic-rich AP would provide a promising economic benefit for algae biorefinery.[17−20] Table 1 presents recent studies (in the last 5 years) on the recovery of nutrients from microalgae AP.

It is noteworthy that most of these studies were performed in batch HTL experiments and organic separation was mostly conducted with dichloromethane (DCM) used as a solvent, 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.[27] However, it has been reported that the addition of an organic solvent could affect the biocrude properties greatly. As an example, organics initially contained in the aqueous phase may be transferred into the biocrude phase, resulting in higher biocrude yields at the expense of higher oxygen and nitrogen content.[28,29] At the same time, the remaining organic solvent in the AP could be harmful for further algae cultivation. After all, the usage of this organic solvent in upscaled production seems not only environmentally unfriendly but also economically unfavorable. The effect of organic solvent on product yields to deliver realistic and meaningful data for scale-up-process development has already been reported; however, the existing studies were mostly approached from the batch experiment level, and due to differences such as stirring type, heat transfer, and mass transfer in batch and continuous systems, their HTL behavior and following AP properties may differ. A related study at a continuous production level is still scarce. As Biller et al. suggested,[18] the use of a continuous reactor and the avoidance of organic solvents for separation would be desirable for a commercial system. The recovery of AP from a continuous HTL process for algae cultivation may further improve the process efficiency and has received increasing attention in recent years,[30] motivating the elaboration of a useful strategy in this study.

The goal of our work is to promote a more economical microalgae biorefinery with hydrothermal liquefaction as the core technology and get a deeper understanding of this process chain. In this work, two consecutive steps have been investigated along one processing route: first to understand the impact of using DCM as a solvent for HTL product recovery, specifically in view of a continuous production mode and subsequent AP recovery for algae cultivation. Different separation methods were applied, the yield and quality of the obtained biocrudes, as well as the composition of the AP, have been examined along with their HTL temperature dependency (from 300 to 400 °C). After a purification treatment, the obtained AP was further evaluated for algae cultivation.

2. MATERIALS AND METHODS

2.1. Feedstock Preparation. Microalgae *Chlorella vulgaris* (Cv), being one of the fastest growing strains with great potential for oil production,[31,32] was 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 prepared homogeneously using motorized stirring for 30 min in a 5 L beaker. The elemental and biocomponent characterization of the feedstock is given in Table 2.

2.2. Continuous Hydrothermal Liquefaction. Microalgae cHTL experiments were performed in a continuous stirred tank reactor (CSTR). As shown in Figure 1, the CSTR is constructed with Inconel 625 and has an internal volume of 190 mL, featuring a magnetically coupled impeller (made of stainless steel EN 1.45471) used for stirring. A detailed description of the reactor and operation of the cHTL experiment can be found in our previous studies.[33,34] In brief, approximately 2 h of preheating time using only water was first conducted for the reactor to reach the target temperature (300, 325, 350, 375, and 400 °C) at a pressure of 24 MPa. A flow rate of 760 mL h^{-1} was applied to maintain a holding time (the average period of biomass remaining inside the stirred tank reactor during a continuous process) of 15 min. After preheating, another 90 min of holding time

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(about 6 times the HTL holding time) was maintained to reach a steady cHTL state before feeding of algae slurry into the reactor was started with a double press pump. Afterward, all samples were collected during the steady cHTL state. For the product yield calculation, a total of 18 samples were taken during the entire cHTL operation period (a total of 100 min, three samples were collected for each time point with a sampling time of 2 min, approximately 25 mL of the liquid product was obtained for each sample) for each temperature investigation, and the mean values with their standard deviation were reported. Product gas was not recovered in this work, while biocrude, aqueous phase, and solid residues were collected into a preweighted tube for further separation.

2.3. Product Separation. To investigate the effect of DCM solvent on cHTL products, two different separation methods were applied in this study. For the nonsolvent method, the biocrude, solid, and AP in the sample tube were first transferred into a falcon tube. Then, the biocrude with the solid product was separated from the AP, applying centrifugation at 8000 rpm for 5 min. The product mass was determined by weighing: the biocrude and solid product were attached to the preweighted centrifugation tube, while the AP was removed and placed into a preweighted tube for mass determination. The dry mass content of the AP and that of the algae slurry were measured using careful water evaporation using an oven at 60 °C as described in our previous study[35] and was calculated according to eq 1. In addition to the direct measurement of AP yield as described above by eq 2, it was also calculated by the dry masses difference for comparison. This allows the ability to check the amount of light organic fraction in the biocrude, which can be evaporated at 60 °C.

$$\text{Dry content}_{\text{AP}} = \frac{\text{Mass}_{\text{tube+APafter60°C-oven}} - \text{Mass}_{\text{emptytube}}}{\text{Mass}_{\text{tube+APbefore60°C-oven}} - \text{Mass}_{\text{emptytube}}} \quad (1)$$

$$\text{Yield}_{\text{AP}} = \frac{\text{Dry content}_{\text{AP}} \times \text{Mass}_{\text{APin2minsampling}}}{\text{Dry content}_{\text{algaeslurry}} \times \text{Mass}_{\text{productin2minsampling}}} \quad (2)$$

The solvent separation method using DCM was performed as follows: 9 mL of DCM (≥99.7% purity) was added into the sample tube and agitated for intensive contact among the different products. Due to the different polarities and densities of the solvent and aqueous phase, complete phase separation was spontaneously achieved after 2 h. Afterward, the upper aqueous phase was carefully removed with a pipet. The DCM phase left in the tube, which contained biocrude oil and solid, was placed under a nitrogen flow for 24 h. After evaporation, the biocrude and solid were determined together by weight. Therefore, the biocrude reported in this study is a mixture of biocrude product and solid product. The aqueous phase yield was calculated as mentioned before for comparison.

2.4. Aqueous Phase Purification. Due to the potentially toxic substances evolved from HTL (rich in nitrogen heterocycles such as pyrroles and phenols[36]), the growth of algae could be inhibited.[37] A drastic dilution with water is usually performed to alleviate the algae growth inhibition; however, this could lead to nutrient limitations. To avoid such phenomena, a pretreatment to remove or reduce the toxic compounds in the AP shall be performed,[38] and a relatively light dilution can be kept. In general, purification methods such as precipitation, membrane filtration, and air stripping have been reported, but their applicability to microalgae HTL-AP is still limited. Supercritical water gasification (SCWG) has been considered for pretreatment because the organic material contained in the water is decomposed into gases (mostly to CO_2, H_2, CH_4) in this process. This technology has also been reported to effectively remove the harmful compounds in the AP for recultivation.[37] The phosphorus accumulation ability is reported to be improved with the SCWG mixed medium[39] in the algae cultivation. Another commonly used purification method is the removal of organics by adsorption, which is widely applied in the removal of pollutants from water, especially employing activated carbon absorption (ACA), because it is cost-effective and easily adaptable. In a recent study,[40] ACA treatment has been shown to remove or convert humic acid into nutrients and water recovery. In our study, SCWG and ACA treatments were applied as purification steps of the aqueous product from microalgae HTL.

The SCWG treatment was performed using 24.5 mL autoclaves, made of stainless steel EN 1.4980. A precalculated quantity of 2.5 mL of AP was injected into the autoclave, and nitrogen was preloaded into the autoclave at approximately 0.5 MPa, reaching supercritical water conditions after heating up to 450 °C by a metal block heater while maintaining a pressure of 23.9 MPa. The heating block was preheated to 450 °C before the autoclave was placed inside for 30 min. The ACA purification was carried out using activated carbon with a maximum particle size of 80 μm purchased from VWR Chemicals (CAS: 7440-44-0). A total of 100 g of activated carbon was added into 1 L of aqueous product and magnetically stirred for 30 min. Afterward, 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 material and aqueous phase. The purification was repeated two times for each sample.

2.5. Algae Cultivation with Recovered AP. Due to the limited amount of AP obtained by SCWG treatment, only ACA-treated AP was used to evaluate algae cultivation. In the previous studies, a dilution ratio range from 50× to 600× was applied to reduce the inhibitory effects derived from the HTL-AP.[18,41] In this work, with the help of the purification step, a lower dilution ratio could be investigated, aiming at a more economical approach of the entire process. The ACA-treated AP was first diluted with distilled water by a factor of 15. On the basis of the characterization results of the treated AP, some additional nutrients were added to achieve a comparable nutrient level to the standard medium, which was also prepared for the comparison, the composition of which is shown in Table 3.

Table 3. Chemical Composition of Standard Medium (Numbers Are Given for 1000 mL Solution)

TAP salt	NH_4Cl	15 g
	$MgSO_4{\cdot}7H_2O$	4 g
	$CaCl_2{\cdot}2H_2O$	2 g
phosphor solution	K_2HPO_4	28.8 g
	KH_2PO_4	14.4 g
Hutner's trace elements	$Na_2EDTA{\cdot}H_2O$	2.5 g
	$ZnSO_4{\cdot}7H_2O$	1.1 g
	H_3BO_3	570 mg
	$MnCl_2{\cdot}4H_2O$	255 mg
	$FeSO_4{\cdot}7H_2O$	250 mg
	$CoCl_2{\cdot}6H_2O$	80 mg
	$CuSO_4{\cdot}5H_2O$	80 mg
	$(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$	55 mg

The cultivation was carried out in a 200 mL flask on a vibration plate (100 rpm) at a temperature of 25 °C. The light intensity during the 8 days of cultivation was adjusted to 125 $\mu E/m^2s$ (LI-250 Light Meter, Li-Cor, USA); approximately 5% CO_2 was continuously fed at a rate of 25 mL/min as the carbon source for autotrophic cultivation, maintaining a pH value of approximately 7.3. Cultivation with a standard medium, with untreated AP, and with ACA treated AP was implemented in parallel, with three cultivation flasks being prepared for each cultivation condition. An overall flowchart of this work is presented in Figure 2.

2.6. Analysis of cHTL Product, Aqueous Product, and Algae Cultivation. The elemental analysis (EA) was performed on biocrude samples using a Vario EL Cube Analyzer; the content of O was calculated by difference (100−C−H−N−S), and Boie's formula is applied for the HHV calculation equation 3.

$$\text{HHV [MJ/kg]} = 0.3516 \times C + 1.16225 \times H - 0.1109 \times O + 0.0628 \times N + 0.10465 \times S \quad (3)$$

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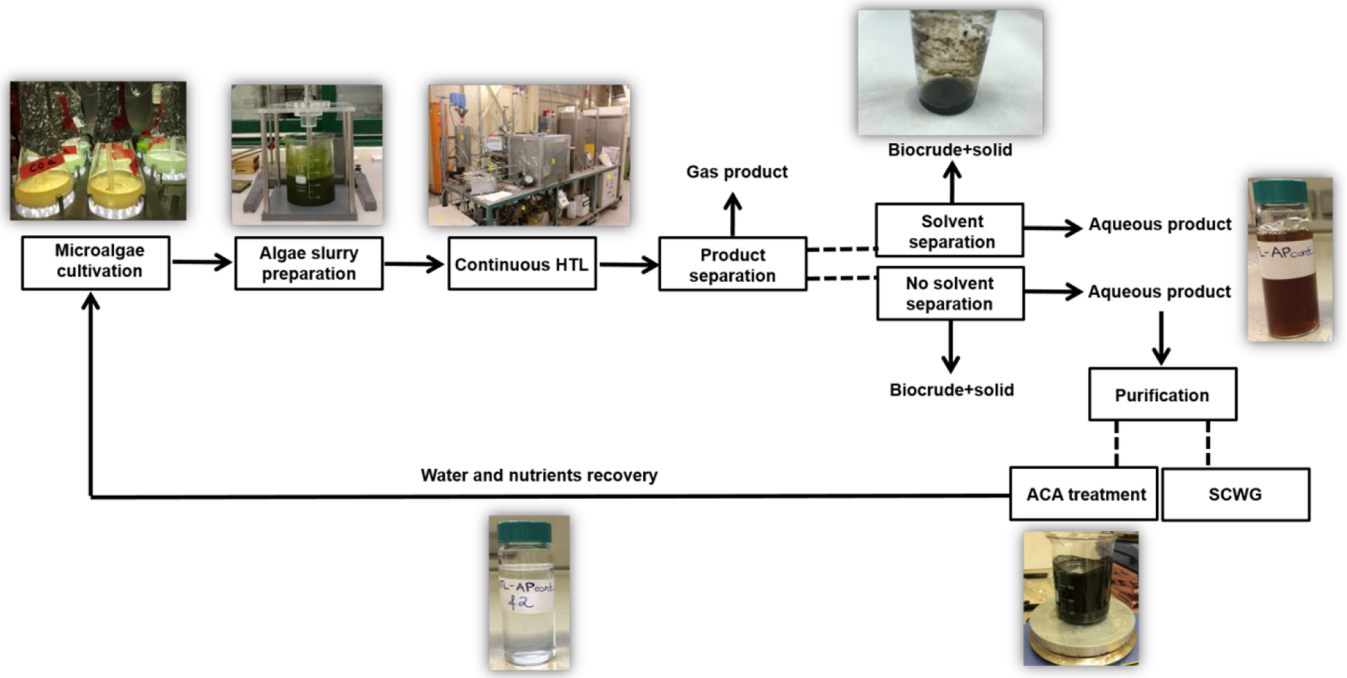

Figure 2. Overall experimental flowchart of this work.

The pH value of the 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 on the cHTL-AP and purified AP using a DIMATOC 2100 instrument, and 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 TOC, TNb, and partial ions was calculated using eq 4

$$\text{Recovery}\,(\%) = \frac{\text{Concentration of target component}_{\text{AP}} \times \text{Volume}_{\text{AP}}}{\text{Concentration of target component}_{\text{algaeslurry}} \times \text{Volume}_{\text{algaeslurry}}} \quad (4)$$

GC-MS analysis (Agilent 6890N equipped with a 5973 network mass selective detector) was performed on the AP before and after the purification step to further understand its composition.

The course of microalgae growth during cultivation was monitored by measuring optical density (OD) at 750 nm (Genesys 10S UV−vis, Thermo Scientific) using a disposable cuvette (ref 634−0676, VWR);[42] the dry biomass content was determined at harvest time. A correlation between OD and dry mass content was used as the basis for the growth curve; the correlation is shown in eq 5. The mean value for each cultivation condition was reported with the standard deviation.

$$\text{Dry biomass concentration}\,(\text{g L}^{-1}) = 0.172 \times \text{OD} + 0.00106,\ R^2 = 0.9975 \quad (5)$$

3. RESULTS AND DISCUSSION

3.1. Continuous HTL Experiments. *3.1.1. Production Yields.* Figure 3 presents the biocrude yields at different HTL temperatures with/without DCM separation. It can be seen

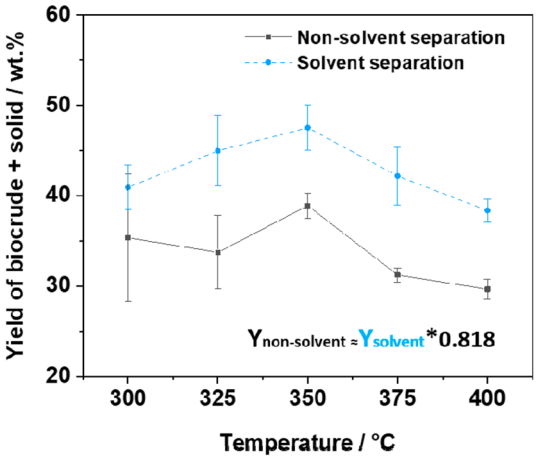

Figure 3. Biocrude yield at different cHTL temperatures (°C) with/without DCM separation. Data are shown as mean values of 18 measurements for each temperature during steady cHTL along with the standard deviation.

that, in general, the biocrude yield increases with rising temperature and reaches the maximum at around 350 °C (about 38.9 ± 1.4 wt % for non-DCM method and 47.5 ± 2.5 wt % for DCM method) before dropping at higher temperatures. This could be explained by the lipids and carbohydrates starting to react at a relatively low temperature and the increasing temperature promoting the degradation of proteins and conversion to the biocrude phase when surpassing the critical point of water; radical-induced cracking of biocrude, repolymerization, and redecomposition of intermediate products were enhanced to form lighter compounds, leading to a decrease of biocrude yield.[43,44] Therefore, it can be suggested that 350 °C could be the optimal temperature for high biocrude yields in the cHTL system. Furthermore, the yields present a relatively low fluctuation at higher temperatures (375 and 400 °C); this indicates that the performance of

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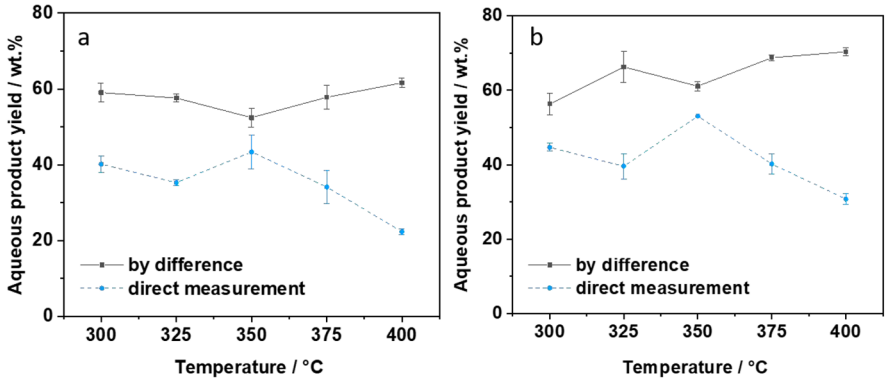

Figure 4. Aqueous product yield at different cHTL temperatures using a solvent (a) and nonsolvent method (b). Data are shown as a mean of 18 measurements for each temperature with the standard deviation.

Table 4. Elemental Analysis (wt %) and HHV (MJ kg^{-1}) of Biocrude + Solid Produced at Different HTL Temperatures (NS, Nonsolvent Separation; SS, Solvent Separation)

temperature	method	C	H	N	S	O[a]	HHV
300 °C	NS	54.6 ± 0.2	7.35 ± 0.05	7.15 ± 0.05	0.5	30.4	24.87
	SS	56.6 ± 2.7	7.6 ± 0.1	8.3 ± 1.1	0.56 ± 0.04	26.94	26.33
325 °C	NS	64.7 ± 1.14	8.07 ± 0.12	5.7 ± 0.37	0.33 ± 0.05	21.2	30.17
	SS	66.9 ± 1.25	8.25 ± 0.15	6.6	0.4	17.8	31.61
350 °C	NS	65.7 ± 2.2	8.8 ± 0.2	5.8 ± 0.3	0.6 ± 0.05	19.1	31.64
	SS	67.2 ± 2.3	8.8 ± 0.2	6.8 ± 0.3	0.6 ± 0.04	16.6	32.5
375 °C	NS	70.9 ± 0.66	8.67 ± 0.12	5.67 ± 0.05	0.37 ± 0.05	14.42	33.79
	SS	69.3 ± 2	8.47 ± 0.21	6.53 ± 0.24	0.57 ± 0.17	15.13	33
400 °C	NS	70.5 ± 0.45	8.95 ± 0.05	5.55 ± 0.05	0.7	14.3	34.03
	SS	69.2 ± 1	9.1 ± 0.2	6.6 ± 0.08	0.75 ± 0.04	14.35	33.81

[a]By difference: 100−C−H-N-S.

the cHTL reactor system is more stable when applying higher temperatures. By using DCM solvent, a 9 wt % mean yield increase can be achieved among the overall temperatures investigated, compared to that of the nonsolvent method. This value is quite close to that achieved using a batch reactor, which was reported to lose approximately 8.4 wt % of the total biocrude if the water-soluble portion was not recovered.[29] The biocrude yield change across the HTL temperatures shows a similar pattern, and a general average biocrude yield estimation can be proposed for Cv algae at the investigated temperature range: $\text{yield}_{\text{nonsolvent}} \approx 0.818 \times \text{yield}_{\text{solvent}}$. Due to environmental and health concerns, the use of DCM as a solvent should be avoided at scale; however, this estimation could help to build up a bridge between reported laboratory results and practical production in the continuous mode.

The aqueous product (AP) from the HTL process is also considered to be a resource which should be recycled in a later step. In this work, we approached the AP data using two calculation methods: the calculation of AP yield by difference and a direct measurement have been performed, and they present opposing trends over temperature, as shown in Figure 4. When the AP yield is calculated by difference, the values in both cases remain practically the same, at approximately 60 wt %, while when using direct measurement, a maximum of AP yield can be observed at 350 °C before a drastic decrease occurs at higher temperatures. This indicates that at higher temperatures, the conversion of intermediate water-soluble products is promoted, and more light organics are obtained in the AP above 350 °C, which are likely to be lost during the 60 °C evaporation step in the direct measurement method. This explains the increasing gap between these two determination methods above 350 °C. In addition, at 350 °C, the yield values resulting from two calculation methods reach the closest, indicating that a temperature of 350 °C converts the least amount of light organics into AP, and possibly the most amount to the biocrude phase, supporting 350 °C being the optimal temperature for running cHTL of *Chlorella vulgaris* (obtaining the highest biocrude and AP yield). A similar conclusion that a mild HTL temperature produces a more suitable AP for algae culture has been found by,[20] therefore, later purification and algae cultivation experiments were done using AP samples at 350 °C.

3.1.2. Elemental Analysis. To better understand the cHTL process and the effect of using DCM on the HTL product, elemental analysis was performed on the biocrude. Table 4 shows the elemental composition and the higher heating value of the biocrude produced at different cHTL temperatures. The HHV of the obtained biocrude covers a range from 24.8 to 34 MJ kg^{-1}, which is substantially higher than that of the dry microalgae feedstock (22.03 MJ kg^{-1}). Generally, higher temperatures increased C and H content in the biocrude while lowering the O content results in a higher HHV, which is in agreement with other studies on batch experiments. However, it can be noted that in cHTL, this pattern is not as significant after 350 °C, indicating that the effect of temperature on the biocrude chemical composition is less influential when surpassing the critical point of water during the cHTL process.

Within the temperature range investigated in this study, approximately 9 wt % higher biocrude oil yield and 1 wt %

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higher nitrogen contents were achieved in the biocrude when separating the products by DCM in cHTL. Also, by using DCM, higher carbon and nitrogen contents were obtained in biocrude, particularly at 300−350 °C. However, this effect on biocrude quality tends to be alleviated at HTL temperatures higher than 350 °C, where the carbon and hydrogen content seems to be stable regardless of the separation method applied. Temperature is believed to play a more significant role in the elemental composition than solvent at a higher temperature range. Since solvents should be avoided in real-life production, this finding indicates that the reported laboratory HTL product data from a high temperature range process could be more meaningful and realistic for production at scale.

3.2. Analysis of Aqueous Product. *3.2.1. Composition of cHTL Aqueous Product.* An examination of the cHTL aqueous product regarding pH value, the content of total organic and inorganic carbon, total nitrogen, ions, and organic acids of cHTL-AP was performed. The pH value of the AP rose constantly from 6.8 to over 8 with increasing cHTL temperature. As Cv algae is a protein-rich strain, this is probably due to the decarboxylation of organic acids and deamination of proteins.[37] Some amine compounds tend to form water-soluble N-containing compounds,[45] which could be utilized by further algae cultivation. Additionally, the recovery of total nitrogen, total organic carbon, and ammonium was calculated as presented in Figure 5. When

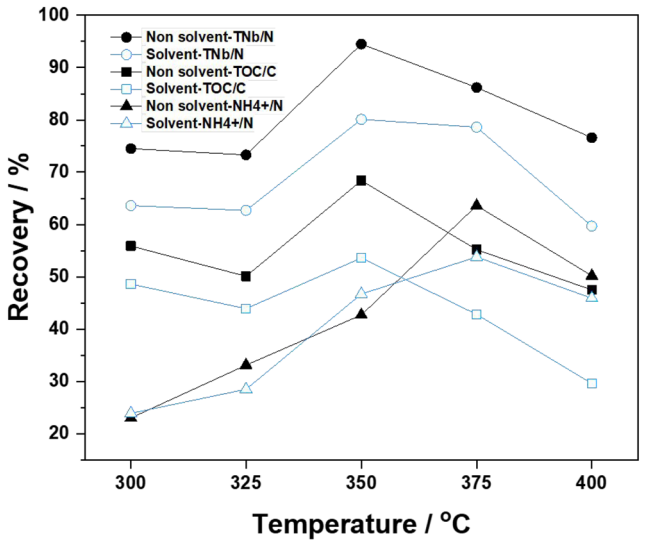

Figure 5. Recovery of total carbon, total nitrogen, and NH_4^+ from AP at different cHTL temperatures. Data are shown as the mean of two measurements.

no solvent is applied, more nitrogen and organic carbon is recovered, confirming that the DCM would absorb a certain amount of nitrogen and carbon into the biocrude phase. The trends in both methods are quite similar. More NH_4^+ can be found in AP produced at higher temperatures, which is also in line with the stronger deamination of proteins with increasing temperature. In this case, there is no significant difference between non-DCM and DCM samples, indicating that DCM has no direct impact on the trace elements such as Mg, P, S, Fe, and Ca ions as they present a decreasing pattern with the cHTL temperature (data not shown). K and Na ions show a stable recovery, regardless of the cHTL temperature or the usage of solvent.

3.2.2. Composition of Purification Treated Aqueous Phase. The nonsolvent applied AP sample produced in cHTL at 350 °C was selected for further supercritical water gasification (SCWG) and activated carbon absorption (ACA) purification treatment. The selection of the samples at this condition is based on the consideration that the usage of organic solvent in real industrial applications is unfavorable, and 350 °C was proven to be the optimal temperature for cHTL, which has been discussed in section 3.1.1. Table 5

Table 5. Composition of AP with SCWG or ACA Purification Treatment (before Dilution)[a]

	original microalgae feedstock slurry	350 °C original nonsolvent AP	350 °C SCWG-AP	350 °C ACA-AP
TC (mg L^{-1})	24930	16970	8599	3230
recovery		68.1%	34.5%	12.9%
TOC (mg L^{-1})	24747	16778	6970	2136
recovery		68.4%	27.7%	8.7%
TNb (mg L^{-1})	4396	4299	3534	1361
recovery		94.5%	81.8%	31.5%
NH_4^+ (mg L^{-1})	4397	1943	2064	1981
recovery		42.7%	47.8%	45.8%
K (μg mL^{-1})	1028	1016	595	2345
recovery		98.8%	57.8%	228%
Na (μg mL^{-1})	138	11	2.3	158
recovery		7.9%	1.7%	113.8%
Mg (μg mL^{-1})	140	7.8	2	116.3
recovery		5.6%	1.4%	83.76%
P (μg mL^{-1})	710	535	90	90
recovery		72.7%	12.9%	12.9%
S (μg mL^{-1})	257	231	160	160
recovery		89.8%	62.2%	62.2%
Fe (mg L^{-1})	78	2.2	5	0.25
recovery		2.8%	6.48%	0.23%
PO_4^{3-} (mg L^{-1})	No data	1370	172	21.38
Cl^- (mg L^{-1})	No data	39	54	97.7

[a]The recovery ratio was calculated based on the ion or elemental amount from original algae feedstock slurry.

contains the composition and recovery ratio of some nutrients in the aqueous products. By both treatments, the TOC and TNb content were drastically reduced, while the ACA treatment shows a stronger reduction, indicating that the absorption can remove a greater proportion of carbon, especially organic carbon. In addition, most of the NH^{4+} remained after both treatments. A huge increase in K, Na, and Mg content was observed after ACA treatment, even higher than that of the original algae slurry, indicating that the AP would absorb some K, Na, and Mg elements from the activated carbon during processing. An ICP-OES analysis has been performed on the activated carbon before and after the treatment, as presented in Table 6. It can be seen that the amount of K and Mg was reduced after the treatment, confirming that the K was dissolved into the aqueous phase. The other elements such as P and S remained at around the same level after the treatment. Most of the organic acids (methanol, ethanol, glycol, and formic acid) were below the detection limits of an HPLC measurement after purification.

To better understand the organic composition of the aqueous product, a GC-MS analysis of the untreated and treated aqueous product has been performed; compounds like

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Table 6. ICP-OES Analysis of Activated Carbon before and after Treatment (Result Shown in Weight Percentage of the Original Carbon)

wt %	K	Na	Mg	P	S	Fe
original activated carbon	0.545	<0.04	0.2	0.05	0.05	0.03
activated carbon after treatment	0.27	<0.04	0.165	0.17	0.04	0.04

pyrimidine, 4-methyl-, 2-propanone, dimethylhydrazone, pyrrolo[1,2-*a*]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-, phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl-, and methylcarbamate can be found in the untreated aqueous phase, while none can be found in the sample after ACA treatment. This supports activated carbon treatment being able to effectively remove phenolic N-containing compounds, which was reported to have an inhibitory effect on algae growth.[46]

3.3. Algae Cultivation with Different Medium. The ACA treated AP and other media were further subjected to the cultivation of Cv algae in flasks. Figure 6 shows the monitoring

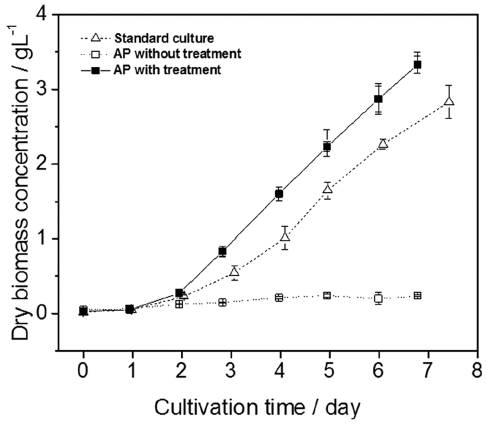

Figure 6. OD test of following algae growth (correlated to dry biomass concentration in the culture).

of algae growth curve over 8 days with the help of OD_{750} measurement. It is clear that without ACA treatment, the algae growth was inhibited since day 2, due to toxic compounds (phenolics, cyclic nitrogenous compounds or heavy metals etc.) that were generated by the HTL process.[47] Nevertheless, the ACA treated AP supported an even better growth than the ones with standard culture, indicating that ACA treatment can remove the undesired compounds effectively and promote algae growth. This is possibly caused by a particularly high content of K and Na after ACA treatment. K can neutralize the organic anions and other compounds to stabilize the pH in the culture,[48] and Na was reported to exert a beneficial effect on the microalgae biomass production. However, it should also be noted that a concentration of Na higher than 3 mM could generate a toxic effect for the growth.[49] Similar results (recycling the aqueous phase for algae cultivation achieved higher biomass yields than in the standard medium) have been reported in ref 50, while the exact reason in our case needs further investigation.

Figure 7 visualizes the OD_{750} measurement of algae growth: it can be confirmed with the result of the OD test that the growth with ACA-treated AP medium and with standard medium exhibited a rapid increase over the cultivation time

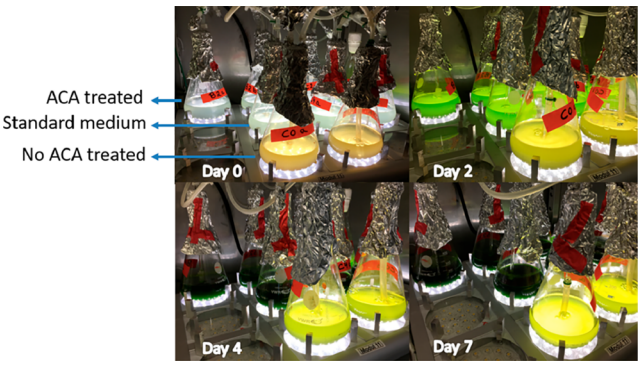

Figure 7. Algae cultivations with different media.

from day 2 on, while the growth with untreated AP medium stopped in the beginning period and did not see any change. This proves that the recycling of the cHTL-AP with ACA purification treatment was successful in the algae cultivation. To run such "algae-HTL-aqueous recycle-algae" for multiple cycles could be meaningful for process feasibility evaluation in the long term. In addition, there is prior literature on using biochar generated from microalgae thermal conversion in an integrated process instead of commercial activated carbon.[51,52] This idea could further promote mass recovery in order to achieve full utilization of microalgae biomass, thus potentially improving cost efficiency of the purification treatment.

CONCLUSIONS

In this study, microalgae *Chlorella vulgaris* was processed via HTL in a continuous stirred tank reactor at different temperatures, and HTL liquid products were separated with/without DCM. 350 °C was proven to be the optimal temperature for cHTL, obtaining the highest biocrude yield, and increasing the temperature above 350 °C seems to have less influence on biocrude elemental content. The addition of DCM induced about 9 wt % higher biocrude yields at the expense of 1 wt % more nitrogen in the biocrude in microalgae cHTL, while this effect on biocrude quality tends to be alleviated at higher cHTL temperatures. Over the temperature range investigated, an average biocrude yield estimation can be proposed: $yield_{non\text{-}DCM} \approx 0.818 \times yield_{DCM}$. The ACA treatment was proven to remove the toxic components effectively as treated AP showed better growth than the standard culture; however, further study is required to clarify the reason for this. Our work proves the principal feasibility of using cHTL-AP to improve the overall nutrient efficiency in a microalgae biorefinery. For future research, applying algae-derived biochar as an active carbon source in this process could be an interesting idea, as it promotes a biorefinery concept "from algae to algae", exploring the application possibility of algae biomass without external material input, which is an important issue for the circular economy in microalgae biorefineries.

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Notes

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