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Biocrude production by hydrothermal liquefaction from seaweed residues

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The development of new technologies to replace conventional fossil fuel energy sources is crucial for transitioning to a sustainable production system that better manages available resources, such as the Circular Economy. One potential solution is the revaluation of organic waste into raw materials through thermal treatment. Hydrothermal liquefaction (HTL) is a technology that produces biocrude from organic matter, through a reaction in an aqueous medium at elevated temperatures and pressures below the critical point of water. The organic matter treated in this case is a microalgae-bacteria consortium from the biological treatment of an effluent loaded with nutrients and organic matter generated by a marine aquaculture facility that produces sole. The aim of this study is to investigate the impact of key variables, namely temperature (240-300 ºC), initial biomass load (5-15 % w/w), and reaction time (20-60 min), on biocrude yields and their higher heating value (HHV). The highest biocrude yield (18.18%) was achieved at 270 ºC, 40 min, and 5 % w/w, while the highest HHV (37.1 MJ/kg) was obtained at 300 ºC, 40 min, and 10 % w/w.

* 1. Introduction

As aquaculture has mainly developed in the last 50 years, environmental sustainability has become a significant concern. Therefore, efforts have been made to raise awareness of current environmental issues and to implement practices to reduce the environmental impact of aquaculture (Boydet et al., 2020). Traditional open aquaculture facilities (continuous inlet and outlet water flow) are being replaced by recirculation aquaculture systems (RAS) to reduce the water footprint of the process. This technology consists of reusing the water circulated in the plant to return it to fish tanks with adequate quality, requiring low water renewal rates, less than 10 % of the total daily volume to make up the evaporation and flush out residues (Calderini et al., 2021). Reduced wastewater flows are generated in RAS compared with open systems and are more concentrated, obtaining a more concentrated flow of pollutants. Consequently, it opens up the possibility of using technology to exploit these resources (nutrients, total suspended solids (TSS), organic matter) and minimise pollution. Therefore, several authors are advancing in removing these nutrients using microalgae technology (Villar-Navarro et al., 2021). Another challenge is the treatment of the solids concentrated stream resulting from mechanical filtration, marine water with high nutrient and biodegradable organic matter load. Some studies have demonstrated the effectiveness of its bioremediation using microalgae in consortium with bacteria (Sirohi et al., 2022). The biomass-sludge generated in this process (microalgae residue) could be treated by hydrothermal liquefaction (HTL), technique that has not been approached until now with this type of raw material.

HTL is an intriguing technique that produces biocrude (a fuel precursor), biochar, water-soluble compounds and some gases using the whole biomass of microalgae in a wet state (Gou et al., 2015). HTL can be performed with or without a catalyst at a temperature range of 200–370 °C and a pressure range of 2–20 MPa (Liu et al., 2021). HTL is seen as the most appropriate thermal conversion technique for aquatic biomass such as microalgae because it performed with the wet biomass, thereby eluding the drying step–related energy costs (Barreiro et al., 2013). In this process, the microalgae biomass undergoes many reactions that depolymerize the materials, such as hydrolysis, dehydration, and decarboxylation, which form intermediate water-soluble compounds (Basar et al., 2021). This is followed by re-polymerization and condensation reactions which form water-insoluble biofuel. Process parameters are the key factors in altering the yield and efficiency of the HTL (Akhtar and Amin, 2011). HTL is linked with several process parameters such as reaction temperature, residence time, choice and application of catalysts and solvents, and finally, the biomass-to-water ratio, which directly alters the yield and properties of biocrude (Mishra et al., 2022). During HTL, water plays three roles by acting as a solvent, reactant, and catalyst (Dimitriadis and Bezergianni, 2017).

In the present study are performed preliminary HTL tests of the microalgae in consortium with bacteria biomass obtained from bioremediation of marine aquaculture effluent. In the experimental design, tests were carried out at different times, temperatures and initial loading of algal biomass (biomass-to water ratio) in order to find the higher yield and heating value of biofuel produced from this raw material. A high pressure reactor of 1 L volume was used for the HTL experiments. Around 250 mL of water were used as reaction medium and an inert nitrogen atmosphere was applied. Biofuel extractions were performed over the solids and aqueous phases obtained in the reaction using dichloromethane as solvent. Final gas phase was also analyzed.

* 1. Material and Methods
		1. Microalgae-bacteria consortium

The consortium culture was conducted using a stream from the RAS system of an intensive marine aquaculture fish farm of *Solea senegalensis* located in the Bay of Cadiz (Southern Spain). The fish farm is designed to produce 500 tonnes of sole per year. The RAS system comprises a rotosieve drum screen, biofiltration for aerobic nitrification, protein skimmer and ozonation. The bioremediated stream is a marine wastewater flow with a high concentration of biodegradable solids and nutrients, obtained as a rejection from the rotosieve. The bioremediation process took place in a 6000 L raceway reactor located outdoors at the fish farm facilities and operated in batch mode. The microalgae biomass was harvested in autumn 2021 using a GEA Westfalia centrifuge (model OTC 2-02-137) and preserved at -24ºC. The moisture content was 65% (w/w).

* + 1. Analysis methods

This section describes the analyses conducted to characterize the raw material and the phases resulting from the hydrothermal liquefaction tests.

*Gas phase:* The composition of the gaseous sample was analysed based on a standard sample containing CO, CO2, N2, CH4, H2 and O2. The standardised closed-loop scrubbing method (Standard Methods, 6040 B) was used to quantify the different volatile components of the sample. This technique uses an inert mobile gas, helium in this case, to displace the remaining components. The experiment employed an Agilent Technologies 6890N gas chromatograph with two columns in series and connected to a valve for separating CO and CO2 and TCD detector. Samples were manually injected at an injection temperature of 120°C. The oven temperature increased from 55°C to 160°C and remained constant for a few minutes. The analysis lasted approximately 15 minutes.

*Aqueous phase*: Chemical oxygen demand (COD) analysis is a measurement parameter for organic matter in aqueous samples. The standardised closed reflux method (Standard Methods, 5220 C) was used, where the amount of Cr (III) formed is determined by spectrophotometry instead of titration. Due to the high amount of salts in the initial biomass, a series of dilutions was necessary as these salts can interfere with the analysis method. For this analysis, Pyrex COD tubes were used and placed in a HANNA HI83900 thermoreactor with a timer and automatic temperature control. The absorbance reading at 584 nm was taken using a MACHEREY-NAGEL PF11 spectrophotometer. To study the COD range, a calibration line was created using glucose standard solutions at specific concentrations. pH is a parameter used to determine the degree of acidity or alkalinity of samples obtained during tests. The pH meter used for this analysis was a pH-Meter BASIC 20+. Conductivity analysis was carried out to measure the electrical conductivity of the sample using a CRISON CM 35 conductivity meter. The total organic carbon (TOC) concentration of the feed samples (algal biomass aqueous solution) and the effluent aqueous phase was measured using the wet oxidation method (Standard Methods, 5310 D) and analysed with a SHIMADZU TOC-SM 5000 A analyser. The IC is measured as the CO2 released when treated with an acid solution in a combustion chamber at 200°C. The sample is then injected into the combustion chamber. The sample is then injected into a combustion chamber at a temperature of 685°C with an oxidising catalyst. This causes all of the CO2 to be entrained in a gaseous stream, and the TC is measured using non-dispersive infrared.

*Solid phase:* The energy released during the combustion of solid phase samples and initial biomass was quantified using the high heating value (HHV) measurement through a substitution procedure. This involved comparing the heat obtained from the sample with the heat obtained from a standardisation material (benzoic pellets) using a Parr 6400 calorimeter.

* + 1. HTL equipment

The hydrothermal liquefaction tests were conducted using a 1 L capacity Parr model 4570 high pressure reactor for batch operation. The reactor consists of a cylindrical electric heater with a power of 2300 W and a temperature probe with a sensor. The temperature probe comprises a thermocouple connected to a controller that measures the temperature inside the reactor. The reactor body is made of 316L stainless steel, and the head, which include the necessary accessories, is also made of the same material. Flanges are used to maintain a hermetic seal on the reactor. The pressure gauge indicates the pressure inside the reactor, which can reach a maximum operating pressure of 27.6 MPa. The equipment features openings that are regulated by manual valves for gas inlet and outlet. The reactor is initially pressurised with nitrogen. The ParrCom application remotely controls the reactor. The temperature controller is a PID type that can maintain a constant operating temperature with an error of ± 3ºC.

* + 1. Experimental procedure

The different experiments aim to determine the biocrude yield (see Ec (1)) and its HHV from microalgal residue as a function of the different LHT operating conditions tested; thus, the effect of temperature, reaction time and initial biomass loading. The experiments listed in Table 1 were planned for this purpose. Specifically, the effect of reaction time was examined within the range of 20 to 60 min at a fixed temperature of 270ºC and 10% loading. Additionally, the effect of loading percentage was studied at 5%, 10%, and 15% at 270ºC for 40 min. Finally, the effect of temperature was analyzed within the range of 240 to 300ºC at a fixed time of 40 min and 10% loading.

After determining the moisture content of the biomass (65% w/w), calculate the amount of water needed to prepare the reactor load (% by weight of dry biomass with respect to dry biomass plus water), taking into account a total volume of 250 mL as the reaction medium. Once the feed is ready into the reactor, it is closed properly to prevent leaks. To do this, place the head on the reactor body and tighten the fastening screws with a flange. The O2 air inside is displaced by purging with N2 for 3 minutes from a high-pressure N2 bottle. Following purging, establish an initial pressure of at least 0.5 MPa gauge. This ensures that the pressure reached during experimentation maintains the liquid state conditions of the feed and allows for proper execution of the HTL. The heating blanket is placed around the reactor cylinder and chamber to prevent leaks or malfunctions, ensuring safe working conditions during the experiment. Temperature and pressure values are recorded using the ParrCom application, and a PID controller regulates the temperature until the desired LHT test value is reached. Upon reaching the designated temperature for the experiment, the experiment's time zero is established and temperature is maintained during the experiment. Once the reaction time has elapsed, the heating mantle is turned off, and the reactor is cooled. The gas phase is then sampled in triplicate after reaching room temperature and the reactor is depressurised. The reactor head is unscrewed, and the flange is separated to access the effluent for further analysis.

The effluent produced by the reaction undergoes vacuum filtration to separate the aqueous phase from the solid phase. Some of the biocrude produced is present in both phases. The solid phase is then subjected to solid-liquid extraction using an organic solvent (dichloromethane, DCM) to extract the biocrude. The remaining solid (biochar) is then dried in an oven to remove any remaining solution and to determine its yield (see Ec (2)) and HHV. The aqueous phase also containing biocrude is extracted using DCM in a separating funnel for liquid-liquid extraction. After approximately 24 hours, phase separation is performed, and the exhausted aqueous phase is subjected to a second liquid-liquid extraction with DCM. The resulting extracts, containing the extracted biocrude and DCM, are then evaporated using a rotary evaporator to recover the biocrude and remove the solvent. The biocrude yield is determined through gravimetry. A sample of the biocrude is then taken to the calorimeter to determine its HHV, as detailed in section 2.2 Analysis methods. The recovered dichloromethane is used for further extractions. Borazjani et al. (2023) present a diagram of the process that is similar to the one used in this work.

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| $$Yield\_{biocrude}=\frac{biocrude (g)}{dry weight of microalgae residue (g)}∙100$$ | (1) |
| $$Yield\_{biochar}=\frac{dry weight of solid (g)}{dry weight of microalgae residue(g)}∙100$$ | (2) |

Table 1: Operating conditions tested with microalgae residue, yields of biocrude (solid and aqueous phases) and biochar, HHV of biocrude (solid phase) and biochar

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| HTL test | Temperature (ºC) | Reaction time (min) | Biomass loading (% w/w) | Yieldbiocrude (%) | Yieldbiochar (%) | HHV (MJ/kg) |
|  |  |  |  | Solid phase | Aqueous phase | Both phases |  | Biochar | Solid phase  |
| 1 | 240 | 40 | 10 | 7.7 | 9.1 | 16.8 | 45.3 | 9.9 | \* |
| 2 | 240 | 20 | 5 | 2.7 | 19.8 | 22.5 | 37.4 | 9.0 | \* |
| 3 | 270 | 40 | 10 | 4.6 | 5.3 | 9.9 | 57.7 | 5.9 | 34.9 |
| 4 | 300 | 40 | 10 | 6.4 | 2.0 | 8.4 | 55.1 | 5.8 | 37.1 |
| 5 | 270 | 20 | 10 | 5.4 | 4.2 | 9.6 | 52.0 | 7.0 | 34.8 |
| 6 | 270 | 60 | 10 | 6.6 | 5.0 | 11.6 | 49.1 | 6.8 | 34.9 |
| 7 | 270 | 40 | 5 | 9.9 | 8.3 | 18.2 | 40.3 | 7.9 | 35.8 |
| 8 | 270 | 40 | 15 | 7.0 | 1.1 | 8.1 | 28.9 | 6.8 | 35.3 |

\* Low amount of biocrude oil available for analysis.

* 1. Results and Discussion

Two preliminary experiments (HTL tests 1 and 2, see Table 1) were conducted in a smaller reactor volume with a stirring speed of 50 rpm. During the experiment, it was noted that a portion of the effluent sample adhered to the stirrer, resulting in difficulties in its recovery and leading to inaccurate biocrude yield measurements. As a solution, the reactor was changed to the current one, which does not have a stirrer. These preliminary tests focused on the use of acetone as an organic solvent for separation operations. DCM was chosen as the organic solvent because the previous solvent did not completely separate the liquid phases.

The biocrude yields of experiments 1 and 2 (see Table 1) are higher than expected due to the occlusion of liquid (water) in the volumetric flask after using the rotary evaporator during the first experiments. This resulted in a higher value of biocrude mass during the calculations. To calculate the biocrude yield, subsequent experiments removed the occluded liquid.

* + 1. Characterization of raw material

The University Institute of Marine Research (INMAR) of the University of Cadiz characterised morphologicaly the microalgae residue by Amnis ImageStreamX Mk II Imaging Flow Cytometer. The analysis identified that the consortium consisted of 95% microalgae, with *Chlorophyceae* and *Diatoms* being the predominant genera. The majority *Chlorophyceae* was subsequently isolated and identified as *Halochlorella rubescens* through bioinformatic analysis. Additionally, an elemental analysis of the microalgae residue was conducted by the Central Service of the University of Seville (CITIUS), which revealed a composition of approximately 35.1% Carbon, 5% Hydrogen, and 4.4% Nitrogen. A calorimetric analysis was conducted on the microalgae residue, which had been dried at 105ºC, resulting in a HHV value of 12.4 MJ/kg.

* + 1. Influence of process parameters

*Effect of reaction temperature*: Temperature is an important factor that affects the distribution of the product and the yield of biocrude oil during the HTL process. It is important to highlight that the operating pressure is primarily determined by chosen temperature (3.5, 6.0, 9.0 MPa at 240, 270 and 300ºC, respectively). In a typical HTL process, the effect of temperature on the biocrude yield follows a sequential manner. Initially, elevating temperature increases biocrude yield by enhancing biomass conversion into fuel fractions through overcoming the decomposition or depolymerisation reactions. Increasing the reaction temperature beyond a certain value decreases the biocrude yield while forming a higher amount of incondensable gas and biochar. Secondary degradation and Boudouard gas reactions are the main mechanisms for reducing biocrude yield at higher temperatures (Brindhadevi et al., 2021). The recombination of free radical reactions at higher temperatures is the reason for the biochar formation (Gopirajan et al., 2023). Figure 1 presents the results obtained by varying the reaction temperature between 240 and 300ºC while keeping the time and biomass loading at 40 min and 10%, respectively. The experiment conducted at 240ºC yielded a higher biocrude yield than expected, as discussed at the beginning of section 3 of Results and Discussion. Figure 1A demonstrates that an increase in temperature from 270 to 300ºC promotes the production of heavy biocrude in the solid phase, which may eliminate the need for extracting light biocrude from the liquid phase. At 300°C, the heavy biocrude had a maximum HHV of 37.1 MJ/kg (see Figure 1B). Ramirez et al. (2015) found that the calorific value is directly proportional to the carbon and hydrogen fraction of the biocrude, while the oxygen and nitrogen content has a negative effect on the HHV. The results suggest that the biocrude's carbon and hydrogen content is higher at the maximum reaction temperature.

*Effect of reaction time:* Eboibi et al. (2014) showed that the effect of short and long reaction times depends on the reaction temperature and leads to products of different yields and quality. Figure 1 displays the results obtained by varying the reaction time from 20 to 60 min while maintaining the temperature and biomass loading at 270°C and 10%, respectively. The data shows that increasing the reaction time to 60 min results in a 17% increase in biocrude yield (see Figure 1A). However, the reaction time does not lead to an increase in HHV (see Figure 1B) or heavy biocrude yield.

*Effect of biomass loading*: Borazjani et al. (2023) showed that in most cases, increasing the biomass loading leads to a decrease in biocrude production yield and carbon content, and an increase in residual solid and gas phase volume. On the other hand, the energy balance of these processes improves by decreasing the moisture content. Figure 1 presents the results obtained by varying the biomass loading from 5 to 15% while maintaining a constant temperature of 270ºC and reaction time of 40 min. The data shows that a lower biomass loading leads to a higher biocrude yield, with a maximum value of 18.2% observed in all experiments. However, it should be noted that the biocrude is equally distributed between the solid and liquid phases (see Figure 1A). With regards to HHV (see Figure 1B), its value is 35.8 MJ/kg, which is 3.5% lower than the highest value recorded in the experiments (37.1 MJ/kg). From a process economics perspective, in order to determine whether it is better to increase the biomass load upper than 10%, percentage considered optimal in HTL (Ellersdorfer, 2020); a more comprehensive experimental design is required.



*Figure 1: A) Yields of biocrude (solid and aqueous phases), B) HHV of biocrude (solid phase), n.d: not date.*

The chemical oxygen demand (COD) and total organic carbon (TOC) of both the initial feedstock and the resultant aqueous phase post-hydrothermal liquefaction (HTL) were examined across all experimental conditions. It was noted that HTL leads to a significant reduction in oxygen content within the aqueous phase, ranging from 60.8% to 75.0%, corresponding to experiments 2 (240°C, 20 min, 10%) and 8 (270°C, 40 min, 15%), respectively. Furthermore, HTL results in a decrease in the concentration of organic carbon present in the aqueous phase, with reductions ranging from 14.8% to 38.9%, observed in experiments 2 (240°C, 20 min, 5%) and 4 (300°C, 40 min, 10%), respectively. Experiment 4 has the highest HHV (37.1 MJ/kg).

Regarding the gas phase, only CO, CO2, N2, and O2 were identified, with no presence of H2 or CH4. The majority of the identified gases were N2, ranging from 69.2% to 93.2%, and CO2, ranging from 4.3% to 25.8%. The presence of N2 is due to its use in establishing an initial pressure of 0.5 MPa inside the reactor (see section 2.4 Experimental procedure).

The average pH and conductivity of the feed were 7.0 and 11.5 mS/cm2, respectively. Upon analysis of both parameters in the aqueous phase, an increase was observed, with mean values of 8.2 for pH and 23.2 mS/cm2 for conductivity.

Finally, HHV of the biocrude obtained in the present study was found to be comparable with the heating value of the biocrude obtained from various lignocellulosic biomass (28-36 MJ/kg) and microalgae (30-48 MJ/kg) (Ramirez et al., 2015).

* 1. Conclusions

The preliminary study of applying hydrothermal liquefaction to a microalgae-bacteria consortium biomass obtained from the bioremediation of marine aquaculture effluents shows that similar HHV values (37.1 MJ/kg) can be obtained as with lignocellulosic biomass and microalgae. Additionally, this value triples the HHV of the initial biomass (12.4 MJ/kg). The highest biocrude yield (18.2%) was achieved at 270 ºC, 40 min, and 5 % w/w with an HHV only 3.5% lower than the highest value obtained at 300ºC, 40 min and 10% w/w (37.1 MJ/kg). The biomass produces a biocrude that is distributed between the solid phase (heavy biocrude) and the aqueous phase (light biocrude). The production of heavy biocrude is favoured by higher temperatures and biomass loads. At the operating conditions of 270ºC, 40 min, 15% w/w, the biochar yield is the lowest at 28.9%. Additionally, 87.5% of the biocrude is distributed in the solid phase. However, this result in a reduction of 55.5% in biocrude yield compared to the maximum obtained. The results obtained for hydrothermal liquefaction of seaweed residues were promising. Further experiments will be conducted in the near future to model the production of biocrude and its quality.

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