

Hydrothermal liquefaction of biomass produced from domestic sewage treatment in high-rate ponds

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ABSTRACT

This study evaluates the application of biomass produced from the treatment of domestic sewage in high-rate ponds (HRPs) as feedstock for the production of bio-oil via hydrothermal liquefaction (HTL). The effects of reaction time, temperature, and biomass/water ratio on the yield of bio-oil were assessed. In addition, a balance of carbon and nitrogen among the products (bio-oil, aqueous phase, solid residue, and gas) was carried out, in order to evaluate the quality of the bio-oil and possibilities for increasing value from the byproducts. In a 15-min operation at 300 °C with biomass/water ratio of 1/10 (w.w⁻¹), the bio-oil yield was of 44.4% (Dry Ash Free - daf-basis). Under every condition tested, the solid residue was the most abundant byproduct, mostly due to the high ash content in the biomass. The minimum nitrogen recovery in the bio-oil was 57%, obtained in the operation at 275 °C, which is considered the main disadvantage of the process. The use of biomass directly after its production may result in an excessive consumption of energy due to the high water content. However, the need for drying is reduced when compared to other microalgal-based bioenergy production processes, potentially achieving a positive energy balance in the HTL.

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1. Introduction

Energy dependence on fossil fuels is associated with the accumulation of atmospheric pollutants including greenhouse gases, which may harm the environment and pose problems to the human health. Therefore, there is an increasing interest in the development of new and sustainable processes for producing energy from renewable sources with the aim of reaching net-zero carbon emissions. In addition, these new energy sources must be capable of meeting the current global energy demand. In this context, the Intergovernmental Panel on Climate Change [1] states that the sustainability of the planet depends on the capacity to provide sufficient clean and sustainable energy to meet the demands of future generations.

Microalgae biomass is widely identified as a promising feedstock for the production of third generation biofuels. Microalgae are organisms that may present autotrophic metabolism with greater

photosynthetic efficiency, growth rate and yield/area ratio than other energy crops [2]. In addition, they can be cultivated throughout the year, in areas unfit for agriculture, and in most climates [3].

Despite such advantages, there are still challenges to the large-scale use of microalgae biofuels, mostly related to its high production costs and to difficulties in obtaining a positive energy balance. The axenic culture of species with high lipid content has high water and nutrients demands, in addition to being susceptible to contamination by species with low lipid content, and bacteria, which present faster growth. Another important aspect is the energy demand of biomass drying, which can be responsible for up to 50% of the energy consumption in the microalgae oil production process [4].

In the search for a solution to the aforementioned issues, several studies addressed the use of wastewater as a cultivation medium for a consortium of microalgae and other microorganisms [5–7], with the objective of reducing the production costs relative to those of water and nutrients. The significant advantage of this is the production of treated effluent as a byproduct of biomass production, in conditions to be used for several non-potable purposes. The

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use of effluents can produce a high rate of microalgae growth; however, the lipid content of this biomass is usually reduced [8,9]. Given this characteristic, associated with no need of microalgae drying before its utilization, makes hydrothermal liquefaction (HTL) a suitable process for bioenergy production from wastewater.

HTL is a thermochemical process characterized by the physical and chemical conversion of biomass under conditions of high pressure and temperature. The biomass is converted into four distinct products: bio-oil, gas, solid residue and materials that are soluble in water, usually consisting of sugar, organic acids and nutrients. In HTL, macromolecules in the biomass are depolymerized into more simple molecules and then the unstable fraction of these products is repolymerized into compounds that constitute the bio-oil [10]. Due to the extreme conditions of temperature and pressure, the HTL converts not only lipids into bio-oil, but also carbohydrates and proteins, which enables the obtention of a high yield of bio-oil [11]. Additionally, the complete drying of the biomass is not necessary, which guarantees greater competitiveness from the energy aspect, making this process more suitable for microalgae biomass than other thermochemical processes, such as pyrolysis and gasification [12]. Moreover, HTL has the capacity to recycle nutrients through its generated byproducts [4].

Therefore, the integration of wastewater treatment and HTL can, at first, resolve several key bottlenecks associated with the production of biofuels from microalgae, namely the high water and nutrients demand, culture contamination, low lipid content of the biomass and energy demand for drying. However, improvement of the operation conditions is essential to optimize the oil yield. Within the HTL temperature range (250 °C–370 °C) there are different hydrolysis and polymerization reactions, and the intensity at which these reactions occur will influence the yield and quality of the bio-oil [2,13]. The reaction time must be sufficient for a satisfactory conversion of organic matter, but on the other hand, reaction times that are too long may favor the production of gases and other soluble materials, and consequently reduce the bio-oil yield [4]. If the biomass/water ratio is too high, it can have a negative effect on the process, since water participates as a catalyst and as a reactant. However, too low biomass concentrations can represent an economic disadvantage.

Many studies have been carried out using macroalgae [14,15] and isolated microalgae species [16–18], but so far few are developed with biomass produced from the treatment of effluents [8,12]. The cultivation of biomass grown during wastewater treatment entails a biomass composed of a microorganism consortia on its constitution. The biomass formed under these conditions may have low lipid concentrations due to the presence of bacteria that generally have less than 10% lipids in their composition [19]. Moreover, ash content may be high, due to ash from the effluent itself. Faced with this, this study is aimed at evaluating the use of biomass produced from the treatment of domestic sewage as feedstock for HTL. Differences in the composition of the biomass in relation to microalgae grown in synthetic media and the possibility of using the constituents of the sewage reinforce the originality of the study. The effects of reaction time, temperature and biomass/water ratio on the bio-oil yield were investigated. Furthermore, the distribution of the carbon and nitrogen present among the products in the biomass was measured in order to assess the characteristics of the bio-oil, to propose bio-oil end-uses and thus explore the potentials of HTL within the concept of biorefinery.

2. Material and methods

2.1. Biomass production

The biomass used in HTL studies consists of a group of

microorganisms and organic matter (mainly microalgae together with bacteria, zooplankton and detritus) that was produced at the experimental sewage treatment and biomass production plant of the Sanitation and Environmental Engineering Laboratory of the Federal University of Viçosa (UFV), Minas Gerais, Brazil (UTM coordinates 722924 E, 7702003 S, zone 23 K). The cultivation was carried out in pilot-scale high-rate ponds (HRPs), with the following dimensions: 1.28 m width, 2.86 m length, and surface area of 3.3 m². A domestic effluent from an upflow anaerobic sludge blanket (UASB) reactor was used. After production, the biomass was lyophilized.

2.2. Biomass characterization

Elemental characterization of the biomass was carried out. The levels of C, H and N were measured using a Perkin Elmer Series II 2400 Elemental Analyzer. The level of S was obtained by turbidimetry using a spectrophotometer at 440 nm. The Cl was determined according to the EN 15289:2011, whereas Si, Na, K, Ca, Mg and P were determined according to the EN 15290:2011; both European norms for solid biofuels. The oxygen level in the biomass was determined by difference.

Moisture and ash levels were determined according to EN 14771–1:2009 and EN 14775:2009, respectively. Carbohydrates were solubilized by quantitative acid hydrolysis and measured by the phenol-sulfuric reagent method [20]. The lipid content in the biomass was determined by the soxhlet extraction method. Hexane and ethanol were used as solvents in the extraction. It used 1 g of biomass. The extraction using hexane was performed first, for 6 h, by adding 100 mL of the solvent to the distillation flask. In sequence, with the same cartridge, ethanol extraction was performed using 100 mL for 3 h. The procedure was carried out in duplicate. Then the solvent was evaporated in a BUCHI Waterbath B-480 rotavapor with thermostatic bath at 50 °C, 500 mbar was used for hexane, and for the ethanol, 350 mbar. After evaporation of the solvents, the lipid content was determined by gravimetry. Determination of the protein content was performed indirectly using the Total Kjeldahl Nitrogen method (TKN) according to [21]. Since this method consists of quantifying the total nitrogen in the biomass, the quantification of protein was carried out considering a nitrogen-to-protein conversion factor of 6.25 g g⁻¹ of total nitrogen [22].

2.3. Hydrothermal liquefaction (HTL)

The HTL tests were performed in batch mode, using 0.16 L autoclaves built in Hastelloy C276 by Parr Instruments.

The autoclave cap consists of a thermocouple inserted into a sheath, which extends to the bottom of the reactor, a stainless steel Bourdon tube pressure gauge, for measuring the pressure inside the autoclave, an inlet and outlet gas valve and a safety rupture disc. The sealing of the autoclave is a graphite gasket allocated between the cap and the body of the autoclave.

The autoclave was heated by an oven that has an oscillation system to provide agitation. During heating of the reactor and throughout the tests, pressure and temperature were measured by a controller connected to both the pressure gauge and the thermocouple.

Three operation parameters were assessed: reaction time, temperature, and biomass/water ratio. Reaction time was assessed considering 15 min, 30 min and 45 min after reaching the operation temperature. The temperatures assessed were 275 °C, 300 °C, 325 °C, and 350 °C. For each temperature, the maximum variation was ±5 °C. The biomass/water ratios were 1/20, 1/10, and 1/5 (w.w⁻¹), which are equivalent to solids concentrations of 4.8%, 9.1%,

and 16.6% (w.w⁻¹). The 1/20 ratio was the smallest ratio used, as very low amounts of biomass may make separation of the products in laboratory scale very difficult, thus increasing losses in percentage terms. The reactor always operated with 77 g of material in its interior, and when the biomass/water ratio was 1/20, 3.7 g of biomass and 73.3 g of water were added; while for the 1/5 ratio, 12.8 g of biomass and 64.2 g of water were added.

Before the beginning of each test, the autoclave was purged three times with nitrogen gas (N₂), and then pressurized with N₂ to guarantee that the operation pressure was within the desired range. Initial pressures were determined from preliminary tests prior to each test. The aim was to ensure that the pressure at the test temperature was within the required range (130–170 bar). Pressure increases with increasing temperature. Thus, the higher the test temperature, the lower the initial pressure, since pressure increase as a function of temperature will be higher. However, the increase in pressure as a function of temperature does not occur linearly. In the tests performed it was observed that from 300 °C the pressure increase occurred at higher rates, which justifies the initial pressures presented in Table 1.

At the end of each test, the reactor was cooled in an ice bath until it reached room temperature of around 25 °C for the collection of the products.

The tests were carried out in a completely randomized design (CRD), with two repetitions for each treatment. The analysis of variance was performed from the F-test. The *t*-test at 5% significance level was used to determine the statistical difference of each treatment. For both tests the ASSISTAT version 7.7 beta statistical software was used.

2.4. Separation and determination of HTL product yield

After cooling, the reactor was weighed. Then all of the gas was released, the reactor was weighed again. The gas mass was calculated by subtracting the N₂ mass added before the test. The calculations were performed according to the ideal gas law, using the initial pressures and temperatures (to determine the N₂ mass) and the final ones.

The other products were diluted in 450 mL of dichloromethane (DCM) and 300 mL of distilled water. This mixture was vacuum filtered with Whatman paper filters with porosity of 1.6 μm, for the separation of solid residues. The filters were weighed before filtration and, after the process they were put into the heater at 80 °C for 8 h. After this period, the filters were cooled to room temperature and were weighted for the determination of solid residue yield.

The biphasic-filtered liquid was transferred into a 1 L separation funnel to isolate the aqueous phase of the bio-oil, soluble in DCM.

Afterwards, the DCM and distilled water were evaporated in the BUCHI Waterbath B-480 rotavapor. For evaporation of the DCM, a thermostatic bath at 40 °C and 500 mbar was set up; for the distilled water, the process was at 60 °C and 100 mbar.

Fig. 1 illustrates the separation process.

The yield of the products on a dry weight basis (d.w.%) was calculated separately using Equation (1):

$$Y_{\text{PRODUCTS}} = \frac{m_{\text{Products}}}{m_{\text{biomass}} - m_{\text{moisture}}} \times 100\% \quad (1)$$

where:

- Y_{PRODUCTS}: yield for each of the products (%);
- m_{Products}: mass of each of the products (g);
- m_{biomass}: mass of the biomass used in the test, dry-basis (g);
- m_{moisture}: mass of water in the sample (g).

In addition, products yield may also be calculated on a dry ash-free basis (daf), according to Equation (2). This calculation is especially useful to compare bio-oil yields with data in literature.

$$Y_{\text{BIO-OIL}} = \frac{m_{\text{BIO-OIL}}}{m_{\text{biomass}} - (m_{\text{ash}} + m_{\text{moisture}})} \times 100 \quad (2)$$

where:

- Y_{BIO-OIL}: yield of bio-oil, daf basis (%);
- m_{BIO-OIL}: mass of bio-oil obtained (g);
- m_{ashes}: mass of ashes in the biomass sample (g).

The determination of yield for the four products, even though the gas was obtained indirectly, allowed the determination of percentages losses for each of the assessed conditions. It is important to highlight that the losses occur during laboratory procedures for the separation of the products. This information is important for the interpretation of the results and conclusions regarding the yields obtained under the different assessed conditions.

2.5. Characterization of the HTL products

The water-soluble products were characterized for TKN and total dissolved carbon (TDC). After water evaporation and the determination of their yield, these products were diluted with 100 mL of deionized water. The determination of the TKN was carried out as described for determining the protein in the biomass. The TDC was analyzed using the Shimadzu TOC – 5050 A. For the TDC analysis, the samples were filtered using 0.45 μm membranes beforehand.

The solid residues were characterized in terms of C, H, N and S, using a Perkin Elmer Series II 2400 Elemental Analyzer, as for the biomass characterization. Oxygen content was determined by difference.

The gas produced during the HTL mostly consisted of CO₂. This result is reported in several studies using different species of macro and microalgae [8,23–25]. Therefore, the gas was characterized in an assay to confirm the predominant formation of CO₂. The characterization was performed by the Agilent/HP GC6890 gas chromatograph, which consists of a valve for gas sampling, and two detectors in series, one for flame ionization (FID) and another for thermal conductivity (DCT). The columns used were a Molecular Sieve 5 A for determination of N₂, H₂, CH₄ and CO, which were analyzed by the DCT, and a Porapak Q for determination of hydrocarbons between C₂ and C₅, which were also analyzed through DCT. The CO₂ was analyzed through FID. The mobile phase of both columns was argon. The calibration of the chromatograph was carried out using mixtures of standard gases.

2.6. Energy balance

The HTL energy balance was determined according to two

Table 1
Initial pressures used at each tested temperature.

T of the test (°C)	Pressure at room temperature (bar)
275	50
300	49
325	38
350	7

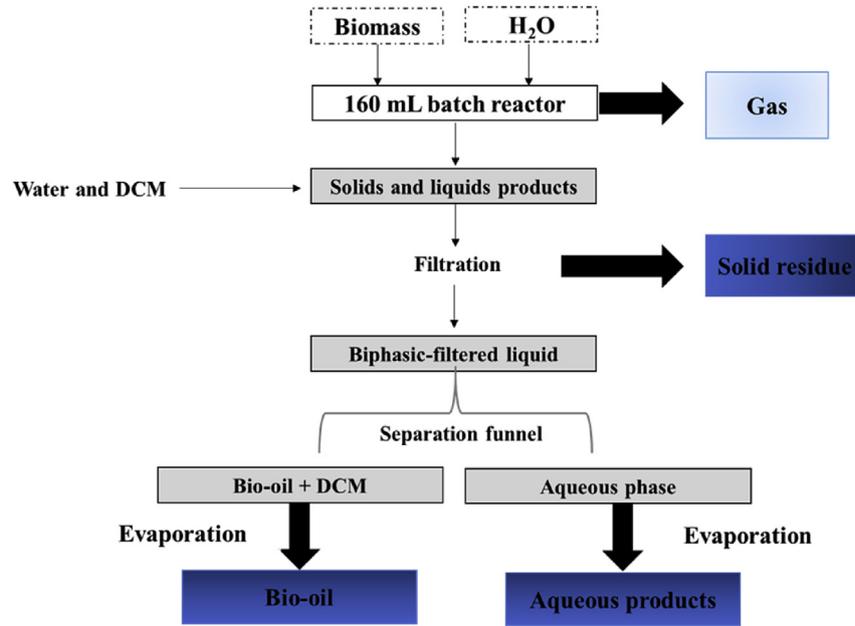


Fig. 1. Processes for separation of the HTL products.

different approaches. The first one is the method of energy recovery (ER), which can be defined as the energy initially present in the biomass and converted into bio-oil, and is obtained from Equation (3).

$$\text{ER bio - oil (\%)} = \frac{\text{HHV}_{\text{bio-oil}} \times m_{\text{bio-oil}}}{\text{HHV}_{\text{biomass}} \times m_{\text{biomass}}} \times 100 \quad (3)$$

where:

HHV_{bio-oil}: higher heating value of the bio-oil (MJ.kg⁻¹);
 HHV_{biomass}: higher heating value of the biomass (MJ.kg⁻¹);
 m_{bio-oil}: bio-oil mass obtained in the test (g);
 m_{biomass}: biomass mass used in the test (g).

The ER was calculated for the test that presented the greatest bio-oil yield. The HHV of the bio-oil was 38.1 MJ kg⁻¹ [8] and that of the biomass was measured using a bomb calorimeter (EN 14918:2009).

The second approach used to determine the energy balance was the energy consumption ratio (ECR), which is defined as the ratio between the energy demand for the HTL and the energy obtained by the combustion of the bio-oil. Thus, the ECR values above 1 indicate a negative energy balance, whereas values below 1 indicate a positive energy balance. This method enables the assessment of the effect of different temperatures and biomass/water ratios during the HTL process. In this study, it was assumed that the energy required for the biomass production process must be attributed to the wastewater treatment in the HRP's [26]. Thus the energy consumption considered was that required for the process of reducing the water content in the biomass. Equation (4) was used to obtain the ECR, and is adapted from other research in the literature [27–29]:

$$\text{ECR} = \frac{E_{\text{collection}} + [w_i C_{pw} (T_f - T_{\text{ini}}) + (1 - w_i) C_{ps} (T_f - T_{\text{ini}})] \cdot [1 - r_h]}{[Y_{\text{bio-oil}} \text{HHV}_{\text{bio-oil}} (1 - w_i)] r_c} \quad (4)$$

where:

W_i is the water content in the reactor, C_{pw} is the specific water heat (4.18 kJ kg⁻¹K⁻¹), C_{ps} is the specific biomass heat (1.25 kJ kg⁻¹K⁻¹ [27]), T_f is the operation temperature in the HTL (K), T_{ini} is the temperature in the beginning of the heating (K), r_h and r_c represent the efficiency of heat recovery and the combustion energy, respectively, both assumed as 0.7, according to Zhou [8]. Y_{bio-oil} is the yield of the bio-oil in the process and HHV_{bio-oil} is the higher heating value of the bio-oil, considered 38.1 × 10³ kJ kg⁻¹ [8].

E_{collection} is the energy consumed in the reduction of the water content at each biomass/water ratio. For the 1/20 ratio gravitational sedimentation can be used, thus, E_{collection} was considered as zero. For the 1/10 and 1/5 ratios, the energy consumption of the centrifugation process was considered: 0.2 MJ kg⁻¹ and 0.8 MJ kg⁻¹ of biomass produced, for 1/10 and 1/5, respectively [26,30].

3. Results and discussion

3.1. Biomass composition

Table 2 presents the composition of the biomass used in the HTL tests.

As can be observed in Table 2, the biomass used had a large percentage of ash. These ashes consist of inorganic matter (including metals) present in biomass (microalgae, bacteria and other microorganisms), but mostly consist of the sand found in the domestic sewage.

The ash content in the biomass produced and collected in effluents is usually between 30 and 50% (d.w.) [29]. Chen [12] characterized the biomass produced from wastewater treatment for use in the HTL and obtained 47.5% of ash. These values are higher than those found in axenic cultivations of algal biomass in culture media

Table 2
Characteristics of the biomass used in the HTL.

Components	Characteristics of the biomass used in the study	
<i>Immediate composition (%)</i>		
Moisture	7.1	
Ash content	40.0	
Volatile matter	47.2	
Fixed carbon ^a	5.7	
<i>Biochemical composition</i>		
	<i>Dry basis (%)</i>	<i>Dry ash-free basis (%)</i>
Lipids	23.3	40.8
Carbohydrates	5.4	9.5
Proteins	28.3	49.7
<i>Ultimate analyses</i>		
C (%)	27.5	51.8
H (%)	5.0	9.5
N (%)	4.2	8.0
S (mg/kg)	0.95	1.8
O ^a (%)	23.2	44.5
<i>Other elemental analyses (%)</i>		
Cl	0.2269	
Si	0.0467	
Na	0.0037	
K	0.0127	
Ca	0.0176	
Mg	0.0065	
P	0.0170	

^a Determined by difference.

other than sewage. Barreiro [24] evaluated *Nannochloropsis gaditana* and *Scenedesmus almeriensis* for the HTL process, and found 12.4% and 20.0% of ashes, respectively. Biller and Ross [28] evaluated the HTL of *Chlorella vulgaris*, *Spirulina*, *Nannochloropsis oculata* and *Porphyridium cruentum*, and found ash contents of 7.0%, 7.6%, 26.4% and 24.4%, respectively. High ash contents, in general, do not contribute to the biomass conversion into bio-oil. However, the way the ashes interact with the other components in the feedstock during the HTL process still needs to be better explained [31].

It was possible to observe that the percentage of carbohydrate in the biomass was relatively low, being only 9.5% (daf). Biller and Ross [28] found concentrations of 9% (daf) for the biomass of *Chlorella vulgaris*, which corroborates the presented result, since this was one of the most abundant species in the used biomass. However, the carbohydrate fraction can be variable, depending on the conditions of cultivation and of the species. Chen [12] found 23.6% (d.w.) of carbohydrates in biomass composed by a consortium of microorganisms cultivated in effluent. Valdez [16] evaluated the algal biomass of *Scenedesmus sp.* and found 31% (d.w.) of carbohydrates.

With respect to lipids, the value shown in Table 2 refers to neutral and polar lipids, since the objective was to study the overall composition of the biomass. Considering only neutral lipids extracted by hexane, the concentration was 12.6% (daf). For comparison, Assemany [32], using an adaptation of the Bligh & Dyer Method with petroleum ether as solvent, found 9.3% of lipids in biomass produced in the same experimental unit. In general, species with high lipid concentrations have lower growth rates than species with reduced lipid concentrations and other microorganisms, such as bacteria [33,34]. Thus, in open pond cultivations with domestic sewage and in association with microorganisms, the neutral lipid concentrations are hardly higher than the values obtained. Chen [12] obtained biomass with only 1.7% (d.w.) of lipids.

The protein concentration was 49.7% (daf), which was the highest percentage in terms of biochemical composition found in the biomass. The high protein content is one of the main differences between algal biomass and other types of terrestrial biomass [4]. Biller and Ross [28] found 55%, 65%, 57% and 43% of protein (daf) in the biochemical composition of *Chlorella vulgaris*, *Spirulina*,

Nannochloropsis oculata and *Porphyridium cruentum*, respectively.

3.2. HTL products yield

Figs. 2–4 present the yields of the products obtained under the different conditions assessed.

For the three operational parameters assessed, the higher fraction of the products was found in the solid residues. One of the main causes of this result is the high ash content in the biomass (40% (d.w.)), which results in the difference between the yields of the bio-oil on a dry basis and on a dry ash-free basis. This behavior was different from other studies [16,24,28], which presented, in general, greater fraction of water-soluble products. There is the theoretical possibility that some minerals could lead to the formation of compounds soluble in the aqueous phase. According to biomass composition the presence of Cl and N could have led to the formation of chlorides and nitrates. Most chlorides and all nitrates are soluble in water. However, according to the high solids yield, it is expected that most mineral matter of initial biomass remained in the solid phase. The partition of mineral matter among the products formed is an interesting and complex issue that is not the core of the present work and that should be studied in a future work.

In this research, the four products were quantified, which enabled us to identify the losses in the separation process. These losses were relatively low, and can be attributed to products that adhere to the reactor and to the laboratory devices used for their separation. Also, at the stage of solvent evaporation, some volatile compounds can be lost [35].

The first experimental parameter assessed was the reaction time, which is the duration of the test from the moment the pre-established temperature was reached. As shown in Fig. 2, the bio-oil yields showed an increasing tendency with the rise of reaction time. Though no great increases were observed when reaction time rose from 30 to 45 min, it should be highlighted that the differences in yields observed among these reaction times were lower than the losses in the tests. The bio-oil yield, dry-basis, did not statistically differ at the 5% significance level, according to the test used. Similar results were obtained in other studies of micro and macroalgae, where reaction times over 15 min did not represent a significant increase in the bio-oil yield. Anastasakis and Ross [35] found that 15 min was sufficient for the formation of bio-oil in the HTL of the macroalgae *Laminaria saccharina*, with a yield of 19.3% (daf) at 350 °C. Valdez [36] studied the HTL of *Nannochloropsis sp.*, and observed higher yields after 10 min of reaction, whereby

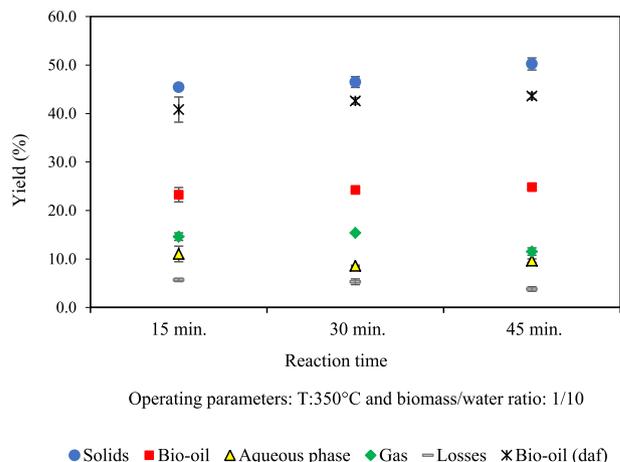


Fig. 2. HTL products yield at different reaction times (vertical bars mean standard deviation).

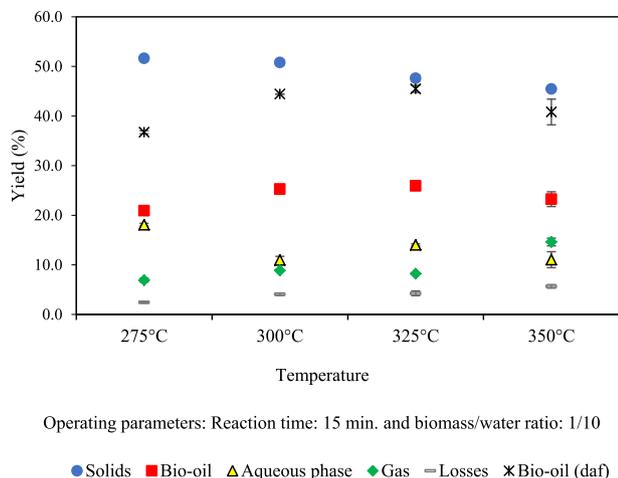


Fig. 3. HTL products yield at different temperatures (vertical bars mean standard deviation).

approximately 40% of bio-oil (d.w.) was obtained at 300 °C. On the other hand, Chen [12] obtained higher bio-oil yields (~40% daf basis) in operations lasting 30 min at 320 °C, also with biomass produced in effluent. Zhou [37] assessed reaction times of 5–60 min for the HTL of the macroalgae *Enteromorpha prolifera* and reported higher yields of bio-oil for reaction times over 30 min at 300 °C. Yu [38] found that extending reaction time only had an apparent effect at temperatures below 220 °C, but above 220 °C, the bio-oil yield did not show a clear relationship with reaction time.

In the beginning of the HTL reactions, the biomass molecules are decomposed into smaller molecules. The reaction time must be sufficient for these compounds to rearrange through condensation and repolymerization to form the bio-oil. However, when reaction times are too long, the bio-oil molecules can continue to react, forming new compounds and reducing bio-oil yield [4]. An important factor related to the reaction time is the total energy input and operation cost of the process. A reduced reaction time, considering that the oil yield is not decreased, is directly related to cost reduction. The results show that, when operating at 350 °C, the bio-oil yield did not increase significantly after 15 min.

Therefore, the reaction time of 15 min was used in the assessment of temperature. The biomass/water ratio was maintained at

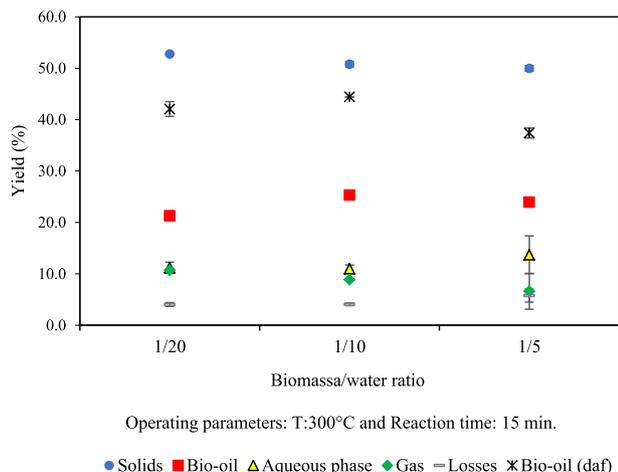


Fig. 4. HTL products yield at different biomass/water ratios (vertical bars mean standard deviation).

the same value as in the previous tests. The bio-oil yield increased with temperature until 325 °C, and then decreased at 350 °C, Fig. 3. However, the values found for 300 °C and 325 °C did not statistically differ at the 5% significance level. Chen [12] observed similar behavior when comparing the results obtained at 300 °C and 320 °C for the HTL of biomass cultivated in effluent. At 320 °C, the yield was equal or below that presented at 300 °C, and the maximum yield observed in the operation at 300 °C and 30 min was approximately 40% (daf). Other studies with distinct biomasses also found a similar baseline yield at temperatures below the maximum assessed. Zhou [37] obtained higher yields at 300 °C with the macroalgae *Enteromorpha prolifera*. Valdez [16] assessed the HTL of *Scenedesmus sp.* and a reaction time of 20 min, and also presented a steady yield after 300 °C. This behavior suggests that the highest temperatures promote the decomposition of bio-oil into other products. As the temperature rises, the bio-oil molecules are degraded, forming more volatile compounds, which in turn, can form gases [2].

The yield of the water-soluble products decreased with the increase in temperature. Valdez [36] suggested that, under such conditions, bio-oil formation from water-soluble products occurs, which is coherent with the results observed in this study. The reduction of the water-soluble products with the increase in temperature is reported in other studies, both for macroalgae and microalgae biomass [12,24,35]. The solid residues also decreased with the increase of temperature, which may explain the increase in bio-oil and in gas yields. The highest increase in gas yield obtained at 350 °C may also be due to the conversion of some bio-oil initially formed into gaseous compounds.

The importance of temperature for the bio-oil yield in the HTL, and also for other thermochemical processes, is related to the reactions that will dominate in a certain range of this parameter. The ionic characteristics of water change with temperature, and thus, a series of reactions are favored. At temperatures up to 220 °C, hydrolysis reactions are dominant; however, after that, repolymerization reactions start to happen with greater frequency, especially after 250 °C, which is the lower range of the HTL [39]. As the critical point is approached, the water has a high ionic product and the H⁺ and OH⁻ dissociated ions can catalyze acid and basic reactions, respectively. Therefore, the organic compounds undergo isomerization, depolymerization and repolymerization, which will eventually produce the bio-oil [4]. In the proximity of the water critical point (218 bar and 374 °C), and mostly above it, decarboxylation, cleavage and gasification reactions are favored, increasing the gas yield [40]. This information supports the justification of the results obtained in this study, or more precisely, the increase in oil yield and the reduction of water-soluble products with the increase in temperature.

The difference between the bio-oil yields at 300 °C and at 325 °C was 1.1%, and, as previously mentioned, did not statistically differ at the 5% significance level. In addition, this difference was lower than the losses obtained in the tests for the evaluation of temperature. Thus, for the assessment of the biomass/water ratio 300 °C and the reaction time of 15 min was selected.

Biomass to water ratios of 1/5, 1/10, and 1/20 (w.w⁻¹) corresponded to biomass concentrations of 16.6%, 9.1%, and 4.8%, respectively; the ratios 1/5 and 1/10 were obtained by centrifugation. In this case, the higher the solids concentration, the greater the energy consumption of the centrifugation process. The bio-oil yields obtained for the ratios 1/5 and 1/10 did not statistically differ at the 5% significance level, Fig. 4. The relationship between the biomass concentration (i.e. the biomass/water ratio) and bio-oil yield is still not evident. Since the water acts as a catalyst and/or as a reagent in the hydrothermal conversion process, high concentrations may, in theory, compromise the yield. On the other hand, the

polymerization reactions of water-soluble intermediate compounds to the compounds that make up the bio-oil is favored at higher concentrations of these intermediate, hence higher biomass concentration yield more bio-oil, potentially. Moreover, the volumetric efficiency and the productivity can be seriously affected if concentrations are too low [13], which may represent a disadvantage in an economic feasibility analysis.

The literature presents a wide range of results. Jena [40] obtained higher bio-oil yields with 20% of biomass in the HTL of *S. platensis*. Valdez [36] found an increase in the bio-oil yield from 36% to 46%, by increasing the biomass concentration of *Chlorella sp.* from 5% to 35%. On the other hand, other studies obtained high yields with reduced biomass concentrations. Neveux [41] studied the HTL of six different macroalgae species and obtained a yield of 39.6% (daf) for *Oedogonium*, using a biomass concentration of 6.6%. Biller and Ross [28] obtained bio-oil yields of approximately 40% for *Chlorella sp.* and *Nannochloropsis sp.*, using 10% of biomass in the mixture feeding the HTL reactor. The divergence in the results may be related to the composition of the biomass used in each of the studies, which results in different behavior during HTL.

The presented results indicate the importance of the method of the biomass separation, mostly regarding the energy balance of the entire process. Although some authors state that biomass concentrations should be around 20% [26], the present study supports the idea that high yields can be obtained from reduced biomass concentrations. The greatest yield obtained was for the biomass concentration of 9.1% (biomass/water ratio of 1/10 (w.w⁻¹)). In this case, the high-energy consumption of the separation process was avoided. The centrifugation, which was mentioned above, consumes nearly 1.0 MJ kg⁻¹ of energy to obtain solids concentration of 15% [26]. Simplified separation processes, such as gravitational sedimentation, or another drying process with lower energy demands (e.g., solar energy) may represent significant advantages. In contrast, Guo [4] stated that in cases of reduced biomass concentrations, the energy required for heating the reactor can be high, and since there is greater water volume, also a greater volume of effluent would be produced in the process. Indeed, the energy consumption is a negative point that must be considered. However, with respect to the effluent production, one of the main advantages of the HTL is that it enables the use of all byproducts, mostly those soluble in water, which usually have high loads of organic carbon and nutrients.

3.3. C and N balance in the HTL products

Figs. 5 and 6 present the C and N distributions in the biomass among the HTL products. Other studies in the literature stated that the gas produced in the HTL is composed almost entirely of CO₂ with other gases in lower concentrations [8,13]. The results of the gas characterization for the tests at 350 °C and 30 min duration showed that it contains 96% of CO₂ and 2% of CH₄, which corroborates this previous information. This result was used in the calculations of the carbon balance of the products.

The distribution of C and N in the HTL products provides important information regarding their quality. For the bio-oil, a high content of C is expected and desired. On the other hand, the goal is to reduce N in order to minimize the formation of NO_x during combustion [35].

The C content in the bio-oil ranged from 43.0% to 62.7% of the C in the biomass. The lowest value was obtained in the 15 min test at 275 °C, whereas the highest one was obtained at 350 °C and 45 min. These values are similar to those obtained by other studies. Zhou [8] obtained a C recovery of 54% in the bio-oil of the HTL of biomass produced in HRP. Chen [12], also studying the HTL of the biomass produced in domestic effluent, obtained a C content in the bio-oil

that ranged between 59.1% and 71.4%, with the highest value being obtained in the most severe operating conditions of 320 °C and 1 h. Anastasakis and Ross [35] found 51% of C in the bio-oil after the HTL of *Laminaria Saccharina* at 350 °C for 15 min. The authors observed that the C content in the bio-oil had the same behavior as its yield, increasing with the increase in temperature and biomass/water ratio. The results obtained in this research show that there was an increase in C recovery of the bio-oil with the increase in the biomass/water ratio and time of reaction. However, for temperature, this increase occurred until 325 °C, and then dropped slightly at 350 °C.

The C recovery in the aqueous phase ranged from 15.0% to 29.6%, with an opposite behavior to the C recovered in the bio-oil. The greatest percentage of recovery in the aqueous phase occurred under the mildest temperature condition (275 °C), whereas the lowest one was obtained at 350 °C and 45 min. The values were similar to those obtained by Zhou [8] for biomass produced in wastewater, which ranged from 15.0% to 24.0%. However, they were lower when compared to other studies using macro and microalgae. Neveux [41] found 40.9% of C in the aqueous phase of the HTL of the marine macroalgae *Ulva ohnoi*. Jazrawi [42] evaluated the HTL of microalgae cultivated in a synthetic medium in open ponds, and found C recoveries in the aqueous phase ranging from 30% to 61%, depending on the biomass concentration in the reactor, with higher values for concentrations of 1% and lower values for concentrations of 10%. The authors attributed this behavior to the possible saturation of the water present in the reactor. It should be highlighted that in this research, the yields of the aqueous phase were lower than those from many studies available in the literature, mostly due to the greater fraction of ash, which increased the solids yield. This may have influenced the low C content in the aqueous phase in this research. Nevertheless, the C recovered in the aqueous phase was significant, and higher than that observed in the solid residue or in the gas. Therefore, there could be an impact on the costs involved in the process, which reinforces the importance of promoting its use.

The C in the solid residue ranged from 8.7% to 17.1%. Similar to the aqueous phase, it presented an opposite trend to that observed for the bio-oil, i.e., it was inversely proportional to the time of reaction, biomass/water ratio, and temperature. Thus, probably the C recovery in the solid residue is related to the bio-oil yield. Operation conditions leading to greater bio-oil yield had most of the organic matter degraded, and consequently, lower recovery of C in the residue. Chen [12] recovered between 30% and 42.3% of C from the solid residue. The authors, assessing the reactions that can occur during HTL, stated that the solid residues can be formed during the decomposition of cyclic oxygenates. However, the results of C percentages obtained in this research do not indicate such an occurrence, due to the relationship with the bio-oil yield previously described.

The N balance shows that, regardless of the operation conditions, its content in the bio-oil was significant. This fact is reported in other research and is the main challenge of the HTL of algae [24,35,36,41]. The high levels of protein in the biomass justify these results. Barreiro [24] evaluated the HTL of algal biomass after protein extraction. In comparison to the process that used biomass in its totality, the authors observed a reduction of 18% in the N of the bio-oil produced from the HTL of *Nannochloropsis gaditana*, and a reduction of 33% when *Scenedesmus almeriensis* was used. However, even with these reductions, the authors considered that protein extraction alone is not sufficient to remedy the presence of N in the bio-oil, as even under these conditions, the N content in the bio-oil was still high. This indicates that the N present in the bio-oil can be originated from other organic molecules besides proteins, which are degraded during the HTL. Barreiro [24] also conclude that

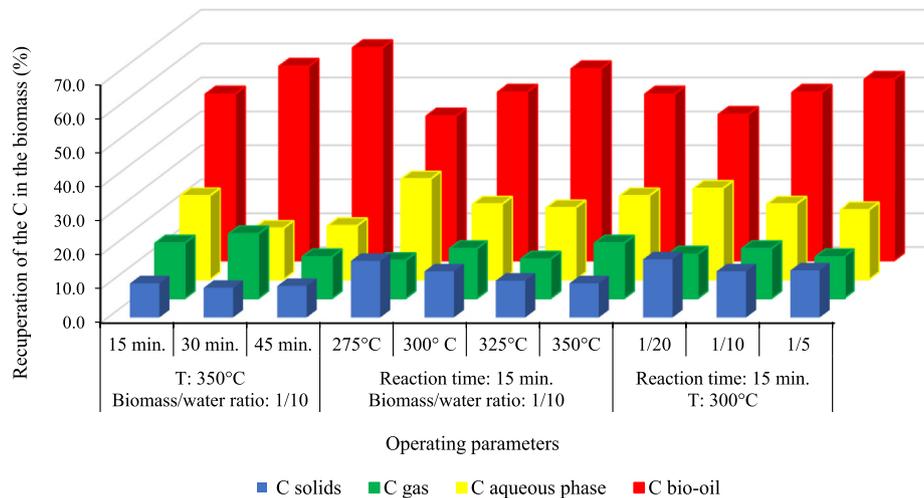


Fig. 5. Balance of C in the biomass among the HTL products.

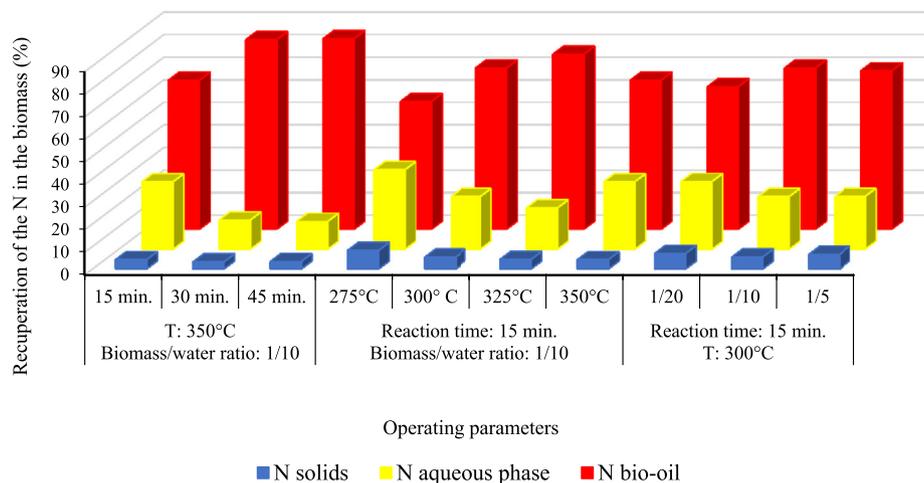


Fig. 6. Balance of N in the biomass among the HTL products.

the protein extraction from the biomass can be considered for the production of high added value products, within the concept of biorefinery, and partially reduce the N content in the bio-oil. On the other hand, an assessment that should be made in this case is whether the loss in mass as a result of the protein extraction will represent an important drop in the organic matter available for energy generation. This is mostly the case for biomass with high protein content, such as that used in this study.

Chen [12] obtained bio-oil with N recovery ranging from 8.4% to 16.8%, from the HTL of biomass produced in wastewater. The authors attributed this result to the high concentration (16.5%) of CaCO_3 in the biomass used in the HTL. Previous research showed that the CaCO_3 could adsorb proteins, and thus precipitate them [43]. The authors reduced the concentration of CaCO_3 in the biomass, from 16.5% to 11.0%, and the N recovery increased to 34.3%. This result corroborates with other researches affirming that the use of catalysts can reduce the N content in bio-oil [8,18,28].

The N recovery in the aqueous phase ranged from 12.3% to 35.2%, and the values were inversely proportional to the reaction time and the biomass/water ratio. For temperature, the values decreased till 325 °C, and then showed a slight increase at 350 °C. The trend of N recovery in the aqueous phase was in the opposite direction to that in the bio-oil yield, as also observed by Anastasakis

and Ross [35]. Conditions that presented greater bio-oil yields had a greater N fraction in the bio-oil, and consequently, less N in the aqueous phase. The values were reduced when compared to those reported by Zhou [8], who found 51% of N recovered in the aqueous phase of the HTL of algal biomass cultivated in open ponds. Neveux [41], when studying six distinct macroalgae species, found N recoveries in the aqueous phase ranging from 41.2% to 65.4%.

Again, the reduced yield in the aqueous phase, obtained in this study should be highlighted. Even under these conditions, it represents an important method to recover nutrients. As opposed to C, the presence of N in the aqueous phase must be increased, instead of in the bio-oil. The results presented here show that the search of means to increase the N recovery in the aqueous phase is as important as studying ways to use the nutrients in this product and a way to get further valorization through other uses such as cultivation of microalgae.

3.4. Energy balance

The ER obtained by Equation (3) was 69.1%. This result indicates that approximately two thirds of the energy available in the biomass was converted into bio-oil. Zhou [8], also evaluating the HTL of biomass produced from effluent treatment, obtained ER of

82%, which can be explained by the higher yield of bio-oil presented in such study, as the heating value was similar to that used in our study. The higher bio-oil yield may have been caused by the greater organic content in the biomass. In their study only 16.0% of ash content was found, whereas the biomass used in our study presented an ash content of 40.0%. Factors that positively influence the bio-oil yield will contribute to a greater ER. Yu [29] obtained an ER of 65.4% for the bio-oil of the HTL of *Chlorella sp.*, when operating at 280 °C and 120 min. The author observed that the rise of temperature up to 300 °C and the longest reaction time favored the increase in ER. Biller and Ross [28] obtained an ER of 66% for the HTL of *Nannochloropsis oculata*, which is slightly higher than that observed for *Chlorella vulgaris*, *Porphyridium cruentum* and *Spirulina*. The authors attributed this result to a greater lipid content of the first species, which may have influenced the bio-oil yield. It is important to highlight that the ER can be optimized by the use of the energetic fraction contained in the other byproducts of the HTL [29], and the results presented in this study did not consider this possibility.

The energy consumption ratio (ECR) is shown in Fig. 7.

The first aspect to be considered is the effect of the biomass drying process, i.e., the reduction of water concentration, in the ECR. The greater the drying efficiency, the greater the difference between the ECR with biomass drying and the ECR without such process. This corroborates the results of other studies that show the high energy demand of processes used to separate biomass [26,44–46]. It should be highlighted that the maximum concentration in this research was 16% of solids (biomass/water ratio of 1/5). Therefore, the energy advantage of the HTL is evident, when compared to processes that require higher levels of biomass drying.

With respect to the different temperatures evaluated, and considering the consumption of the biomass drying process, only at 350 °C there was a negative energy balance. Higher operation temperatures resulted in greater energy consumption of the reactor. However, when compared to other temperatures (300 °C and 325 °C) the yield of the bio-oil at 275 °C was compensatory in relation to this consumption, which resulted in lower ECR values.

The results of the different biomass/water ratios evaluated provide interesting interpretations regarding the intervening factors on the energy balance of the HTL. Without the energy demand of the biomass separation process, the ECR is inversely proportional to the solids concentration. This is explained by the lower water content in the operation conducted with a 1/5 biomass/water ratio.

The heating of the amount of water inside the reactor significantly influences the energy demand of the process. For the results considering only the HTL process, the operation conducted with more water inside the reactor (biomass/water ratio of 1/20) presents a negative energy balance. This result is consistent with that observed by Yu [29], who discovered that moisture over 93% leads to an ECR higher than 1. However, when the energy demand for separation was included, the ECR of the operation with 1/5 ratio was also higher than 1. The energy consumption required to concentrate the biomass in 16% of solids was enough to cause the energy balance to be negative. The relationship between energy demand and the water content in the reactor is mentioned in numerous studies. Hognon [47] stated that, during the process, the HTL can consume up to three-times more energy than pyrolysis, due to the water content. Guo [4] mentioned the importance of assessing the water content in the reactor. According to the authors, the lack of water can impair the HTL reactions, but, on the other hand, the excess of water can result in high energy costs, making the process unfeasible. The 1/10 ratio resulted in a positive energy balance, even when considering the energy consumed in the reduction of the water content. This ratio implies a solids concentration of 9%. The evaluation of processes with lower energy demand to reach such a concentration can contribute to improving the energy balance of the entire process. The drying of the water content using solar energy, for instance, can represent a potential option that should be evaluated [46].

4. Conclusion

The HTL technique represents a possibility for the energy conversion of biomass produced from domestic sewage treatment. Bio-oil yields of up to 44% were obtained. The increase of the reaction time above 15 min did not provide significant yield gains, and temperatures higher than 300 °C favored the bio-oil formation. The biomass/water ratio presented the greatest bio-oil yield at 1/10. The byproducts presented characteristics that offer the potential for use within the cycle of biomass production and energy generation, mostly those soluble in water, with significant amounts of N and organic C. The N recovery in the bio-oil was high, which constitutes the main challenge for the optimization of the process. The use of biomass directly after its production in the HRP can lead to an excessive consumption of energy due to the high water content. However, the need for drying is reduced when compared to other

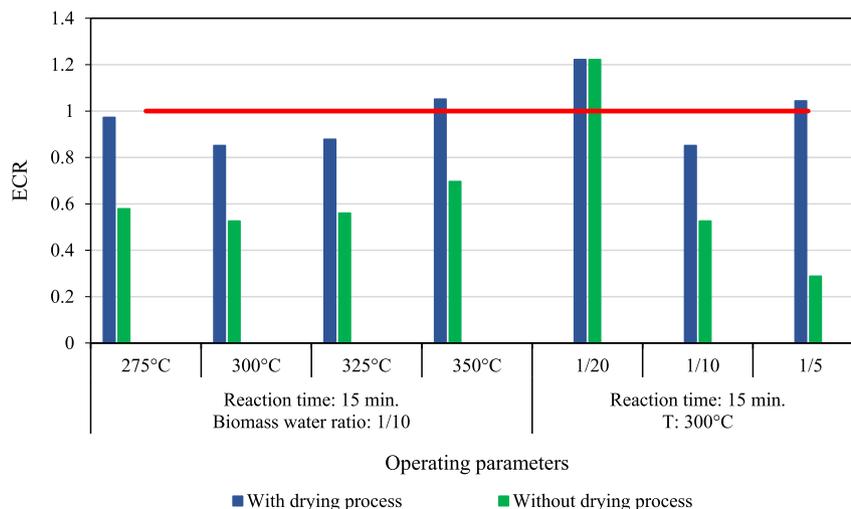


Fig. 7. Energy consumption ratio for the conditions assessed in the HTL.

energy production processes, which guarantees a positive energy balance under experimental conditions with higher bio-oil yields in the HTL.

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