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A Mini Review of the Potentials of *Chlorella* Species Microalge for the Production of Biocrude by Hydrothermal Liquefaction (HTL) Operation

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ABSTRACT

The operation of hydrothermal liquefaction (HTL) of biomass is an enticing technique for the production of biocrude, which can serve as a viable alternative energy source to the growing universal energy demand and the pollution problem caused by fossil fuels. This article reviewed research publications that describe the elemental composition of Chlorella species biomass, biochemical analysis of the feedstock, the effects of the conditions of operating the Chlorella species biomass conversion, hydrothermal liquefaction mechanisms, separation technique, and the products of hydrothermal liquefaction operation. Nevertheless, the modification of the chemical and biochemical compositions of Chlorella specie biomass to yield higher-quality biocrude was not researched. Also, other biocrude separation techniques, such as mechanical separation, were not researched after the hydrothermal liquefaction of the Chlorella specie biomass. This information can help in making decisions on the route where research should be directed, towards in-depth study, improvement of yield of the biocrude more than other products, and quality of the derived biocrude by the operation of hydrothermal liquefaction of Chlorella species biomass.
Keywords: Biocrude; *Chlorella* specie; hydrothermal liquefaction; microalgae; separation technique

1.0 INTRODUCTION

About 88% of energy around the world that is utilized by humans is obtained from fossil fuels (Guo et al., 2015), but fossil fuels are non-renewable, produce greenhouse gases during combustion, and pollute the environment (Song et al., 2015). The utilization of microalgae globally for consumption and for microalgae products to be used as food supplements, dietary supplements, medicine, cosmetic industries, functional foods, and biofuels has greatly increased its production (Tang &Suter; Bitoet al., 2020). The rapid urban development and industrialization in the past decades have resulted in the degradation of water quality, which has become a threat to public health (Gollakota et al., 2018), hence the need for alternative sources of fuel that are renewable (Tareket et al., 2022). Chlorella species are among the most studied microalgae, and they are widely cultivated for biotechnological applications (Aigneret et al., 2020). Because of the properties of rapid growth rate, high oil content, high productivity of biomass, and renewability associated with Chlorella species, they are being considered as possible feedstock for the production of biocrude in comparison to other terrestrial biomass (Eboibi, 2019). The genus Chlorella is a single-cell microalgae, and the matured cells of Chlorella species are spherical and sometimes oval (Aigneret al., 2020), which contain a huge number of various functional compounds such as 12–18% lipid (Phusuntiet al., 2017), 50–60% protein, and 15–20% carbohydrate, zinc, growth hormones, calcium, potassium, vitamin E, B_1, B_2, C, B_6, magnesium, folic acid, free biotin, and chlorophyll (Tianet al., 2022). Chlorella microalgae can be found virtually in all geographic locations (Aignere et al., 2020). Presently, Chlorella species are made up of three varieties, which are Chlorella lobophora, Chlorella vulgaris, and Chlorella sorokiniana (Bito et al., 2020). Chlorella sorokiniana is a smaller species that was first isolated in 1953 by Sorokin but was originally thought to be a thermo-tolerant Chlorella pyrenoidosa, which is strange. Over 100 strains of Chlorella species have been described, with more than 20 characterized (Lizzulet et al., 2018). Chlorella pyrenoidosa, referred to as the subject of many scientific research studies, is now called Chlorella sorokiniana (Bito et al., 2018). Chlorella strains are reported to possess the ability to adapt to different environmental conditions and several carbon forms because they can be cultivated heterotrophically, autotrophically, mixotrophically, and photoheterotrophically (Piasecka & Baier, 2022).

Hence, renewable energy, where biomass is used as a source of energy, has attracted widespread attention (Hao et al., 2021). The B_{12} content of Chlorella species products reported in the literature varies greatly from <0.1 to 400 g/100 g dry weight. Among all chlorella species grown in an open culture, the B_{12} content is much higher in Chlorella pyrenoidosa than in Chlorella vulgaris. The B_{12} is not essential for Chlorella species growth (Bito et al., 2020). Biomass refers to organic substances like animals, plants, and microorganisms, as well as materials derived from the metabolism and excretion of these organisms, with the exception of fossil fuels and their derivation (Rabacalet et al., 2017). Microalgae biomass has a higher calorific value and a lower density and viscosity than biomass obtained from plants. It accounts for the world's fourth-most consumed energy source after crude oil, natural gas, and coal (Cao et al., 2017). The organic composition of the biomass of Chlorella specie can be converted, extracted, and processed into fuels by chemical and physical techniques (Hao et al., 2021). This conversion can be carried out by hydrothermal operation, but there are several techniques that are used for the conversion of microalgae biomass into liquid fuels; these can be either biochemical or thermochemical techniques (Kumar et al., 2018). The types of these techniques are presented in Figure 1. Thermochemical methods include hydrothermal carbonization (HTC), hydrothermal gasification (HTG), and hydrothermal liquefaction (HTL), depending on the ranges of pressure and temperature at which the reaction is carried out (Duo et al., 2019). Hydrothermal operation involves the biomass undergoing different thermochemical pathways at different temperature and pressure conditions to form solid fuel, liquid fuel, and gaseous fuel in hot compressed water (Hao et al., 2021).

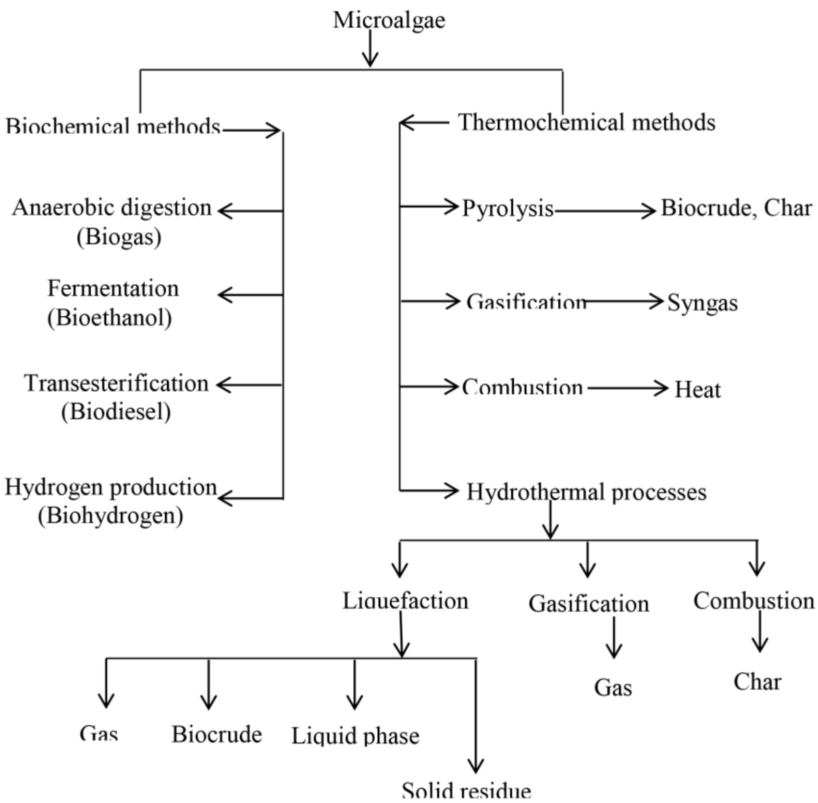

Figure 1 Flow chart of the biochemical and thermochemical methods

HTL produces biocrude, gases, solid residue phase, and aqueous phase in the presence of compatible catalyst and solvent at about 200–400 °C temperature (Xue *et al., 2016*; Hao et *al., 2021), 0–28* MPa pressure (Hu *et al.,* 2019), and 5–60 minutes of residence time (Eboibi, 2019). The process of converting biomass to bio-crude involves the hydrolysis operation, degradation process, decarboxylation process, deamination, repolymerization, and other possible reactions (Hao et *al.,* 2021). The desired liquid product of HTL, which is biocrude, has high carbon and energy values that are similar to those of conventional petroleum (Eboibi, 2019), but biocrude has a high heteroatom content, a large acid value, a low higher heating value (HHV), and a high viscosity (Hao et *al.,* 2021).

Zhang *et al. (2013) worked* on biocrude production from the biomass of *Chlorella pyrenoidosa* by hydrothermal liquefaction using ethanol and water as solvents. The operation was carried out at 280 °C for about 60 minutes and 0.69 MPa. They reported a bio-crude yield of about 41.7% with an HHV of 31.1 MJ/kg when water was used as a solvent, but when ethanol was used alone as a solvent, the bio-crude yield increased to 51.1% with an HHV of 31.7 MJ/kg. Hague *et al. (2022)* reported the atmospheric effect and modified plasma on the HTL of *chlorella* using DCM as a solvent. The HTL operation was carried out at about 240–250 °C with a residence time of 0–15 minutes using SiO2/Al2O3 as a catalyst. They carried out the HTL operation at different conditions, giving an average total yield of biocrude of 49.83%. He *et al. (2018)* worked on the production of biocrude from the biomass of *Chlorella* by hydrothermal liquefaction before upgrading it catalytically. They used a 415-ml reactor, 53.208g of *Chlorella* microalgae, 212.8 ml of deionized water, DCM as a solvent, and Co Ni Mo W/γ.Al_2O_3 as a catalyst. The reactor was operated at a temperature of about 340 °C, a pressure of about 22 MPa, and a residence time of about 140 minutes. The total bio-crude yield was obtained as 28.2 wt%, and its higher heating value (HHV) was obtained as 31.9 MJ/kg. Jin *et al. (2017) carried* out the utilization of optimum biochemical components in *Chlorella* specie KR1 for biocrude production by hydrothermal liquefaction. The researchers used 95 ml of distilled water and 0.5g of lyophilized chlorella species KRI with DCM as solvent in a 42 ml high-pressure reaction vessel operated at 150 °C under autogenous pressure condition

They obtained a maximum biocrude yield of about and a higher heating value (HHV) of 27.62–33.16 MJ/kg. Nwanya *et al. (2021) worked* on the potential of the biofuel they produced from *Chlorella vulgaris.* They used 200g of dried *Chlorella* vulgaris and 300 ml of n-hexane in a Soxhlet extraction unit. The extraction was carried out for about six hours before recovering the oil in a rotary evaporator. They obtained a 37% yield of the biofuel, 45.125 MJ/kg as the calorific value, -7.667 °C as the pour point, and 56.67 °C as the cetane number. Chakraborty *et al. (2013) carried* out the isolation of α-glucan, a co-product of the derived biocrude, through the hydrothermal liquefaction operation of the *Chlorellasorokiniana* species with ethanol or water as solvents to extract lipid and polysaccharide from the microalgae. They performed sequential hydrothermal liquefaction at an optimum temperature of about 160 °C for the ethanol-insoluble polysaccharide and 200 °C for the water solvent, with a retention time of 10 g of alginate and the solvent (1:9). Rinanti and Purwadi (2019) researched the increasing yield of carbohydrates and lipids in *Chlorella vulgaris* ($CH_{0.0052}N_{0.0013}O_{0.01472}P_{0.0589}$) biomass, used for the production of biofuel. In their work, they used various concentrations of CO_2 ranging from 0.03% to 50% in the culture, and they obtained a carbon content of 0.57 g carbon⁄g biomass of *Chlorella* specie AG10002. They observed that the internal cellular energy storage of oil instead of carbohydrates actually slows down the reproduction rate of the microalgae. They used a photobioreactor for the microalgae cultivation, and the harvesting was carried out using the bioflucculation technique at room temperature, using microalgae as flocculants. The Fourier transform infrared (FTIR) patterns of the biomass of *Chlorella sorokiniana* were recorded to define the variety of functional groups involved in the microalgae. The waveband at approximately 3276.33 cm^{-1} corresponds to O-H and N-H stretching vibrations in *Chlorella sorokiniana* biomass. The peaks obtained at 1636.14 cm^{-1} and 1537.94 cm^{-1} were attributed to amino group N-H stretching, while the C-OH stretching was assigned the band at 1031.52 cm^{-1} (Embaby et *al.,* 2022). All these researchers used solvent for the extraction of the biocrude after the hydrothermal liquefaction operation, but none of these researchers used the mechanical method (gravity separation technique) for the recovery of the biocrude without the use of solvent. Also, none of them studied how the yield and quality of the biocrude can be enhanced by modifying the composition of the biomass. Among these researchers,Zhang *et al.* (2013) obtained the highest biocrude yield at lower operating temperatures and pressures.

2.0 Elemental compositions of Chlorella species biomass

Generally, biomass consists of elements such as carbon (C), hydrogen (H), nitrogen (N), sulfur (S), and oxygen. The higher heating value of biomass can then be obtained with different models using the elemental compositions. These elements are explained below, and their percentage weight present in different *Chlorella* species is presented in Table 1 (Gollakota et *al.,* 2018; Gita *et al.,* 2019). The elemental composition of *Chlorella* species presented in Table 1 shows that there are similarities concerning carbon content (always above 40 wt%), hydrogen content (always above 5 wt%), and oxygen content (always above 20 wt%), with the exception of the work done by Rizzo *et al. (2013)*, but there is some variance for the nitrogen content, especially for *Chlorella sorokiniana*, carried out by Chenet et *al. (2014).* In general, the elemental composition of the *Chlorella* species biomass reported falls within the standard range for all microalgae (see Table 1).

2.1 Carbon content of *Chlorella* specie biomass

This is the most significant constituent of the *Chlorella* species biomass. Carbon obtained from the CO_2 in the atmosphere is taken in by the plant and becomes part of its composition during photosynthesis (Gollakota et *al.,* 2018). Carbon is the major contributor to the heating value of *Chlorella* specie biomass, and during combustion, the carbon is converted into CO2, which is released and can be channeled back to the cultivation system, where it becomes part of the photosynthetic process. Majorly, the content of carbon in the different biomasses is estimated through the compositions of hemiscellulose, lignin, and cellulose. Biomass rich in lignin has a carbon content that is higher than 50 wt% (Gollakota et *al., 2018).* Different sources of carbon and their concentrations influence the structural composition of microalgal cellular lipids. When the CO_2 concentration is high, it promotes the accumulation of saturated fatty acids, but at low concentrations of CO2, unsaturated fatty acid accumulation is induced (Jaiswal*et al.,* 2020). Jabeenet et *al.* (2019) carried out thermogravimetric analysis on *Chlorella* algae using a Netzsch STA 449 F3 instrument, and they obtained a carbon composition of 52.1 wt%. Chang *et al. (2014) worked* on the characterization of *Chlorella* biomass activated carbon and reported that the carbon present is about 50.5 wt%. Zhenget et *al. (2019)* reported that the carbon/nitrogen ratio of *Chlorella vulgaris* cultivated in manure-free piggery wastewater had a significant effect on cell viability. They added that cell viability was improved when glycerol in the carbon/nitrogen ratios of 5:1, 25:1, and 125:1 were added compared with the control carbon/nitrogen ratio of 17:20. Their results show that the cell viability of the *Chlorella* species is enhanced by balancing the carbon/nitrogen ratios. Some microalgae species have the ability to use organic carbon sources instead of CO2. *Chlorella* species heterotrophic growth can synthesize up to 45 wt% more carbohydrates and about 280 wt% more lipids compared to Chlorella specie autotrophic growth (Jaiswal*et al.,* 2020).

Jazrawi*et al. (2015) worked* on a double-staged hydrothermal liquefaction operation of a good protein-containing chlorella species microalga. They obtained the carbon content of the biomass as 53.5 wt%. Gao et *al. (2021) worked* on the cultivation of mixotrophic and anaerobic hydrolysis for the treatment of *Chlorella* species wastewater in an anaerobic membrane photobioreactor and membrane photobioreactor. They obtained carbon contents of 0.38 and 0.47 g/g dry biomass for the anaerobic membrane photobioreactor and membrane photobioreactor, respectively. All these researchers at times determined the composition of carbon in the different *Chlorella* species biomass and also talked about the importance of carbon in the biomass and in the derived biocrude, but they did not research on how the carbon composition can be modified genetically for a higher heating value of the *Chlorella species* biomass and that of the derived biocrude.

2.2 Hydrogen content of *Chlorella* species biomass

This is another significant constituent of the *Chlorella* species biomass as an organic substance, as can be observed in carbohydrates, phenolic polymers, etc. During the combustion process of the *Chlorella* species biomass, the hydrogen component present is converted to water (H2O), and this contributes greatly to the overall higher heating value (HHV) of the *Chlorella* species biomass. The content of hydrogen is normally lower in herbaceous biomass, that is, 5.5–6%, than in woody biomass, which is about 6–8% (Gollakota *et al.,* 2018). Microalgae biomass has been regarded as an important feedstock for hydrogen production because of its high growth rate and good protein and carbohydrate contents (El-Dalatony*et al.,* 2017). The use of hydrogen in the past decades has gained attention increasingly because of its high specific energy (142 MJ/kg) and its environmental friendliness. Jabeenet et *al.* (2019) carried out thermogravimetric analysis on *Chlorella* algae using a Netzsch STA 449 F3 instrument, and they obtained a hydrogen composition of 6.5 wt%. Giang*et al. (2019) reported* that hydrogen production from *Chlorella species* biomass increased significantly from 1188 ml/l to 3055 ml/l when the biomass concentration was increased from 10 to 20 g/l. When the concentration of the biomass was further increased to 50 g/l, there was no significant improvement in hydrogen production, as it fluctuated within a narrow range of 2992 to 3180 ml/l. Jazrawi*et al. (2015), who* worked on a double hydrothermal liquefaction of a good protein-containing chlorella species microalga, obtained the hydrogen content of the biomass as 7.4 wt%. The works of these researchers show that the significance of hydrogen composition in *Chlorella* species biomass cannot be overemphasized, both in the production of hydrogen and in increasing the HHV of *Chlorella* species biomass and the derived biocrude. Nevertheless, these researchers did not consider how the composition of hydrogen can be further enhanced genetically in the *Chlorella* species biomass.

2.3 Nitrogen content of *Chlorella* species biomass

Nitrogen constitutes a vital nutrient that is obtained from the *Chlorella* species biomass. The amount of nitrogen element present in the biomass contributes greatly to the process of degradation in fermentation or digestion, but during combustion of the biomass, nitrogen does not oxidize and therefore does not contribute to the overall heating values (Gollakota et *al.,* 2018). It is applied in compound form to the soil as fertilizer to enhance plant growth and its overall yield. The nitrogen element plays a significant role in the production of lipids and during cell growth. The total dry weight of the biomass of microalgae is composed of about 1–10% of nitrogen.The production of lipid in *Chlorella* species biomass increases under the condition of starvation of nitrogen element, which in turn reduces the total biomass of the *Chlorella species,* which reduces the overall yield of lipid (Ru*et al.,* 2020). Jabeenet et *al.* (2019) carried out thermogravimetric analysis on *Chlorella* microalgae using a Netzsch STA 449 F3 instrument, and they obtained a nitrogen composition of 9.95 wt%. Jazrawi*et al. (2015) worked* on a double-staged hydrothermal liquefaction operation of a good protein-containing chlorella species microalga. They obtained the nitrogen content of the biomass as 11.0 wt%. Gao et *al. (2021) worked* on mixotrophic cultivation and anaerobic hydrolysis for the treatment of *Chlorella* specie wasterwater in an anaerobic membrane photobioreactor. They obtained biomass nitrogen content of 0.140 and 0.092 g (dry biomass) for the anaerobic membrane photobioreactor and membrane photobioreactor, respectively. These different researchers, having reported that the nitrogen starvation condition increases the lipid production of microalgae while reducing the total biomass production, which will eventually affect the overall lipid yield, did not consider how the biomass production of Chlorella *microalgae* can be increased under the nitrogen starvation condition.

2.4 Sulfur content of *Chlorella* specie biomass

Sulfur is another significant nutrient similar to nitrogen in the structure of amino acids, enzymes, and proteins for improved plant growth. The sulfur content of the biomass of herbaceous crops is higher than that of woody biomass, thereby contributing to their high growth rate. The sulfur content of wood can be below the detection limit (0 wt%) and can be up to 1 wt% in exceptional cases, while it can be as high as 0.2 wt% or higher in herbaceous biomass but is most significant in gaseous emissions, corrosion, and syngas cleaning in gasification processes (Gollakota et *al.,* 2018). Jabeenet et *al.* (2019) carried out thermogravimetric analysis on *Chlorella* species of microalgae using a Netzsch STA 449 F3 instrument, and they obtained a sulfur composition of 0.55 wt%. Jazrawi*et al. (2015) worked* on a double-staged hydrothermal liquefaction of a good protein containing *Chlorella* specie microalga. They obtained the sulfur content of the biomass as 0.5 wt%. Since it was reported by these researchers that the presence of sulfur in microalgae enhances the growth rate, they did not work on how the sulfur content of the *Chlorella* species can be further enhanced for a higher growth rate and possible ways that the oxide formation of sulfur can be reduced when it occurs. Ferreira & Soares-Dias (2020) studied the pyrolysis of *Chlorella* vulgaris over carbonate catalysts and obtained the sulfur content of the microalgae biomass as 0.7 wt%.

2.5 Oxygen content of *Chlorella* specie biomass

When considering the biomass chemical composition of *Chlorella species, the* oxygen element is very significant. The amount of oxygen present in the biomass influences the heating value. The oxygen content in phenolic compounds is difficult to break so as to enhance the heating values. The measurement of the oxygen content is not done directly; it is estimated by subtracting the concentrations of carbon (C), hydrogen (H), nitrogen (N), sulfur (S), and ash from 100, whether wet or dry biomass (Gollakota et *al.,* 2018). Jazrawi*et al. (2015) worked* on a double-staged hydrothermal liquefaction operation of a *Chlorella* species microalga containing a good amount of protein. They obtained the oxygen content of the biomass as 27.6 wt%. Jabeenet et *al.* (2019) carried out thermogravimetric analysis on *Chlorella* algae using a Netzsch STA 449 F3 instrument, and they obtained an oxygen composition of 30.9 wt%. Khoo*et al. (2020) reported* that the amount of oxygen decreases when the temperature of the hydrothermal liquefaction is elevated from 180 to 250 °C, with the raw microbial biomass having an oxygen content of 59.7 wt%. Ferreira &Soares-Dias (2020) researched the pyrolysis of *Chlorella* vulgaris over carbonate catalysts and obtained the oxygen content of the *Chlorella vulgaris* biomass as 35.1 wt%. Zhang *et al.* (2019) researched the property variation of microalgal biomass, its comparison, and its characterization, and they obtained the *Chlorella* specie biomass oxygen content as 31.4 wt%.

Table 1. Elemental composition of Chlorella species

Species	Carbon (wt%)	Hydrogen (wt%)	Nitrogen (wt%)	Surphur (wt%)	Oxygen (wt%)	HHV (MJ/kg)	Citation
Microalgae	35-57	6 – 9	2 - 12	~1.5	24-35	-	Gollakota et al., (2018)
Chlorella	52.31	6.99	10.14	0.86	29.70	-	Jin et al., (2017)
Chlorella KR1	53.62	8.10	2.59	0.61	35.08	24.61	Jin et al.,(2017)
C. Pyrenoidosa	51.4	6.6	11.1	-	30.9	-	Zhang et al., (2013)
C. Pyrenoidosa	51.0	6.6	11.0	-	31.4	-	Gai et al., (2015)
Chlorella	40.31	5.99	9.14	0.76	27.74	17.31	Xu et al., (2019)
C. Pyernoidosa	50.99	7.83	9.48	1.08	30.62	-	Peng et al., (2016)
C. Sorokiniana	50.42	7.89	2.91	0.23	37.44	21.63	Mao et al., (2012)
C. Vulgaris	47.7	7.5	8.4	0.50	35.9	22.03	Guo et al., (2019)
C. Vulgaris	48.5	7.0	8.5	0.2	35.0	20.2	Yang et al., 2018)
Chlorella sp.	46.1	6.1	6.7	0.4	19.1		Babich et al., (2011)
Chlorella sp.	50.2	7.25	9.30	nr	33.2	nr	Babich et al., (2011)
C. vulgaris	52.6	7.1	8.2	0.5	32.2	nr	Biller & Ross, (2011)
C. vulgaris	45.8	7.9	7.5	nr	38.7	nr	Chakinala et al., (2009)
C. vulgaris	42.51	6.77	6.64	nr	27.95	nr	Wang et al., (2013)
C. vulgaris	48.3	7.3	3.0	nr	32.9	nr	Figueira et al., (2015)
C. vulgaris	50.39	6.01	14.77	6.05	22.78	nr	Raheem et al., (2015)
C. vulgaris	50.0	7.1	5.8	0.53	33.8	nr	Peng et al., (2017)]
C. vulgaris	44.8	6.8	7.0	1.0	40.4	nr	Lopez-Gonzalez et al., (2014)
C. vulgaris ESP-31	53.01	8.67	3.26	nr	35.05	nr	Bach et al., (2017)
C. pyrenoidosa	51.2	6.8	11.3	0.7	30.7	nr	Gai et al., (2015)
C. sorokiniana CY1	40.32	7.38	2.61	nr	44.50	nr	Chen et al., (2014)
Chlorella	51.34	7.29	9.35	nr	31.45	23.09	Tarek et al., (2022)

3.0 Biochemical analysis of the Chlorella species biomass

The biochemical analysis of the *Chlorella species* biomass includes the determination of carbohydrate content, lipid content, moisture content, ash content, protein content, and other valuable bioactive compounds such as carotenoids (Safafar*et al.,* 2016). These components are presented in Table 2. Zhang *et al. (2014) determined* the biochemical composition of *Chlorella pyrenoidosa* by Fourier transform infrared (FTIR) spectroscopy. They washed the cell pellets with deionized water twice and then suspended the cell pellets in fresh deionized water again at a concentration of approximately mg/mL (dry weight). A total of 200 µl of suspension was dropped on a KRS-5 window (30 x 5 mm) and dried at 40 °C in the vacuum oven. The transmittance of the spectra was determined between 400 and 4000 cm^{-1} at a resolution of 4 cm^{-1} with 32 scans on the equipment. Rani & Ojha (2021), in their research on *Chlorella sorokiniana,* obtained biomass yields of about 126 and 158 mg⁄(l.d.) in synthetic wastewater and 319 and 548 mg⁄(l.d.) in real tertiary wastewater under 12:12 h and 24:0 h light and dark regimes, respectively. Sakarika and Kornaros (2017) researched the lipid accumulation and kinetics of the growth of the cells of chlorella *vulgaris* and obtained a biomass yield of about 2.69 g/l. Huang *et al. (2016),* in their research work, achieved a stable biomass productivity of 103 g/m^2 after cultivation of *Chlorella vulgaris* in an attached system, which is about 30.4% higher than that cultivated when the system is suspended. Gao et *al. (2021) worked* on mixotrophic cultivation and anaerobic hydrolysis for the treatment of *Chlorella* specie wastewater in an anaerobic membrane and membrane photobioreactor. They obtained biomass production rates of 32.55 and 91.10 for the anaerobic membrane and membrane photobioreactor, respectively.

3.1 Lipid content of *Chlorella* species

Lipids constitute 5 to 40% of the *Chlorella* species biomass, which is mainly glycolipids, phospholipids,waxes, hydrocarbons, and fatty acids, which, when produced in the chloroplasts, are directed to the cell walls and cell membranes of the several organelles, as well as the chloroplasts themselves and the mitochondria. When *Chlorella* specie is cultivated in an unfavorable environment, the production of lipid may be up to about 58% of the total composition of the *Chlorella microalgae* (Mobinet et *al.,* 2019). Lipids form the main component that transforms into biocrude after the hydrothermal liquefaction operation of microalgae. Figure 2 shows the possible lipid chemical reaction pathways in the hydrothermal liquefaction operation. Lipids may be hydrolyzed by a non-catalytic method to quickly produce higher fatty acids and glycerols under hydrothermal liquefaction operations. Some of these higher fatty acids transform into hydrocarbons with long chains through the process of decarboxylation, which then transform into alkanes through hydrogenation. Hao et *al.* (2021) Some of the higher fatty acids transform into alcohols through the process of deoxygenation; the alcohols are further esterified with fatty acids to produce esters of fatty acids. Also, some of the higher fatty acids may react with the ammonia that is produced in the conversion process of amino acids to produce fatty acid amides. All these components make up the derived biocrude from biomass hydrothermal liquefaction operations (Kumar *et al.,* 2016). The lipids of microalgae usually take the form of triacylglycerols (TAGs), which mostly have an aliphatic character (Bareiro *et al.,* 2013). They are mostly non-polar, and they are made up of a glycerol backbone that is bonded to three fatty acids (Barreiro *et al.,* 2013; Gollakota et *al.,* 2018). Some of the major compositions of *Chlorella* species lipids are palmitic, oleic, and linolenic acids. The composition and content of the lipids of microalgae vary with different species, temperature, geographical location, season, salinity, light intensity, or a combination of these factors. Mobinet et *al.* (2019). *Chlorella vulgaris* and *Chlorella emersonii can* have more than 50% weight lipid content (Rani *et al., 2018).* At room temperature, the fats are generally not soluble in solvents, and as temperature changes, they tend to be polar. When TAGs are hydrolyzed, glycerol is one of the products obtained, and this glycerol can be processed to produce acetaldehyde, methanol, propionaldehyde, acrolein, ethanol, allyl alcohol, and formaldehyde, as well as a gas mixture (CO, CO_2 and H_2), when subjected to hydrothermal liquefaction (Gollakota et *al.,* 2018). Within the profiling of lipids that has been carried out on *Chlorella* species, a higher concentration of fatty acids with 16 and 18 carbon atoms has been found (Table 2), and the specific heat capacity of some of the fatty acids is presented in Table 3.

It can be observed from Table 4 that most of the fatty acids present have carbon atoms and are not short-chain. These *Chlorella* species fatty acids with carbon atoms are favorable for health when consumed. It has been identified that 70.18% by weight of the *Chlorella* species fatty acids corresponds to saturated fatty acids (SFA), 16.85% by weight corresponds to monounsaturated fatty acids (MUFA), and about 8.72% by weight corresponds to polyunsaturated fatty acids (PUFA), while account of the balance was not reported, therefore fatty acids give a wide range in their lipid profile [69]. Zhang *et al.* (2014) researched the effects of carbon from organic sources on the biochemical composition and growth of *Chlorella pyrenoidosa*. In their report, the addition of 3.0 g⁄l galactose and glucose greatly reduced the content of lipid by 27.9 and 27.5%, respectively, while the addition of 5.0 g⁄l and 0.5–1.0 g⁄l galactose and glucose and 0.5–5.0 g⁄l fructose, starch, and disaccharide did not affect the lipid content significantly. They further reported that the effect glucose has on the lipid content of *Chlorella* species might be dependent on the specific strain and also dependent on the concentration of glucose.

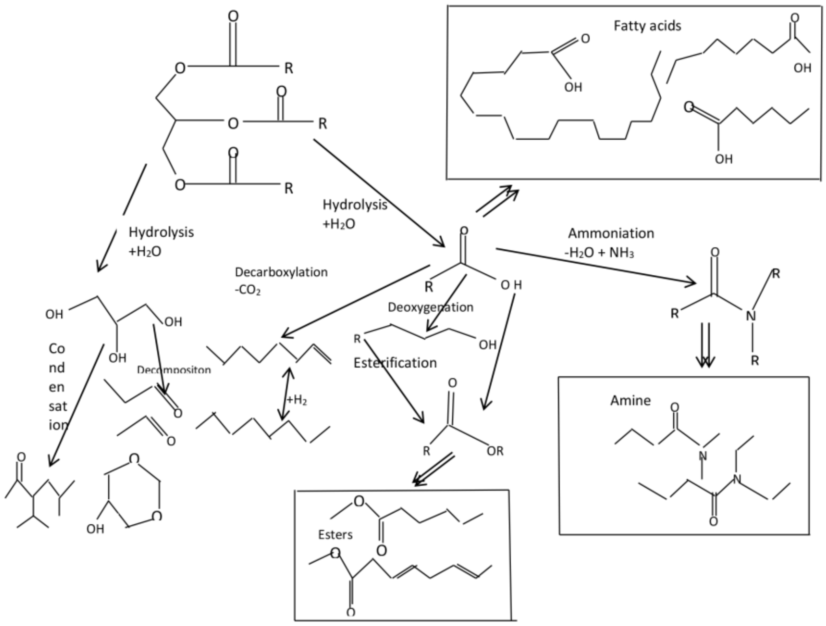

Figure 2: Lipids chemical reaction pathways in HTL

Table 2 Biochemical composition of *Chlorella* species (Safafar et al., 2016)

Species	Starting concentration	Nitrogen (mmol⁄l)	Biomass Conc. (g⁄l)	Lipid content (%dry weight)
Chlorella vulgaris	33.6 mM KNO_3	33.6	11	15
Chlorella vulgaris	1.5 g⁄l $NaNO_3$	17.6	nr	5.9
Chlorella sp.	0.025 g⁄l urea	0.8	0.464	66.1
Chlorella vulgaris	5 mM KNO_3	5.0	0.4	15.9
Chlorella vulgaris	0.0003% KNO_3	0.03	0.017	57.9
Chlorella vulgaris	10 mg⁄l nitrate	0.2	0.72	20
Chlorella vulgaris	70.02 mg⁄l nitrogen	5.0	0.86	30
Chlorella vulgaris	40 mg⁄l nitrate	0.6	0.48	60

Table 3 Specific heat capacity of triacylglycerols (Zhu et al., 2018)

Triacylglycerols	Specific heat capacity kJ/kg
Tristearin	1.938
Triolein	1.886
Trilinolein	1.813
Trilinolenin	1.765

Table 4 Fatty acids composition in *Chlorella* species (Coronado-Reyes et al., 2022)

Fatty acids	Carbon number	Percentage weight
Palmitic cid methyl ester	C_{16}:0	17.5
Palmitoleic acid methyl ester	C_{16}:1	3.27
Stearic acid methyl ester	C_{18}:0	15.39
Oleic acid methyl ester	C_{18}:1	13.87
Linolelaidic acid methyl ester	C_{18}:2n6	9.18
Lignoceric acid methyl ester	C_{24}:0	22.11

3.2 Protein content of *Chlorella* species

This is one of the significant constituents of microalgae biomass, and it is made up of many chains of peptides that grade into polymers of amino acids (Gollakota et *al.,* 2018). High protein content is one of the nutritional characteristics of these microalgae species, i.e., about 60%, which also includes most of the essential amino acids. This amount of protein in *Chlorella* species is three times more than what is available in beef, which is considered to be one of the most concentrated sources of protein (Rani *et al.,* 2018). The yield of amino acids when subjected to HTL is low because they undergo decarboxylation, which produces carbonic acids and amines; they also undergo deamination to form organic acids and ammonia (Hao et *al.,* 2021). These products from the reactions may then be repolymerized to form aromatic ring structures such as nitrogen heterocyclics (pyrole or indole), phenols, and long-chain hydrocarbons. Proteins are the major source of nitrogen in the derived biocrude (Barreiro *et al.,* 2013). Since the protein of microalgae has a low molecular weight, once the cells of the *Chlorella* species have been disrupted, the protein can be easily digested, but peptides extracted from *Chlorella species* have significant protection against cell damage (Rani *et al.,* 2018). The total protein composition of *Chlorella vulgaris* is about 42-48% dry weight in the biomass produced. Of this percentage, about 20% adhere to the cell wall and serve as structural and transport roles for the cell. About 50% of the proteins are intracellular proteins, which function mainly as enzymes, while the remaining 30% develop the microalgae after being secreted into the extracellular medium. The composition of the amino acids of the Chlorella specie that has been identified contains isoleucine, histidine, leucine, lysine, threonine, methionine, valine, and phenylalanine (Coronada-Reyes *et al.,* 2022). The chemical reaction pathways involve the breakdown of the amide bonds (Figure 3) in the peptide chain before it combines with water molecules, which will generate amino acids (Hao et *al.,* 2021). Some of the amino acids formed (-COOH) decarboxylate to carbon dioxide (CO_2) and amine compounds. Part of the amine compounds produced tends to form water-soluble compounds containing nitrogen, and they are partitioned in the aqueous phase of the hydrothermal liquefaction. These processes improve the quality of the biocrude produced effectively by removing the oxygen and nitrogen contents present in it. Furthermore, some of the compounds of amine are also converted into compounds that are soluble in water, such as pyrazine and pyrrole in biocrude (Zhang *et al.,* 2017). The total protein content present in the *Chlorella* species microalgae can be estimated by the standard Lowry method. In this method, bovine serum albumin (BSA) can be used as a standard. A spectrophotometer is then used to measure the absorbance of the sample using a maximum wavelength of 750nm. The protein concentration present in the chlorella species biomass can be calculated by using the equation below:

$$\text{Total protein }\%(\text{w/w}) = \frac{\text{CVD}}{\text{X}} \text{ x } 100 \qquad (1)$$

Furthermore, some of the compounds of amine are also converted into compounds that are soluble in water, such as pyrazine and pyrrole in biocrude (Zhang *et al.,* 2017).

The total protein content present in the *Chlorella* species microalgae can be estimated by the standard Lowry method. I

Where *Cre represents* the concentration of protein in the Chlorella species biomass, *V represents* the volume of lysis buffer added to the *Chlorella* species biomass that has been freeze-dried for suspension, d represents the dilution factor, and X represents the amount of biomass (mg) (Rani & OJha, 2021). El-Sheekh*et al. (2020) worked* on the *Chlorella vulgaris and* obtained the estimated protein content as 39.85 wt% of dry mass, which lies in the range of 33–46% when the *Chlorella vulgaris* was cultured under different light regimes. They also obtained the protein content as 23.7 wt% by mass when cultured in Bold basal medium (BBM), but under nitrogen limitation conditions. In this method, bovine serum albumin (BSA) can be used as a standard. A spectrophotometer is then used to measure the absorbance of the sample using a maximum wavelength of 750nm. The protein concentration present in the chlorella species biomass can be calculated by using the equation below:

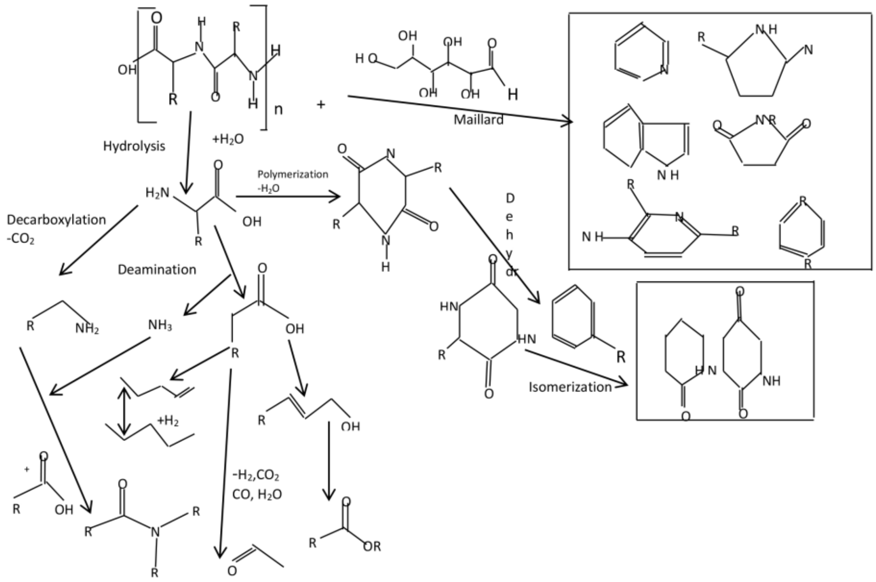

Figure 3: Protein chemical reaction pathways in hydrothermal liquefaction

3.3 Carbohydrate content of *Chlorella* specie

Chlorella specie contains carbohydrates in different forms, such as starch, cellulose, and some reducing sugars. The starch is found in the chloroplasts of the *Chlorella* species, consisting mainly of amylose and amylopectin. Cellulose is part of the cell wall of *Chlorella* species whose glycosidic bond is β1-3; consequently, this can be used as input for the formation of functional products that contribute to human health (Coronada-Reyes *et al.,* 2022). Carbohydrates are mostly decomposed into materials that are polar and water-soluble, rather than non-polar hydrocarbons that eventually dissolve in the aqueous phase of the hydrothermal liquefaction process of the biomass (see figure 4). Consequently, a small amount of carbohydrates converts into biocrude during the process (Hao et *al.,* 2021). The total content of carbohydrate present in the *Chlorella* microalgae biomass can be evaluated by the method of phenol-sulfuric acid. In this method, glucose can be used as a standard for phenol-sulfuric acid. This is followed by the use of a spectrophotometer to measure the absorbance of the sample using a maximum wavelength of 490nm. The carbohydrate concentration present in the *Chlorella* species biomass can be calculated by using the equation below:

$$\text{Total (\%) carbohydrate content} = \left(\frac{X}{0.1}\right) x\ 100\ mg \text{ of glucose} \quad (2)$$

X (mg of glucose) is the absorbance at 0.1 ml of the sample test (Rani &Ojha, 2021).

The characterization and identification of the sugars present in *Chlorella* species is best carried out with high-performance liquid chromatography (HPLC), and with this equipment, the composition of the *Chlorella* species cell walls has been identified to result from a mixture of rhamnose, xylose,glucose, arabinose, galactose, and mannose, where rhamnose is the most abundant (Coronada-Reyes *et al.,* 2022).

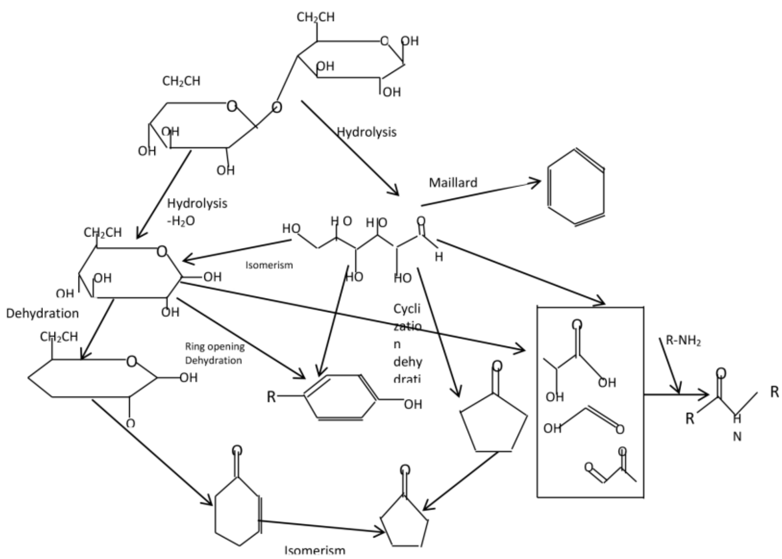

Figure 4: Carbohydrates chemical reactions pathways in hydrothermal liquefaction

Table 5: Biochemical analysis of *Chlorella species*

Species	Ash (wt%)	Moisture (wt%)	Proteins (wt%)	Lipids (wt%)	Carbohydrate (wt%)	Citation
Chlorella	6.5	5.7	60	2.8	25	He et al., (2018)
Chlorella KR1	5.13	-	22.17	-	36.12	Jin et al., (2017)
Chlorella Pyrenoidosa	5.6	-	71.3	-	22.0	Zhang et al., (2013)
Chlorella Pyrenoidosa	5.6	6.3	71	-	22	Gai et al., (2015)
Chlorella	-	-	60.1	2.9	20.2	Xu et al., (2019)
Chlorella Pyernoidosa	5.81	-	70.65	-	21.75	Prng et al., (2016)
Chlorella Sorokiniana	-	-	16.8	24.7	-	Mao et al., (2012)
Chlorella Vulgaris	6.8	3.6	51.9	23.6	9.2	Guo et al., (2019)
Chlorella Vulgaris	3.0	-	54.0	18.7	24.3	Yang et al., (2018)
Chlorella sorokiniana	nr	nr	40.50	22–24	26.8	Hossain et al., (2019)
Chlorella sp. FC2IITG	nr	nr	22–41	15–54	18–46	Sajjadi et al., (2018)
Chlorella vulgaris	nr	nr	51–58	14–22	12–17	Rinanta & Purwadi, (2019)
C. sorokiniana	5.4	8.5	51.7	22.7	15.5	Niccolai et al., (2019)
C. vulgaris Allma	9.3	4.9	56.8	16.9	5.9	Niccolai et al., (2019)
Chlorella vulgaris	nr	nr	nr	9.23	5.7	Rinana & Purwadi, (2019)
Chlorella vulgaris	9.50	5.83	51.45	12.18	11.86	Coronada-Reyes et al., (2022)

4.0 Effects of operating conditions on the HTL process of *Chlorella* specie biomass

Commercialization and industrialization of biocrude production by hydrothermal liquefaction require a reduction in the production of gaseous phase, aqueous phase, and solid residue to obtain high yields and high-quality biocrude. Consequently, because of the difference in reaction parameters, there is considerable variation in the percentage yield of the biocrude obtained from biomass and the quality of the biocrude (Xue et *al.,* 2016). Therefore, the effects of biomass/water ration, holding time, effect of biomass feedstock, effects of reaction temperature on the operation, heating rate, effect of operating pressure, effect of catalyst on the biomass, and effect of atmosphere (Tianet et *al., 2014)*

4.1 Temperature effect on the HTL operation

One of the deciding parameters in the hydrothermal liquefaction operation of chlorella species microalgae is temperature (Xue et *al., 2016).* At subcritical temperature conditions, an increase in the reaction temperature will increase the yield of biocrude by fracturing the chemical bonds of the biomass (Kumar *et al.,* 2016). A typical subcritical temperature at which hydrothermal liquefaction is performed is in the range of 200–400 °C. Xue *et al.,* 2016). The role of temperature in hydrothermal liquefaction is defined by the competitive reaction between hydrolysis and depolymerization. At temperatures below 220 °C, the dominant reaction is hydrolysis, but the highest yield of biocrude is normally observed in the 250–375 °C temperature range, while at temperatures >375 °C, gasification becomes active, which promotes gaseous product formation (Hu *et al.,* 2019). Duanet et *al.* (2013) presented in their report that the maximum yield of biocrude was obtained from Chlorella pyrenoidosa at 350 °C; this corroborates the report by He *et al. (2018)* for biocrude production from *Chlorella species* at 340 °C. Zhang *et al. (2013) obtained the* maximum yield of biocrude at 280 °C for *Chlorella pyrenoidosa*, which is close to the report by Hague *et al. (*2022), which reported the optimum yield of biocrude from *Chlorella* specie to have occurred in the temperature range of 240–250 – 250℃.Therefore, the reaction temperature of biocrude production by hydrothermal liquefaction operations of microalgae varies largely among feedstock species.

4.2 Effect of residence time on HTL

This can be defined as the duration at which the hydrothermal liquefaction operation will be maintained at a designated temperature, outside the heating and cooling times. Higher temperatures require a shorter residence time. Generally, the residence time for the hydrothermal liquefaction operation is (Barreiro *et al.,* 2013). Several studies have been carried out on the residence time effect on the hydrothermal liquefaction of *Chlorella* species of microalgae for the production of biocrude. Duanet et *al. (2013) worked* on the residence time effect on the conversion of *Chlorella pyrenoidosa* to biocrude at 350 °C and obtained maximum yield after 70 minutes. Zhang *et al. (2013) obtained the* maximum yield of biocrude from *Chlorellapyrenoidosa* after 60 minutes at 280 °C. Miao *et al. (2012) reported the* effect of reaction conditions on biocrude production and polysaccharide production from *Chlorella sorokiniana* by the operation of hydrothermal liquefaction. They obtained the maximum yield of biocrude at 240 °C after 20 minutes.

4.3 Effect of heating rate on HTL

This is the ratio of the operating temperature to the time taken for the operation. The effect of the rate of heating the microalgae is not mentioned in the literature, as only a few studies have been done on it; therefore, its effect on the yield of biocrude is still unclear (Barreiro *et al.,* 2013). Studies on *Chlorella vulgaris* by Biller *et al. (2011)* show that increasing the rate of heating from 10 to 25 °C/min slightly decreases the percentage yield of biocrude. Zhang *et al. (2013) reported* that when the rate of heating increases from 5 to 140 °C/min, the yield of biocrude increases by 30% for the conversion of grassland perennials to biocrude by HTL. Consequently, there is a need for further studies on the heating rate of microalgae biomass conversion to biocrude by hydrothermal liquefaction.

4.4 Effect of *Chlorella* biomass/water ratio on HTL

The presence of water in the operation of hydrothermal liquefaction of *Chlorella species* biomass promotes pyrolysis and dehydration, which actually enhance the breakdown of intermediates and subsequently repolymerize the small molecules (Xue et *al., 2016).* Water also helps in stabilizing free radicals while improving the quality of the biocrude. In most investigations carried out, the ratio of biomass to water used varies. Eboibi (2018) used about 1:5 for biocrude production by hydrothermal liquefaction of Tetraselmis specie; Hague *et al. (2022) used* 1:5 for biocrude production from *Chlorella* by HTL; Kumar *et al. (2018) used* 1:6, 1:7; 1:8; 1:9; and 1:10 for the hydrothermal liquefaction of microalgae and obtained maximum biocrude yield at a ratio of 1:9. However, excess water concentration decreases biocrude yield because, when the water concentration is in excess, it easily splits the macromolecules of the algae into small gas molecules.

4.5 Effect of Catalyst on HTL of *Chlorella* species

Catalysts are significant substances in chemical reactions. Undoubtedly, they are very important in the rate of conversion of *Chlorella* specie biomass to bio-crude and in the composition and quality of the bio-crude obtained by hydrothermal liquefaction (Tianet et *al.,* 2014). In hydrothermal liquefaction (HTL) operations, the catalysts used can either be heterogeneous or homogenous catalysts (Guo et *al.,* 2015). Heterogeneous catalysts such as Pd, Pt, or Ru that are supported on C, Co Mo, Ni, Pt, Ni/SiO_2 supported on Al_2O_3, and zeolite present many setbacks like sintering, dissolution, poisoning, and intraparticle diffusion limitations (Barreiro *et al.,* 2013). Homogenous catalysts in the form of acids such as phosphoric, acetic, sulfuric, hydrochloric, and perchloric acids are the most commonly used acid catalysts for biomass hydrothermal liquefaction. Weak acids like formic and acetic acids are usually preferred as solvents rather than catalysts in HTL, but they lead to the production of biocrude with a high content of oxygen (Xue*et al.,* 2016), while the corrosive nature of strong acids hinders their applications in the industry. Homogenous catalysts in the form of alkali salts like KOH and Na_2CO_3,have ae positive effect onmicroalgale

Table 6 Catalyst used for the production of biocrude by HTL

Catalyst used	microalgae species	microalgae HHV (MJ/kg)	Biocrude yield (wt%)	Reference
Na_2CO_3	Botryococcus branunii	34.2	64	Ross et al., (2010)
Na_2CO_3	Chlorella vulgaris	24.6	20	Biller et al., (2011)
Pt/Al_2O_3	Chlorella vulgaris	22.2	34	Eboibi, (2018)
Na_2CO_3	Chlorella vulgaris	22.2	35	Biller & Ross, (2011)
Pd/C	Chlorella sorokiniana	21.7	30	Mao et al., (2012)
–	Chlorella pyrenoidosa	21.3	39	Yu et al., (2011)

4.6 Effect of pressure on HTL of *Chlorella* species

Another significant factor that affects the production yield of biocrude from the biomass of *Chlorella* species is pressure. Pressure helps to keep water in a single phase during hydrothermal liquefaction under supercritical or subcritical conditions, thereby avoiding the enthalpy required for phase change in water (Xue et *al.,* 2016). The acid-catalyzed effect is increased as the water density increases, which in turn increases the release of more hydrogen ions (H^+) from compressed water at high temperatures (Guo et *al.,* 2015). A common way of doing this is by using a vacuum pump to evacuate the air from the reactor and substituting it with nitrogen,making it oxygen-free (Tianet et *al.,* 2014).

4.7 Effects of the physical state and load of *Chlorella* microalgae

The initial state of *Chlorella species microalgae* at the point of loading affects the yield of biocrude production by hydrothermal liquefaction. The microalgae physical state can be in dried pulverized form (Nwanya et *al.,* 2021; He *et al.,* 2018), dried freeze pulverized cells mixed with water (Biller *et al.,* 2011), or the state of the microalgae cells as received after harvest (Mao *et al.,* 2012; Gai et *al.,* 2015). When the pulverized sample is used, the rate of extracting constituents may be changed, therefore affecting the total yield of the biocrude and its composition, but the reported data of the HTL experiment of microalgae as received from harvest is more meaningful for the total yield and quality of the biocrude (Barreiro *et al.,* 2013).

5.0 Hydrothermal liquefaction mechanism

The operation of hydrothermal liquefaction is carried out at a subcritical temperature of about 200–400 °C and a pressure of about in the presence of water to produce biocrude, biogas, aqueous phase, and solid residue as represented in Fig. 5, where bio-crude is the desired product and others as by-products (Hao et *al.,* 2021). During the operation, the biomass decomposes and depolymerizes into monomers or smaller units in the subcritical water through a catalytic or non-catalytic path to produce small molecules that rearrange through cyclization, condensation, and polymerization to produce biocrude (Mathimani & Mallick, 2019). The hydrothermal liquefaction pathway is made up of three major steps: the de-polymerization process, which occurs first, followed by the decomposition process, and the recombination process (Gollakota et *al.,* 2018). The selectivity of these hydrothermal liquefaction mechanisms can differ depending on the pH of the medium, the severity of the HTL operating conditions (temperature, pressure, ramping, and retention time), the type of solvent used for the extraction and its concentration, and the type and nature of the catalyst used (Basar*et al.,* 2021). Hague et *al.* (2022) researched the plasma modification effect on the hydrothermal liquefaction of *Chlorella* species using a catalyst. The *Chlorella* species they used was purchased from Stakich incorporated, while the catalyst (zeolite) with a surface area of 780 m^2/g, was purchased from Alfa Aesar, and they used ethanol and dichloromethane as solvents. They carried out the hydrothermal liquefaction operation in a batch reactor fitted with a stirrer and an electric heater. They introduced 4.5g of the *Chlorella* species into the reactor, followed by the addition of 22.5 ml of deionized water and 17.75 ml of ethanol, respectively. 0.225g of the catalyst was added, and the sample tube was corked for the hydrothermal liquefaction (HTL) operation. After reaching 240 or 250 °C, it was allowed to remain for about 15 min. They carried out the extraction of biocrude from the final product with the addition of 25 ml of dichloromethane (DCM) to extract organic components from the liquid and solid products. They kept the two phases, which are immiscible (organic solvent-soluble and water-soluble), in a separating funnel overnight. The DCM was evaporated at 45 °C for 20 min, and the ethanol was evaporated at 78 °C for 40 min. They obtained a total biocrude yield at different conditions with an average of about 50.0 wt% with a dark color and high viscosity. A major drawback of the HTL is the high operating temperature and pressure, which result in high capital costs for the operation. The authors reported that biocrude can be produced economically by adding alcohol to the HTL water to reduce the high temperature and pressure and modifying a suitable heterogeneous catalyst. Nevertheless, the researchers did not report whether any other solvent can be used as a substitute for the high efficiency of biocrude production at a low cost.

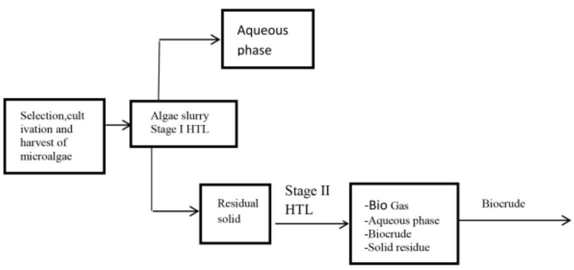

Figure 5: Diagrammatic representation of the entire process

5.1 Biomass depolymerization

Depolymerization is the first stage in which the bonds of the biocrude are broken by the substitution of molecules based on their chemical and physical properties, and the long-chain hydrocarbons produce the short-chain hydrocarbons (Mathimani & Mallick, 2019). Depolymerization solves the problems and inexorable properties of biomass from lignocellulose that imitate the natural process of fossil fuel formation (Gollakota et *al.,* 2019). In these depolymerization or hydrolysis reactions, lipids are decomposed into glycerol and fatty acids; the proteins present are broken down to produce amino acids, while carbohydrates are broken down to produce monosaccharides (Huang *et al.,* 2016). At this stage of hydrothermal liquefaction, no appreciable formation of biocrude takes place. If the factors affecting the HTL operation are adjusted in such a way that only the depolymerization step is affected, the effluent obtained can be further used in the fermentation process to produce fuels such as methane, hydrogen, and ethanol (Basar*et al.,* 2021). Depending on the characteristics of the substrate, the temperature range required for depolymerization at this level of HTL varies between 150 °C and 250 °C (Tianet et *al.,* 2014). The depolymerization operation in the hydrothermal process is a widely researched technology in the pretreatment section of the hydrothermal field (Fan *et al.,* 2016). This technology is primarily used to enhance the lignocellulosic hydrolysis of biomass or increase the biodegradability of municipal sludge (Cantero et *al.,* 2015). Most substrates used in hydrothermal liquefaction, such as herbaceous plants and woody plants, cyanobacteria, microalgae, macroalgae, and manure, all contain various ratios of lignocellulose (Basar*et al.,* 2021). Depolymerization of the biomass of lignocellulosic produces different pentoses and hexoses together with monolignols. The monosaccharides produced from the depolymerization of *Chlorella species* biomass by hydrothermal means can contribute to the formation of biocrude by reacting with amino acids (Terrell *et al.,* 2020).

5.2 Deposition of *Chlorella* species biomass monomers

This step of deposition of *Chlorella* specie biomass monomers involves the loss of carbon dioxide molecules by decarboxylation, dehydration (which is the loss of water molecules), and withdrawal of the content of amino acids by deamination (Jena *et al.,* 2015). The decarboxylation and dehydration processes aid in the oxygen withdrawal in the form of depolymerization of *Chlorella species* biomass and water, respectively, from the *Chlorella species* biomass, thereby reducing the oxygen content (Kumar *et al.,* 2018). The *Chlorella* specie biomass hydrogen bond is broken at the subcritical temperature and pressure of the water, forming polar monomers (Mathimani & Mallick, 2019). Smaller molecules of *Chlorella species* biomass that are hydrolyzed at high temperatures go through a series of thermal breakdowns such as decarboxylation, dehydration, decarbonylation, deamination, dehydrogenation, and some cleavages of the bonds. This decomposition reaction of various compound groups in HTL takes place in the 180–340 °C range (Hom-Diaz *et al.,* 2015). In the operation of hydrothermal liquefaction, the first group of compounds to start decomposition is carbohydrates at about 180 °C. When the temperature increases above 200°C, proteins and lipids begin to breakdown, and their decomposition will be total at about 300 and 640 °C, respectively (Chen *et al.,* 2018). Total breakdown of lipids is not required in the hydrothermal liquefaction operation since some lipids, that is, fatty acids with long chains, are directly included in biocrude formation. The balance of the decomposition reactions is directly related to the efficiency of the HTL. The strength of the operating conditions of the hydrothermal liquefaction should be sufficiently high to aid the organic compounds in breaking down into biocrude-forming compounds. Also, it should not be too high to avoid decomposing biocrude into gases. In the application of the treatment of wastewater, decomposition reactions in the hydrothermal operation allow for the treatment of most pharmaceutical materials and the conversion of microplastics into biocrude (Dimitriadis & Bezeraianni, 2017). Carbohydrates, which are the first group to degrade in the hydrothermal operation, have different decomposition pathways that end with volatile basic alcohols, short-chain fatty acids, furan compounds, ketones, furanic alcohols, and furanic acids. With the exception of large furanic molecules, the breakdown of carbohydrates does not really contribute significantly to the formation of biocrude. However, the products from the decomposition of carbohydrates can form bigger molecules during recombination reactions. The second group that decomposes mostly in hydrothermal liquefaction operations is proteins. This decomposition pathway consists primarily of amino acid deamination and decarboxylation reactions (Basaret et *al.,* 2021). The deamination reaction of amino acids is the primary source that produces ammonia in the aqueous phase of hydrothermal liquefaction. Consequently, protein-rich substrates in the hydrothermal liquefaction operation aqueous phase can have up to about 16 g⁄lammonia with pH levels over 8 (Changi*et al., 2015).* The end products of the decomposition of proteins are mostly simple amines, amides, fatty acids with short chains, aldehydes, and N-heterocyclic compounds (Watson *et al.,* 2020). N-heterocyclic molecules of protein decomposition are the only significant direct contributor to the formation of biocrude (Basar*et al.,* 2021).

5.3 Recombination of the reactive fragment of *Chlorella* specie biomass

The recombination reaction is the last step in the hydrothermal liquefaction pathway. In this step, the fragments of the *Chlorella* specie are repolymerized due to the absence of hydrogen compounds, and the large molecules formed in this recombination reaction contribute to the formation of biocrude at a temperature above 300 °C (Kumar *et al.,* 2018; Basar*et al.,* 2021). Most of the organic molecular groups present in the biocrude, which are formed in the recombination process of long-chain fatty acids, are: amides, amines, aromatics, ketones, and esters. Also present in the biocrude are complex alcohol molecules, which come from the cyclization and hydration reactions of alkenes (Denielet et *al.,* 2015). At this subcritical condition, the absence of hydrogen compounds is excessively large, and then the reactive fragments are repolymerized to produce char with a high molecular weight, which is referred to as the formation of coke (Gollakota et *al.,* 2018). In the HTL operation, the Maillard type of reaction is one of the most significant recombination pathways for biocrude production. In this Maillard reaction, reducing sugars' carbonyl groups react with the free amino groups present in amino acids, which form nitrogenous polymers and melanoidins, which contribute to the production of biocrude (Qiu et *al.,* 2019). Research carried out on model compounds revealed that if the Maillard reaction did not take place in a hydrothermal liquefaction operation, the biocrude yield from proteins and carbohydrates would be in the range of 7–18 wt%, while a biocrude yield of about 40 wt% was reported when the Maillard reaction occurred with the same carbohydrates and proteins in the hydrothermal liquefaction operation (Fan *et al.,* 2018). Recombination of the small fragments also includes some of the biocrude compounds that form coke and eventually contribute to the formation of hydrochar (Basaret et *al.,* 2021). But this coke formation can be reduced with the introduction of high-pressurized hydrogen gas during the recombination step (Barreiro *et al.,* 2013). At this point, when the hydrothermal liquefaction conditions become severe, the biocrude begins to crack again, resulting in a decrease in process efficiency (Basaret et *al.,* 2021).

5.4 Products of HTL from *Chlorella* species biomass

The end products of the hydrothermal liquefaction (HTL) of *Chlorella* specie are the biogas, the aqueous phase, the biocrudefraction, and the solid residue. The total mass of these products is properly accounted for by carrying out material balance on them, together with the feedstock. These end products need to be separated in such a way that biocrude organic liquid, which is the desired product, will be obtained without much negative effect on its quality. The biocrude has an energy content of about 30 to 40 MJ/kg (Eboibi, 2019). The solvent extraction method is widely used for the separation operation, using a separating funnel. Dichloromethane (DCM), acetone, and chloroform are some widely used organic solvents for the extraction of biocrude from the aqueous phase and solid residue (Shanmugamet et *al.,* 2020). Hargue*et al.* (2022) found out that the yield of biocrude increased up to about 43.76% when treated with zeolite as a catalyst at about 240 °C, but the weight percentages of the gas phase, aqueous phase, and solid residue were not reported.

5.4.1 Gaseous product of the HTL of *Chlorella* specie biomass

A gaseous product is a co-product of hydrothermal liquefaction, which is usually obtained first, immediately after the system cools to room temperature. The gaseous fraction represents about 10% of the feedstock that was originally fed into the system (Hu *et al.,* 2019). It is primarily made up of CO_2 and a small amount of C_2H_4, C_2H_6, CH4, and H_2 (Tianet et *al.,* 2014). Depending on the hydrothermal liquefaction conditions, part of the nitrogen in the feedstock can be converted to ammonia, and both the ammonia and CO_2 produced can be recycled into algae cultivation medium (Tianet et *al.,* 2014; Wang *et al.,* 2018). Gai*et al. (2015) presented* in their report that hydrothermal liquefaction of *chlorellapyrenoidosa* at varying temperatures shows that the gas yield increased continuously as temperature increased but did not report the percentages of gas yield at the various temperatures. Zhang *et al. (2013)* reported that the gas phase of the HTL of *Chlorella pyrenoidosa* increases from to, respectively.

5.4.2 Aqueous phase of the HTL of *Chlorella* specie biomass

In the operation of all three types of hydrothermal processes, which are liquefaction, gasification, and carbonization, aqueous products are produced because they all involve the use of water (Tianet et *al.,* 2014). The composition of the aqueous phase produced from *Chlorella microalgae by* hydrothermal liquefaction includes PO_4^{3-}, NH_4^+, CH_3COO^- and some minerals such as K^+, Na+ and Mg^{2+}. Therefore, its recirculation pplays asignificant role in the *Chlorella*microalgae cultivation (Hu *et al.,* 2019). Zhang *et al.* (2014) did not report the percentage yield of aqueous phase produced from*Chlorella pyrenoidosa*hydrothermal liquefaction operation, butGai*et al.* (2015) presented that the percentage yield of the aqueous layer increases with increaseintemperature, from 200 – 320 ℃when *Chlorellapyrenoidosa*was subjected to hydrothermal liquefaction.

5.4.3. Biocrude phase of the HTL of *Chlorella* specie biomass

Biocrude is a dark viscous liquid similar to petroleum, but it is produced by hydrothermal liquefaction of biomass (Hu *et al.,* 2019). The chemical and physical compositions of biocrude are largely influenced by the properties of the feedstock and the operating conditions of the system. Bio-crude is the desired product in the hydrothermal liquefaction operation, and the yield varies largely, even among the same strains and the same operating conditions (Barreiro *et al.,* 2014). Biocrude is a complex mixture involving a large number of compounds and several distributions of molecular weight, and its analysis is commonly performed with the use of gas chromatographic equipment fitted with mass spectrometry (GC-MS) (Hu *et al.,* 2019). Zhang *et al. (2014) reported* that the biocrude yield obtained by hydrothermal liquefaction of *Chlorella pyrenoidosa* using 50 wt% ethanol as solvent was 57.3 wt%, while Gai et *al. (2015) also* reported that the biocrude yield fell after an initial rise in the HTL of *Chlorella pyrenoidosa* from 200 to 320 °C. Xue *et al. (2014)* reported that when HZSM-5 was used as a catalyst, the biocrude yield of *Chlorella pyrenoidosa* was 34.02 wt%, and when Ce/HZSM-5 was used, it was 49.87 wt%, while Zhang *et al. (2013) reported* that the yield of biocrude from the hydrothermal liquefaction of Chlorella *pyrenoidosa* was 70.8 wt% at 240 °C and 47.1 wt% at 300 °C.

5.4.4 Solid residue of the HTL of *Chlorella* specie biomass

This is primarily made up of inorganics and trace organic matter remaining after the HTL operation (Tianet et *al.,* 2014). The solid residue percentage yield of HTL is largely determined by the content of ash in the feedstock, and it is usually observed to be less than 10 wt% because of the low ash content of *Chlorella microalgae.* HTL solid residue might contain products of protein that are degradable, which suggests it might be useful as a feed additive for animals because of its possible high nutritional value (Hu *et al.,* 2019). Zhang *et al. (2014) reported* that the solid residue yield obtained by HTL of *Chlorella pyrenoidosa* using different concentrations of ethanol as a solvent produces solid residue ranging from 9.42 wt% to 17.4 wt%. Gai *et al. (2015) reported* that the percentage yield of solid residue decreases gradually as the temperature of the operation increases from 200 to 320 °C, while Zhang *et al. (2013) reported* that for non-catalytic HTL at about 200 °C and 220 °C, the solid residue obtained was 59.5 wt% and 37.5 wt%, respectively.

6.0 Challenges in the Production of Biocrude from *Chlorella* Species Biomass and Future Directions

The unique advantages of the production of biocrude from the *Chlorella species* feedstock and the nature of its environmental friendliness make the hydrothermal liquefaction operation of *Chlorella microalgae* a promising technology for biorefinery. The prospects and challenges for the HTL of *Chlorella microalgae* studies and production are as listed:

1. Inability to improve the amount of chemical and biochemical compositions such as carbon, hydrogen, which contributes to the *HHV* of the biomass, and lipids, which are the main components that transform into biocrude, while reducing the amount of the components, does not favor the production of biocrude nor enhance its quality. This can be achieved by genetic modification of the *Chlorella* species during cultivation.

2. Application of solvent extraction techniques for the separation of the biocrude, which require the use of chemicals and additional techniques for the separation of chemicals. Mechanical separation techniques, such as gravity separation, can be employed.

7.0 CONCLUSION

The hydrothermal liquefaction operation of *Chlorella* species appears to be a very attractive technology for the production of biocrude, but this technology is still in its early stages. Several studies are being undertaken to make biocrude produced from *Chlorella* species of microalgae an economically competitive substitute for conventional petroleum. In this work, literature research was conducted to bring together several studies that have already been published. Consequently, all the works done to present that were brought together show that significant areas were not covered in the work the researchers carried out,the data they obtained from their experiment, or in the analysis of their results. Most of the authors reported the effects of operating conditions on the percentage yield of the biocrude from the biomass of *Chlorella* species, the biocrude quality, the elemental percentage composition of the biocrude, and its heating value. However, attention was not focused on how the relevant chemical and biochemical compositions can be modified to improve the biocrude yield and quality while reducing the amount of the compositions that do not favor it. Also, the data currently available for the extraction of biocrude after the hydrothermal liquefaction of *Chlorella* species were obtained by solvent extraction experiments. No comparative study of extraction methods is available, especially mechanical means of extracting the biocrude. The assessments of the composition of the biomass, the operating conditions, and the separation process indicate that hydrothermal liquefaction of *Chlorella* species could become an economically feasible technology for biocrude production,but much work still needs to be done in this area of study for a comprehensive understanding of the conversion process.

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