EFFECT OF OPERATING CONDITIONS ON THE CONVERSION OF DEFATTED MICROALGAE BIOMASS TO BIO-OIL THROUGH HYDROTHERMAL LIQUEFACTION

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ABSTRACT

The growing demand for sustainable energy has increased interest in bio-oil production from microalgae via hydrothermal liquefaction (HTL). However, limited research exists on utilising defatted microalgae biomass, the residue after lipid extraction. Therefore, this study investigates the effects of different operating conditions on the conversion of defatted Chlorella sp. microalgae biomass into bio-oil through HTL. Defatted biomass undergoes HTL at varying temperatures (210–270°C) and reaction times (30–90 min). Gas chromatography-mass spectrometry (GC-MS) analysis shows that reaction conditions influence bio-oil composition, which consistently contains aliphatic acids, amides, amines, pyrazine, pyridine, and phenolic compounds. Aliphatic acids, crucial for biofuel applications, are most abundant at 250–260°C and 45 min, indicating optimal conditions. Future work will explore broader parameters, including pressure and catalysts, to enhance bio-oil properties.

Keywords: Biomass, sustainable biofuel, hydrothermal liquefaction, reaction temperature, reaction time, lipid-extracted microalgae

INTRODUCTION

At present, burning fossil fuels such as coal, oil, and natural gas is the primary energy source, accounting for 80% of global energy consumption. It has impacted the environment through greenhouse gas emissions, global warming, and the depletion of fossil fuels [1]. In addition, the world's demand for energy has been increasing significantly over the years due to commercialisation and industrialisation, rapid population growth, limited fuel resources, and rising environmental concerns about the burning of fossil fuels [2]. This high energy requirement has led to multiple studies on alternative, sustainable renewable energy resources to ensure future energy demand is met and to reduce the effects of climate change.

In recent years, biofuel has gained attention as an alternative energy source due to its environmental friendliness and renewability, offering a promising solution to the global energy crisis and the environmental issues caused by burning fossil fuels. This is because biofuels and petroleum liquid fuels derived from fossil fuels are comparable in terms of energy density, chemical structure, and combustion performance [3]. However, current biofuel production from first-generation feedstocks such as food and oil crops has caused many issues, including impacts on global food security and environmental pollution from the use of fertilisers and pesticides. Occasionally, second-generation feedstock from non-edible crops, such as forest and agricultural wastes, is introduced for biofuel production. However, more land and freshwater are required for processing the biomass, and the conversion technology has not been scaled up for commercial exploitation [2],[4]-[5].

Due to limitations in first- and second-generation biofuel feedstocks, biofuel produced from microalgae biomass, a third-generation biofuel, is of high interest for further study [3]. Recently, many studies have focused on extracting energy-dense lipids from microalgae for biofuel production, leaving behind a large amount of residual biomass, known as defatted microalgae biomass.

After lipid extraction, these defatted microalgae biomass still contain significant amounts of organic material, including proteins and carbohydrates, which can be utilised as animal feed and fertiliser or fermented for ethanol production [5]. Instead of disposing of these waste materials, they can be further processed through thermochemical conversion, such as HTL, to generate valuable products, such as bio-oil. HTL is a process that converts defatted microalgae biomass into bio-oil as the main product, along with by-products, including solid residue such as biochar, gas such as carbon dioxide and aqueous phase by-products, which usually operate at moderate to high temperature between 280–370°C and pressure within 5–25 MPa with the presence of water as the reaction medium [2],[6]. Despite growing interest in microalgae biofuel production, there has been a lack of comprehensive research on optimising the HTL process for converting lipid-extracted microalgae biomass

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residue. Thus, a comprehensive literature review is conducted. From previous research articles, the suggested temperature range for the HTL is 250 to 370°C, an operating pressure of 10 to 25 MPa, and a reaction time of 30 to 60 min [7]-[10]. However, most research articles discussed the operating conditions of the HTL process, focusing only on microalgae and other biomass, rather than lipid-extracted microalgae biomass.

In this paper, the effects of different operating conditions, such as reaction temperature and HTL time, on the conversion of defatted microalgae biomass into bio-oil are investigated through GC-MS characterisation which are significant to the sustainability of microalgae-based biofuel production.

METHODOLOGY

The *Chlorella sp.* microalgae powder was purchased from a local store, along with other high-purity chemicals, including chloroform, methanol, and dichloromethane (DCM). About 50 g of *Chlorella sp.* microalgae powder was weighed on an electronic balance. The weighed microalgae powder was transferred into a 500 mL conical flask and mixed with 200 mL of methanol. The microalgae-methanol mixture was left to soak overnight. Afterwards, the soaked microalgae were filtered through filter paper to remove excess methanol, then mixed with 300 mL of a methanol–chloroform mixture (2:1, v/v) for lipid extraction. The mixture was then continuously stirred at 1000 rpm for 30 min at room temperature. The resulting mixture was filtered to remove residual solvents, and the filtered microalgae biomass was dried in the oven at 100°C for 24 hours.

About 40 g of dried defatted *Chlorella sp*. microalgae biomass was mixed with 160 mL of deionised water to obtain a microalgae slurry with 80 wt.% moisture content. The wet microalgae biomass was then transferred to the HTL autoclave reactor, which was sealed tightly. The reactor was then placed in a preheated oven at 210°C and allowed the reaction to proceed for 1 hour. After the reaction, the oven was turned off, and the reactor was removed and cooled to room temperature. The reactor was then unsealed, and the reaction mixture was filtered under vacuum to remove solid residues. The collected filtrate was transferred to a 500 mL conical flask and mixed with 200 mL of DCM. The mixture was stirred for 2 min and then transferred to a separatory funnel for separation of the aqueous by-product from the bio-oil recovered in DCM. The lower layer separated from the mixture was collected, and characterisation analysis was conducted. The process was repeated at operating temperatures ranging from 220°C to 270°C, with 10°C intervals. In addition, another set of experiments was conducted at different reaction times of 30 to 90 minutes, with 15-minute intervals, at a constant reaction temperature of 240°C. Figure 1 illustrates the step-by-step procedure for the synthesis of bio-oil from lipid-extracted microalgae biomass.

RESULTS AND DISCUSSION

Based on the GC-MS chromatogram, the amount of each compound in the produced bio-oil is represented by the peak area; the larger the peak, the greater the quantity of that compound in the bio-oil. Each compound is distinguished by its retention time, which is the time it takes to travel along the gas chromatographic column from the injection point to the detector. The formation of each chemical component in the bio-oil produced from HTL of microalgae biomass varies with increasing reaction temperature, as shown in Figure 2, which is also used as a reference for component selection in this experiment [11].

The selected components for analysis are derived from the breakdowns of lipids, proteins and carbohydrates. Although lipid extraction has been conducted on the microalgae *Chlorella sp.* biomass, there is also residual lipid present in the defatted microalgae biomass, which will undergo reaction to produce fatty acids. In addition to lipid breakdown, disintegration of leftover proteins and carbohydrates in defatted microalgae during the HTL process produces compounds such as amides, amines, pyrazines, phenolic compounds, and carboxylic acids [12]-[13],[15]. Based on comparisons of various research articles, seven compounds commonly found in microalgae-derived bio-oil were selected for

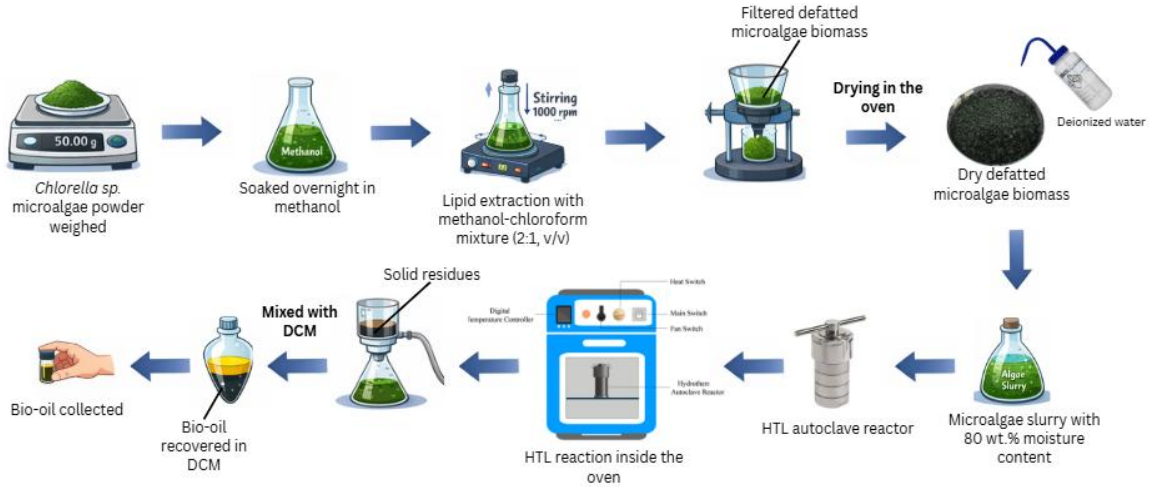

Figure 1 The experimental steps in synthesis of bio-oil from lipid-extracted microalgae biomass

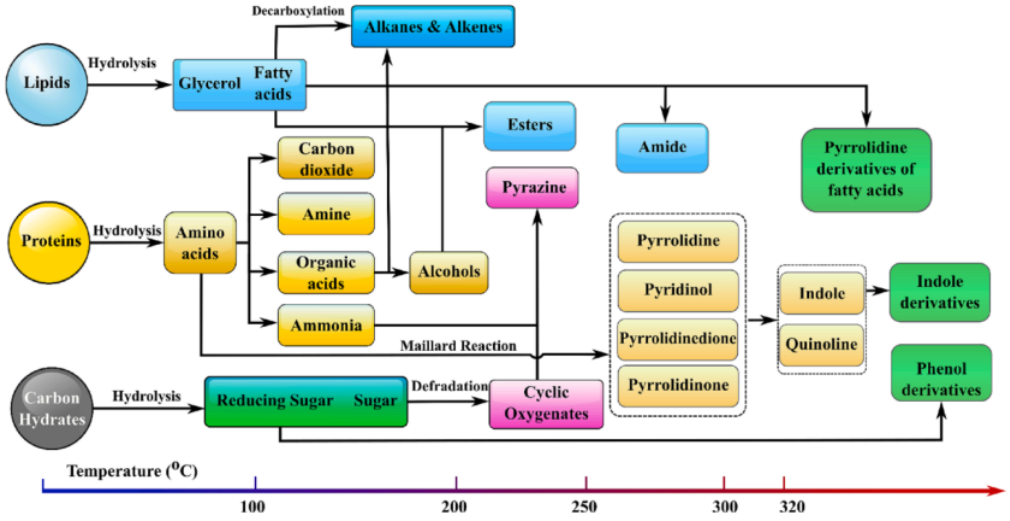

Figure 2 Formation of different compounds at increasing reaction temperature

analysis of bio-oil composition: hexadecanoic acids, octadecenoic acids, amides, amines, pyrazines, pyridines, and phenolic compounds and their derivatives, which will be further discussed.

Effect of Reaction Temperature

The percentage area for each selected chemical compound present in the bio-oil produced from HTL of defatted microalgae biomass at different reaction temperatures is shown in Figures 3a–3d. From the graphs, it is observed that temperature variation affects the composition of bio-oil produced from defatted Chlorella sp. biomass via HTL. For aliphatic acids in Figure 3a, the plot shows that both hexadecanoic and octadecenoic acids follow a similar trend. The highest percentages of hexadecanoic acid and octadecenoic acid are observed at 250°C and 260°C, respectively. In the case of amines and amides in Figure 3b, both selected compounds show a similar trend, with the highest percentage areas at 270°C. For nitrogen-containing compounds in Figure 3c, pyrazine is not detected at lower temperatures between 210°C and 230°C. However, its percentage area increases significantly as the temperature increases from 240°C to 270°C, with the highest

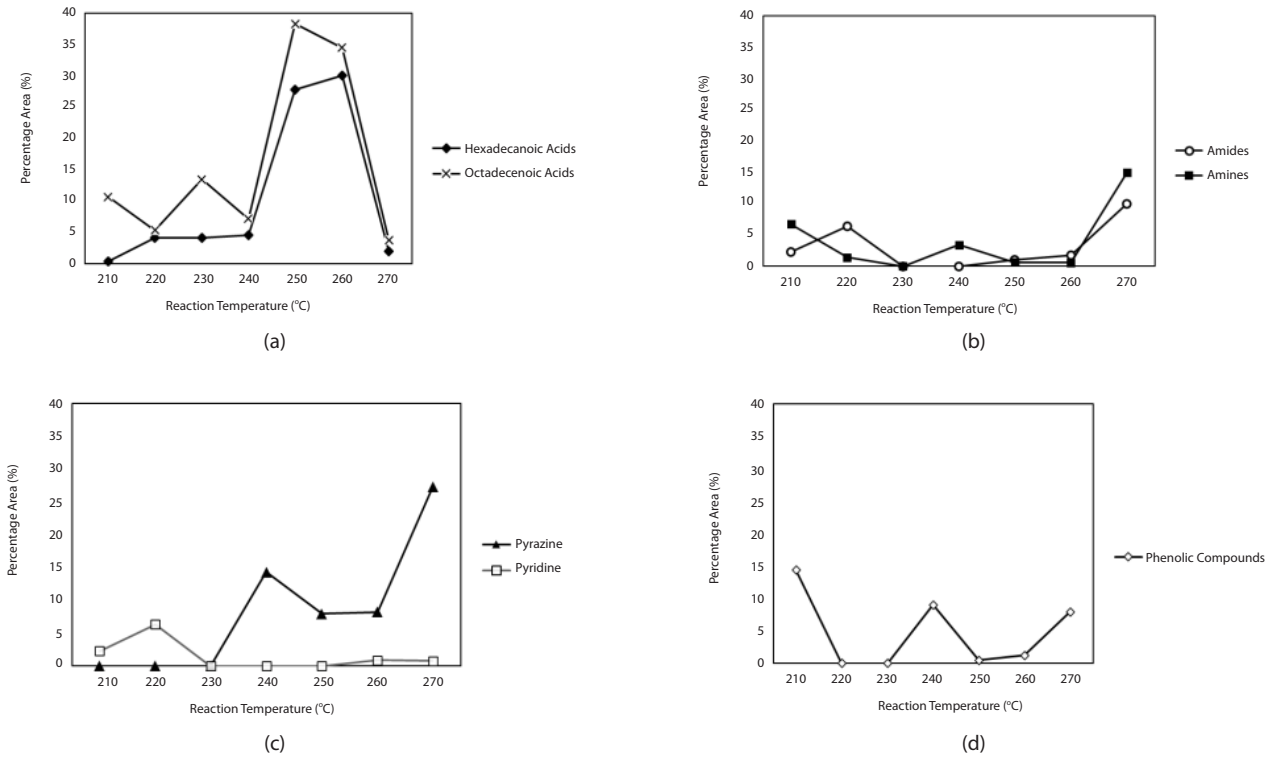

Figure 3 Graphs of percentage area from chromatogram at different reaction temperatures for (a) aliphatic acids, (b) amides and amines, (c) pyrazine and pyridine, and (d) phenolic compounds

concentration observed at 270°C. For pyridine, it is detected in small amounts at lower reaction temperatures, but its percentage area drops below 1% at temperatures above 230°C, indicating its near absence at higher temperatures [7]. For phenolic compounds in Figure 3d, the data show an inconsistent data trend, with the highest percentage area observed at the lowest temperature of 210°C. Overall, the bio-oil yield increases significantly with rising temperature and begins to decrease beyond a certain point [6],[12],[16]. To determine the optimum reaction temperature, aliphatic acids were selected as reference compounds due to their relevance as precursors for biofuels and their role in improving bio-oil quality [17]. According to the experimental data, the optimum temperature range is 250°C to 260°C, where the highest percentage of aliphatic acids was observed.

Effect of Reaction Time

The percentage area for each selected chemical compound in the bio-oil produced from HTL of defatted microalgae biomass, as shown in Figures 4a–4d, indicates that variations in reaction time affect the bio-oil composition. For aliphatic acids in Figure 4a, both hexadecanoic and octadecenoic acids follow a similar trend, with the highest percentage areas observed at a reaction time of 45 min. In Figure 4b, amines and amides are absent at shorter reaction times, with the highest percentage areas recorded at longer reaction times of 75 and 90 min for amines and amides, respectively. However, the data for amines show an unstable trend at reaction times above 60 min. For nitrogen-containing compounds in Figure 4c, both pyrazine and pyridine are absent at reaction times between 30 and 60 min. The percentage area of pyridine increases significantly at 75 min and slightly decreases at 90 min. Similarly, the percentage area of pyrazine increases with reaction time, peaking at 75 min before slightly decreasing at 90 min. Thus, the highest percentage areas for both pyrazine and pyridine occur at a reaction time of 75 min. For the phenolic compounds in Figure 4d, the data show an unstable pattern with an overall increasing trend. The highest percentage area is observed at the longest reaction time of 90 min. In general, bio-oil yield is expected to increase significantly with longer reaction times [12]. However, it is also reported that temperature plays a more significant role than reaction time in enhancing bio-oil yield recovery using HTL [12]. Therefore, to determine the optimum reaction time for the process, a similar method used to evaluate reaction temperature was applied, selecting aliphatic acids as the reference components due to their desirability for biofuel production [10],[17]. Based on this criterion, the optimum reaction time is determined to be 45 min from the experimental results, as this condition yields the highest percentage of the aliphatic acid area.

CONCLUSION

This study has successfully investigated the effect of operating conditions on the conversion of defatted microalgae biomass to bio-oil via HTL by analysing the bio-oil composition obtained from characterisation. It is determined that the variability in reaction temperature (210°C to 270°C) and reaction time (30 to 90 min) has resulted in different bio-oil compositions. By selecting aliphatic

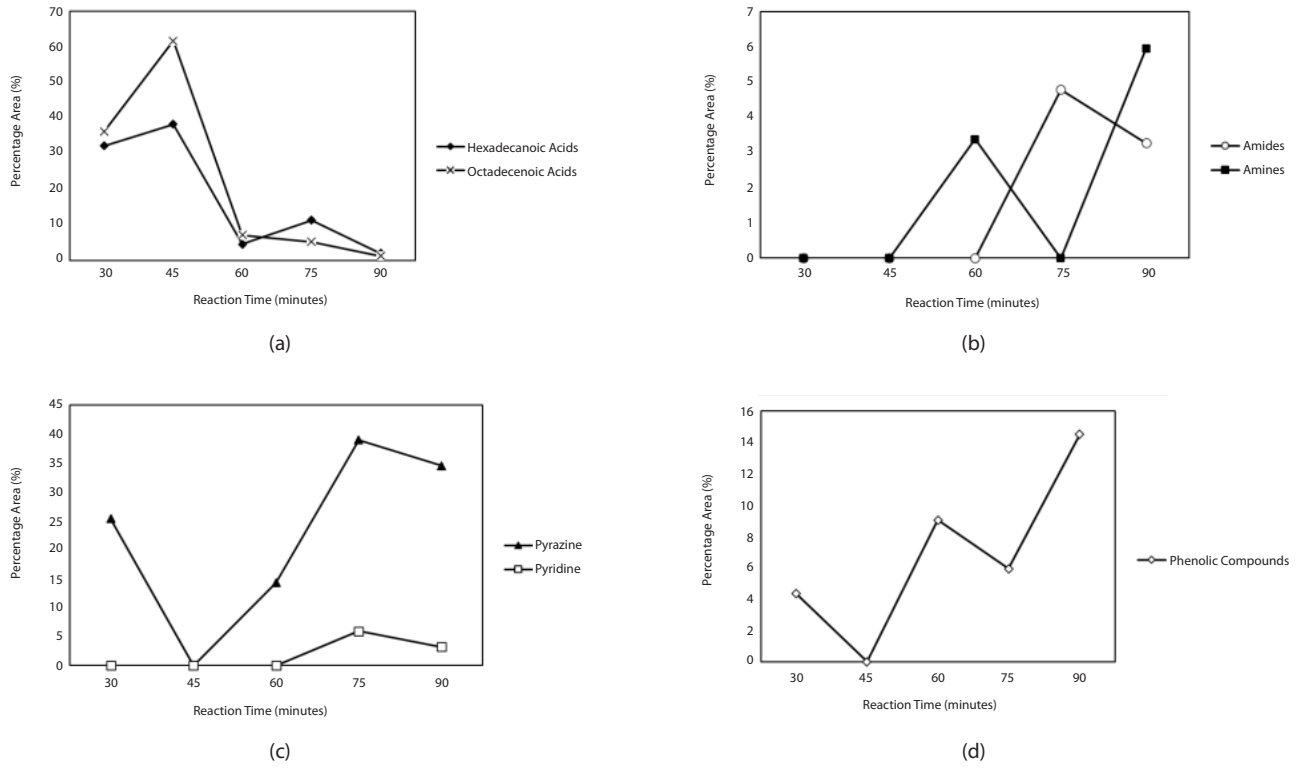

Figure 4 Graphs of percentage area from chromatogram at different reaction time for: (a) aliphatic acids, (b) amides and amines, (c) pyrazine and pyridine, and (d) phenolic compounds

acids as the reference component due to their desirability for biofuel applications, the optimum reaction temperature for HTL is determined from the resulting data to be 250–260°C, with a reaction time of 45 min, and the highest percentage of aliphatic acids is recorded. Future research should implement a wider range of reaction parameters, as suggested in the literature review, and introduce additional parameters, such as operating pressure and the presence of a catalyst for the HTL, to determine optimal conditions for bio-oil yield and enhance bio-oil properties. By expanding the scope of study, this research will provide better insights into high-quality, high-yield bio-oil production as a sustainable biofuel resource for the future.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

AUTHORS' CONTRIBUTION

Muhammad Khairul Hilmi Mohd Zaki and Shafirah Samsuri conceptualised the study and critically reviewed and edited the manuscript for intellectual content. Muhammad Khairul Hilmi drafted the manuscript, designed the methodology, conducted the experimental work, collected and analysed the data. Shafirah Samsuri validated the methodology and supervised the study. All authors have read and approved the final version of the manuscript.

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