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Bioconversion of Microalga *Chlorella* sp. as an Alternative Energy for Biofuel in Fishery Waste Media

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ABSTRACT

Carbon dioxide emissions from burning fuel oil have been increasing, resulting in increasingly severe climate change. The use of microalgae as raw materials for biofuel production is an environmentally friendly alternative. Microalgae *Chlorella* sp. can be sustainably converted into alternative biofuels. Fishery liquid waste, such as that resulting from fish farming and fish processing, can be used as a nutrient medium for microalgae. This study aimed to analyze biomass production from *Chlorella* sp. using fishery waste in an algae reactor. The working principle of fisheries wastewater processing is related to microalgae in the reactor. The surface of the media forms a biological layer that breaks down organic compounds in the air, thereby reducing the organic content. The compounds resulting from metabolic processes are in the form of solids and gases. The results show that the bioethanol produced from the thermostat *Chlorella* sp. with fishery waste media can produce 20,000 L/ha. Microalgae contain approximately 20–50% dry weight of carbohydrates, which can be converted into bioethanol. *Chlorella* sp. can reduce chemical oxygen demand and ammonia by up to 70% by converting carbon dioxide into carbohydrates, fats, and proteins. The application of bioconversion to all companies in the fisheries sector will provide benefits and help the government reduce fossil fuel subsidies.

Keywords: bioconversion, biofuel, fisheries, microalgae, waste

INTRODUCTION

This rapid human growth will cause energy needs to increase by 50% by 2030. Petroleum can no longer meet current consumption; therefore, it is necessary to create natural materials as alternative energy sources (Shuba and Kifle 2018; Huang *et al.* 2017). In addition, fossil use can negatively impact the environment through greenhouse gas emissions and global warming (Hosseini *et al.* 2019). Fossil fuels have been a driving force behind global economic growth since the Industrial Revolution. First-generation biofuels were derived from vegetable oils, animal fats, and food crops, which can have negative consequences for the food crisis (Karthikeyan *et al.* 2018; Alaswad *et al.* 2015). Microalgae are micro-scale in size, have fast reproducibility, good adaptability, excellent carbon sinks, and high conversion rates, and are considered third biological resources and fourth-generation biofuels with great potential (Ahmad *et al.* 2014; Ramadhan *et al.* 2021).

Large population growth results in the excessive use of non-renewable resources. The biowaste approach plays a fundamental role in environmental recovery. Biological waste in the form of fishery waste can be a valuable resource (Coppola *et al.* 2021). The nutritional content of fishery waste supports the production of renewable energy. The by-products of the fishing and aquaculture industries contain primary macronutrients in the form of N, P, and K. The high protein and lipid content in fish waste can be utilized as amino acids and peptides as fertilizers, which have the same or even better performance, superior to conventional synthetic fertilizers (Zhang *et al*. 2023).

Utilizing waste materials in production encourages a circular and sustainable approach. Overall, algae biodiesel promises to be a cleaner and more resilient alternative energy source (Gaurav *et al*. 2024). Some waste contains organic and inorganic components that can function as nutrients for algae growth, thereby reducing cultivation media costs and overall process costs (Silva *et al*. 2021). Cultivated microalgae are converted into biomass to produce biofuels sustainably to increase CO_2 emissions and treat wastewater (Rony *et al*. 2023; Mirzayanti *et al*. 2024). Sustainable and high-quality biofuel production can reduce dependence on fossil fuels (Thirugnanasambantham *et al.* 2020).

Microalgae are a good and sustainable source of biofuel and require further research. Microalgae fuel

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has biofilm-based growth with a higher biomass content (Ennaceri *et al*. 2023). These species are rich in lipids and represent the most important link between global warming and the need for clean energy to replace petroleum-based fuels (Hosain 2019). The development of microalgae as a renewable raw material requires the use of a Rotary Algae Biofilm Reactor (RABR). The reactor processes fishery waste and functions as a place for the formation of microalgae (Elystia *et al*. 2024). Therefore, this study aimed to identify the microalgae *Chlorella* sp. for biofuel production using fishery waste as nutrients. The results obtained are consistent with previous research on lipid content and biomass. We hope that this will provide an overview of the prospects for utilizing fishery waste as a clean energy alternative in the future. This comprehensive overview of biofuel production is expected to reduce carbon emissions to zero, which has an impact on the environment while providing profits for fishing companies. According to Krisdayanti *et al*. (2024), the large number of fossils whose existence cannot be denied will run out in the future. Therefore, there is a need for environmentally friendly transportation efforts that utilize alternative energy sources to replace fossil fuels.

METHODS

This research uses a review method by searching for previous journals from various process methods and understanding the essence of the themes or topics listed in journals. In scientific journals, reviews are conducted to understand the research process and the results contained therein (Wafi and Budianto 2022). The journal review method is interesting for discussing biofuel production using raw fishery waste materials. Production using microalgal raw materials is a promising and sustainable alternative to fossil fuels.

Data were obtained online, including the design stage, literature review search stage, and results of various journal reviews (Natsha *et al*. 2024). It is necessary to carry out an analysis that underlies this research, namely the lipid content of microalgae, and the relationship between the use of fishery waste as a medium for cultivating microalgae. The review stage included data searches on Google Scholar, ScienceDirect, DOAJ, and Link Springer websites. Data were selected from articles of good quality according to the required problems. The compilation of these results can provide information on the biomass content of microalgae that utilize fishery waste to produce biofuel.

RESULTS AND DISCUSSION

Biofuels derived from living organisms benefit the environment by reducing harmful CO_2, SO_x, and hydrocarbon emissions. The transition to third-generation biofuels, such as microalgae cultivation, is a promising source. This alga can efficiently convert sunlight, CO_2, and water into biofuel products (Shuba and Kifle 2018). Utilization of fisheries wastewater can provide nutrients for the growth of microalgae, thereby improving the environment by reducing toxic emissions. Biodiesel produced from lipids derived from microalgae offers important environmental benefits, such as reducing the greenhouse effect through the utilization of CO_2 emissions or waste processing (Krasowska *et al*. 2013; Bisht *et al*. 2023). This fishery waste can be converted into renewable energy by utilizing the available nutrients for the growth of microalgae (Figure 1).

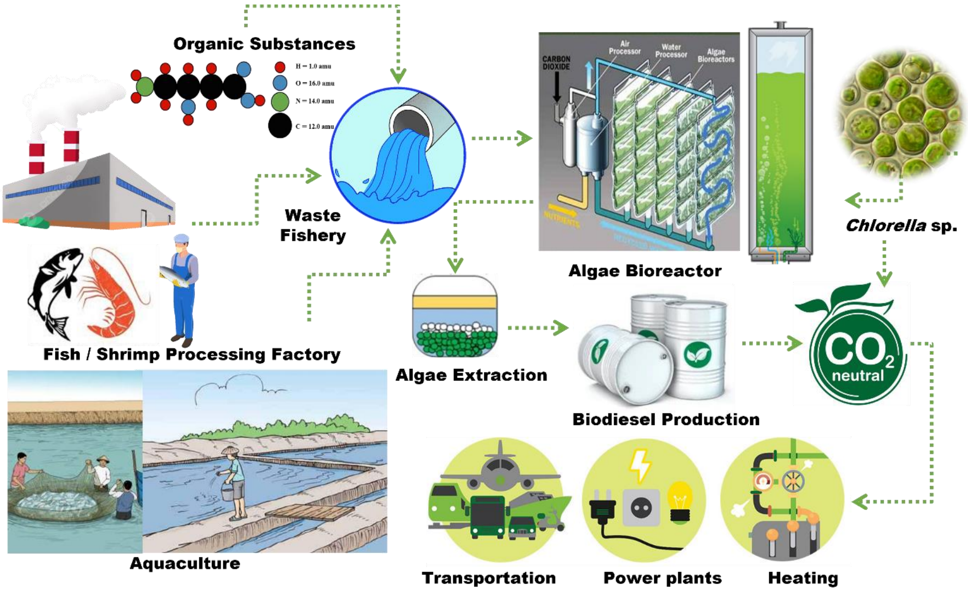

Figure 1 Processing of fishery waste as a nutrient medium for microalgae for biofuel production.

The prospect of utilizing fishery waste from fish cultivation and processing is highly profitable. Proper processing of fishery waste will generate additional profits for companies and help governments tackle climate change. This requires structured, systematic, and massive collaboration between companies and the government. Fishery waste still contains nutrients that can be used as microalgae nutrition. The organic substances it contains are carbon, hydrogen, oxygen, and nitrogen, which can still be used as nutrients. The growth process was carried out in a bioreactor containing fishery wastewater and *Chlorella* sp. microalgae. with optimal condition parameters. The results of microalgae growth are biomass, which is extracted and converted into biofuel. Biofuels can be used as substitutes for fossil fuels, which are useful for transportation, electricity generation, and heating. Microalgae conversion is a sustainable clean energy effort that must be implemented immediately.

Microalgae *Chlorella* sp.

Bioethanol produced from renewable biomass, such as sugar and starch, is believed to be an alternative energy source and is currently widely used. However, the use of sugar and starch as raw materials for bioethanol production creates great competition with the food market, in this case, land for cultivation, making bioethanol from these sources less economically attractive (Shuba and Kifle 2018). Algae are aquatic photosynthetic organisms with diverse species and growing conditions that vary greatly in nutritional composition, with the amounts of protein, carbohydrates, and lipids varying based on species and growing conditions (Gaurav *et al*. 2024).

Microalgae have several advantages: they do not depend on light and season, can efficiently remove organic carbon, and can be cultured at high cell densities to produce intracellular products (Silva *et al*. 2021). Microalgae have the potential to provide much higher yields than other oil-producing plants and can grow in freshwater, saltwater, and even wastewater. In addition, microalgae do not compete with productive agricultural land; therefore, there is no competition with the food chain (Krasowska *et al.* 2013). Microalgae have clear advantages over energy crops in biofuel production. Despite their high growth rates and large lipid (triacylglycerol) yields, microalgae can grow in wastewater (animal, municipal, and mining wastewater) and efficiently remove major nutrients (C, N, and P), heavy metals, and micropollutants, and do not compete with food crops for fertile land (Miranda *et al.* 2017).

Chlorella microalgae cells are green with an oval shape. The initial growth of microalgae experiences an adaptation phase that occurs in a short time, marked by an increase in the number of cells that occur from the beginning of the seeding process, thus allowing the microalgae to adapt to the cultural environment, reach the exponential phase, and then undergo the acclimatization stage. If the nutrients in the reactor are well supplied, the cell growth rate will increase; therefore, the waste replacement period every 4 days is the right time to replace some of the growth culture. This indicates that the nutrients in the reactor were well supplied, and the cell growth rate increased. The aim of replacing wastewater is to add nutrients such as nitrogen and glucose as a carbon source to the growth media so that the nutrients are well supplied and support the lipid synthesis process through maximum microalgae growth, which can be observed from the increase in cells density. The surface of the reactor disk can produce a dark green biofilm layer with a thickness of 2.5 mm (Elystia *et al*. 2024).

Fishery Waste

Waste processing is accompanied by the production of high-value-added products (Silva *et al*. 2021). In addition to being able to utilize wastewater, it can also reduce odors, emissions, and harmful gases (Rony *et al.* 2023). Fish processing industry waste is generated from used fish washing, which contains protein, fat, and dissolved substances (Oktavia *et al.* 2012). The organic material content, especially the protein content, is still very high; therefore, fishery waste can become a serious problem if not managed properly. This is because the number will continue to increase every year, which can cause environmental pollution (Hakim *et al.* 2017). The utilization of water in fish farming activities on land (ponds, tanks, and aquariums) produces wastewater as a byproduct. Fish farming wastewater is relatively abundant and contains high amounts of organic matter. This condition is caused by food waste and fish metabolism products, such as urine and feces. Direct and continuous disposal of liquid waste by environmental institutions causes pollution (Febrianto *et al.* 2016). The potential for fish farming waste also has nutrients for microalgae obtained from leftover feed that has undergone a metabolic process (Ardana *et al.* 2018).

Bioreactor

The use of this bioreactor is advantageous for producing high-value products. This tool is a better controller of cultivation and growth parameters (pH, temperature, CO_2, and O_2) than the conventional tools. Additionally, it offers a safe environment, prevents evaporation, and minimizes invasive contamination. The provision of nutrients such as N and P is more accurate using this bioreactor. It is a flexible system that can be adapted to the biological and physiological characteristics of algal species (Shuba and Kifle 2018).

The main tool used is a Rotary Algae Biofilm Reactor (RABR) made of acrylic with dimensions that can be customised. This reactor was designed to process liquid waste with a volume of 20 L. The disk media used in this research was hydrophobic to facilitate microalgae adhesion to form a biofilm layer and was made of thin circular acrylic glass that was installed parallel to it. The depth of the disk media in this study was 60% of the disk diameter to maximize the growth of the *Chlorella* sp. biomass. Other supporting tools include a microscope to check the development of microalgae, a hemacytometer to count the number of microalgae, a pH meter to check the acid-base, a thermometer to check the temperature so that it is stable, a 1000 mL Erlenmeyer flask, and others. The main ingredients used were 20 L of fishery waste at one collection point, which was deposited for 6 h, *Chlorella* sp. microalgae seeds, Dahril Solution media, and distilled water. Using RABR technology, the average lipid content in the reactor was influenced by the growth of *Chlorella* microalgae cells. which occurs suspended in wastewater and the biofilm attaches to the disk media thereby increasing the amount of biomass and lipid content, where the higher the amount of microalgae biomass, the higher the lipid content produced. This is because the microalgae cells that are formed when they have reached the maximum number in the growth medium will experience a phase of decreasing growth rate due to lack of nutrients so that the microalgae cells will change the use of carbon from the growth process into energy reserves such as lipids (Elystia *et al.* 2024).

Biofuel Manufacturing Process

Energy production from microalgae biomass involves biochemical, thermochemical, chemical, and direct combustion processes. The steps begin with the production of microalgal biomass using light, carbon dioxide, water, and inorganic nutrients. The next step is harvesting, where the cells are separated from the water and remaining nutrients. In the third step, algal oil is extracted from the biomass. In the fourth step, most of the biomass undergoes anaerobic digestion, producing biogas to generate electricity. Most of the electricity generated from biogas is consumed in the biomass production process, and excess energy can be sold to the electricity grid. In this process, carbon dioxide emissions from the power generation stage are incorporated into biomass production. Thus, the entire process is carbon neutral. The power required to produce and process algae will come from the biodiesel itself and from the methane produced by the anaerobic digestion of the biomass residue left after the oil is extracted (Shuba and Kifle 2018).

Microalgae cultivated in bioreactors not only increase CO_2 emissions and treat wastewater but also meet the biomass needs for sustainable biofuel production. If the biomass contains less than 50% moisture, direct combustion is recommended to produce energy. This is the largest amount of energy consumed for biomass drying. If the water content in wet biomass is higher than 50%, a biochemical conversion process is recommended to convert it into biofuel (Hosain 2019). Factors that influence the growth of microalgae include a light intensity of 2000–4000 lux, light duration of 12:12 or 16:8 (light-dark cycle), a maximum temperature of 20–30°C, a CO_2 concentration of 2–10%, an optimal pH of 7.0–9.0, and nutrients in the form of nitrogen, phosphate, potassium, and micronutrients. Therefore, choosing the right method and optimizing conditions is very important for successful cultivation (Gaurav *et al.* 2024).

Production of *Chlorella* sp. Cultivation

This supports the economic reduction of raw material prices, which usually account for 55–80% of the final purchase cost of alcohol (Hosain 2019). Microalgal raw materials have great potential for sustainable biofuel production. Thermochemical processes, biochemical conversion, and transesterification are used for producing biofuels. In particular, the transesterification of lipid molecules into fatty acid alkyl esters is widely used for biodiesel production. In the extractive transesterification process, biodiesel is produced from the extracted microalgae oil. Meanwhile, *in situ* (reactive) transesterification allows the direct conversion of microalgae into biodiesel by avoiding sequential stages, which reduces production costs (Karpagam *et al.* 2021).

Biomass residues can be used for bioethanol production. This lipid-free microalgal biomass residue will be produced through industrial-scale microalgae-based biodiesel production. In addition, the remaining protein-rich microalgal biomass after biodiesel and bioethanol production can be used effectively for biogas production (Karpagam *et al*. 2021; Ismail *et al*. 2020). According to research by Utomo *et al.* (2015), microalgae production in fishery waste has a bioethanol potential of 20,000 L/ha. This effort can be made to produce renewable alternative fuels (renewable energy) as a substitute for non-renewable fossil fuels (nonrenewable energy). These efforts are made to guarantee the availability of sustainable energy supplies. Renewable fuels have several advantages, including being relatively low-cost, carbon-neutral, and generally non-polluting.

The resulting lipid content was directly proportional to the respiration rate of the *Chlorella* sp. cells. In the respiration process, cells will break down glucose to produce energy in the form of ATP, and if there is excess energy in the glycolysis process, this energy is then converted into lipid compounds and stored as energy reserves, so that there are more cells. During processing using RABR, an increasing number of levels accumulate. lipids in microalgae cells will be higher. The highest lipid content was found using the Rotary Algae Biofilm Reactor (RABR) method, reaching 52.66%. The lipid levels in this study were higher than those in other studies because of the differences in the microalgae culture system and technology used. In a semi-continuous cultivation system, the aim of changing wastewater is to add nutrients such as nitrogen and glucose as a carbon source to the growth medium so that the nutrients are well supplied and support the lipid synthesis process (Elystia *et al.* 2024; Chi *et al.* 2018; Makarevičienė *et al.* 2011).

Effect of Wastewater on COD

Wastewater turnover time affects the level of chemical oxygen demand (COD) removal. The best COD removal results were obtained during the wastewater replacement period, which varied every 4 days, reaching 90.24% with a final COD concentration of 64 mg/L. This shows that by replacing wastewater at the right time, microalgae can utilize organic compounds contained in fishery waste as nutrients for good growth, so it was optimal for reducing COD concentrations in the reactor. This is because the longer the wastewater change interval, the greater the cell division of the *Chlorella* sp. through the absorption of nutrients in the form of organic compounds contained in fishery waste. However, if the optimal wastewater replacement period is exceeded, namely every four days, microalgal growth will decrease, as indicated by a smaller decrease in COD. The nutrient content in wastewater gradually decreases with the accumulation of biomass when microalgae are cultured in wastewater. This shows that there is a process of decomposition and utilization of organic compounds by microalgae for their growth, resulting in an increase in the efficiency of COD removal during processing (Elystia *et al*. 2024). This is supported using *Chlorella* sp., which has good bioremediation capabilities because it can live in polluted waters (Dongoran *et al*. 2024). The use of fishery waste as a nutrient medium for microalgae is also a part of bioremediation, which utilizes living organisms for their growth.

CONCLUSIONS

Biofuel production from fishery waste, in the form of residual cultivation water and fishery processing water from factories, can be used as a microalgal culture medium. Utilization of fishery waste also reduces COD content as part of bioremediation by utilizing living microalgae. Fishery waste contains carbon, hydrogen, oxygen, and nitrogen as nutrients for microalgae. Microalgae *Chlorella* sp. contains sufficient nutrients for growth produce high biomass. Biomass through the extraction process produces lipids, which are then converted into biofuels. The resulting biofuel can be used as an alternative to fossil fuels. Biofuels can be produced sustainably, thereby reducing pollution and climate change and distributed to fuel stations to help the government reduce subsidies. Cooperation in processing fishery waste produced by fishing companies and the government will benefit both parties.

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