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The Effect of Waste Molasses on the Growth and the Amount of Lipid and Protein of *Chlorella vulgaris*

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Abstract

In recent years, microalgae have become the focus of attention because they are used in different fields (biodiesel, protein extraction, etc.). One disadvantage of microalgae is that their production costs are pretty high. This paper aimed to reduce the cultivation costs of *Chlorella vulgaris*, which is an important species in terms of protein and lipid content. Molasses solutions at different concentrations were used as media for the cultivation of *C. vulgaris*. Molasses is a byproduct of the extraction of sucrose from sugar beets. A Jaworski's medium was used as the control group. *C. vulgaris* was inoculated into molasses media (0.5 g/L, 1 g/L, 2 g/L, and 4 g/L). Growth and protein, and lipid content were calculated for ten days. *C. vulgaris* had the highest growth in 4 g/L molasses medium on day five. It had the highest protein content in 2 g/L molasses medium on day five. It had the highest lipid content in 4 g/L molasses medium on day seven. The molasses media promoted the growth and the protein and lipid content of *C. vulgaris*. The results show that molasses media help significantly reduce microalgae cultivation costs.

Key words: Molasses, Lipid amount, *Chlorella vulgaris*

1. Introduction

The global energy demand is rising due to rapid population growth and technological advances, resulting in intense competition among countries. However, air pollution due to fossil fuel emissions causes serious social and economic problems (Badar et al., 2018). Since the 1950s, researchers have been attempting to discover alternative sources of protein, lipids, carbohydrates, and energy against food shortages expected in the near future. There has been extensive research into yeasts, fungi, bacteria, and microalgae as alternative protein and energy sources. The primary focus has been on microalgae because they have significant advantages over other sources in producing biodiesel and protein. Microalgae do not compete with other products because microalgal cultivation does not require arable land. Therefore, they are ideal organisms for obtaining biofuel and protein. Another advantage of microalgae is their high growth rate and protein and lipid yield (Gonzáles-Garcununo, 2014; Ruangsomoon, 2015; Arora et al., 2016).

However, microalgal cultivation has one disadvantage; high costs, which we need to reduce. Recent research has focused on waste materials for microalgal cultivation to produce biomass in an eco-friendly fashion. Microalgae growing in nutrient-rich wastewater help reduce pollutants in water, thus making significant contributions to environmental sustainability (Jalilian et al., 2020; Mustafa et al., 2021).

It is expensive to obtain metabolites, protein, and lipids from microalgae. However, we can turn it into an advantage. We can reduce microalgal cultivation costs and dispose of waste products by using them as media for cultivation. We need to determine the ideal medium in which to cultivate microalgae in a cost-effective and eco-friendly way. The choice of media mainly depends on various factors, including the chemical composition of the medium. Research shows that growth media and chemical compositions vary across algae species (Ilavarasi et al., 2011; Sangapillai and Marimuthu, 2011; Yeh and Chang, 2012).

We need to determine the best media for maximum microalgal growth and rapid metabolite production. Ma et al., (2017) were the first to focus on molasses for microalgal cultivation. Molasses is a dark brown byproduct of sugar production and refining. Molasses is produced via the separation of sucrose crystals after water evaporating from clarified sugarcane or beet juice during the production of crystal sugar (Lino et al., 2018). Molasses rich in elements and vitamins are inexpensive and renewable carbon sources in various industrial processes (Mordenti et al., 2021). Molasses contains many nutrients (polysaccharides, nitrates, and phosphates) needed for microalgal growth (Becker, 2007). *Chlorella vulgaris* is a green algae found in most bodies of fresh water. Microalgae contain high amounts of protein, carbohydrates, and lipid (Ilıman et al., 2000). *C. vulgaris* (2-10 microns) is a single-celled, eukaryotic, and vigorous organism that grows rapidly in different media (Yamamoto et al., 2004; Yamamoto et al., 2005).

This study had two objectives: (1) Cultivating *C. vulgaris* in molasses medium cost-effectively and (2) preventing environmental pollution by recovering nutrients in molasses wastewater.

2. Material and Methods

2.1. Medium and culture condition of C. vulgaris

C. vulgaris specimens were collected from the Keban Dam Lake. They were isolated from phytoplanktons and grown in Jaworski's medium (control group) [80 mg $NaNO_3$, 20 mg $Ca(NO_3)_2.4H_2O$, 36 mg $Na_2HPO_4.12H_2O$, 12.4 mg KH_2PO_4, 50 mg $MgSO_4$. $7H_2O$, 2.25 mg EDTAFeNa, 15.9 mg $NaHCO_3$, 2.25 mg $EDTANa_2$, 2480 µg H_3BO_3,1390 µg $MnCl_2.4H_2O$, 1000 µg $(NH_4)_6Mo_7.4H_2O$, 40 µg biotin, 40 µg cyanocobalamin (B12), and 40 µg thiamin (B1)] (Thompson et al., 1988), which was sterilized in an autoclave at 121 °C for 15 minutes. Molasses media (0,5 g/L, one g/L, two g/L, and four g/L) were also sterilized in the autoclave at 121 °C for 15 minutes.

Both Jaworski's and molasses media (200 mL) were inoculated into sterile flasks (1000 mL), which were then left to grow at a light intensity of 55 µmol photon $^{m-2}sec^{-1}$ at 23±1 °C in a climate cabinet under a 16h light and 8h dark regime for ten days. The flasks were shaken three times a day.

2.2. Growth, protein, and lipid analysis

Growth was evaluated every 24 hours via optical density (OD) at 550 nm using a UV-Vis spectrophotometer (Jenway 6105 UV/Vis). All measurements were made in triplicate.

Lipid content was determined using a modified Bligh and Dyer method (Blight and Dyer 1959). Methanol (40 mL) and chloroform (80 mL) were added onto a specimen (0.2 g). Calcium chloride (20 mL 0.4% CaCl2) was added into the mixture, which was then filtered through filter paper and kept in the dark for a night. The next day, methanol and water were separated in a separator. Chloroform was evaporated in a water bath at 60 °C. The rest was kept in an oven at 90°C for one hour to evaporate all the chloroform. The remaining part was then weighed.

Total protein content was measured using the Lowry method (Lowry et al., 1951). Dissolved organic carbon (DOC) solution (0.1-mL) was added to a 1-mL specimen, which was then kept at room temperature for ten minutes. Afterward, trichloroacetic acid (0.1 mL TCA) was added to the specimen, which was then centrifuged at 7500 rpm for ten minutes. Following the removal of the supernatant, 1-mL Lowry solution was added to the precipitate, which was then kept at room temperature for 20 minutes. Later on, 1-mL folin reagent was added to the specimen and kept for 30 minutes. Lastly, absorbance was plotted at 750 nm to generate a standard curve, which was used to evaluate the results.

3. Results and Discussion

Jaworski's medium is generally used to culture green algae. We cultivated *C. vulgaris* in Jaworski's (control) and molasses (experimental) media and observed the growth rates and protein and lipid contents in both media for ten days. Both cultures had an optical density of 0.050 on inoculation day. There was a significant increase in optical density in all cultures from day three of inoculation. The molasses media at 0.5 g/L, 1 g/L, 2 g/L, and 4 g/L concentrations had optical density values of 0.152, 0.196, 0.255, and 0.082, respectively. The control group had an optical density of 0.128. The optical density continued to increase until day five. *C. vulgaris* had the highest growth in 2 g/L molasses medium. The optical density started to decrease in all cultures after day 6 (Figure 1).

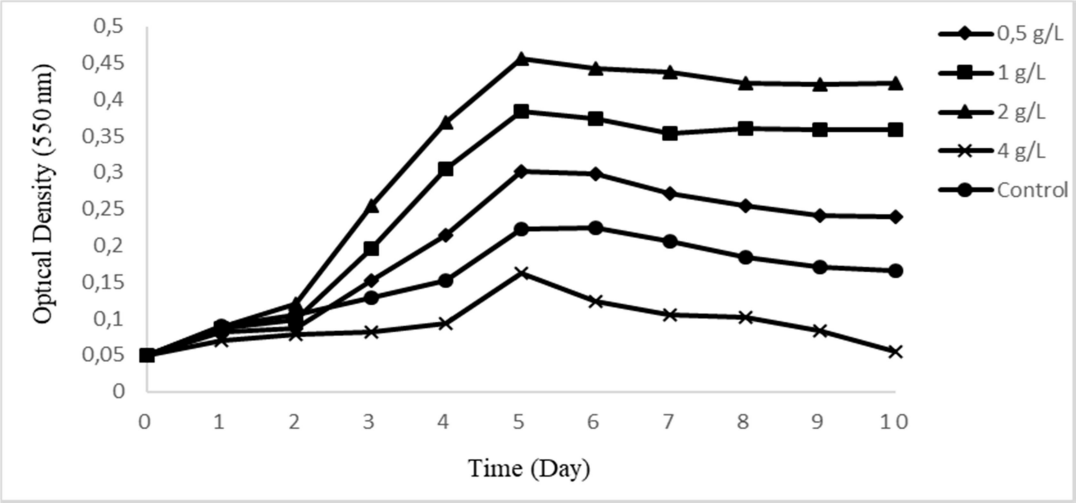

Figure 1. Daily changes in *C. vulgaris* cultured in Jaworski's (control) and molasses (experimental) media.

There was an increase in protein content in all cultures until day five. The molasses media at 0.5 g/L, 1 g/L, 2 g/L, and 4 g/L concentrations had a protein content of 135.20 µg/ml, 180.50 µg/ml , 205.4 µg/ml, and 58.12 µg/ml, respectively. The control group had a protein content of 75.35 µg/ml. *C. vulgaris* had the highest protein content in 2 g/L molasses medium (Figure 2).

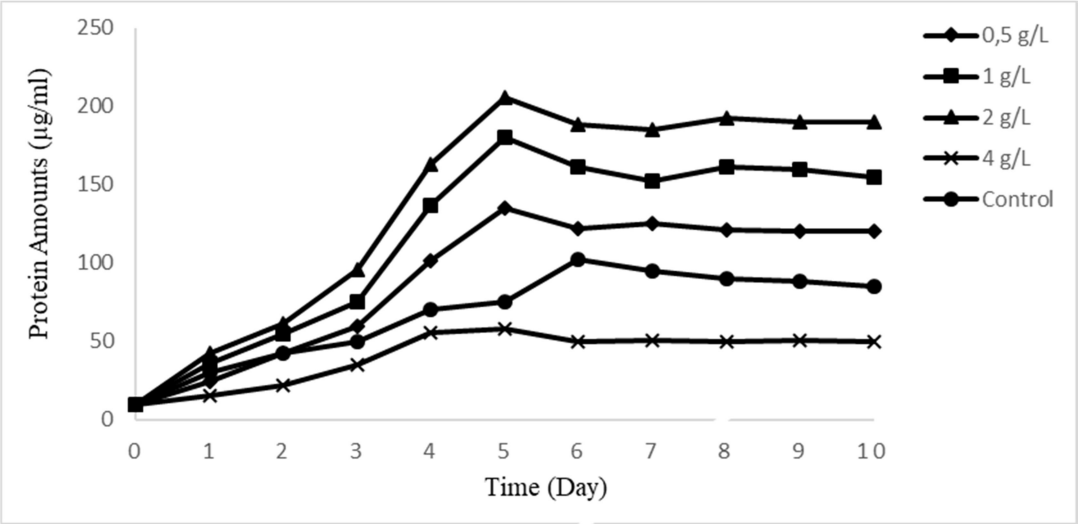

Figure 2. Daily changes in protein content.

There was an increase in lipid content in all cultures until day 6. The molasses media at 0.5 g/L, 1 g/L, 2 g/L, and 4 g/L concentrations had a lipid content of 40.36%, 46.12%, 57.15%, and 62.85%, respectively. The control group had a lipid content of 40.15%. *C. vulgaris* had the highest lipid content in 4 g/L molasses medium on day six (Figure 3).

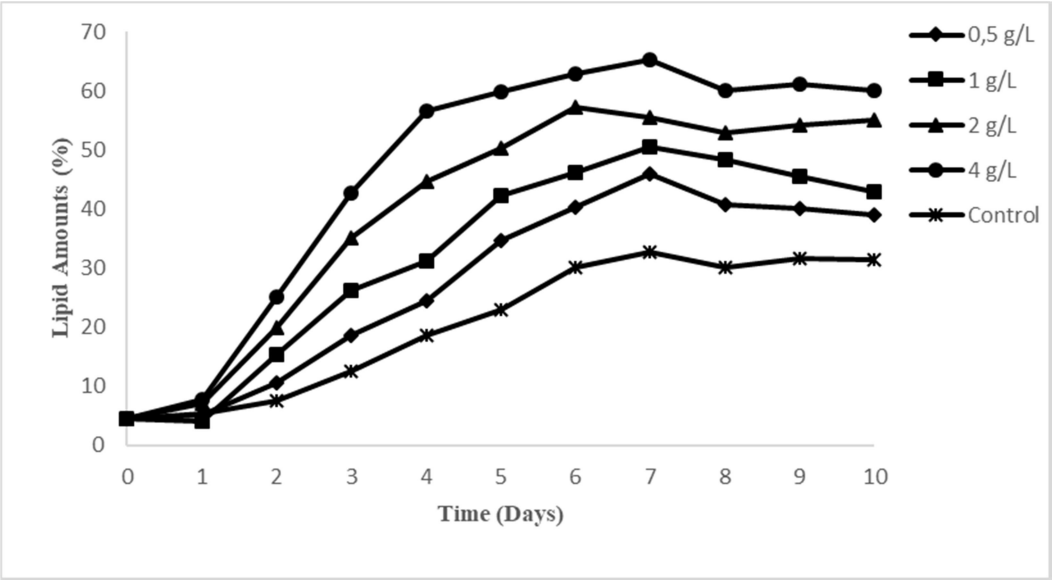

Figure 3. Daily changes in lipid content.

Many researchers have investigated microalgal growth and biochemical content in different media (Ilavarasi et al., 2011; Niccolaia et al., 2019; Leasing et al., 2011). In recent years, researchers have developed a wide variety of strategies to expand the use of microalgae. They have concentrated particularly on cost-reducing strategies in mass cultures. Research shows that molasses is a suitable medium for the cultivation of some microalgae (Liu et al., 2012; Gautam et al., 2013; El-Sheek et al., 2014; Gaurav et al., 2016; Mondal et al., 2017; Yew et al, 2020). However, some studies point out that molasses concentration may limit microalgae growth. Our results showed that *C. vulgaris* had the highest growth rate in 0.2 g/L molasses medium on day six and the highest lipid content in 0.4 g/L molasses medium on day seven.

4. Conclusion

This study investigated the growth rate and protein and lipid contents of *C. vulgaris* inoculated in molasses media at different concentrations. The results showed a negative correlation between molasses concentration and *C. vulgaris* growth rate. Depending on the molasses concentration, the lower the light transmittance, the less the growth of *C. vulgaris*. The higher the molasses concentration, the higher the lipid content to some extent. Our results indicate that culturing *C. vulgaris* in molasses media helps reduce cultivation costs and contributes to waste management.

Conflicts of Interests

Authors declare that there is no conflict of interests

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