



RESEARCH ARTICLE

Determination of growth conditions for *Chlorella vulgaris*

İrem Deniz^{1*} 

¹ Manisa Celal Bayar University, Faculty of Engineering, Bioengineering Department, 45119, Muradiye, Manisa, Turkey

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ABSTRACT

Microalgae known as third generation technology for biofuel in nature are used as a renewable bioenergy source. Microalgae and crop plants have common for the production of organic compounds by using sunlight and carbon dioxide. In addition, microalgae are capable of being reproducing in the whole year allowing the more product yield than those of plants. Therefore, microalgae are more favorable feedstock since they have some advantages such as photosynthetic efficiency, biomass productivity and oil content. In this study, the most proper conditions for the growth of microalgae *Chlorella vulgaris* were studied. After medium optimization, at 25°C and pH of 9 using 24-hour-illuminating/day, the best growth conditions for *C. vulgaris* giving the maximum biomass productivity was found to be 205 mg/L for 10 days. Determination of optimal microalgal growth conditions may lead to an increase in the industrial applications of *C. vulgaris*.

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Introduction

Microalgae are micrometer-sized aquatic organisms, generally in the range of 1–400 µm, and they are one of the oldest forms of life on the planet (Tijani et al., 2015). They have chlorophyll to be responsible for photosynthesis, their cells are covered with pigment-reproducing cells as well (Tan et al., 2018). Moreover, they account for approximately 50% of the whole photosynthetic activity on Earth. Because, some microalgae have rapid growth such as double in number within hours, thus, having a short harvest time as less than 10 days (Bach and Chen, 2017).

Microalgae particularly require optimum light, carbon dioxide, temperature, pH and nutrients to grow. While the light gives energy, carbon dioxide provides the carbon source for the production of biomass. Microalgae are able to produce different types of renewable biofuels such as biomethane with the anaerobic digestion process, biohydrogen, biodiesel with transesterification process and biochar production with pyrolysis process (Tan et al., 2018). Thus, microalgae can be accepted as an alternative and promising feedstock for energy production along with the overuse of fossil fuels. A progressive decline of resources of fossil fuels and an increase in climate change caused an obligatory situation to search for a renewable and sustainable energy source. Therefore, biofuels are

* Corresponding author
E-mail address: iremdenz@gmail.com (İ. Deniz)



considered as the best choice for that purpose (Jankowska et al., 2017). The fossil fuel energy is approximately 78.3% of the whole global energy consumption. Moreover, fossil fuel is a great contributor to CO₂ emissions which are directly linked to global warming. Biodiesel is a clean-burning fuel and is obtained from plants such as soybeans, oil palms, jatropha and microalgae (Elrayies, 2018). The development stages of biofuels are classified into three generations in terms of their feedstock. The first generation of biofuels is obtained from food crops such as sugar cane, sugar beets, rapeseed, soybeans, oil palms and corn. However, the undesirable competition had occurred between food and fuel in time. This is due to the place intended for the production of food that was shifted to the production of food crops used for biofuels. The second generation biofuels are obtained from lignocellulosic agriculture, forest residues and non-food crop feedstocks which also termed as energy crops such as jatropha and pongamia. However, some deficiencies had been occurred such as low productivity of biomass, need for excess water and arable land, and also low energy required pretreatments. In recent years, a new biofuel generation had been appeared and known as the third generation biofuels which are obtained from microalgae. Microalgae culture as feedstock has more advantages compared that the second generation needs the more land, such as having high growth rate, the need for less amount of water for the growth as compared to terrestrial crops, having a high photosynthetic efficiency; having more oil productivity (Faried et al., 2017; Jankowska et al., 2017; Elrayies, 2018). In this study, the proper growth conditions of *Chlorella vulgaris* was investigated.

Materials and Methods

Microalgae

Chlorella vulgaris was provided by courtesy of Prof. Dr. Meltem Conk Dalay from Ege University Bioengineering Department, Turkey. The strain was preserved in the BG11 medium which has composition as follow: NaNO₃ (1.5 g/L), K₂HPO₄·3H₂O (0.04 g/L), MgSO₄·7H₂O (0.075 g/L), CaCl₂·2H₂O (0.036 g/L), Na₂CO₃ (0.02 g/L), citric acid (0.006 g/L), Ferric ammonium citrate (0.006 g/L), EDTA (0.001 g/L), and A5 + Consolation (1 mL/L) that consists of H₃BO₃ (2.86 g/L), MnCl₂·H₂O (1.81 g/L), ZnSO₄·7H₂O (0.222 g/L), CuSO₄·5H₂O (0.079 g/L), Na₂MoO₄·2H₂O (0.390 g/L) and Co(NO₃)₂·6H₂O (0.049 g/L) (Suthar and Verma, 2018). The BG11 medium was used as a growth medium in each experimental set-ups.

Medium Optimization in Erlenmeyer Flasks

Obtaining the most proper growth conditions was performed with 100 mL of BG11 medium within 250 mL of Erlenmeyer flasks in shaker-incubator (Microtest, Turkey). The different range of pH (5, 7, 9), temperature (25, 35, 45 °C) and illumination period (24 h light/day, 12h-dark-and-12-h light/day, 24 h dark/day) were used using one variable at a time method. Microalgal cell biomass was separated from the medium by centrifugation at 5000 rpm for 15 min. Dry cell weight was used as a marker for biomass production.

Analysis

Microalgal cell biomass was separated from medium by centrifugation (Centurion, England). BG11 culture medium containing microalgae was centrifuged at 5000 rpm for 15 min, washed twice with distilled water then, the cell pellet was dried at 105°C until a constant weight was observed. Dry weight was measured by filtering a 10 mL of the culture by preweighted filters. After rinsed with ammonium bicarbonate, the filters were dried at 105°C overnight. Dry cell weight was used as a marker for biomass production.

For cell analysis, the culture broth was sampled daily and absorbance was recorded spectrophotometrically at 650 nm.

Results and Discussion

Optimization of pH

In order to determine the optimum pH for *C. vulgaris*, the microalgae production has occurred in Erlenmeyer flasks in the range of pH of 5-9. In this study, the suitable value of pH with a maximum biomass concentration of 150 mg/L after 10 days for *C. vulgaris* was found as 9. Once the pH goes up and the sufficient amount of CO₂ is injected into the microalgal growth medium, pH would back to optimum and lead to an increase in the microalgal biomass. In another study, it was reported that the best growth pH of *C. vulgaris* was 9. It was interpreted that H⁺ ion gradients control transport processes throughout the cellular membranes and perform the metabolic activities in the cytoplasm and organelles (Suthar and Verma, 2018).

Optimization of Temperature

The temperature range (25, 35, 45 °C) was used to determine the optimal temperature for *C. vulgaris*. The optimum temperature for the highest biomass concentration of 182 mg/L after 10 days was decided as 25°C. The less growth has appeared at 35 and 45°C. Microalgal growth rapidly decreased when the temperature exceeding the optimum temperature. Therefore, the growth did not occur above 35°C.

It was previously reported that the growth rates for *C. vulgaris* at 28°C and 25°C are not greatly different (Serra-Maia et al., 2016). It was also discovered that temperature below 30°C enhanced the solubility of CO₂ in growth medium thus, dissolving the CO₂ resulted in higher biomass production because of an increase in the growth rate of culture (Mathimani et al., 2017). Moreover, it was declared that the growth rate of *C. vulgaris* at 35°C exhibited a 17% decrease according to those at 30°C and further temperature caused a sharp interruption of microalgal growth and eventually cell deaths (Converti et al., 2009).

Optimization of Illumination Period

The microalgal growth was observed during different illumination conditions at 25°C and a pH value of 9. Continuously illumination (24 h light/day) of the microalgal culture provided more rapid growth and higher biomass concentration of 205 mg/L for *C. vulgaris* after 10 days in this study. Previously, it was remarked that the lipid percent and biomass productivity were higher in 18:6 h light compared to 12 h and 24 h light when keeping the other conditions constant (Ebrahimian et al., 2014). However, it was also reported that there was no significant change in the lipid content of *C. vulgaris* when the dark cycle increases from 8 h to 12 h (Luangpipat and Chisti, 2017). In another study, it was shown that the best light:dark cycle for *C. vulgaris* was 16:8 h/day (Sasi, 2009). The cell growth at optimized conditions (pH=9, 25°C and continuous illumination) was shown in Figure 1.

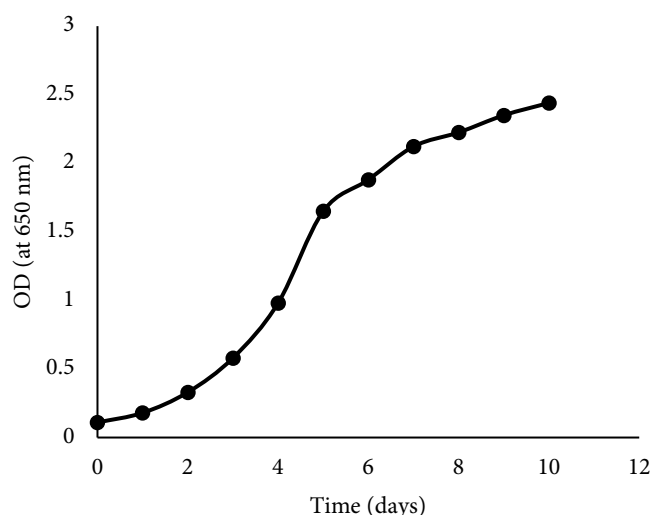


Figure 1. Cell concentration at optimized conditions

Conclusion

In this study, the best conditions with the highest biomass concentrations were determined to be pH 9, 25°C and 24 hour light for the biomass growth of *C. vulgaris* regarding a biomass

concentration of 205 mg/L. Microalgae are one of the important sources for the production of biodiesel due to they possess high oil content and there is a limitation about keeping important places of plant-based oil such as safflower, canola and peanut in the food industry. This study focused on the biomass concentration of *C. vulgaris* providing its optimum growth conditions. In the future, the utilization of microalgae as a feedstock of renewable fuels is considered to increase.

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Compliance with Ethical Standards

Authors' Contributions

Author ID designed, performed and managed statistical analyses of the study and wrote the manuscript. The author read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

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