The growth potential of single-celled marine Chlorophycean microalgae in modified saline karst water culture media

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Abstract. Chlorella sp. and Nannochloropsis sp. as two common single-celled marine Chlorophycean microalgae were cultivated in modified saline karst water culture media. The survival and growth of those two microalgae were the objectives of this study. There were three different modified nutrients in saline karst water applied for Chlorella sp. and Nannochloropsis sp. cultivation. The result shows that the highest density of Chlorella sp. was shown on the fourth day, but dropped sharply to the tenth day, and had the potential to increase at the end of the observation. The highest density of Nannochloropsis sp. was reached on the sixth day, then decreased slightly and stabilized, even showing an increase at the end of the observation. Overall, Chlorella sp. and Nannochloropsis sp. showed different adaptation patterns for survival and growth on modified saline karst water culture media.

Keywords: modified saline karst-water; nutrient; single-celled Chlorophycean

1. Introduction

Chlorella and Nannochloropsis are two single-celled marine microalgae that are highly utilized for any purpose. These microalgae are used as natural food, raw materials for health food supplements, pharmaceuticals, and biofuels. Therefore, many studies have been carried out to explore the growth potential of these microalgae in various culture media containing various nutrient sources, both commercial and those that the researchers have tried to mix. Both types of microalgae can be grown in different cultural media [1-11]. Various types of commercial culture media are always being developed on a manufacturing scale [12].

The main factors that must be considered when growing microalgae are the conditions of the cultural environment and the type of cultivation media used. Microalgae show different responses when grown in different temperature [13], pH, and salinity [14]; influenced by the concentration of nitrogen and phosphorus [15], and also alkalinity and hardness [16]; and especially for several species of Chlorella, they can grow photoautotrophically or heterotrophically under different culture conditions, with different composition of carbon source [7].

On the other hand, microalgae growing has been tried in the media with chemical or organic fertilizers, either using specific commercial fertilizers, such as Bold’s Basal Medium, Guillard F/2, Wallace, Conway, or others [4, 16, 18], technical fertilizers [19], specific organic compounds as carbon sources [20, 7, 19], organic waste [20], and fermentation products [9]. This is one of the triggers for the idea to use karst water as a nutrient source in the cultivation of Chlorella sp. and Nannochloropsis sp.

Karst water sourced from diluted calcareous ground-water contains a high concentration of nutrients
[21-23]. There is a specific and unique characteristic of Ciseeng karst water which is known as saline karst water with about 30-35 ppt of salinity value. It means that the Ciseeng saline karst water is the potential to use as a substitute culture medium for marine microalgae, such as Chlorella and Nannochloropsis.

As relatively quickly growing types [24] the experimental treatment of cultivation in the saline karst water could be applied to those microalgae. The growth of microalgae was influenced by the concentration of nitrogen and phosphorus [15]. To ensure the proper composition of macronutrients, the technical fertilizes must be added with a specific ratio. The growth pattern of the microalgae will indicate whether those microalgae can grow in modified saline karst water as a culture medium. This study aimed to study the survival and the growth of Chlorella sp. and Nannochloropsis sp. in modified saline karst-water.

2. Materials and methods
There was a field observation of the natural condition of karst water, especially minerals as the key parameters for microalgae life conditions in a culture medium. Those are hardness, chloride, bicarbonate, sulfate, natrium, calcium, magnesium, kalium, silicate, iron, orthophosphate, ammonia, nitrite, and nitrate.

Furthermore, the experiment to understand the productivity of Chlorella sp. and Nannochloropsis sp. in modified saline karst water was set as simple random in times design [25]. However, firstly, the new karst water was boiled to reduce the hardness.

There were three treatments of nutrient application in this study; Walne media as control. The application of Urea as the source of nitrogen and TSP (Triple Super Phosphate) as a source of phosphorus was set with several dosages. The dosages were set in a fixed of P concentration about 0.5 mg/L with different N concentrations in each treatment. Nitrogen was applied 4, 10, and 20 times the P concentration and Walne culture media. Those treatments terms as Dosage 1 (D1), Dosage 2 (D2), Dosage 3 (D3), and Control (C). The culture was run in 12 days.

The determination of culture density can be carried out through optical density (OD) by using colorimetry, cell count examination using a haemacytometer, or a dry weight approach [10]. In this study, the colorimetry approach was used to determine the algal cell density as applied in several studies; the determination of density using optical density shows the best result when applied to monocolture spherical cells. Furthermore, for green algae, the range of wavelength is 550-750 nm [1, 26]. However, in this study, the densities of microalgae were observed using optical density at 680 nm wavelength which showed second-best performers.

3. Result and discussion
The boiled karst water showed a lower value of minerals than the seawater, except for hardness, iron, and orthophosphate. Furthermore, after adding nutrients, the main nutrient parameters were measured; those were ammonia, nitrite, nitrate, and orthophosphate, as shown in table 1. The added nutrient composition is expected to provide an overview of the growth potential of microalgae according to the prepared N/P ratio.

The presence of microalgae during the observation showed an increase and decrease in density, which means that the microalgae can utilize the available nutrients in the culture media. The optical density shows the dynamic of the microalgae population. The densities of Chlorella and Nannochloropsis showed different patterns along with the observation (figure 1 a and b). The growth of Chlorella spp. was influenced by the concentration of nitrogen and phosphorus [15].

The result shows that the peak of Chlorella sp. in Walne medium was 1.3 x 10^7 cell/mL. The highest density of Chlorella sp. in the treatment medium was shown on the fourth day, but dropped sharply to the tenth day, and had the potential to increase at the end of the observation. The highest density of Nannochloropsis sp. was reached on the sixth day, then decreased slightly and stabilized, even showing an increase at the end of the observation. The microalgae show the capacity to uptaking the nutrients
supported in the modified saline karst water medium. Microalgae show specific behavior in nutrient uptakes, especially for nitrogen [27].

Table 1. Nutrients measured in modified saline karst water treatments.

| N/P Ratio | Chlorella sp. | Nanochloropsis sp. |
|-----------|---------------|-------------------|
| 4:1       | 0.241, 0.022, 0.739, 0.142 | 10:1, 0.252, 0.152, 1.205, 0.146 |
| 20.01     | 0.290, 0.180, 2.260, 0.140 | 0.01, 0.032, 0.781, 0.130 |
| 04.01     | 0.227, 0.032, 0.177, 0.144 | 0.01, 0.177, 1.242, 0.144 |
| 10.01     | 0.226, 0.032, 0.177, 0.144 | 0.01, 0.177, 1.242, 0.144 |
| 20.01     | 1.287, 0.232, 1.607, 0.143 | 0.01, 0.232, 1.607, 0.143 |

Figure 1. The optical densities of Chlorella and Nanochloropsis showed different pattern along the observation; a. Chlorella sp., b. Nanochloropsis sp.
As resulted in other studies, microalgae sometimes achieved high density, but in another study found in low population. For example, *Nannochloropsis oculata* showed the highest density (8.6 x 10^7 cell/mL) in the culture medium with Conway formulation and the lowest density (5.4 x 10^7 cell/mL) in medium with the combination of N_P and 25% of Conway formulation [11]. The growth of *Chlorella* sp. shows a high density of about 8.53 x 10^7 cell/mL in Guillard f/2 medium [3]. The highest density in pro-analyze fertilizer medium was reached to 2.62 x 10^7 cell/mL and 1.08 x 10^7 cell/mL in technical fertilizer medium within nine days [4]. *Chlorella ellipsoidea* shows higher density (2.0 x 10^7 cell/mL) when cultivated in the supernatant of 50% digested sugar mill effluent (DSME) on the 10th day than that cultured in Bold basal medium (BBM) with the cell number of (1.9 x 10^7 cell/mL) [6]. The highest density was achieved in a culture medium with 0.9% concentration of vermicompost with around 1.7 x 10^7 cell/mL [28]. Meanwhile, the highest density of microalgae was in the 4th day with 50 mL cassava hydrolysate’s addition medium was relatively low, about 172 661 cell/mL [9]. The growth of microalgae is supported by optimum environmental conditions and the sufficiency of nutrients [29, 30, 1].

Compare to those results, the density of *Chlorella* sp. and *Nannochloropsis* sp. that were cultivated in modified saline karst water were not as high as achieved in those many kinds of medium, except the medium with cassava hydrolysate’s addition. However, there was a unique phenomenon that at the end of observation the density of those microalgae was tent to stable or even increase. It was shown, by the fresh color of the culture of *Chlorella* sp., for example, at the end of the observation (figure 2). This raises the assumption that there is a slow release of nutrients from mineral compounds in karst water.

![Figure 2](image_url)

*Figure 2.* Culture perform at the end of observation.

Usually, in a batch culture, along with the growth of microalgae, the high density at the peak condition could be inhibited by the auto-inhibitory compounds secretion or specific nutrient depletion in the medium [5]. Sometimes, the algae take less than one day for the stationary stage [1]. Furthermore, the growth of microalgae decreases when the nutrient requirements for new membrane compounds synthesis reduces along with limiting nutrient condition [31]. This is because each membrane requires a compound with a certain determinant. Therefore, the low content of the essential elements making up the membrane-forming compounds can inhibit, even negate the membrane formation process.

Within a controlled laboratory scale, *Chlorella* sp. and *Nannochloropsis* sp. could grow and survive along with the observation. The dynamic of densities indicated the dynamic of their capacity in nutrient uptakes and also support by the capacity of karst water to release minerals slowly. This raises the desire that studies on the binding and releases abilities of minerals in karst water are very necessary to study the capacity of karst water to support nutrient availability in microalgae culture.
4. Conclusion
Overall, *Chlorella* sp. and *Nannochloropsis* sp. showed different adaptation patterns for survival and growth on modified saline karst water culture media. However, both of them had the potential to increase at the end of the observation.

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