Application of MSSIP-2 nutrient in marine phytoplankton culture to support the production of biomass for biofuel industry

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Abstract. A research on the application of MSSIP-2 nutrient in marine phytoplankton culture has been conducted to produce biomass to be used as raw material for biofuel. Marine phytoplankton was cultivated using the nutrient media and the growth rates were studied by measuring the cell solidity at various growth times. Seven phytoplanktons; Isochrysis aff galbana, Spirulina sp., Thalassiosira sp., and Nitzchia sp., Chlorella vulgaris, Chaetoceros calcitrans and Isochrysis tahiti were used in the research. The experimental temperature, salinity, and pH of the media were measured and the specific growth rates of phytoplanktons were determined using the first order rate equation. Results showed that the highest specific growth rate was given by Chlorella vulgaris (0.0322 cells/hour) and the lowest one was by Thalassiosira sp. (0.0277 cells/hour). The highest biomass weight was obtained from Isochrysis aff galbana (0.329 g), whereas the lowest one (0.27 g) was from Nitzchia sp. The carbohydrate content was various, the highest content was 34.07% found in Isochrysis aff galbana and the lowest was 28.16% in Thalassiosira sp.

1. Introduction
Fossil fuels (coal and oil) become a primary energy nowadays. Their contribution as a source of energy is around 80% where more than 50% of the total energy was for transport sector [1]. The fossil fuel is non-renewable resulting in the drastically decrease of its sources. This kind of fuel can produce harmful gases that have a negative impact. Furthermore, the high use of the fossil fuel can affect the global economic activities due to the increase in the prices of crude oil. The high-speed modern world travels by both industrialization and motorization and it is being the main cause for the unpredictable fuel demand [2]. Therefore, many efforts have conducted in order to find alternative energy sources which are renewable. Biofuels are alternative energy sources that are renewable and can be produced from biomass. Researchers are constantly trying to find the biofuel production from the sustainable biomass since it is being an efficient alternative to replace non-renewable fuels [3].

There are several advantages of using biofuels compared to petroleum fuels. It is easy to extract them from the biomass obtained from various sources, they have biodegradable property for the sustainability purpose, its combustion based on carbon-dioxide cycle, and they are more environment-friendly. The use of biofuel in automobile market will enhance rapidly for the next decade because of...
its environmental merits. Therefore, the strong growth in the agricultural sector for more production and associated by-products will occur [4,5].

Biofuels produced mainly from biomass can be in the form of solid, liquid and gaseous fuels. They are classified into first, second and third generations depending on the chemical composition and the natural complexity of the biomass. The first one, biodiesel and vegetable oils have been produced from the crop plants and the second, bioethanol and biohydrogen have been produced from agricultural by-products and energy plants which requires fertile lands for growth. The sources of the third generation which are attractive sources are marine algae [5,6].

It has been reported that biomass is one of the most important energy sources of the world [7]. It can also be considered as the natural and inexpensive source of energy and that energy could be utilized at any time [8,9]. The biomass was usually generated from major agricultural crops. However, the use of agricultural crops as resources of biofuels can compete with the need of food. Production of biofuels predominantly dependent on terrestrial plants, which have the limitation of cultivable land exploitation, marine biomass include seaweeds are new sources of biofuel feedstock produces the highest percentage of biomass productivity in lesser time and therefore such bioresources can be exploited for renewable biomass-based energy production. The various terrestrial and marine resources for sustainable and renewable resources for biofuel production can be used. The photosynthesis fuel has been initiated as another blooming field of biofuels. The plants and algae effectively utilize the atmospheric CO₂ and storing the energy as biomass which is converted into any form of energy in reverse. The photosynthetic microbe cyanobacteria have the ability to convert the CO₂ directly into ethylene a fuel chemical without production of biomass.

Biofuel (bioethanol dan biodiesel) become expectation sources that can open the new work field, help to decrease the number of poor people and to protect the damage of forestry as well as the lack of food material. Therefore, the effort to find biofuel sources have to be focused to the marine resources of Indonesia because 2/3 part of Indonesia is watered with the longest beach line, i.e. ± 80 791.42 km, having microscopic unit cell plants called phytoplankton about 35,000 species [10]. Utilization of phytoplankton for biofuel is environmentally friendly. Another advantage of the source is that phytoplankton can absorb CO₂ and convert it to oxygen gas. About 90% of phytoplankton’s dried weight can absorb CO₂ that can decrease the gas to 1000 tonne/ha/year.

To produce biofuel, materials containing carbohydrate are required. Phytoplankton can be one of the sources because it can contain 29 – 30% carbohydrate that can be converted to bioethanol. In addition, it can also be a source of biodiesel because of its lipid content, i.e. 21% [10]. To produce 1 liter of bioethanol, it required about 6.5 kg of cassava. However, with the same amount of phytoplankton, bioethanol can be produced 100 times because harvesting can be conducted once in 7-10 days.

Besides the light, the phytoplankton growth also depends on the availability of nutrient for its growth. In this research, the excellent nutrient called MSSIP-2 was used to cultivate phytoplankton under investigation.

2. Experimental

2.1. Materials

Materials used was MSSIP-2 (Muh. Syahrul, Syahruddin Kasim, Indah Raya and Paulina Taba) consisting of sodium chloride (Merck), MgSO₄·7H₂O (Merck), KNO₃ (Merck), KH₂PO₄ (Merck), CaCl₂·6H₂O (Merck), H₂BO₃ (Merck), ZnSO₄·7H₂O (Merck), MnSO₄·4H₂O (Merck), CuSO₄·5H₂O (Merck), CoCl₂·6H₂O (Merck), (NH₄)₂MoO₄·2H₂O, NaFeEDTA (Merck), NaSiO₃·9H₂O (Merck), Mixture of vitamin, HCl (Merck), NaOH (Merck), Marine product waste, organic waste mixture, urea, ZA and KCl fertilizer, 1M Fe²⁺ ion, 1M Ni³⁺ ion, and distilled water.

2.2. Instruments

Instruments used were haemocytometer, High-Performance Liquid Chromatography (HPLC).
2.3. Procedure

2.3.1. Preparation of MSSIP-2 Nutrient. The MSSIP-2 nutrient was prepared using components listed in table 1.

| Components                   | Concentrations (ppm) | Components                   | Concentrations (ppm) |
|------------------------------|----------------------|------------------------------|----------------------|
| NaCl                         | 250                  | NaFeEDTA                     | 25                   |
| MgSO₄·7H₂O                   | 5                    | NaSiO₃·9H₂O                  | 10                   |
| KNO₃                        | 2 (3 mL)             | Mixture of Vitamin           | 1                    |
| KH₂PO₄                      | 5                    | HCl                          | 50                   |
| CaCl₂·6H₂O                   | 5 (10 mL)            | NaOH                         | 60                   |
| H₃BO₃                       | 200                  | Marine product waste         | 3 mL                 |
| ZnSO₄·7H₂O                   | 1                    | Mixture of organic waste     | 3 mL                 |
| MnSO₄·4H₂O                   | 1                    | Urea, ZA, and KCl            | 3 g each             |
| CuSO₄·5H₂O                   | 0.1                  | Fe²⁺ (1M)                    | 3 mL each            |
| CoCl₂·6H₂O                   | 0.2                  | Ni²⁺ (1M)                    | 1 mL each            |
| (NH₄)₆Mo₇O₂₄·4H₂O            | 0.2                  |                              |                      |

All components were dissolved in distilled water up to a total volume of 1000 mL as nutrient stock for culture, its use was 1% - 20% depending on the culture volume.

2.3.2. Cultivation of marine phytoplankton and analysis of density. Phytoplanktons used in this study were Isochrysis aff galbana, Spirulina sp., Thalassiosira sp., and Nitzchia sp., Chlorella vulgaris, Chaetoceros calcitrans and Isochrysis tahiti provided by the Laboratory of Plankton, Shrimp Seeds Centre, Maros. Phytoplankton was cultured in a medium with superior nutrient, then put into a 100 mL Erlenmeyer. After 4 days, the culture was transferred to a 500 mL bottle. During the cultivation, the physicochemical parameters were maintained including lighting of 40 watt with TL lamps continuously, CO₂ gas aeration, temperatures of between 25-27°C, sea medium pH of between 8.3-9.0; and medium salinity for the optimum condition of phytoplankton species. All equipment and materials used in culture were sterilized. To find out the growth pattern of phytoplankton, calculation of cell count per mL of medium was observed every 12 hours with a haemositometer using a microscope.

At this culture, the specific growth was calculated using equation (1):

\[ \mu = \frac{\ln N_t - \ln N_0}{t} \]  

\( N_t = \) cell population density at the time of \( t \) (cell mL⁻¹)
\( N_0 = \) cell population density at the early time (cell mL⁻¹)
\( \mu = \) fixed flow of specific growth (h⁻¹)
\( t = \) time (h).

2.3.3. Culturing biomass of chosen phytoplankton and determination of the biomass Weight. Based on physical chemistry properties, the parameters affecting the growth of phytoplankton in the field were measured in situ, a great amount of phytoplankton chosen was cultivated in the 60 L aquarium. After the optimal density of phytoplankton obtained, 1 L of the culture was taken to determine its biomass by using gravimetric method through weighing with suitable filter paper. The light and aeration were stopped for about 3 days followed by separation of phytoplankton from water by sedimentation.

2.3.4. Analysis of the carbohydrate content in the chosen phytoplankton. The carbohydrate and monosaccharide content was analyzed using HPLC following a procedure developed by Mannheim,
1987. The total amount of 4 grams biomass of selected marine phytoplankton species was inserted into a 250 mL measuring flask, then added with 40 ml doubly distilled water + 2 mL of TCA 3M, and left for 10 minutes, neutralized with 1 M NaOH, added with 200 mL of doubly distilled water, filtered with Whatman no. 1 followed by syringe 0.45 μm. The clear filter solution was used for HPLC measurement. The sample solution was inserted into the venojek which was first sterilized, then sealed tightly and immediately analyzed by HPLC using an Aminex HPX-87H column. All the data obtained were then analyzed quantitatively by comparing the chromatogram results with the standard chromatogram.

3. Results and discussion

3.1. Physico-chemical parameter of various types of marine phytoplankton

Physico-chemical parameters in the research location were taken in-situ, then combined with culture parameters such as lighting, aeration rate blower for the CO₂ gas pump, and initial density of marine phytoplankton. The result is given in Table 2.

| Phytoplanktons       | Parameters of culture growth of marine phytoplankton |
|----------------------|-------------------------------------------------------|
|                      | Salinity (%o) | pH | Temperature (°C) | Lighting | Aeration | Initial density (cell/mL) |
| Isochrysis aff galbana | 30           | 8-9 | 27-30 TL lamp, 40 watt | Air     | 30,000    |
| Spirulina sp.        | 30           | 8-9 | 28-30 TL lamp, 40 watt | Air     | 30,000    |
| Thalassiosira sp.    | 30           | 7-9 | 29-30 TL lamp, 40 watt | Air     | 30,000    |
| Nitzchia sp.         | 30           | 8-9 | 27-30 TL lamp, 40 watt | Air     | 25,000    |
| Chlorella vulgaris   | 30           | 7-9 | 28-30 TL lamp, 40 watt | Air     | 30,000    |
| Chaetoceros calcitran | 33           | 8-9 | 29-30 TL lamp, 40 watt | Air     | 25,000    |
| Isochrysisi tahiti   | 33           | 8-9 | 28-30 Sda | Air     | 30,000    |

3.2. Cell Density and the specific growth rate of Phytoplanktons

After cultivation using MSSIP-2 nutrient, the density of phytoplankton was determined using a haemacytometer. The results are given in Table 3.

The cell density of samples of marine phytoplankton as a function of the growth time is given in Figure 1.

It is clear that the cell density of marine phytoplankton generally increases with the increase of the cultivation time until achieving the maximum. All species of phytoplankton show similar growth patterns consisting of 3 stages, i.e. adaptation phase, exponential phase and stationary phase. The optimum density was achieved at 8 – 10 days. After the optimum growth of phytoplankton, the cell density decreases. The lowest cell density at the optimum growth time is given by Nitzia sp. (1.18 x 10⁷ cells/mL) and the highest cell density is shown by Spirulina sp (2.029 x 10⁷ cells/mL). The specific density rate of the marine phytoplankton is given in Table 4.
Table 3. Cell density of phytoplankton.

| No | Types of phytoplankton | Growth density (x 10^4 cell/mL) |
|----|------------------------|----------------------------------|
| 1  | Isochrysis aff galbana | 3.0 37 94 217 587 904 1221 1305 1449 1415 1360 1206 1079 918 |
| 2  | Spirulina sp.           | 3.0 39 96 207 453 881 1308 1565 1813 2029 1978 1619 1479 1315 |
| 3  | Thalassiosira sp.       | 3.0 18 58 93 262 672 911 1019 1110 1186 1020 969 811 737 |
| 4  | Nitzchia sp.           | 2.5 21 106 243 333 523 819 1045 1097 1180 1128 930 721 619 |
| 5  | Chlorella vulgaris      | 3.0 23 104 317 782 994 1185 1370 1293 1102 1008 896 672 415 |
| 6  | Chaetoceros Calcitrans | 2.5 15 37 90 263 598 962 1209 1152 835 703 514 472 395 |
| 7  | Isochrysis tahiti       | 3.0 18 89 237 562 803 1048 1196 1265 1149 1027 831 650 317 |

Figure 1. Growth density rate of 7 types of marine phytoplankton.

Table 4. The specific growth density rate of marine phytoplankton.

| Species of marine phytoplankton | The optimum growth time (day) | Cell density | \( \mu (\text{hour}^{-1}) \) |
|---------------------------------|-------------------------------|--------------|-----------------|
| Isochrysis aff galbana          | 9                             | 3 \times 10^4 | 1.448 \times 10^7 | 0.0321 |
| Spirulina sp.                   | 10                            | 3 \times 10^4 | 2.029 \times 10^7 | 0.0302 |
| Thalassiosira sp.               | 10                            | 3 \times 10^4 | 1.186 \times 10^7 | 0.0277 |
| Nitzchia sp.                    | 10                            | 2.5 \times 10^4 | 1.180 \times 10^7 | 0.0284 |
| Chlorella vulgaris              | 8                             | 3 \times 10^4 | 1.370 \times 10^7 | 0.0322 |
| Chaetoceros Calcitrans         | 8                             | 2.5 \times 10^4 | 1.209 \times 10^7 | 0.0365 |
| Isochrysis tahiti               | 9                             | 3 \times 10^4 | 1.265 \times 10^7 | 0.0315 |

It is obvious that the lowest specific growth rate is given by Thalassiosira sp, whereas the highest one is by C. vulgaris.

3.3. Biomass of marine phytoplankton
Table 5 shows the dried weight of the phytoplankton biomass under investigation.
Table 5. The dry weight of phytoplankton biomass.

| No. | Types of Phytoplankton | Biomass dry weight (mg L\(^{-1}\)) |
|-----|------------------------|-----------------------------------|
| 1.  | I. aff galbana          | 0.329                             |
| 2.  | Spirulina sp.           | 0.325                             |
| 3.  | Thalassiostra sp.       | 0.315                             |
| 4.  | Nitzchia sp.            | 0.270                             |
| 5.  | C. calcretans           | 0.285                             |
| 6.  | C. vulgaris             | 0.320                             |
| 7.  | I. tahiti               | 0.299                             |

From the data in table 5, it can be concluded that the highest biomass obtained from I. aff galbana, whereas the lowest one is from Nitzchia sp.

3.4. Carbohydrate content of marine phytoplankton

The carbohydrate content of various types of marine phytoplankton is given in table 6.

Table 6. Carbohydrate content in phytoplankton.

| No. | Types of Phytoplankton | Carbohydrate content (%w/w) |
|-----|------------------------|-----------------------------|
| 1.  | I. aff galbana         | 33.98                       |
| 2.  | Spirulina sp.          | 32.85                       |
| 3.  | Thalassiostra sp.      | 28.16                       |
| 4.  | Nitzchia sp.           | 29.74                       |
| 5.  | C. calcretans          | 32.63                       |
| 6.  | C. vulgaris            | 34.07                       |
| 7.  | I. tahiti              | 33.78                       |

All types of phytoplankton cultivated in the MSSIP-2 nutrient contain high carbohydrate (28.16 – 33.98 %w/w). There are several factors affected the biochemical content including carbohydrate content [11] such as the intensity of light [12], the composition of the species [13] and growth steps [14]. However, the most factor affected the biochemical content were nutrient availability especially nitrogen source [15,16]. From the data of the carbohydrate content in this study, it can be said that MSSIP-2 is a good nutrient for growing phytoplankton which are potential to be used in producing biofuel.

4. Conclusion

The MSSIP-2 nutrient has good ability to grow all types of phytoplankton used in the study. The optimum growth time of all types of phytoplankton is in the range of 8 – 10 days. The highest biomass produced is given by I. aff galbana with the carbohydrate content of 33.98 %w/w. The lowest biomass is produced by Nitzia sp with the carbohydrate content of 29.74 %w/w.

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