Study on the medium effect for the growth rate of Spirulina, *Arthrospira platensis* in natural seawater

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Abstract. *Spirulina, Arthrospira platensis*, is the most important microalgae for the production of biomass as health food for human and animal food. In this experiment, the growth rates of *A. platensis* were tested at seven different media (F-2 with CaCO$_3$, F-2 with NaHCO$_3$, Z-1 with CaCO$_3$, Z-1 with NaHCO$_3$, Z-2 with NaHCO$_3$, urea and T-super with NaHCO$_3$, and PES with NaHCO$_3$) at pH 9 to 10 and salinity 30‰. The culture was started at the optical density (OD) 0.20 and the experimental period was ended after 10 days in the laboratory room. Among the media urea and T-super with sodium bicarbonate were recorded the best. The optimum growth of *A. platensis* (OD 0.69) was observed in urea and T-super with sodium bicarbonate. In addition, urea and T-super with sodium bicarbonate gave rise to the maximum growth of *A. platensis* and then followed by Z-1 medium with NaHCO$_3$ and PES medium with NaHCO$_3$. The minimum OD 0.44 was recorded in F-2 medium by using CaCO$_3$. The results of present investigation revealed the potential use of seawater with some nutrients for laboratory culture of *A. platensis*.

1. Introduction

*Spirulina, A. platensis*, was the dominance of life on earth for more than 3.5 billion years ago. The name “Spirulina” is derived from the Latin word for “helix” or “spiral” denoting the physical configuration of the organism when it forms swirling, microscopic stands [1].

*Spirulina* is not a nitrogen-fixing cyanobacteria (blue-green algae) [2]. It has a long history of safe human consumption, known to be safe and nutritious. It can play an important role in human and animal nutrition, environmental protection through wastewater recycling and energy conservation [2, 3]. *Spirulina* is rich in proteins (60-70%), vitamins and minerals used as protein supplement in diets of undernourished poor children in developing countries [2]. One gram of *Spirulina* protein is equivalent to one kilogram of assorted vegetables [4].

The length of cell is 125-225 μm and the cell’s width is 25-30 μm. Ends of trichomes are not or very slightly attenuated, terminal cells broadly rounded. Cells of trichomes are 10-12 μm in diameter and 2-5 μm long. The filaments are motile, gliding along their axis and have no heterocyst. Reproduction is accomplished by fragmentation of a mature trichome into many shorter segments through destruction of specialized multiple intercalary cells.

There are about 1.386 x 10$^{12}$ (one trillion three hundred eighty-six billion) cubic meters of water on Earth. About 97% of it is in the oceans and seas. Of the remaining water, about 3% is freshwater. Nearly 70% of that freshwater is frozen in icecaps of Antarctica and Greenland. Most of the remainder is present as soil moisture or lies in deep underground aquifers not accessible to human use. Of the 1%
freshwater, agriculture (including irrigation) uses 70%, industry 20% and other 10% including drinking water for 7 billion people. In the year 2025 at least 2.5 billion people won’t have enough water to drink [5]. So, we will have to use seawater for agriculture.

Spirulina media contain generally high concentrations of nutrients that impact their cost [6]. The cost of nutrients is considered to be a major factor for A. platensis biomass production [7]. The advantages of A. platensis production in seawater medium are: (i) lower fertilizer cost; (ii) saving farmland by using waste sea beach; (iii) seawater culture is not easily polluted by heavy metals and contaminations. The utilization of seawater media for the cultivation of A. platensis will be reduced the production cost considerably [8].

High pH and temperature are the key factors for large scale A. platensis culture indoors. The optimum temperature for A. platensis culture is in the range of (28-33°C) [9]. A. platensis is a cyanobacterium growing at elevated pH. In addition A. platensis requires relatively high pH values (9.5 to 11.0 with an optimum at 10.5) and is, therefore, less subject to contaminations [10]. A. platensis is the edible microorganisms which grow naturally in tropical regions inhabiting alkaline lakes containing sodium carbonate or sodium bicarbonate other materials and a source of fixed nitrogen of tropical and subtropical regions. These lakes are found near volcanoes or even within the caldera of the volcano [11].

In nature lakes of the world, the limited supply of nutrients usually regulates growth cycles. New nutrients come from an upwelling from inside the earth, when rains wash soils into the lakes, or from pollution. The alga grows rapidly and reaches a maximum density, and then dies off when nutrients are exhausted. A new seasonal cycle begins when decomposed algae release their nutrients or when more nutrients flow into the lake [12, 13]. Zarrouk (1966) introduced the famous alkaline medium to enable the analysis of natural habitat [14]. The nutrient source for A. platensis is nitrate. However, Stanca and Popovici [5] proved that the use of urea as source of nutrient was increased the A. platensis biomass production.

Spirulina was known to grow naturally in the Lake Bodu (Chard, Africa), Lake Chad (Chard, Africa), Lake Johann (Chard, Africa), Lake Rombu (Chard, Africa), Lake Aranguadi (Chard, Africa), Lake Elementia (Ethiopia, Africa), Lake Nakuru (Kenya, Africa) Lake Rudolf (Kenya, Africa), Lake Taung Pyauk (Myanmar, Asia), Lake Twyn Ma (Myanmar, Asia), Lake Twyn Taung (Myanmar, Asia), Lake Ye Kharr (Myanmar, Asia), Lake Chenghai (China, Asia), Lake Texcoco (Mexico, North America) and Lake Buccacina (Peru, South America [15]). This present study aims to observe the best media for A. platensis culture and to provide good ideas for mass cultivation in natural seawater and to give knowledge local people to cultivate A. platensis for domestic economy.

2. Materials and methods
A. platensis cultivation was studied in the laboratory of Marine Science Department of Pathein University. The strain which has 20 % of salinity and 10.3 of pH value was collected from Yay Hkar Innn (Lat. 22° 02.598’ Nand Long. 95° 54.545’ E) in Sagaing Township, Sagaing Region. In the laboratory, the stock algal culture was maintained in one litter plastic bottles using urea and T-super. The culture bottles were placed on the shelf and illuminated with 40-watt fluorescent lamps. For the culture experiments, the natural seawater was collected from Ngwe Saung or Chaung Tha coast. Seawater was boiled and then filtered with Whatman No. 540 filter paper. During the experimental period, the laboratory apparatus (pipettes, conical flasks, beakers, drinking plastic bottles), refractometer, pH meter, aerators, digital balance, and digital photo colorimeter was used for culture. A. platensis were cultivated in three different pH values (9.0, 9.5 and 10.0) which were adjusted by using calcium carbonate and sodium bicarbonate with modified F-2 medium (Table 1), two of modified Zarrouk’s media [14]; Z-1 medium (Table 2) and Z-2 medium (Table 3), Provasoli’s enriched seawater (PES) medium [6] (Table 4) and urea and T-super medium. The nutrient urea and T-super medium was prepared as the ratio 5:1 that 10 g of urea and 2 g of T-super were separately solute with each one litre of distil water which 10 ml of each nutrient solution was daily feed into the culture bottles. Other nutrients were fed once when the culture was started.
Table 1. Composition of modified F-2 medium.

| Nutrient Elements       | g/L of seawater |
|-------------------------|-----------------|
| NaNO₃                   | 0.075           |
| NaH₂PO₄.2H₂O            | 0.005           |
| Na₂SiO₃.9H₂O            | 0.030           |
| Thiamine HCl (B₁)       | 0.0001          |
| Biotin (B₆)             | 0.0000005       |
| Vitamin (B₁₂)           | 0.0000005       |

Table 2. Composition of modified Zarrouk’s (Z-1) medium.

| Nutrient Elements       | g/L of seawater |
|-------------------------|-----------------|
| K₂SO₄                   | 1.148           |
| NaNO₃                   | 2.500           |
| NaH₂PO₄                 | 0.344           |
| KOH                     | 0.227           |
| Na₂EDTA                 | 0.080           |

Table 3. Composition of modified Zarrouk’s (Z-2) medium.

| Nutrient Elements       | g/L of seawater |
|-------------------------|-----------------|
| K₂SO₄                   | 1.148           |
| NaNO₃                   | 2.400           |
| NaH₂PO₄                 | 0.344           |
| KOH                     | 0.227           |
| Mg(NO₃)₂.6H₂O           | 0.192           |
| Na₂EDTA                 | 0.080           |

Table 4. Composition of Provasoli’s Enriched Seawater Medium (PES Medium).

| Nutrient Elements       | g/L                      |
|-------------------------|--------------------------|
| NaNO₃                   | 0.7                      |
| NaH₂PO₄. 6H₂O           | 0.1                      |
| Fe (EDTA: 1:1 molar)*   | 0.05                     |
| P II metal**            | 0.05                     |
| Tris Buffer             | 0.001                    |
| Vitamin B₁₂             | 0.00002                  |
| Biotin (B₆)             | 0.00001                  |
| Thiamine HCl (B₁)       | 0.000001                 |

* Fe (EDTA: 1:1 molar) Amount

distilled water 500 mL
Fe(NH₄)₂(SiO₄)₂.6H₂O 0.351 g
Na₂EDTA 0.3 g

** P II metal solution Amount

distilled water 100 mL
H₃BO₃ 114.0 mg
Na₂EDTA 100 mg
MnSO₄.4H₂O 16.4 mg
FeCl₂.6H₂O 4.9 mg
ZnSO₄.7H₂O 2.2 mg
COSO₄.7H₂O 0.48 mg
3. Results

3.1. Effects of F-2 medium with CaCO$_3$

Growth rates of *A. platensis* in different pH values with F-2 medium were illustrated in Figure 1. Increasing or decreasing cell densities were found within age for all different pH values at salinity 30% of initial Optical Density (OD) 0.2 by using F-2 medium with CaCO$_3$.

In pH value 9.0, the growth was gradually increased within the experiment and then gradually reduced to OD 0.31 at the end of the experiment. The maximum OD 0.40 was reached 4 or 5 days of the experiment and the color was yellowish-green.

In pH value 9.5, the growth was gradually increased within the experiment for six days and then gradually reduced to OD 0.33 at the end of the experiment. The maximum OD 0.41 was obtained on 6$^{th}$ day of the experimental period. The color was pale-green at the end of the culture.

In pH value 10.0, the growth was gradually increased within the experiment for six days and then gradually reduced after to reach the maximum point of OD 0.44. It was reduced to nearly double time of the initial OD 0.20 at the experimental period end.

In this experiment, the maximum growths were occurred on days 5 and 6. Doubling times occurred on day 5 in all pH. The reducing time was found on day 7 in pH 9.5 and 10, and day 6 in pH 9.0. If the pH values were increased, the growth of *A. platensis* was good. This result suggested that *A. platensis* preferred to grow in high pH values and the best pH was 10.

![Figure 1. Comparison of the growth of *A. platensis* in three different pH values by using F-2 medium with CaCO$_3$.](image)

3.2. Effects of F-2 medium with NaHCO$_3$

Growth rates of *A. platensis* in F-2 medium with NaHCO$_3$ at different pH values were illustrated in Figure 2. Cell densities both increased and decreased within age for all different pH values at salinity 30% with initial OD 0.20 in F-2 medium with NaHCO$_3$.

The cells were gradually grown for seven days in pH value 9.0 and nearly steadied at maximum OD 0.40 and then slowly reduced to OD 0.32.

The growth of *A. platensis* was gradually increased in pH 9.5 and steadied at 6$^{th}$ and 7$^{th}$ days, and then slowly reduced at the end of the experiment. In pH 9.5, the maximum OD was 0.43 and the color was pale-green.

The *A. platensis* growth was increased within the experiment of pH 10.0 for eight days and then slowly reduced in last two days. Double time of cell growth was observed within 4$^{th}$ day. The culture of *A. platensis* was light-green in color and the maximum OD was 0.47.

In this medium, the maximum growth rates were found on 7$^{th}$ and 8$^{th}$ days. Doubling times of cell growth occurred on day 7 in pH 9.0 and day 6 in another pH. The reducing time occurred on 8$^{th}$ day in pH 9.0 and on 9$^{th}$ day in other pH. If the pH value was increased, the growth of *A. platensis* was good and the best pH was 10.0. This result suggested that *A. platensis* preferred to survive in high pH values.
3.3. Effects of Z-1 medium with CaCO₃
Growth rates of A. platensis in Z-1 medium with CaCO₃ at different pH values were illustrated in Figure 3. Increasing or decreasing cell densities were found within age for all different pH values at salinity 30% of initial OD 0.20 in Z-1 medium with CaCO₃.

In this medium, the best growth rate (OD 0.54) was observed in pH value 9.5. The cells were gradually grown for one week and then rapidly reduced after the nutritional discomfort. Over doubling, times occurred in all pH values. The reducing date occurred on 9th day in pH 9 and others on 8th day. According to this result, A. platensis do not prefer higher and lower pH values to grow.

3.4. Effects of Z-1 medium with NaHCO₃
Growth rates of A. platensis in Z-1 medium with NaHCO₃ at different pH were shown in Figure 4. Cell densities increased with age for all pH values with initial optical density 0.20 in Z-1 medium with NaHCO₃.

The growth of A. platensis was gradually increased in all pH using Z-1 medium with NaHCO₃. Cell decreasing was occurred only in pH 9.0 on day 7. The best growth rates in each pH value were OD 0.55, OD 0.57 and OD 0.62 respectively which were found at the end day of the culture. Over doubling, times were observed on 5th day in all pH values. Tripling time (OD 0.6) was found on 9th day in only pH 10. In this medium, A. platensis grew up until the culture end. The color was dark-green at the end of the culture. This experiment suggested that A. platensis preferred to survive in high pH values.
3.5. Effects of Z-2 medium with NaHCO₃

Growth rates of *A. platensis* in Z-2 medium with NaHCO₃ at different pH values were as shown in Figure 5. Cell densities increased with age for all different pH values at salinity 30‰ with initial optical density 0.20 in Z-2 medium. The culture of *A. platensis* was dark-green in color at the end of the experiment.

For three different pH tested, pH 10 in Z-2 medium with NaHCO₃ was found doubling time superior to other pH values (pH 9.0 and pH 9.5). The doubling times were observed on 4th day of the experimental period in pH 10.0 and 5th day in other pH. The maximum OD 0.61 was reached in pH 10.0 within 10 days of the experimental period.

In this experiment, the reducing time did not occur during the culture period. The growth increased and doubling time was observed faster with increasing pH. From this result, pH 10.0 was suitable for production of *A. platensis* in small volume culture.

3.6. Effects of Urea and T-super medium with NaHCO₃

Growth rates of *A. platensis* in urea and T-super medium with NaHCO₃ at different pH values were as shown in Figure 6. Cell densities increased with age for all different pH values with initial optical density 0.20 in urea and T-super medium. The culture of *A. platensis* was dark-green in color at the end of the experiment.

In pH value 9.0, the growth of *A. platensis* was gradually increased and maximum point of OD was three times the initial. The culture of *A. platensis* was dark-green in color.
In pH value 9.5, *A. platensis* growth was gradually increased within the experiment and nearly double time was observed 4\textsuperscript{th} day of the experiment. The maximum OD 0.67 was reached within 10 days of the experiment. The color was dark-green at the end of the culture.

In pH value 10.0, the growth of *A. platensis* was gradually increased and over doubling time was observed 5\textsuperscript{th} day of the experiment. The color was dark-green and the maximum OD 0.69 was reached within 10 days of the experiment.

In this experiment, *A. platensis* grew up the culture end. Over doubling times started from day 5. If the pH value was increased, the growth of *A. platensis* was also increased. This experiment suggested that *A. platensis* may be to survive in high pH values.

![Figure 6. Comparison of the growth of *A. platensis* in three different pH values by using Urea and T-super medium with NaHCO\textsubscript{3}.](image)

### 3.7. Effects of PES medium with NaHCO\textsubscript{3}

Growth rates of *A. platensis* in PES medium with NaHCO\textsubscript{3} at different pH values were as shown in Figure 7. Cell densities increased with age for all different pH values at salinity 30‰ with initial OD 0.20 PES medium. The culture of *A. platensis* was dark-green in color at the end of the experiment.

In this culture, the growth of *A. platensis* was gradually increased to maximum points of OD which were OD 0.56 in pH 9.0, OD 0.62 in pH 9.5 and OD 0.61 in pH 10.0. The doubling times were occurred at 5\textsuperscript{th} day in all pH tested except in pH 9.0.

During this experiment, there was no found cell growth reduced within the culture period. This result suggests that *A. platensis* survive in high pH values. If the pH value was increased, the growth of *A. platensis* was good.

![Figure 7. Comparison of the growth of *A. platensis* in three different pH values by using PES medium with NaHCO\textsubscript{3}.](image)
### Table 5. List of nutrient composition used in the experimental culture.

| Nutrients       | F-2 with CaCO₃ | F-2 with NaHCO₃ | Z-1 with CaCO₃ | Z-1 with NaHCO₃ | Z-2 with NaHCO₃ | Urea and T-super with NaHCO₃ | PES with NaHCO₃ |
|-----------------|----------------|-----------------|----------------|----------------|-----------------|-----------------------------|----------------|
| CaCO₃           | +              | -               | +              | -              | -               | -                           | -              |
| NaHCO₃          | -              | +               | -              | +              | +               | +                           | +              |
| NaNO₃           | +              | +               | +              | +              | -               | -                           | -              |
| NaH₂PO₄·2H₂O    | +              | +               | +              | +              | -               | -                           | -              |
| Na₂SiO₃·9H₂O    | +              | +               | -              | -              | -               | -                           | -              |
| Thiamine HCl (B₁) | +            | +               | -              | -              | -               | -                           | +              |
| Biotin (B₆)     | +              | +               | -              | -              | -               | -                           | +              |
| Vitamin (B₁₂)   | +              | +               | -              | -              | -               | -                           | +              |
| K₂SO₄           | -              | -               | +              | +              | +               | +                           | -              |
| Mg(NO₃)₂·6H₂O   | -              | -               | -              | +              | +               | +                           | -              |
| KOH             | -              | -               | +              | +              | +               | +                           | -              |
| Urea            | -              | -               | -              | -              | +               | -                           | +              |
| Triple superphosphate | -         | -               | -              | -              | +               | -                           | -              |
| Sodium          | -              | -               | -              | -              | -               | +                           | -              |
| glycophosphate 6H₂O | -          | -               | -              | -              | -               | +                           | -              |
| Tris Buffer     | -              | -               | -              | -              | -               | -                           | +              |
| Fe(NH₄)₂(SiO₃)₂·6H₂O | -          | -               | -              | -              | -               | +                           | -              |
| H₃BO₃          | -              | -               | -              | -              | -               | -                           | +              |
| Na₂EDTA        | -              | -               | +              | +              | +               | -                           | +              |
| MnSO₄·4H₂O      | -              | -               | -              | -              | -               | -                           | +              |
| FeCl₂·6H₂O      | -              | -               | -              | -              | -               | -                           | +              |
| ZnSO₄·7H₂O      | -              | -               | -              | -              | -               | -                           | +              |
| CoSO₄·7H₂O      | -              | -               | -              | -              | -               | -                           | +              |
| Symbols: + = presence; - = absence |

**Figure 8.** Comparison of the growth of *A. platensis* in various nutrients with pH (9.0, 9.5, 10.0) of salinity 30‰ at the end of the culture.

The effect of media and pH on growth of *A. platensis* at the end of the culture was shown in Figure 8. *A. platensis* survived in various pH and media. The highest growth was found at pH 10 and urea and T-super medium. Therefore, the yield of *A. platensis* is influenced by pH and medium.

Richmond and Grobbelaar [10] observed that the optimal pH for the growth of *A. platensis* was 10.5. Mahadevaswamy [16] said that the yield of *A. platensis* could decrease when pH values were decreased.

Florian et al. [17] and Yean-Chang C [18] reported that the best medium to reduce the cultural cost and increase the biomass of *A. platensis* cultures was modified by Zarrouk’s medium. Dineshkumar et
al. [19] stated the highest yields of *A. platensis* culture by using Zarrouk’s medium and F-2 medium were found on 30th day and 21st day as 1.86 dw/L and 0.52 dw/L, respectively.

Table 6. The growth of *A. platensis* in various nutrients with pH (9.0, 9.5, 10.0) at salinity 30‰.

| Media                      | pH    | Days |          |          |          |
|----------------------------|-------|------|----------|----------|----------|
|                            |       | 8    | 9        | 10       |          |
| F-2 with CaCO₃             | 9.0   | OD 0.34 | OD 0.34 | OD 0.31 |          |
|                            | 9.5   | OD 0.36 | OD 0.35 | OD 0.33 |          |
|                            | 10.0  | OD 0.41 | OD 0.38 | OD 0.37 |          |
| F-2 with NaHCO₃            | 9.0   | OD 0.39 | OD 0.36 | OD 0.32 |          |
|                            | 9.5   | OD 0.43 | OD 0.41 | OD 0.38 |          |
|                            | 10.0  | OD 0.47 | OD 0.45 | OD 0.44 |          |
| Z-1 with CaCO₃             | 9.0   | OD 0.51 | OD 0.49 | OD 0.43 |          |
|                            | 9.5   | OD 0.51 | OD 0.47 | OD 0.46 |          |
|                            | 10.0  | OD 0.52 | OD 0.48 | OD 0.45 |          |
| Z-1 with NaHCO₃            | 9.0   | OD 0.48 | OD 0.52 | OD 0.55 |          |
|                            | 9.5   | OD 0.51 | OD 0.54 | OD 0.57 |          |
|                            | 10.0  | OD 0.57 | OD 0.60 | OD 0.62 |          |
| Z-2 with NaHCO₃            | 9.0   | OD 0.49 | OD 0.51 | OD 0.53 |          |
|                            | 9.5   | OD 0.55 | OD 0.57 | OD 0.59 |          |
|                            | 10.0  | OD 0.57 | OD 0.59 | OD 0.61 |          |
| Urea and T-super with NaHCO₃ | 9.0  | OD 0.54 | OD 0.57 | OD 0.60 |          |
|                            | 9.5   | OD 0.55 | OD 0.60 | OD 0.67 |          |
|                            | 10.0  | OD 0.57 | OD 0.61 | OD 0.69 |          |
| PES with NaHCO₃            | 9.0   | OD 0.53 | OD 0.55 | OD 0.56 |          |
|                            | 9.5   | OD 0.58 | OD 0.60 | OD 0.62 |          |
|                            | 10.0  | OD 0.57 | OD 0.59 | OD 0.61 |          |

4. Discussion and conclusion

*A. platensis* production plants for mass cultivation could be done in areas with suitable climatic conditions, particularly with the sunshine throughout the year. It is difficult to have an ideal growth due to different environmental factors like solar radiation, rain, wind, temperature fluctuation, etc. In this research, *A. platensis* was studied in laboratory culture.

According to the report of Mahadevaswamy [16], pH values between 8.5 and 9.5 decreased *A. platensis* growth due to the contamination of bacteria and protozoa. Richmond [20] stated that *A. platensis* grow well at pH values between 9 and 11 and a specific growth rate is also independent of pH between 8.5 and 10.5. Hill [11] observed that the growth of predators can be prevented by maintaining pH levels near 10.5. May Yu Khaing [15] reported that the optimum pH for the growth of *A. platensis* biomass was 8.5 to 9.5.

Khin Mar Soe [21] studied the growth of *A. platensis* on seawater-based Provasoli (PES) medium, seawater-urea medium, seawater-based medium I and seawater-based medium II and seawater-based medium III in the laboratory. The optimal growth of *A. platensis* was found in seawater-based medium III which contained a low concentration of phosphate, a small amount of bicarbonate, nitrate, and Fe-EDTA. She reported that the maximum OD 0.79 with initial OD 0.2 was obtained on 15th day of the experimental period.

In the present study, the effects of culture media on the growth of *A. platensis* in seawater were studied in different three pH values (9.0, 9.5, and 10.0) at salinity 30‰. Among this experiment, the highest optimum density (OD 0.69) was observed in urea and T-super nutrient medium with NaHCO₃ at pH 10.0 at the end of culture. The lowest was in modified F-2 media at pH 9.0. Therefore, *A. platensis* possesses a high tolerance of alkaline pH for cultivation.

Dineshkumar et al. [19] and Bharat et al. [22] said that *Spirulina* was survived in seawater but growth was not flourished, achieving a maximum dry weight of 1.86 dw/L on 30th day [19] and 0.28
dw/L on 18th day [22] of cultivation. Natural seawater fortified with different amounts of NaHCO$_3$ and NaNO$_3$ did not show significant impact on *Spirulina* growth. In this study, *A. platensis* was also survived in seawater and growth contained NaHCO$_3$ in media was flourished during cultural period.

In F-2 and Z-1 media, the growth rate of *A. platensis* was found that content of sodium bicarbonate media was higher than containing calcium carbonate media in seawater. From this study, using sodium bicarbonate into culture media may be one of the important factors for *A. platensis* growth. Calcium carbonate rapidly raised the pH values but the responding growth of *A. platensis* was not achieved to a suitable rate in seawater media. Sodium bicarbonate was suitable to use in seawater because it slowly increased the pH values and nearly steadied at the required pH.

Florian et al. [17] used a modified Zarrouk’s medium to reduce Spirulina production cost. The modified Zarrouk’s medium was diluted up to five times without impacting the growth rates in 28-days batch cultivation. Higher dry weights (1.21 g/L and 0.84 g/L) were observed after 21 days of batch cultivation. In the present study, maximum growth rates in modified Zarrouk’s medium were observed on 10th day in pH 10.0.

*S. platensis* could grow well in the modified Zarrouk (Z9) and Provasoli’s enriched seawater (PES) media were re-modified into five media by adding varying concentrations of NaCl, NaHCO$_3$, and seawater (salinity). The Z9 medium provided the best growth rate at the end of culture and the PES medium provided the best at the initial days [18]. In the present study, both modified Zarrouk’s and Provasoli’s enriched seawater (PES) media provided the best growth rates at the end of the culture.

Z-1 medium which was absent of Mg(NO$_3$)$_2$·6H$_2$O nutrient was higher the growth of *A. platensis* than Z-2. The problem with seawater is the high level of Calcium and Magnesium ions which precipitate out the low level of Phosphorus in seawater, a vital component that must be supplied to promote microalgae growth. To counteract this precipitation of Phosphorous and other elements, most of these authors and other researchers have used large quantities of sodium or potassium carbonate to precipitate the Calcium and Magnesium as carbonates [4].

In all media urea and T-super obtained the highest growth rate and followed by PES medium and Z-1. This may be the effect of feeding method. In this experiment urea and T-super were fed daily. So, the plants obtained the nutrients for growth. In other culture media, the increasing growth rates gradually slow down due to the exhausted nutrients. With an increase in age of culture, cell density increased, light penetration decreased, the new cells may absorb nutrient leading depletion of nutrient in the culture as well as the medium leading to reduction of growth. Therefore, age and medium have a substantial effect on the culture.

PES and Z-1 media are suitable for culturing in seawater but the treatments of *A. platensis* are expensive. Others, Z-2 and F-2, are not suitable to use in seawater culture because the growth rates of *A. platensis* in them were poor and the costs are expensive. Therefore, urea and T-super medium was the most suitable medium in seawater.

It may be concluded that urea and T-super medium was the most suitable medium in seawater for the best growth of *A. platensis* because the nutrients are cheaper than other media. Therefore *A. platensis* culture with pH 10.0 at salinity 30‰ by using urea and T-super medium was the most suitable condition in natural seawater. The results were to provide good ideas for mass cultivation in natural seawater.

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