LABORATORY TO OUTDOOR CULTIVATION OF *ARTHROSPIRA PLATENSIS* IN NATURAL SEAWATER

Sit Thu Aung[1], Sein Than Lin [2] and Min Oo [3]

Abstract

In the experiment of laboratory, the growth rates of *Arthrospira platensis* were tested at five different media (modified F-2 medium with $CaCO_3$, modified F-2 medium with $NaHCO_3$, modified Zarrouk's medium with $CaCO_3$, modified Zarrouk's medium with $NaHCO_3$, and urea and T-super with $NaHCO_3$) at pH 8.5 to 10.5 and salinity 30‰ and 35‰. Then they were also tested with urea and T-super with $NaHCO_3$ medium at pH 10 and salinity 30‰ for outdoor culture. The optimum growth of *A. platensis* (OD 0.56) was observed in modified Zarrouk's medium with $NaHCO_3$ at pH 10.5 in both salinities and at pH 10 in salinity 30‰ and also observed in urea and T-super with sodium bicarbonate at pH 10.5 of salinity 30‰ in the laboratory cultivation. For outdoor culture the optimum growth was OD 0.47. The minimum growth rate was occurred in modified F-2 medium with $CaCO_3$ at pH 8.5 of salinity 30‰.

Keywords – *Arthrospira platensis*, medium, seawater, pH, laboratory culture, outdoor culture

Introduction

Spirulina are multicellular and filamentous blue-green microalgae belonging to two separate genera *Spirulina* and *Arthrospira*, consisting of about 15 species (Habib *et.al* 2008). It is a primitive organism originating some 3.5 billion years ago that has established the ability to utilize carbon dioxide dissolved in seawater as a nutrient source for their reproduction (Hill 1980). *Arthrospira* is a photosynthesizing cyanophyte (blue-green algae) that grows vigorously in strong sunshine under high temperatures and highly alkaline conditions. It is found in soil, marshes, freshwater, brackish water, seawater and thermal springs. Alkaline, saline water (>30 g/l) with high pH (8.5–11.0) favour good production of *Arthrospira* (Habib *et.al* 2008).

It grows naturally in tropical regions inhabiting alkaline lakes containing sodium carbonate or sodium bicarbonate, especially the place where there is a high level of solar radiation at altitude in the tropics. These lakes are found near volcanoes. The natural lakes of the world are found in Chad, China, Ethiopia, Kenya, Mexico, Myanmar and Peru. In Myanmar, there are four natural lakes namely Twin Taung, Twin Ma, Taung Pyauk and Yae Kharr which are located at Sagaing Region in upper part of Myanmar. Spirulina is motile but do not have heterocyst. The new cells are reproduced by separating into a number of shorter segments.

Arthrospira platensis contains high levels of protein (50-70%), lipids (7-16%) and vitamins, especially vitamin A and B complexes which make it suitable for animal feeding (Cohen 1997). In many countries of Africa, it is used as human food as an important source of protein and is collected from natural water, dried and eaten. It has gained considerable popularity in the human health food industry and in many countries of Asia it is used as protein supplement and as human health food. *Arthrospira* has been used as a complementary dietary ingredient of feed for poultry and increasingly as a protein and vitamin supplement to aqua feeds (Habib *et.al* 2008).

Cultivations of *A. platensis* are influenced by physical and chemical variable such as temperature, light intensity, nitrogen and carbon sources and pH. In particular, the success of *A. platensis* growth depends on maintenance of both cultivation temperature near the optimum and light intensity below a photoinhibition threshold (Vonshak and Richmond 1988)

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The conventional nitrogen sources for the production of *Arthrospira* are sodium or potassium nitrates. 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). In addition, *A. platensis* requires relatively high pH values which may effectively inhibit the contamination of most algae in the culture (Henrikson 2010). The ability to flourish in extreme pH is a strategy of cyanobacterial species to avoid contamination by other micro-organisms (Touloupakis *et.al* 2016).

The mass cultivation of *Arthrospira* depends on a number of factors, including the availability of nutrients, temperature, and light. *Arthrospira* also requires a relatively high pH, which inhibits the growth of other algae in the system. In order to maintain high pH and avoid fluctuations, high amounts of sodium bicarbonate must always be there in the culture medium (Soni *et.al* 2017). Moreover, the light and temperature are the main factors for growth in nutrient-operated outdoor pond (N, P, CO2, etc.) and well mixed conditions because the specific growth rate of the selected strain must be determined based on these two variables (Huesemann *et.al* 2016)

The first successful culture of *Arthrospira* using untreated seawater in laboratory condition was reported in Italy in 1984 by Materassi *et.al* (1984). The culture technique developed in the laboratory has been successfully applied to outdoor mass culture of *Arthrospira*. The climatic condition is very suitable for *Arthrospira* culture especially in south of Italy throughout the year (Tredici *et.al* 1986).

There are about 1.4 trillion cubic meters of water on Earth. Among them, about 97% is in the oceans and seas. The remaining, about 3% is fresh water. In fresh water, nearly 70% is frozen in icecaps of Antarctica and Greenland. Most of the remainder is present in soil. Only about 1% of fresh water is used for 7 billion people and animals. In 2025 at least 2.5 billion people won't have enough water to drink (Gershwin & Belay 2007). So, we will have to use seawater for *Spirulina* cultivation. The two advantages of *Spirulina* cultivation in seawater are: 1) lower fertilizer cost and 2) saving farm land by using waste sea beach. The aim of this present study is to observe the suitable media, pH and salinity for *A. platensis* culture and to know whether or not cultivation on coastal regions with natural seawater.

Materials and Methods

For laboratory condition, the growth rates of *Arthrospira platensis* were tested with mainly five media which are two modified F-2 media: medium (1) F-2 with $CaCO_3$ and medium (2) F-2 with $NaHCO_3$, two of modified Zarrouk's medium: medium (3) Z-1 with $CaCO_3$, medium (4) Z-1 with $NaHCO_3$, and medium (5) urea and T-super, and medium at five different pH values ranged from 8.5 to 10.5 and two salinity values were 30‰ and 35‰. For outdoor culture condition, the growth rate was only tested with urea and Triple super phosphate medium, 10 of pH value and 30‰ of salinity.

The initial cell density of all experiments was OD 0.2. The cultivations were carried out for a period of 7 days. The materials and apparatus used in this experiment were (8′×3.5′×8″) pond, cultural shelf, 40-watt fluorescent lamps, aeration pump, digital photocolorimeter (Model – 312), microscope, heater, Whatman No. 540 filter paper, refractometer, pH meter, four-digit digital balance and laboratory apparatus (pipettes, conical flasks, beakers). The culture designs for our researches were shown in figures 1 & 2 as flow chats.

Table 1 List of nutrients used in the experimental culture.

Nutrients	Medium				
	1	**2**	**3**	**4**	**5**
$CaCO_3$	+	-	+	-	-
$NaHCO_3$	-	+	-	+	+
$NaNO_3$	+	+	+	+	-
$NaH_2PO_4.2H_2O$	+	+	+	+	-
$Na_2SiO_3.9H_2O$	+	+	-	-	-
Thiamine HCl (B_1)	+	+	-	-	-
Biotin (B_6)	+	+	-	-	-
Vitamin (B_{12})	+	+	-	-	-
K_2SO_4	-	-	+	+	-
KOH	-	-	+	+	-
Urea	-	-	-	-	+
Triple super phosphate	-	-	-	-	+
Na_2EDTA	-	-	+	+	-

\+ present
\- absent

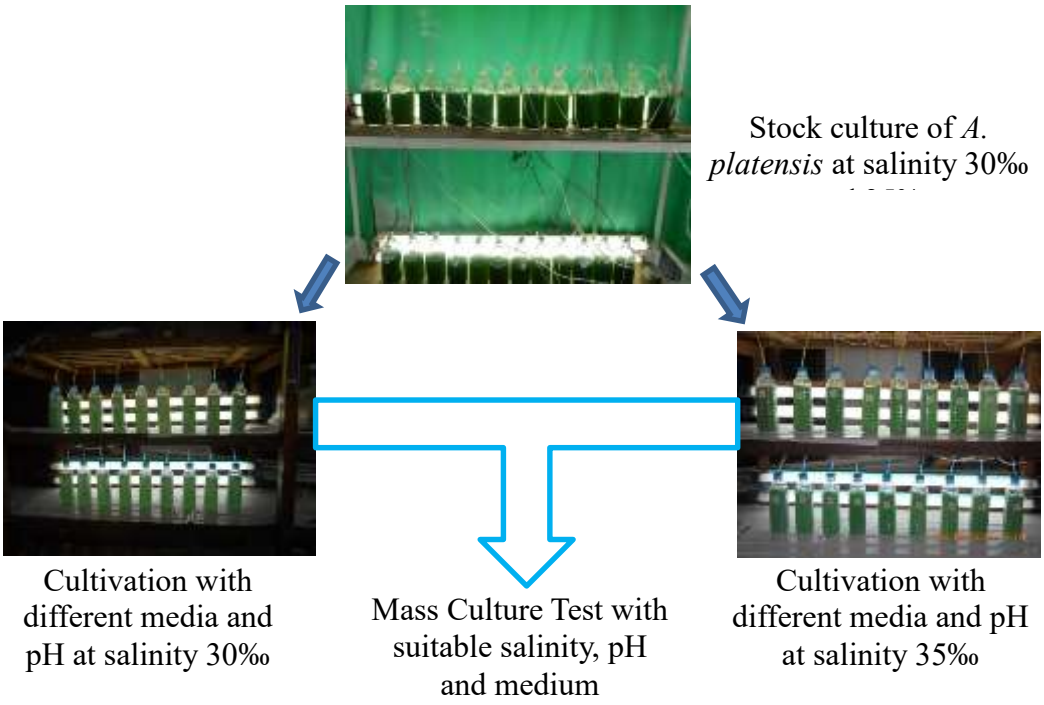

Figure 1 Flow Chat of *A. platensis* cultivation for Laboratory.

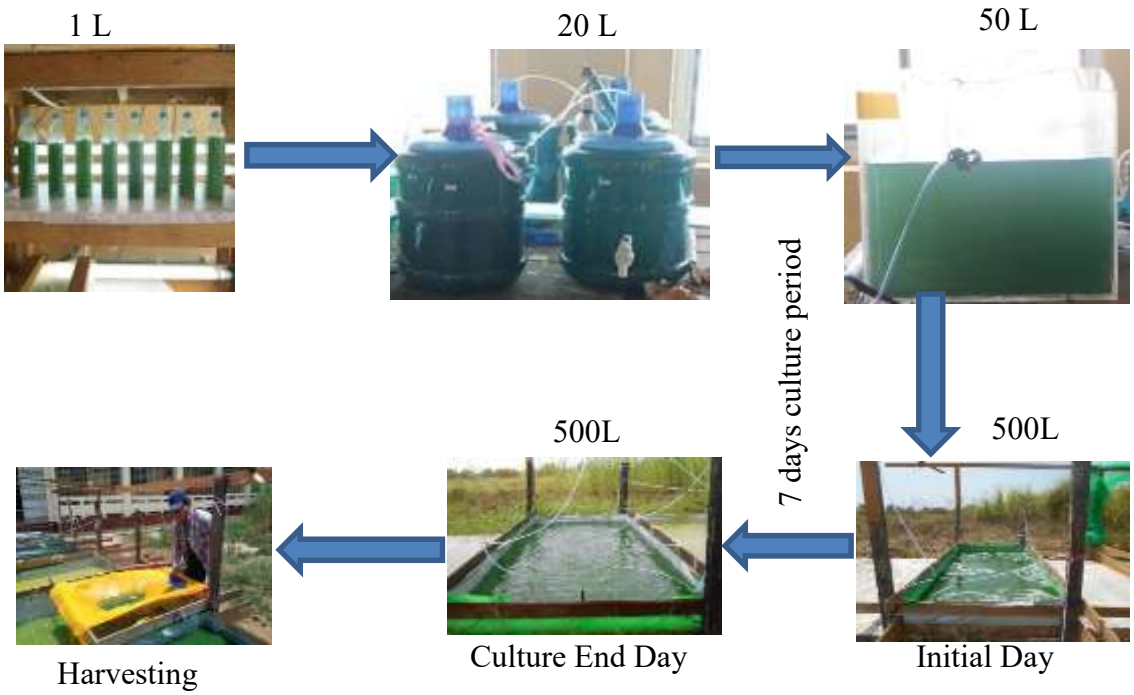

Figure 2 Flow Chat of *A. platensis* cultivation for outdoor.

Results

Laboratory Experiments on the Growth Rate of *Arthrospira platensis* in modified F-2 medium

Arthrospira platensis was cultivated in five different pH values (8.5, 9.0, 9.5, 10.0 and 10.5) which were adjusted by using calcium carbonate or sodium bicarbonate at salinities of 30‰ and 35‰ with modified F-2 medium (Table- 2). Total 20 of 1 litter plastic bottle capacity containing 800 ml were cultured with nearly same amount of aeration. All bottles were kept at room temperature.

Among the 20 bottles, ten bottles were prepared at salinity 30‰ and others were at salinity 35‰. Each three bottles of salinities 30‰ and 35‰ were adjusted to reach the require pH value with calcium carbonate ($CaCO_3$) or sodium bicarbonate ($NaHCO_3$). Growth rates of *A. platensis* were illustrated in Figures (3-6).

Table 2 Chemical composition of modified F-2 medium.

Nutrient Elements	**g/L of seawater**
$NaNO_3$	0.075
$NaH_2PO_4.2H_2O$	0.005
$Na_2SiO_3.9H_2O$	0.030
Thiamine HCl (B_1)	0.0001
Biotin (B_6)	0.0000005
Vitamin (B_{12})	0.0000005

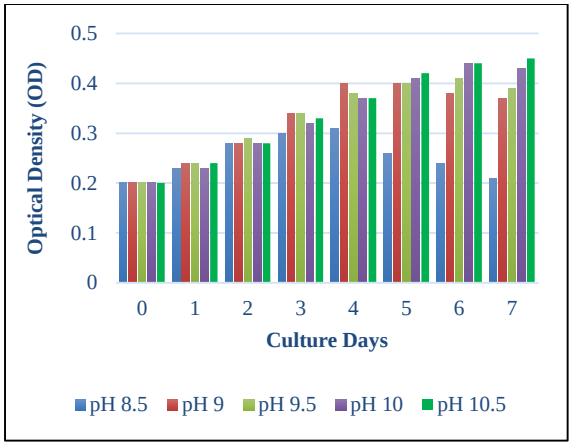

Figure 3 Comparison of the growth of *A. platensis* in different pH by using $CaCO_3$ with F-2 medium at salinity 30‰.

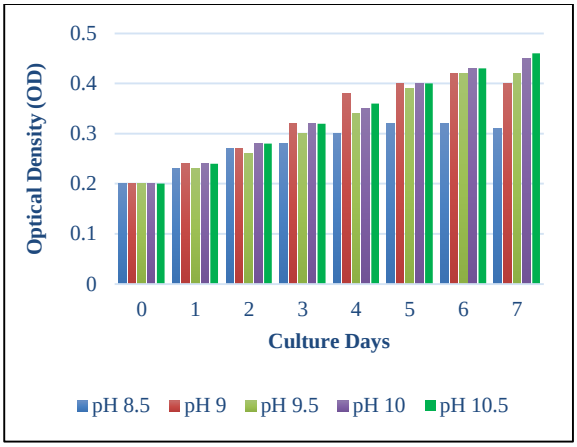

Figure 4 Comparison of the growth of *A. platensis* in different pH by using $CaCO_3$ with F-2 medium at salinity 35‰.

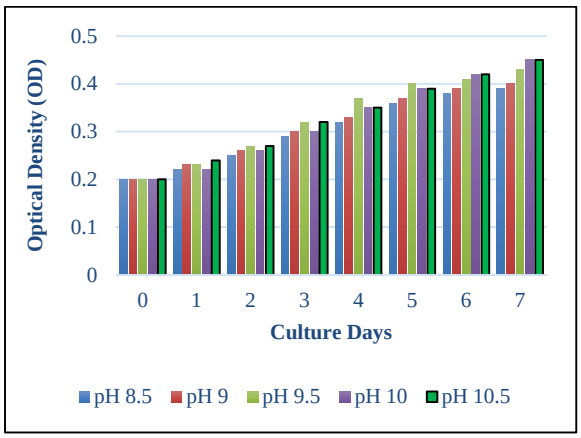

Figure 5 Comparison of the growth of *A. platensis* in different pH by using $NaHCO_3$ with F-2 medium at salinity 30‰.

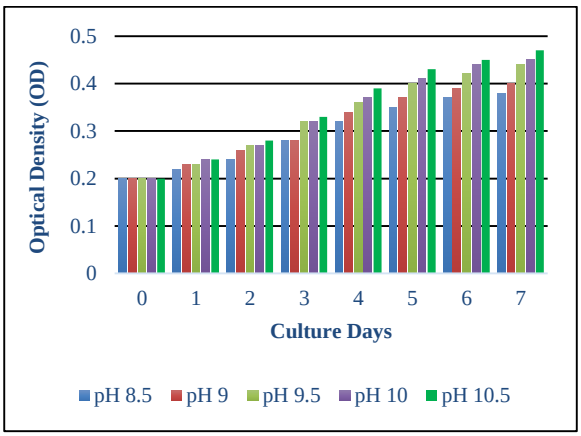

Figure 6 Comparison of the growth of *A. platensis* in different pH by using $NaHCO_3$ with F-2 medium at salinity 35‰.

Laboratory Experiments on the Growth Rate of *Arthrospira platensis* in modified Zarrouk's medium

Arthrospira platensis was cultivated in five different pH values (8.5, 9.0, 9.5, 10.0 and 10.5) which were adjusted by using calcium carbonate or sodium bicarbonate at salinities of 30‰ and 35‰ with modified Zarrouk's medium (Table- 3). Total 20 plastic bottles of one litter capacity containing about 800 ml of the medium were cultured with nearly same amount of aeration. All bottles were kept at room temperature. Among the 20 plastic bottles, ten were prepared at salinity 30‰ and others were at salinity 35‰. Growth rates of *A. platensis* were shown in Figures (7-10).

Table 3 Chemical composition of modified Zarrouk's medium.

Nutrient Elements	g/L of seawater
K_2SO_4	1.148
$NaNO_3$	2.500
NaH_2PO_4	0.344
KOH	0.227
Na_2EDTA	0.080

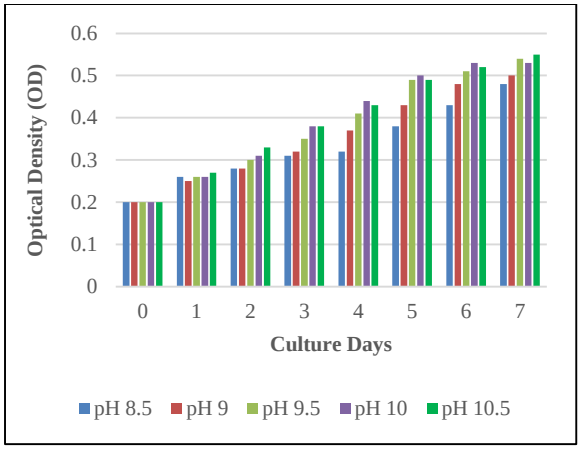

Figure 7 Comparison of the growth of *A. platensis* in different pH by using $CaCO_3$ with modified Zarrouk's medium at salinity 30‰.

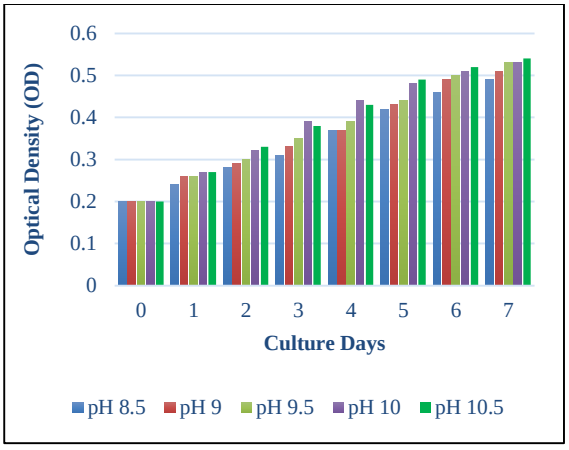

Figure 8 Comparison of the growth of *A. platensis* in different pH by using $CaCO_3$ with modified Zarrouk's medium at salinity 35‰.

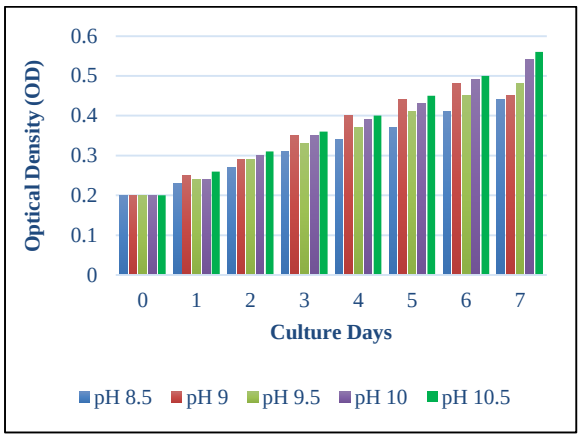

Figure 9 Comparison of the growth of *A. platensis* in different pH by using $NaHCO_3$ with modified Zarrouk's medium at salinity 30‰.

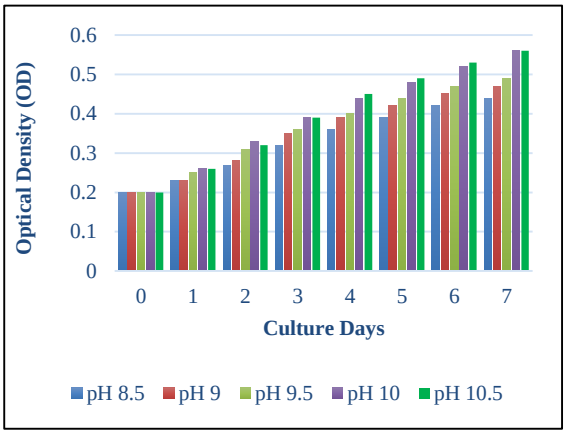

Figure 10 Comparison of the growth of *A. platensis* in different pH by using $NaHCO_3$ with modified Zarrouk's medium at salinity 35‰.

Laboratory Experiments on the Growth Rate of *Arthrospira platensis* in Urea and Triple Super Phosphate medium

Arthrospira platensis was cultivated in five different pH values (8.5, 9.0, 9.5, 10.0 and 10.5) which were adjusted by using only sodium bicarbonate at salinities of 30‰ and 35‰ with urea and T-super as the ratio 5:1. The nutrient medium was prepared that 10 g of urea and 2 g of T-super were separately soluted with each one liter of distil water. Total 10 plastic bottles of one litter capacity containing about 800 ml of the medium were cultured with nearly same amount of aeration. All flasks were kept at room temperature.

Among them, five bottles were prepared at salinity 30‰ and others were at salinity 35‰. Each five bottles of salinity 30‰ and 35‰ were adjusted to reach the require pH value with sodium bicarbonate. In this test, 10 ml of each nutrient solution was daily feed into the culture bottles. The growth rates of the cells were described in figures11 and 12.

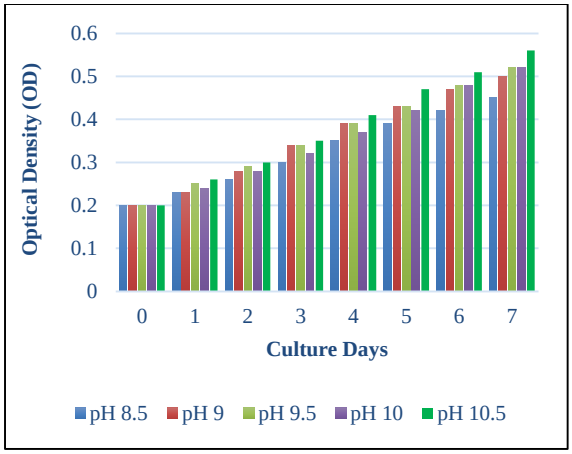

Figure 11 Comparison of the growth of *A. platensis* in different pH by using $NaHCO_3$ with urea and T-super medium at salinity 30‰.

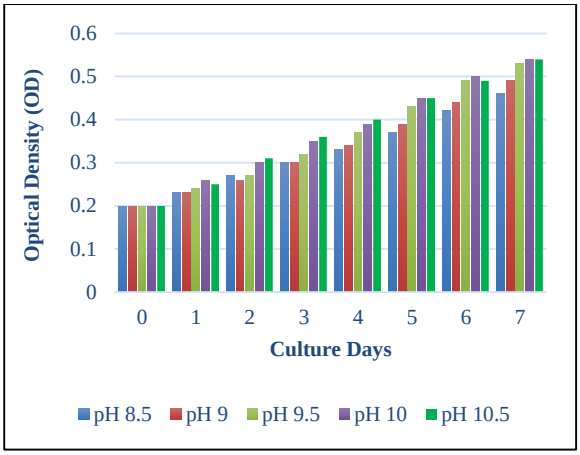

Figure 12 Comparison of the growth of *A. platensis* in different pH by using $NaHCO_3$ with urea and T-super medium at salinity 35‰.

Outdoor Experiments on the Growth Rate of *Arthrospira platensis*

Arthrospira platensis was cultivated in seawater by using sodium bicarbonate ($NaHCO_3$) to control the pH value. The salinity of this experiment is 30‰. The nutrient medium was urea and T-super as the ratio 5:1. The cultivated water volume was 0.5 ton. The growths of *A. platensis* in seawater for outdoor culture were shown in figure 14. In this culture period, the doubling time for the growth was observed at second day. The maximum OD 0.47 was occurred at the end of the culture.

Figure 13 Small scale mass culture of *A. plantensis* in seawater.

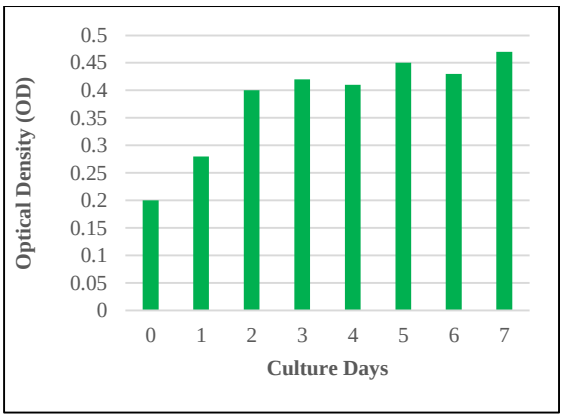

Figure 14 Comparison of the growth of *A. platensis* in outdoor culture by using $NaHCO_3$ with urea and T-super medium at salinity 30‰.

Production of *Arthrospira platensis*

Arthrospira platensis was harvested by using (300μm) screen and then were dried into the oven (70°C) for 24 hours. The yield was 4 kg (wet weight) and 0.3 kg (dry weight). The process for production was shown in figure 15.

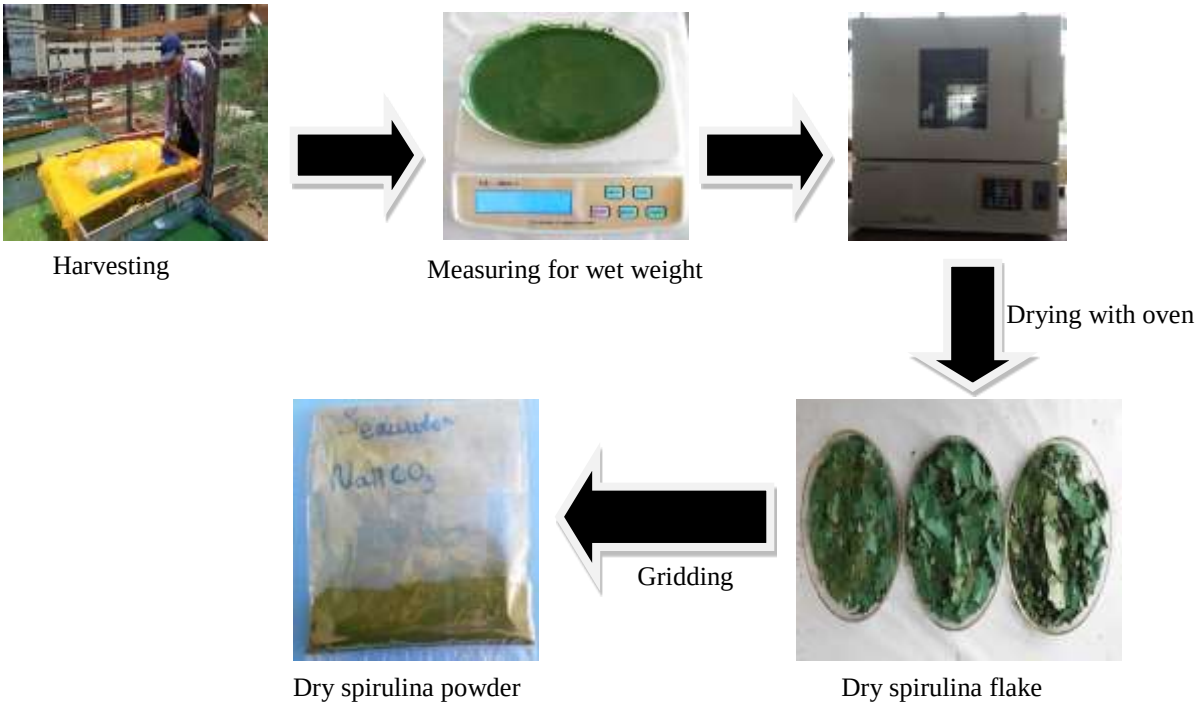

Figure15 Flow chart of the processing of *A. platensis* in sea water.

Discussion and Conclusion

Ciferri (1983) said that eight major environmental factors such as luminosity, temperature, inoculation size, stirring speed, nutrient, salinity, pH and water quality influence the productivity of spirulina.

Arthrospira shows an optimum growth between 35 and 37 °C under laboratory conditions. Outdoors, it seems that an increase in temperature up to 39 °C for a few hours does not harm the blue-green alga, or its photosynthetic ability. Thermophilic or thermotolerant strains of spirulina can be cultivated at temperatures between 35 and 40 °C. Such a property has the advantage of eliminating microbial mesophilic contaminants. The minimum temperature which growth of *Arthrospira* takes place is around 15 °C during the day. At night, *Arthrospira* can tolerate relatively low temperatures (Richmond 1988). In this study *A. platensis* was cultivated at room temperature in the laboratory and the water temperature ranges for outdoor condition were between 32℃ and 35℃.

Khin Mar Soe (2009) 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 at 15th day of the experimental period.

In the present laboratory study, the growth of *A. platensis* in seawater were studied in different five pH values (8.5, 9.0, 9.5, 10.0 and 10.5) at salinity 30‰ and 35‰ with various media. Among this experiment, the highest optimum density (OD 0.56) was observed in modified Zarrouk's medium and urea & T-super nutrient medium with $NaHCO_3$ over pH 10.0 at the end of culture. The lowest was in modified F-2 medium with $CaCO_3$ at pH 8.5. Therefore *A. platensis* possesses a high tolerance of alkaline pH for cultivation.

Faintuch *et.al* (1991) also studied the influence of the nutritional sources on the growth rate of cyanobacteria. They reported that there is very significant influence of mixtures of defined proportions of KNO_3, urea and ammonia on the growth of *Arthrospira*. The most favourable growth rates of *A. platensis* occurred in the presence of 2.57 g/litre KNO_3 with growth rate of 0.3-0.4/day. Chang *et.al* (1999) studied the possibility of using nitrifying bacteria for the fulfilment of nitrogen fertilizer in *Arthrospira* mass culture. They first adapted the nitrifying bacteria with pH 8-10, 0.6-2.2 percent salt and 6-12 mg/litre of $NaHCO_3$ in the culture solution. They found that the concentration of NO_3 reached over 20 mg/litre after the nitrifying bacteria was inoculated in the *Arthrospira* culture solution and then incubated for 6 days at 25–35 °C. In the present study we mainly used nitrogen sources as $NaNO_3$ or urea. The growth rates of *A. platensis* were observed OD 0.56 at pH 10.5 in salinity 30‰ when using $NaNO_3$ or urea for laboratory cultivation and OD 0.46 was occurred in outdoor cultivation by using urea.

Growth performance of *A. platensis* was studied in three different concentrations of banana leaf ash added with 0.4 g/litre jackfruit seed powder and 0.2 g/litre with urea in the laboratory (Toyub *et.al* 2005). Rice husk ash (RHA) and $NaHCO_3$ were used as a source of carbon in *Arthrospira* culture (Akhter *et.al* 1996). They reported that the addition of 2.0 g $NaHCO_3$/litre every two days supported better growth of *Arthrospira* than 1.0 g RHA/litre every day, although this might not be supported on economic grounds.

May Yu Khaing (2007) reported that the optimum pH for the growth of *A. platensis* biomass was 8.5 to 9.5. In the present study salinity ranges (30 g/l and 35 g/l) with pH (8.5-10.5) for laboratory condition and 30 g/l of seawater with pH 10 for outdoor culture were used.

In modified F-2 and Zarrouk's media the growth rate of *A. platensis* was found that content of sodium bicarbonate medium 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.

Lagoon water can be used with some nutrient supplementation to grow *A. platensis* (Costa *et.al* 2004). They used Mangueira lagoon water rich with carbonates and a high pH, and in addition of 1.125 or 2.250 mg/litre of urea and 21 or 42 mg/litre of $NaHCO_3$ during fed-batch culture of *A. platensis*, respectively using a 32-factorial design. They found that lagoon water in addition of 1.125 mg/litre of urea resulted in a 2.67-fold increase in the final biomass of spirulina.

The first successful culture of *Arthrospira* using untreated seawater in laboratory condition was reported in Italy in 1984 by Materassi *et.al* (1984). The culture technique developed in the laboratory has been successfully applied to outdoor mass culture of spirulina. The climatic condition is very suitable for *Arthrospira* culture especially in south of Italy throughout the year (Tredici *et.al* 1986). The mean annual yield of biomass on sea-water plus urea was 7.35 g (dry weight)/m2/day, which was slightly lower value than that obtained on the standard sodium bicarbonate medium with sea-water (8.14 g/m2/day) under controlled pH, ranged from 8.0 to 8.3.

Dineshkumar *et.al* (2016) and Bharat *et.al* (2011) said that *Arthrospira* was survived in seawater but growth was not flourished, achieving maximum dry weight of 1.86 dw/L on 30^{th} day and 0.28 dw/L on 18^{th} day of cultivation. Natural seawater fortified with different amount of $NaHCO_3$ and $NaNO_3$ did not shown significant impact on *Arthrospira* growth. In this study, *A. platensis* was also survived in seawater and growth contained $NaHCO_3$ in media was flourished during cultural period.

Florian *et.al* (2017) used modified Zarrouk's medium in order to reduce the 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 at culture end day in pH 10.5.

From this study, the growth rate of *A. platensis* was found that containing sodium bicarbonate media were higher than containing calcium carbonate media in seawater. So, using sodium bicarbonate into culture media may be one of the important factors for *A. platensis* growth.

Modified Zarrouk's medium was suitable for culturing in seawater but the treatments of *A. platensis* are expensive. Other, modified F-2, are not suitable to use in seawater because the growth rates of *A. platensis* were poor and the costs are expensive. Therefore, urea and T-super medium was the most suitable medium in seawater. These results will be expected to provide good ideas for *A. platensis* production in seawater and to cultivate in coastal region.

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