

CHAPTER II

LITERATURE REVIEWS

2.1 Blue Green Algae

Blue green algae were prokaryote with gram negative cell wall. It can be classified as cyanobacteria because nucleus of these algae was similar to bacteria nucleus and some blue green algae also had capabilities to fix nitrogen. These algae were considered as a largest photosynthetic microorganism in prokaryotes group. Blue green algae can be found in freshwater, salt water, hot spring and soil both in hot climate and cold climate. It had single cell or filament cell. Biological interactions that can be found in these algae were neutralism or symbiosis which mutually interacts with animals, plants, fungi or other bacteria.

Blue green algae not had chloroplast but it had thylakoids which can be found scattered throughout the cell without membrane and layer. These thylakoids were contained with chlorophyll A. The other pigments were perched on the surface of thylakoid that called "Phycobilisome" which can be shown in Figure 2.1 (Citerri, 1983).

Blue green algae were phototrophic microorganisms that carry out oxygenic photosynthesis similar to that of higher plants, use light as a food source. Most photoautotrophs cannot use sugar contents as a food source because they do not had "Enzyme Permease" to intake sugar into cell but while algae photosynthesis, they store sugar in cell as glycogen which can be metabolized as a food source when there was no sufficient light source.

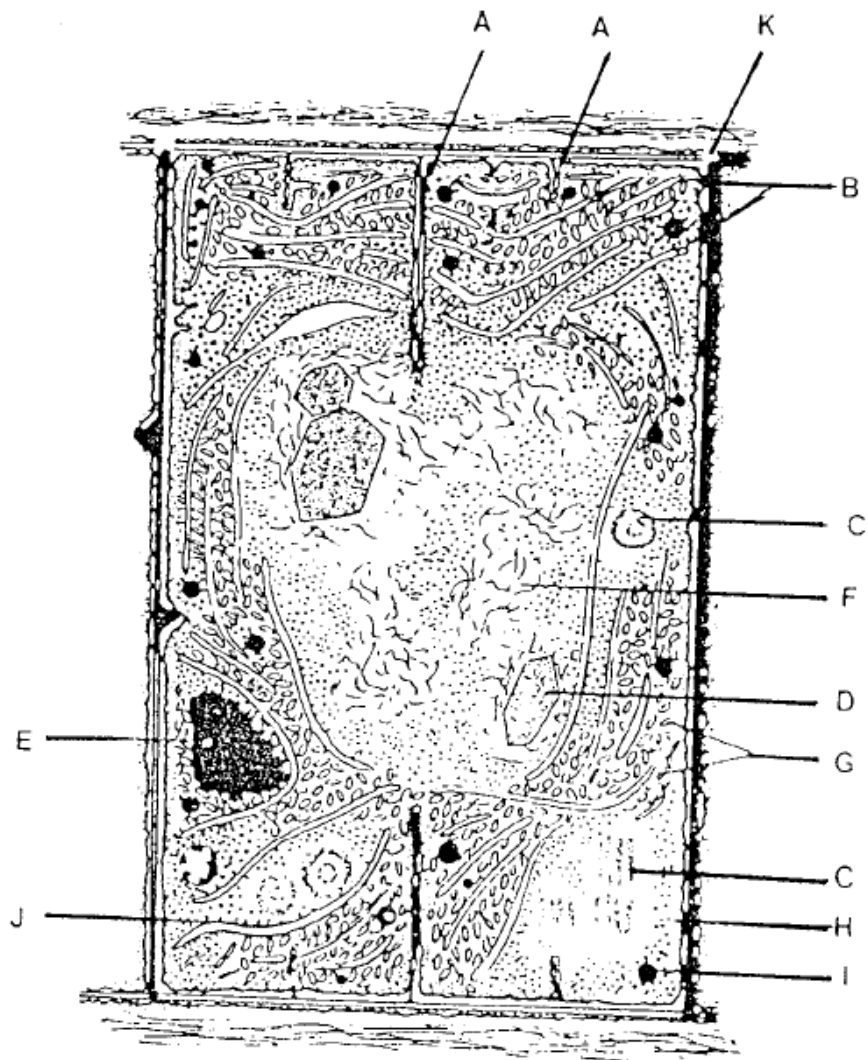


Figure 2.1 Cell structures of blue green algae.

(A) cross wall, (B) Thylakoids or photosynthetic membranes, (C) Cylindrical Body seen in cross section (upper) longitudinal section (lower), (D) Polyhedral Body, (E) Structured Granule, (F) DNA strands in cytoplasm, (G) Glycogen Granule, (H) Ribosomes, (I) Lipid Globule, (J) Phycobilisome, and (K) Pore.

Source: *Spirulina*, the edible microorganism (Citerri, 1983).

2.2 General Characteristics of *Spirulina spp.*

2.2.1 Morphology and Taxonomy

Spirulina spp. was spiral-shaped blue green that can be found commonly in freshwater and brackish water environment, especially in high salinity water. Due to the high nutrition and spiral characteristic, Thai common name for these algae was “Sarai Kliao Thong” which means spiral algae that had same value as gold.

Spirulina spp. was filamentous cyanobacteria that can be classified in the oscillatoriaceae family. Taxonomy of *Spirulina platensis* can be shown as followed; (Peerapornpisarn, 2003)

Kingdom Monera

Division Cyanophyta

Class Cyanophyceae

Order Oscillatoriales

Family Oscillatoriaceae

Genus *Spirulina*

Species *Spirulina platensis*

Spirulina spp. had multi-cell which forms filament structure called trichome. These trichomes were coiled, unbranched and had similar structure to spring shape. It had helix diameter at about 35-50 micrometer and pitches around 60 micrometers. Size of trichome was ranged from 4-8 micrometer and 50-500 micron in length. Each cell was formed filaments that were coiling similar to spring shape. The length of these filaments was depended on age and environment (Citerri, 1983). The structure of these algae can be shown in Figure 2.2

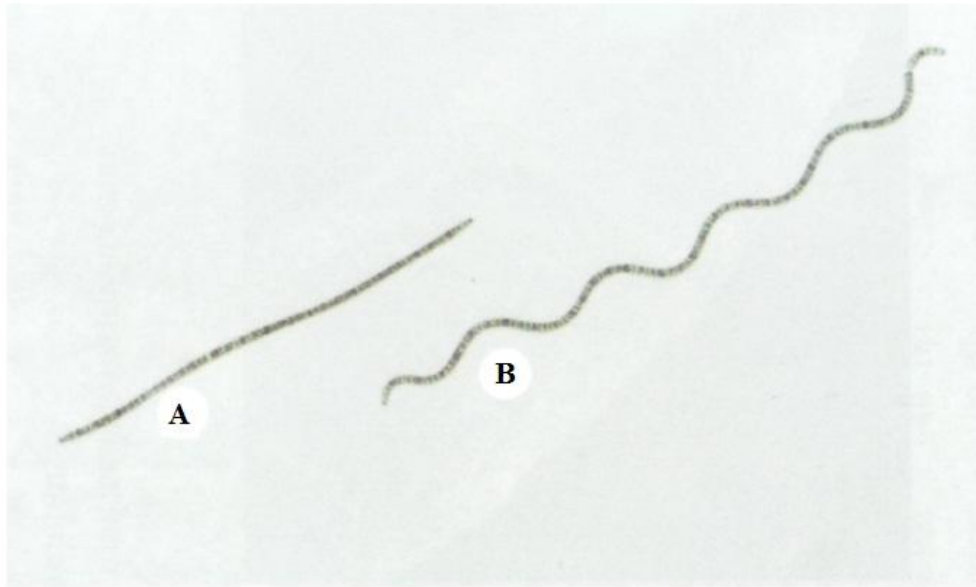


Figure 2.2 Structure of *Spirulina platensis*: (A) Straight Line, (B) Normal Spiral.

Source: Adapted from the book “*Spirulina*” (Peerapornpisarn, 2003).

Spirulina spp. had amino acids which were similar to amino acids from. It also had high amount of protein (55-77 % of dry weight). It had variety of amino acids that not only meets the FAO food standard, but also had vitamins, steroids, enzyme and other nutrient. In addition, it was found that *Spirulina spp.* had pigments (chlorophyll and carotenoid) that can be changed to vitamin A and high amount of phycocyanin that can be used for food coloring and cosmetics (Henrikson, 2009). *Spirulina spp.* had high heat tolerance. It can be growth in high temperature (35-40 °C). Due to this reason, it can withstand contamination from other microorganisms. Large *Spirulina* specie had high floating capability because it had granule in cytoplasm that contains with gas vacuole (Venkataraman, 1983).

Present, there were about 35 species of *Spirulina spp.* which size, length and shape were different in each type as shown in Figure 2.3

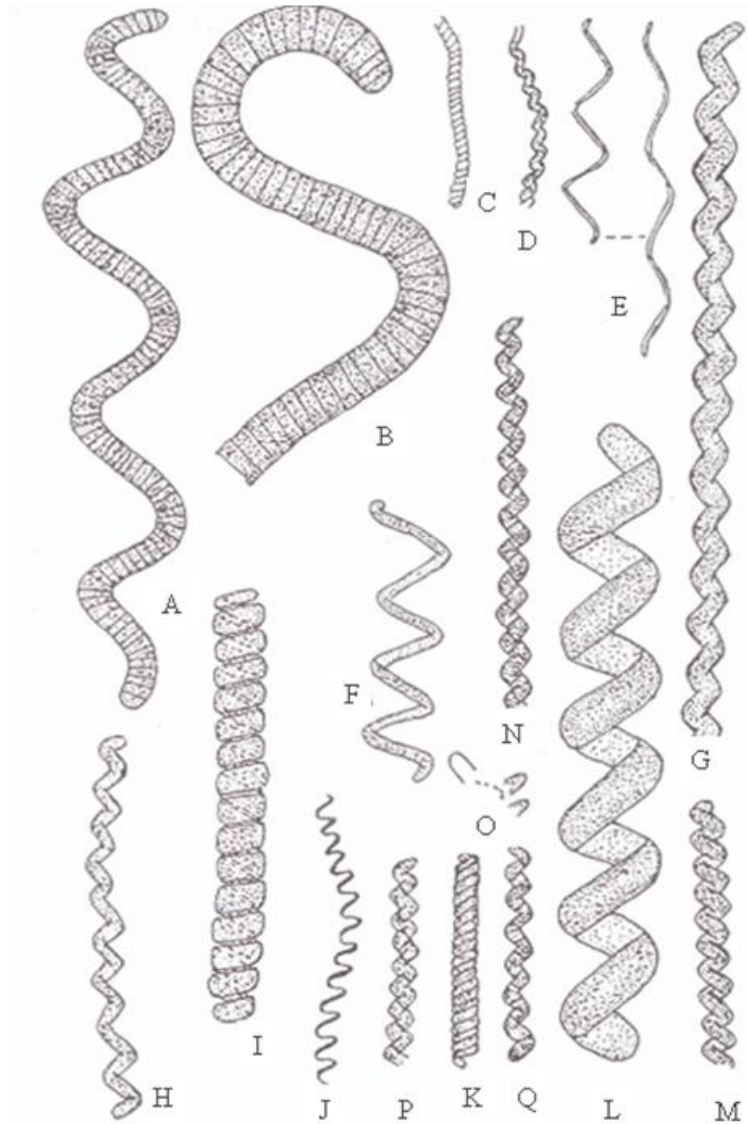


Figure 2.3 Different types of *Spirulina* spp.

(A, B) *Spirulina platensis*, (C, I) *S. subsalsa*, (D, H) *S. meneghiniana*, (E, F) *S. laxissima*, (G) *S. princeps*, (J) *S. subtilissima*, (K) *S. labyrinthiformis*, (L, N-Q) *S. gigantea*, (M) *S. major*.

Source: Adapted from the book “*Spirulina*” (Peerapornpisarn, 2003).

2.2.2 Reproduction

Spirulina spp. was reproduced asexually. It uses hormogonia for reproductions by starting from trichomes differentiate called hormogonia and these will be growth into new filaments or new trichomes which had same characteristics. This reproduction can be called fragmentation which can be shown in Figure 2.4

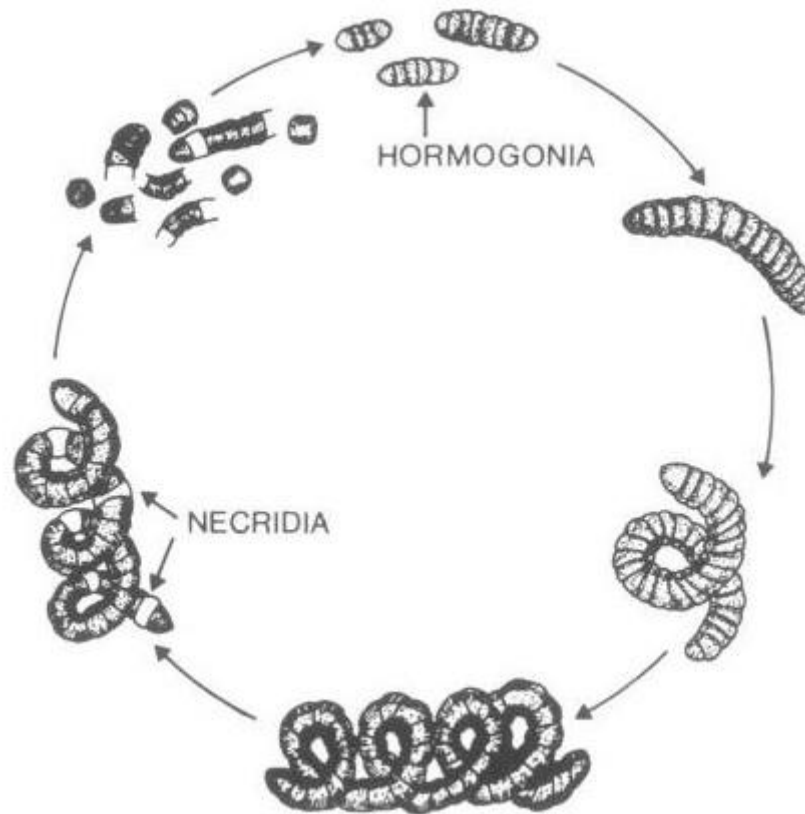


Figure 2.4 Reproduction of *Spirulina spp.*

Source: *Spirulina*, the edible microorganism (Citerri, 1983).

2.2.3 Habitat and environment

Spirulina spp. can be found commonly in freshwater and brackish water environment. It can be growth rapidly in shallow water that had high salinity or had small quantity of salt. Generally, *Spirulina spp.* can be found in soil, swamp, sea, hot spring and freshwater. The most common specie was *S. platensis* and *S. maxima* (Citerri, 1983).

Spirulina spp. can be growth in freshwater that had high concentration of bicarbonate ion or water that had salt concentration from 20-70 gram per liter, such as lake in the Republic of Chad, lake in East Africa, water sources in mountains, along the coastal lake and hot spring in Yellowstone Park which had temperature around 55°C. For Thailand, these algae can be found commonly in water sources.

2.2.4 Nutrition benefits

Spirulina spp. can be used as protein supplement (Single Cell Protein; SCP) because it had low nucleic acid and cellulose but high nutrition. More importantly, it had high amount of protein at about 60-70 % of dry weight which was higher than the protein contents in meat or egg two to three times (Venkataraman, 1983). From the recent study, it was found that *Spirulina spp.* in laboratory scale had protein 64-72%, carbohydrate 9-14%, ash 4-6% of weight. In commercial scale, it had protein 55-77%, carbohydrate 10-16%, fat 5-8%, ash 4-7% and moisture 3-7% and amino acids that can be found in these products were similar to essential amino acids and nonessential amino acid that can be found in animals (Citerri, 1983). Table 2.1, 2.2 and 2.3 shown the comparison of amino acids and other organic contents that can be found in *Spirulina spp.* and other sources.

Table 2.1 The comparison of amino acids contents that can be found in *Spirulina spp.* and standard from Food and Agriculture Organization (FAO) and other sources (gram per 100 gram of protein)

Amino Acid	FAO	<i>Spirulina</i> (Sun - Dried)	<i>Scenedesmus</i> (Drum - Dried)	Egg
Isoleucine	4.0	6.7	3.6	5.8
Leucine	7.0	9.8	7.5	9.0
Valine	5.0	7.1	6.0	7.8
Phenylalanine	6.0	5.3	4.8	5.9
Tyrosine	-	5.3	3.2	4.2
Lysine	5.5	4.8	5.6	6.6
Methionine	3.5	2.5	1.5	4.0
Cystine	-	0.9	0.6	2.2
Tryptophan	-	0.3	0.3	1.9
Threonine	-	6.2	5.1	5.0
Alanine	-	9.5	9.0	-
Arginine	-	7.3	7.1	6.2
Aspartic Acid	-	11.8	8.4	11.0
Glutamic Acid	-	10.3	10.7	12.6
Glycine	-	5.7	7.1	4.2
Histidine	-	2.2	2.1	2.4
Proline	-	4.2	3.9	4.2
Serine	-	5.1	3.8	6.9
Available Lysine	-	3.7	3.0	-

Source: Blue green Alga: *Spirulina* (Venkataraman, 1983)

Table 2.2 The comparison of organic contents in *Spirulina spp.* and other sources

Organic contents	<i>Spirulina</i>	<i>chlorella</i>	soybean
Protein	69.5 – 71%	40-56%	39%
Carbohydrate	12.5%	10-25%	36%
Fat	8%	10-30%	19%
Vitamin	Provitamin A, B, B2, B6, B12, E Pantothenic acid Nicotinic acid Folic acid	Provitamin A, B1 B2, B6, B12 Nicotinic acid	B1, B2, B6
Pigment	Chlorophyll Carotenoid Phycocyanin	Chlorophyll Carotenoid	

Source: Blue green Alga: *Spirulina* (Venkataraman, 1983)

Table 2.3 The comparison of protein contents in *Spirulina spp.* and other sources (Dry weight)

Sources	Protein percentage
Meat	18-20%
Egg	10-25%
Wheat	6-10%
Rice	7%
Soybean	33-35%
Mackerel	20%
<i>Chlorella</i>	40-56%
<i>Spirulina</i>	69.5-71%

Source: Blue Green Alga: *Spirulina* (Venkataraman, 1983)

2.3 Product from *Spirulina spp.* and Utilization

2.3.1 Medical benefits

Due to high nutrition in *Spirulina spp.*, it can be used as food and medicine. It can prevent human health from many diseases, such as diabetes, gastric disorders, liver disease, hemorrhoids, eye diseases and anemia. It also can be used for pus treatment because it contains high amount of protein and cysteine. Due to high concentration of chlorophyll, it can treat and prevent gastric disorders. It can be used as food for patients and elderly because *Spirulina spp.* had cell wall that was not made from cellulose and it was easy to digest. It had low calories and unsaturated fatty acid as a main component which was able to used as diet food. Other reports were also shown that it can treat eye diseases and diabetes (Henrikson, 2009).

2.3.2 Human Food

Since 16 century, human had been used *Spirulina spp.* as food. Aztec people who lived in area of Mexico country nowadays used *Spirulina spp.* from lake and cooking as a food named "Tecuitat". Mayan in Mexico used *Spirulina spp.* to make cake or soup and used as a main course in everyday life. Nowadays *Spirulina spp.* had been used and produced as a human food globally. In USA, Sosatexcoco Company, UN and National Food Research of Mexico were cooperated and produce algae pellet and component for crispy bread and desserts from *Spirulina spp.* These algae had been consumed as pellet or powder in USA and Japan. In 1976, Nanpaoresin Company produced and exported *Spirulina spp.* to USA, Japan and Western country as a healthy food and received good response from the public (Vonshak & Richmond, 1988). Nembu tribe who lives in Sahara desert and Northern West of Chad Lake dry *Spirulina spp.* on the desert called "Dihe" and uses for rice seasoning or making cooking sauce. In Asia, Chinese uses *Spirulina spp.* from freshwater lake and water sources for cooking called "Lan". Indonesian uses dried *Spirulina spp.* for cooking called "Ke-Klap" Indian uses *Spirulina spp.* as protein supplementary. In Thailand, December 6, 1977, Siam algae co. ltd received support from The Board of Investment (BOI) of Thailand to build factory in Bangpli, SamutPrakarn and produce *Spirulina spp.* powder for exporting to Japan (Bunsom, 1988).

2.3.3 Animal feed

Apart from using as human food, it also can be used as feed. From recent studies, it was found that it had higher protein than grass. *Spirulina spp.* had protein at about 24,300 kilogram per dried weight/hectare/year. In contrast, grass had protein only 670 kilogram per dried weight/hectare/year. If compared using grass as feed and using *Spirulina spp.* as feed, protein in animal meat that uses grass will lower than protein in animal meat that uses *Spirulina spp.* for feeding (60 kilogram per dried weight/hectare/year and 2,180 kilogram per dried weight/hectare/year, respectively). Using *Spirulina spp.* as mixing in animal feed had many benefits, such as weight increasing; increased the rate of laying egg in duck and chicken; enhanced yolk color because of high beta carotene that were converted into vitamin A; increased the reproduction rate and sizing of shrimp and fish (Richmond, 1986).

In addition, it had been used as feed mixing for Japanese quail because it enhanced yolk color. It uses as feed mixing for ornamental fish, such as Carp. Pigment in *Spirulina spp.* will enhance fish coloring (Bunsom, 1988).

2.3.4 Wastewater treatment

From recent studies, *Spirulina spp.* can be used for wastewater treatment because *Spirulina spp.* can produce oxygen into wastewater from photosynthesis and it also had capability to utilize nitrogen and phosphorus in wastewater. Food and agriculture organization constructed factory in Singapore to utilize animal waste for feeding algae and uses these algae for wastewater treatment and animal feeding. In India, wastewater had been used for algae feeding and these algae were used for food (Peerapornpisarn, 2003).

2.3.5 Other benefits

Spirulina spp. also can be used in many purposes, such as agriculture purpose. It can be used to created organic fertilizers. *Spirulina spp.* can be extracted essential nutrients, such as beta-carotene which had high production rate up to 14 percent.

Phycocyanin in *Spirulina spp.* was blue coloring pigment that had phycobilin 12-15% of dried weight (Santillan, 1982). This can be used in food

coloring and cosmetics. Dippon Ink Company produce product from *Spirulina spp.* named “Linablue”. However, producing food coloring and cosmetics from *Spirulina spp.* were still not commercially because of high costs (Peerapornpisarn, 2003).

In recent days, *Spirulina spp.* had been produced commercially in many countries which can be summarized in Table 2.4

Table 2.4 *Spirulina spp.* production in different countries in 1990

Company	Location	Type and Size of Production (hectare)		Production (Ton of dried weight)
		Natural	Man-made	
Sosatexcoco	Texcoco lake, Mexico	12	-	300
Earth Rice Farm	California, USA	-	5	90
Siam algae co. ltd	Samut Prakarn, Thailand	1.8	2	100
Blue Continent	Taiwan	-	4 - 15	300
<i>Chlorella</i>				
Nippon <i>Spirulina</i>	Japan	-	1.5	30
Cyanotech	Havana	-	2	40

Source: A review on culture, production and use of *Spirulina* as food for humans and feed for domestic animals and fish. (Habib et al., 2008)

2.4 Factors Affecting Productivity of *Spirulina spp.*

Different algae had different growth rate and different tolerance to changing of environment. Growth requirement of algae can be summarized as follows;

2.4.1 Nutrients Requirement

Nutrients that were required for algae growths were consist of;

2.4.1.1 Carbon Sources

Algae need carbon as a source for photosynthesis. *Spirulina spp.* can able to use carbon salt or carbon dioxide as carbon source. *Spirulina spp.* cans growth well in Zarrouk's media that contains with high bicarbonate up to 17 gram per liter. It can maintain growth rate even the concentration of bicarbonate reduce to 4.5 gram per liter but must add sodium hydroxide to increase pH to 9.0 (Venkataraman, 1983).

Spirulina spp. requires high carbon source for growth up to 47% by weight. Therefore, algae cultivation needs to add large amount of carbon throughout the process. Carbon dioxide (CO₂) had been used as a carbon source because it had same amount of carbon as carbon in plants. Most plants uses carbon dioxide in atmosphere for growth (0.33 % of air at sea level was CO₂ (0.0033 x amount of air). Carbon dioxide in water and soil that plants use as a carbon source was in carbonate (xCO₃) and bicarbonate (xHCO₃) form.

2.4.1.2 Nitrogen Sources

Concentration of nitrogen was influenced the cell dried weight. It was depended on types of algae. Microalgae can be used both organic and inorganic nitrogen as nitrogen source. Algae that were prokaryotes, such as cyanobacteria can be able to fix nitrogen. Most algae can use nitrate (NO₃⁻), nitrite (NO₂⁻) or ammonia (NH₄⁺) as nitrogen source and the growth rate was not different.

Nitrates were the main nitrogen source assimilated by *Spirulina spp.*, but ammonium salt may be used as long as the NH₄⁺ concentration was less than 100 milligram nitrogen per liter (Laorawat, 1995). At higher concentration, the pH of the media may fall sharply, causing undesirable side effects. Urea could be used with no ill effect at pH 8.4, as long as its concentration was kept below approximately 1.5 gram per liter. Nitrite can also used as nitrogen source for *Spirulina spp.* but only at low concentrations, approximately 1 mM. At higher concentrations, nitrite inhibits growth (Morris, 1974).

2.4.1.3 Phosphorus

Phosphorus was an essential nutrient required for the normal growth of algae. It plays an important role in many cellular processes, especially those involved in energy transfer and nucleic acid synthesis. Algae receive phosphorus as inorganic phosphate ($\text{H}_2\text{PO}_4^- + \text{HPO}_4^{2-}$). The phosphorus requirement for optimum growth varies greatly by species, although other external factors were not limited. Under phosphorus deficient conditions, protein content, chlorophyll-a, RNA, DNA and cellular ATP content decrease, while carbohydrate content increase in eukaryotic and prokaryotic algal cells (Laorawat, 1995).

2.4.1.4 Sodium and Potassium

Sodium was essential nutrient for only some algae. Saltwater cyanobacteria need sodium for growth more than freshwater cyanobacteria. If there was no sufficient potassium, it will replenish potassium with sodium.

Potassium was co-factor of ribosome in protein synthesis and control osmotic pressure. It was expected that potassium role in algae was similar to bacteria. Blue green algae can replenish potassium with rubidium and it required only small amount of potassium which can be found abundantly in environment, such as tin, chromium or cadmium.

In addition to carbon, nitrogen and phosphorus, other macronutrients that were required by algae were sulfur, calcium, magnesium, sodium, potassium and chloride. These nutrients were indispensable for the growth of algae.

2.4.1.5 Micronutrients

Mineral elements that were as important as macronutrients, but needed in lower quantities. The elements in this type will be in the concentration range of microgram to milligram per 1,000 mL of culture media. The essential nutrients for algae growth can be shown in Table 2.5

Table 2.5 Amount of essential nutrient for algae growth

Element		Zarrouk (g/L)	Sea water (g/L)	20% Sea Salt (g/L)
C	Carbon	2.29	0.031	0.05
Na	Sodium	5.44800281	10.56	2.112
Cl	Chlorine	0.632995	18.98	3.7962
N	Nitrogen	0.411003145	0.0009	0.023
O	Oxygen	-	-	-
K	Potassium	0.672096	0.38	0.0917
H	Hydrogen	-	-	-
P	Phosphorus	0.0895	0.00011	0.00063
S	Sulfur	0.21139352	0.884	0.1777
Mg	Magnesium	0.0195	1.27	0.254
C	Calcium	0.0144	0.40	0.08
Fe	Iron	0.00201	0.00002	0.001574
B	Boron	0.000498	0.0146	0.00292
Mn	Manganese	0.000614	0.00001	0.000167
Zn	Zinc	0.00000498	0.000014	0.00000028
Cu	Copper	0.000021	0.00009	0.000018
Mo	Molybdenum	0.00000666	0.000002	4×10^{-7}
V	Vanadium	0.00001004	3×10^{-7}	6×10^{-8}
Cr	Chromium	0.0000124	trace	-
Ni	Nickel	0.0001	5×10^{-7}	1×10^{-7}
W	Tungsten	0.00001121	Trace	-
Co	Cobalt	0.000000868	1×10^{-7}	2×10^{-8}
Br	Bromine	-	0.065	0.013
Al	Aluminium	-	0.00199	0.000398

Source: *Spirulina*, Production & Potential (Ripley, 1996)

2.4.2 Environment Factors

2.4.2.1 Light intensity

When nutrients and temperature were not limiting to growth, the most important environmental factor for mass alga culture was light, the most effect of which concerns photosynthesis. In 1987, Vonshak studied the biological limitations for algal mass culture, and found that light intensity was the main effect to mass production. Consistent with studies of Laorawat in 1995, the intensity and duration of the light irradiance determines the growth rate and the output rate.

Spirulina spp. needs light source for photosynthesis. It was found that light intensity that suitable for *Spirulina spp.* growth was influenced by temperature (Venkataraman et al., 1985). In open pond, light intensity that suitable for algae growth was ranged from 20,000-30,000 Lux. In laboratory scale, suitable light intensity was varied from 8,000-10,000 Lux (Citerri, 1983).

2.4.2.2 Temperature

Temperature had both direct and indirect influence to algae environment. It can affect growth rate and reproduction of algae. *Spirulina spp.* prefers temperature between 30-34 °C. If temperature was lower than 20 or higher than 37°C, it can heavily affect the growth rate.

Temperature also influence the capability of dissolved carbon dioxide into water that was carbon dioxide can dissolve in hot water more than cold water. When temperature was increased, carbon dioxide in water will be decreased. This can restrict the growth rate, even though the temperature was decreased (Ripley, 1996).

Temperature had a significant influence on the chemical composition of algae. In 1988, Tomaselli et al. studied the effects of temperature on growth and biochemical composition in *Spirulina spp.* strain M2; found that the chemical composition of algae was different when algae grow at different temperatures. At 42 °C, protein (22%) decreased while lipids (43%) and carbohydrates (30%) increased significantly.

Temperature at night was another very important factor in determining the rate of production of algae biomass. In 1991, Torzillo et al. studied the effect of temperature on productivity and night biomass loss in *Spirulina spp.* in outdoor tubular photo-bioreactors during May to September. They found that temperature had a significant influence on biomass production and biochemical composition of *Spirulina spp.* To get a better yield of *Spirulina spp.*, it seems important to keep the temperature as close to the optimum for growth as possible.

2.4.2.3 pH

Spirulina spp. can be growth well in high alkalinity that was pH between 9-11. By using Zarrouk's culture, pH will be ranged from 8 to 8.5. If pH was lower than 8 or higher than 11, the growth rate will be reduced. To adjust pH in algae culture can be performed by increasing or reducing dissolved carbon dioxide in water. Carbon dioxide can reduce pH in the culture by reducing carbonic acid. In contrast, algae uses carbon dioxide for photosynthesis and pH will be increased (Peerapornpisarn, 2003).

2.4.2.4 Dissolved oxygen

In wastewater treatment, oxygen from photosynthesis will be utilized by microorganisms for degrading organic matters. The amount of oxygen in ponds that contains with algae can be high up to 35 mg/l or 400 % saturation. In ponds and lakes, algae, especially cyanobacteria group can be growth and die rapidly this circumstance called algae boom. This rapidly death was called "Photooxidative Death" due to the super saturation of oxygen and high light intensity (Murray et al., 2013).

2.4.2.5 Size and Density of algae

The suitable density of algae was crucial for cultivation. This was corresponded with light intensity. If density of algae was low and light intensity was high, algae can be died because of Photo-Oxidation process. On the other hand, if density of algae was high, it will receive insufficient light and less efficient because light was obstructed by the number of algae. As a result, the most suitable density for

Spirulina spp. was ranged between 225-250 mg/L of dried weight. It also found that low density causes low growth rate and growth rate will increase when density was high (Ripley, 1996).

2.4.3 Mixing and Turbulence

Mixing and turbulence in cultivation was also an important measure for algae cultivation. It was important to control the flow rate in order to completely mix during cultivation for dispersed food and light exposure. It also prevents algae accumulation during the cultivation. It helps oxygen to disperse in the reactor and cause every cell to receive food and nutrients throughout the reactor. This increases the efficiency of cultivation and reduces the problem of temperature difference from the depth of reactor. If there was no mixing mechanism, algae cells will stick together and growth rate will be reduced. In addition, temperature will be increased and it can cause photo-inhibition when temperature rises to a certain point.

Mixing and turbulence also corresponds with light intensity that was when light intensity was high and no mixing mechanism, it can cause photolysis or photo-destruction which can cause a photoelectric effect. This effect was the destruction of electrons by light. On the other hand, if there was high light intensity and high mixing rate, this was suitable for algae growth. Algae receive sufficient light but light may be obstructed by other algae to protect from light destruction. In another case, if there was a low light source and low mixing rate, growth rate will be slow but pigment will be increased, such as dark green color and blue color of phycocyanin (Ripley, 1996).

2.5 Microalgae Cultivation Methods

Cultivation methods for microorganisms can be separated into two methods; Batch culture and Continuous culture.

2.5.1 Batch culture

The main principle of batch culture was to add inoculum and substances into the fermentation tank under suitable conditions. It had a mixing mechanism within the tank. For a higher production rate of microorganism, an all-time mixing system was

necessary in order to completely mixed microorganism and substances in the tank. Growth phase of microorganisms can be defined as followed;

- **Lag phase**

In this phase, cells were in incubation period. Necessary enzymes for degraded substances were produced. The production of the cells was slowly increased and the concentration of RNA per cell was increased significantly. The duration of this phase was depended on microorganisms, environment in the tank and methods of cultivation.

- **Exponential (Log) phase**

The production of cell in this period was increased significantly and constantly. The ratio of RNA per cell was stabilized. Different cells will had same generation time and the growth in log phase was exponential rate. The duration of this phase was depended on the volume and concentration of substances and the volume of generated waste from the microorganism. When enter the end of log phase, the ratio of RNA per cell will be reduced and the growth rate was constantly reducing.

- **Stationary phase**

This phase, the number of cells was in the highest and constant. The production rate was nearly zero that was death rate and growth rate was equal till the start of decline phase which death rate was higher than growth rate. When the production of cell reaches this phase, the production rate will be declined and the death rate will be increased.

- **Death phase**

In this period, the number of cells will be declined in exponential rate due to the lack of substances, the generation of waste from the cell and the changing of environment in the tank, such as pH and temperature.

The changing of production rate in different times can be called "Growth Cycle". Characteristic the growth of microorganisms in batch culture can be shown in Figure 2.5

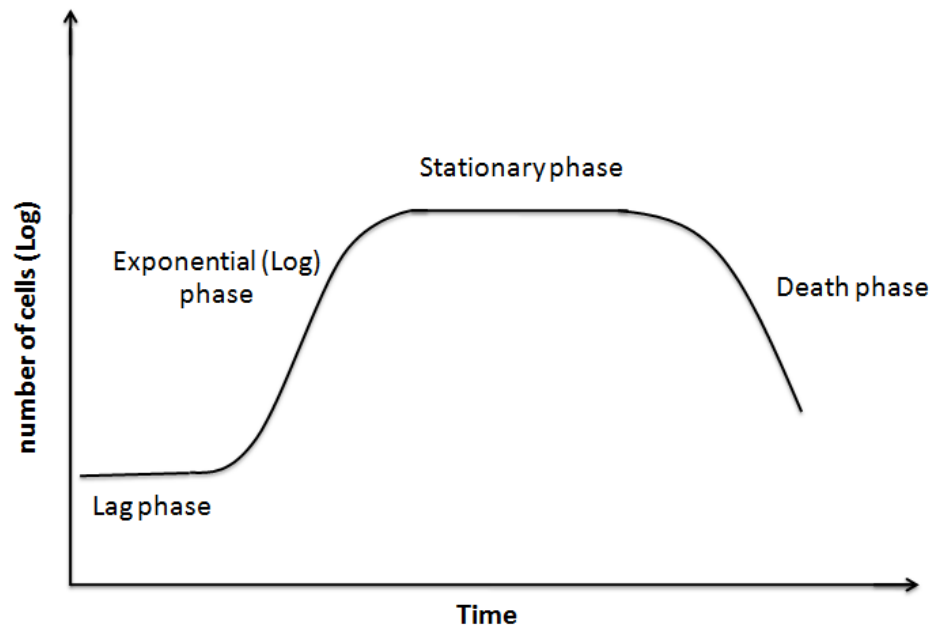


Figure 2.5 Characteristic the growth of microorganisms in batch culture

Source: Adapted from “The Growth of Bacterial Cultures” (Monod, 1949)

Generally, the rate of production was directly varied on the concentration of substances and the concentration of cells.

$$\frac{dx}{dt} = f(x, s)$$

Where; x = the mass of microorganisms per one unit volume

s = the concentration of substances (limiting substance)

$$\frac{dx}{dt} = \text{the rate of production}$$

It also can be defined as $\frac{dx}{dt} = \mu x$

$$\text{Therefore, } \mu = \frac{1}{x} \left(\frac{dx}{dt} \right) = \frac{d \ln x}{dt} \quad (2.1)$$

Where; μ = the specific growth rate of cell, time^{-1} (min^{-1} or day^{-1})

During the specific growth rate of cell and specific environment, it can be defined that “ t_d ” was the required time for the production of the cells was increased two times.

From equation 2.1; $dt = \frac{d \ln x}{\mu}$

Therefore, $t_d = \frac{\ln 2}{\mu} = \frac{0.693}{\mu}$ (2.2)

In 1949, Monod had studied the characteristic of bacteria growth during continuous culture. It was found that specific growth rate (μ) was directly varied on the concentration of required substances which were limited. Monod equation can be shown in equation 2.3

$$\mu = \mu_{\max} \left(\frac{S}{K_s + S} \right) \quad (2.3)$$

Where; μ_{\max} = the maximum of μ when the concentration of substance was not limited

K_s = saturation constant which was equal to the concentration of substance that influences μ equal to the half of μ_{\max}

Substitute μ in equation 2.3;

$$\frac{dx}{dt} = \mu_{\max} \left(\frac{S}{K_s + S} \right) x \quad (2.4)$$

This Monod equation (2.4) was shown the relation of the growth rate of microorganism, the limited substances and the concentration of cells in the specific period. This equation was well-know and it had been used as fundamental components for designing continuation culture method.

From the data of concentration of cells and the concentration of substances in different period during the fermentation, it can be used to find the specific growth rate which can be seen that the specific growth rate was varied throughout the fermentation time.

2.5.2 Continuous culture

This method was cultivated the cell and the products from the cells continuously. The concentration of cell and the necessary substance were controlled in the suitable level which can be performed by sterile feed media into the fermentation tank and removing the cells in the same ratio. The completely mixing was required for

this system and the ratio of cells removing must be suitable to the production of cell that was the rate of production was equal to the dilution rate of the cell. The feeding rate was equal to the sum of the utilization rate of substances by microorganisms and the nutrient that was removed from the tank.

This method uses the “limiting substrate”, such as carbohydrate components which was the main carbon and energy sources for microorganisms, nitrogen, vitamin or oxygen to control the changing rate of microorganisms. These substances were essential for growth of microorganisms, especially when the concentration of microorganisms was low. The changing of these substances concentration can influences the growth rate, the rate of substances usage and the production rate of microorganisms which was according to the Monod equation in equation 2.3 and equation 2.2.

$$\mu = \frac{\mu_{\max} S}{K_S + S} \quad (2.5)$$

In continuous culture, feeding rate into the tank and removing rate must be controlled equally. The volume of media must be constant. If feeding rate into tank was “F” and volume of the media was “V”, the ratio of “F” and “V” can be called dilution rate (D)

$$D = \frac{F}{V}, \text{ time}^{-1} \quad (2.6)$$

If considering the concentration of microorganisms,

“Inlet cell” + “Increased cell from the growth” = “Outlet cell” + “Accumulated cell in the system”

$$\text{Or; } Fx_0 + \mu xV = Fx + V \frac{dx}{dt}$$

In this system, there was no “Inlet cell”, therefore; $x_0=0$

$$\text{Therefore; } \frac{dx}{dt} = \mu x - Dx = x(\mu - D) \quad (2.7)$$

From equation 2.7, when D was higher than μ , cell will be washed out and the concentration of cell (x) was decreased in logarithmically. This influences the increasing of substances concentration (S) and the growth rate of microorganisms (μ). However, if “D” value was too high, “S” will be rapidly increased and overwhelm the

capability of microorganisms to utilize these substances. This can cause washout effect which cell was washout gradually from the system. Therefore, it was necessary to control conditions in the cultivation to the suitable level or “D” was not higher than μ_{\max} ($D \leq \mu_{\max}$)

At the stable condition of microorganisms concentration $\frac{dx}{dt} = 0$

Substitute in equation 2.7;

$$\mu = D \tag{2.8}$$

From the Monod equation;

$$D = \frac{\mu_{\max} S}{K_s + S} \tag{2.9}$$

If considering the concentration of limiting substrate (S);

“Changing rate of substrates concentration” = “Substrates inlet” – “Substrates outlet” – “Utilized substrates by microorganisms”

Or;
$$\frac{ds}{dt} = DS_0 - DS - \frac{\mu x}{Y_{x/s}} \tag{2.10}$$

When; $Y_{x/s} = \frac{\text{rate of growth}}{\text{yield of growth}}$

S_0 = initial concentration of substrates

From the equation 2.10, the cultivation process will be stable when $\mu=D$ and $\frac{ds}{dt} = 0$

In order to control the cultivation process that had higher production rate in stable condition for the long period, it was crucial to control dilution rate to suitable level ($D < \mu_{\max}$) that not causes the washout effect.

The advantages and disadvantages of batch culture and continuous culture showed in table 2.6

Table 2.6 The advantages and disadvantages of batch culture and continuous culture

Cultivation method	Advantages	Disadvantages
Batch culture	<ol style="list-style-type: none"> 1. Not complex and easier to operate than continuous culture method 2. Do not required extensive knowledge to operate 	<ol style="list-style-type: none"> 1. Need breaking time and start cultivated from the beginning after some period of cultivation 2. Preparation of initial microorganisms is required 3. Required more space than continuous culture 4. Required more workforce to operate than continuous culture
Continuous culture	<ol style="list-style-type: none"> 1. Continuously produce 2. In theoretical, produce higher yield and more efficient than batch culture 3. Do not required the preparation of initial microorganisms because it was remained in the system 4. Save space and workforce 5. Can operate automatically 	<ol style="list-style-type: none"> 1. In long period cultivation, contamination from other microorganisms may occur or mutation of microorganisms 2. Required extensive knowledge to operate the system 3. More expensive equipment than batch culture

2.6 Design criteria for photo-bioreactor cultivation

Design of photo-bioreactor for microorganism cultivation was influenced by many factors, such as types of microorganisms, type of substances and if it was operated under open condition, changing of weather and seasons also influence the suitability of photo-bioreactor construction.

The components of bioreactor that had been used for microorganism cultivation must be made of transparent component to allow sunlight penetration through the bioreactor. The media had capability to transfer the heat to atmosphere and had high tolerance under high pressure. Pressure may be required to apply for high flow rate in water circulation. It also should be made of materials that can prevent microorganisms stick on the surface.

Due to the growth rate of microorganism was influenced by the sunlight, the design of bioreactor was considered sunlight as a main component which can be defined as followed;

2.6.1 The exposure area from sunlight

During the cultivation, it was crucial to design bioreactor that maximized the utilization of sunlight by maximize the ratio between the exposure area per volume of media in the system. As a result, bioreactors should be thin panel or tubular type and not had high diameter. Thin panel type had one main constraint that it was required large operation area and cause difficulty to operate. It also cannot control flow rate of substances in the bioreactor which cause a “space area” from incomplete mixing. It was difficult to design large bioreactor that high efficiency while maintaining suitable costs. Due to these reasons, tubular type was more preferable in many cases because it can control the flow rate and easier to operate than panel type.

2.6.2 Increase the exposure to the sunlight

To increase the exposure of sunlight and reduce the accumulation of cells, mixing system may need to accommodate the microorganisms and increase the exposure to the sunlight. It may be performed by using propellers which were similar to other fermentation tanks or using magnetic bars in laboratory scale. For panel

bioreactor and tubular bioreactor, circulation pump may be used to create the recycle of media or using high flow to create turbulent flow.

For tubular type, turbulent flow can be calculated from Reynold's number (NR);

$$N_R = \frac{VD}{\nu}$$

Where; ν = Kinematic viscosity (For water flow through the reactor)

$$= 8.03 \times 10^{-7} \text{ m}^2 \cdot \text{sec}^{-1} \text{ of H}_2\text{O at } 30^\circ\text{C}$$

D = diameter of the tube; m

V = liquid velocity; m.sec⁻¹

For entering the turbulent flow, NR was at about 2000 and full turbulent, NR was at about 4000. This may achieve by changing liquid velocity in the tube. To investigate the result of turbulent flow, it may performed by changing liquid velocity that influences NR > 2000. Turbulent flow was not only increasing the exposure rate, but also reduces wall growth effect in the same time (Pirt et al., 1983)

In continuous culture, recycle ratio (R) will be equal to Recycle flow rate (V_r) divided by Feed rate (V_o)

$$\text{Or; } R = \frac{V_r}{V_o}$$

If the ratio of "R" was high that was "V_r" was larger than "V_o", the cultivation system will be similar to the continuous flow stirred tank reactor or "CFSTR". In contrast, if the "R" ratio was not high, the cultivation will be plug flow reactor or "PFR". As a result, it was important to control the circulation rate (V_r) as high as possible to cultivate in CFSTR system which had completely mixing.

The position of the reactor also can influence the exposure rate of microorganisms. For example, put the reactor vertically had high exposure rate than put the reactor horizontally. For tubular reactor, put the reactor with small slope also reduce the accumulation of cell and increase the flow rate within the reactor and it can help the gas-byproducts to flow upward more easily.

2.6.3 Light intensity

Different microorganism required different wavelength and light intensity. For algae cultivation, light source that had been used was come from fluorescent light because it provides light that had suitable wavelength and intensity to the growth of algae. For bacteria cultivation, light in infrared length was more preferable etc.

In 1983, Pirt et al. found that lack of light concentration can cause wall growth effect and this wall growth will reduce the concentration of light in the same time, therefore, the growth rate of microorganism will be reduced. It was crucial to control light source and provide enough concentration for the growth of microorganisms.

2.6.4 Radiation effect on the temperature of bioreactor

Temperature of bioreactor was influenced by the radiation effect from light source, heat storage within the biomass and heat losses to the atmosphere. This balance can be shown as following equation;

“Net heat input” = “Radiation input” – “Radiation stored” – “Heat lost to environment”

Under laboratory scale, light bulbs were the main light source and the temperature was constant in each days. It may be varied from changing seasons, winter or raining etc. But for open area, light source from the sun was not constant. This causes the changing of temperature in the bioreactor. In addition, heat release system may be required if the outside temperature was too high than the operation.

Apart from these criteria, other equipment may be required depends on the types of microorganisms. For example, for bacteria that does not required oxygen and does not significant gas by-products releasing, gas tube may not require for this cultivation. For algae cultivation, air was required and large amounts of gas by-products were produced, therefore, gas tube was required for ventilation within the system and also prevents interference of process in bioreactor.