

Outdoor Closed System of Algal Mass Culture: In Sight of Comparison on Vertical and Horizontal Photobioreactor for Cultivating the *Spirulina* sp.

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Abstract

Spirulina are multicellular and filamentous blue-green algae that has gained considerable popularity in the health food industry and increasingly as a protein and vitamin supplement to aquaculture diets. The challenge for economically and fulfill the requirement for food and medical purposes has create many ways for mass - growth production, that possibly cultivated in the open - system (such as a raceway pond) or closed - system photobioreactors (such as tubular, bubble-column, airlift, flat-panel, and vertical). The cultivation of the *Spirulina* on the vertical and horizontal photobioreactor has been studied. The photobioreactor, namely BJVP and BJHP, has a design to be less energy consumption using the air bubbling or circular paddle. The observation was conducted in a whole year with parameters of rainfall, temperature, light intensity, pH, and salinity. Results showed that cultivation of *Spirulina* on the vertical photobioreactor growth faster than the horizontal photobioreactor systems and the yield of biomass was about 0.94 gDW/L. Average of temperature ranges of BJHP were 31.0°C - 35.5°C, salinities were 35 per mil level, pH were 8.55 - 10.86, and light intensity were 427 - 2001 $\mu\text{mol photon s}^{-1} \text{m}^{-2}$. Whereas the BJVP has averages temperature range of 31.4°C - 33.9°C, salinity 33 - 35 per mil level, pH 8.46 - 10.75, and light intensity 532 - 2062 $\mu\text{mol photon s}^{-1} \text{m}^{-2}$. The proximate analyses of biomass from BJVP cultivation shows has tendency higher protein content compared to BJHP. The optimization of both reactors has continuing evaluated in order to get the optimum parameters required for economically *Spirulina* cultivation systems.

Keywords: *Spirulina*, BJVP, BJHP, outdoor mass cultivating system, photobioreactor

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INTRODUCTION

Spirulina are multicellular and filamentous blue-green algae which grow under high salinity, pH and temperature conditions. The cell length is about 50 to 500 μm and can form colonies. It is forming populations in freshwater and brackish lakes and some marine environment, mainly alkaline-saline lakes (Vonshak, 1997). From the biotechnological viewpoint, *Spirulina* is one of the most important cyanobacteria because it is able to produce high concentrations of protein (up to 70%) inside its cells, antioxidant pigments (chlorophyll a, phycocyanin, phycobiliproteins, and carotenoids), fatty acids (α -linolenic acid/GLA), essential amino acids, minerals, vitamins (especially B₁₂), and polysaccharides (Belay *et al.*, 1993). Nowadays, *Spirulina* is cultivated in large areas and commercial that has gained considerable popularity in the health food industry, human food supplements, animal feed, and pharmaceuticals (Madkour *et al.*, 2012). Moreover, this microalgae has been successfully employed in integrated systems for wastewater treatment (Kosaric *et al.*, 1974), recovered and re-utilised (Vonshak *et al.*, 1982), also as absorbent material for heavy metals (Solisio *et al.*, 2006). *Spirulina* is one of the prime candidates for the controlled ecological life support system (CELSS) because it proliferates with a high ratio of edible to non-edible biomass, contain many nutrients, and have gas exchange characteristics compatible with human body standard requirements (Minoo & Bernhard, 1991).

The cultivation systems of microalgae can be done in an open system (lakes, ponds) and in a controlled closed system called photobioreactors (PBR). The merits of open system are relatively economical, easy to clean up after cultivation, suitable for mass cultivation of algae, although has demerit parts are lack in control of culture conditions, difficulty in growing algal cultures for long periods, less productivity, occupy large land and water sources, limited to few strains of algae, and easily get contamination. For overcoming the obstacles on open reactors some scientists and algal industries propose controllable closed reactors. It is suggested that closed photobioreactors have many benefits such as better control of the cultivation conditions than open systems, higher biomass productivities and contamination can be easily prevented (Ciferri, 1983).

The design and development of photobioreactors for maximum production of algae are very fundamental. Major environmental factors for high productivity of *Spirulina* biomass that should be considered for designing the photobioreactors are: luminosity (photo-period 12/12, 40 Kluxes), temperature (30°C), inoculation size, stirring speed, dissolved solids (10 - 60 g/liter), pH (8.5 - 10.5), water quality, macro and micronutrient presence (Ugwu *et al.*, 2007). Furthermore, other factors such as design, cost-effectiveness of the bioreactor, purity of the algae produced, user-friendly, low maintenance, and space

convenience need to be optimized. Several bioreactor types which are used for growing cyanobacteria including *Spirulina* are bubble column photobioreactor, airlift photobioreactor, flat panel bioreactor, horizontal tubular photobioreactor, and stirred tank photobioreactor. These bioreactors have their own advantages and disadvantages (Singh & Sharma, 2012). Biomass growth in a PBR is a complex process, which is the result of multiple effects such as photosynthetic light capture, light attenuation of the suspension and reactor hydro-dynamics. In the case of non-limiting nutrient conditions, light is the most relevant factor for autotrophic growth. Light attenuation could strongly influence the photosynthetic biomass productivity (Sforza *et al.*, 2014).

We have study on open and close reactors for algal biomass production in outdoor condition. In this research is focusing on the utilization of a closed reactor for effective growth of *Spirulina* in order to compare between vertical or horizontal reactors. The main purpose is to get the fast-growing the algae with high-density cells and less contaminated condition and edible.

MATERIALS AND METHODS

Organism and Culture Medium

An axenic strain of the cyanobacteria *Spirulina* was cultured in modified Zarrouk's medium (Zarrouk, 1966), containing per liter 2.5 g NaNO₃, 1 g Na₂SO₄, 0.2 g MgSO₄·7H₂O, 0.04 g CaCl₂·2H₂O, 0.01 g FeSO₄·7H₂O, 0.08 g Na₂EDTA, 0.5 g K₂HPO₄, 16.8 g NaHCO₃, 1 ml of trace solution A5. One liter of trace solution A5 contained 2.86 g H₃BO₃, 1.81 g MnCl₂·4H₂O, 0.222 g ZnSO₄·7H₂O, 0.075 g CuSO₄·5H₂O and 0.0818 g Na₂MoO₄·2H₂O. All nutrients were dissolved in distilled water and sterilized in an autoclave at a temperature of 121°C for 15 minutes before use. *Spirulina* was isolated at Semarang Port, Central Java, Indonesia (2005) and therefore maintained at Research Center for Biotechnology, Indonesian Institute of Sciences.

Cultivations in vertical photobioreactor (BJVP)

The material for BJVP was molding acrylic tubes and designed to stand on the compact rack vertically with a slight inclination and placed on two parallel planes so that they were not superimposed each other and could feel the same light intensity (Figure 1).

The culture was continuously mixed by aeration system from the bottom of the reactor with an air pump, blowing air into a vertical pipe used as a riser. It is made up of vertical tubing that is naturally transparent to allow the penetration of light.

Sparger is attached at the bottom of the reactor which converts the sparged gas into tiny bubbles. Sparging with gas mixture provides overall mixing, mass transfer of CO₂ and also removes O₂ produced during photosynthesis (Sforza *et al.*, 2014). An 8 liter

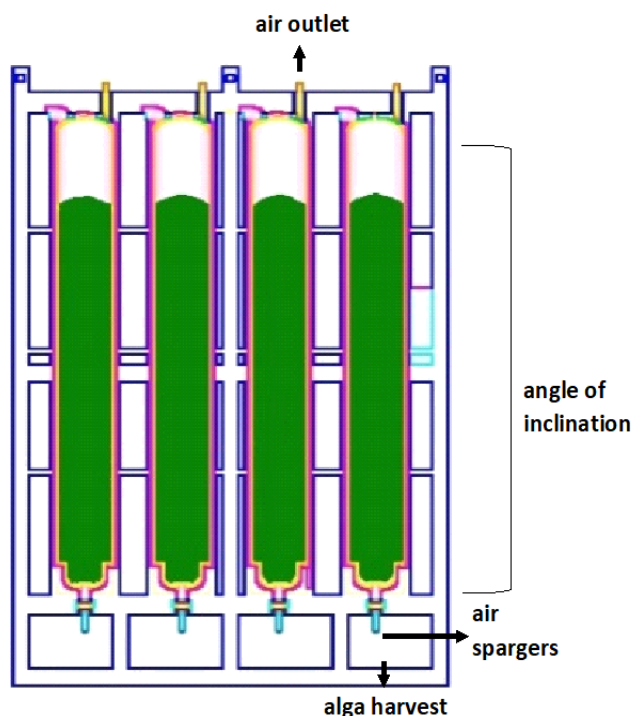


Figure 1. Design of vertical photobioreactor (BJVP)

of culture is used as stock, and it will be added to 80 liter of media, then it is cultivated for seven days.

Cultivations in horizontal photobioreactor (BJHP)

Specific materials of mix polyurethane, plastic and acrylic were molding for making the knock-down horizontal reactors. Horizontal tubular reactors are placed horizontally, giving the design of a parallel set of tubes and loop shape (Figure 2). This mechanism gives advantage in outdoor culture for their orientation towards sunlight resulting in high light conversion efficiency. Propeller set up with speed 5 rpm for mixing microalgae. A 100 liter of culture is used as stock, and it will be added to 1000 liter of media, then it is cultivated for 7 days.

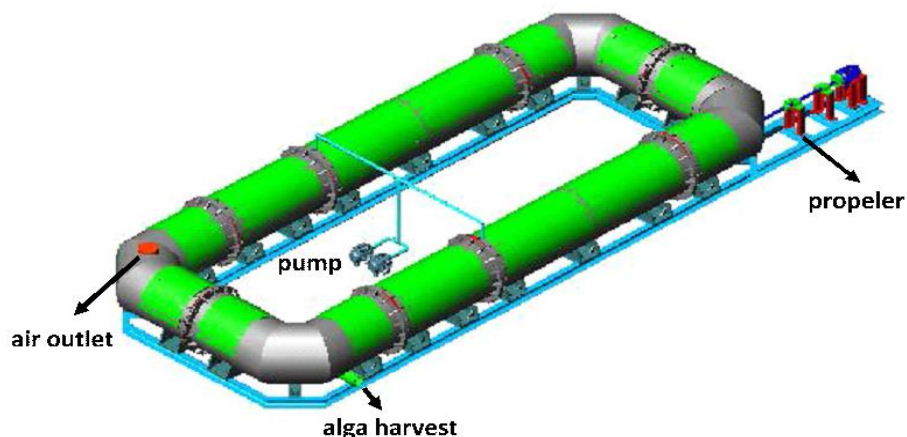


Figure 2. Design of horizontal photobioreactor (BJHP)

An Observation on Biomass Production

Quality control of the cultures

Spirulina biomass productivity was determined by dry weight of cells measurement and in triplicate culture samples (100 ml each). The biomass productivity was monitored every day. The filtered cells were washed with distilled water and dried at 50°C into a constant mass. The pH and temperature were measured using a pH meter Horiba Navi D-55S. The light intensity measurements were done using a Fisher Scientific Light Meter 0666263, and the salinity was measured using a refractometer (Atago ATC-S/Mill-E). Optical density was measured by UV-VIS spectrophotometer (Shimadzu, UV-1700) at 680 nm.

Protein determination

Proteins were determined with the Bradford protein assay (Bradford, 1976) that measured by spectrophotometric at 595 nm. Bovine serum albumin (BSA) was used as the standard protein for the calibration curve. Total proteins were expressed in mg of protein per ml of a culture. Additionally, total protein concentration was also determined by a modified method of Bradford in the centrifugation tests with BSA as standard.

Lipid determination

The wet biomass of *Spirulina* sp. from culture volume 200 ml were extracted by modified Bligh and Dyer method (Bligh & Dryer, 1959). The extraction of microalga biomass was use mixed solven of methanol : chloroform (1:1). After vigorously vortex the biomass for 30 second, and than additional water on sample, sentrifuge sample 2000 rpm 10 minutes. Biomass with the solven resulting three layer of organic, aqueous layers and settling biomass containing the water. The aqueous (contain chloroform) layer were collected and evaporated to dryness, resulting the cruide oil (lipid). Repeated the method until the biomass turn white. Gravimetrically the dry lipid was storage.

RESULTS AND DISCUSSIONS

Growth and properties of *Spirulina* biomass

Biomass productivity

Growth duration for cultivating *Spirulina* is set on seven (7) days cycles. Growth rate that observing by cells density of *Spirulina* was mirroring the biomass productivity. The algal biomasses production on the each photobioreactors are shown in Figure 1. In general the growth pattern of *Spirulina* follows usual growth pattern, which are starting from lag phase and following by exponential phase. After 7 days cultivation, the cells are harvested by simply process of filtration. At the beginning of the cultivation period, algal growth rate was slow, because most of energy resources seems to be allocated for adaptation to the environment condition. However, after 3 days of inoculation, the algae cells began to replicate faster. The results of observation on vertical photobioreactor showed the biomass rate axcelerating from 0.14 g (dry weight) L⁻¹ to 0.94 g (dry weight) L⁻¹ [1-7 days cultivation]. On the other hand, cultivation on horizontal photobioreactor resulting the biomass production from 0.12 g (dry weight) L⁻¹ to 0.62 g (dry weight) L⁻¹ [1-7 days cultivation]. It is suggested that cultivation on the vertical photobioreactor have biomass production higher than cultivation on the the horizontal photobioreactor.

The biomass productivity of the helical tubular photobioreactor in semicontinuous basis is higher than biomass productivity obtained in batch basis. The maximum productivity value was 0.40 g l⁻¹ d⁻¹ (8.52 g reactor⁻¹ d⁻¹ or 6.45 g m⁻² d⁻¹) using a dilution of 1

: 5 and a dilution rate $D = 0.0078 \text{ h}^{-1}$ (Travieso *et al.*, 2001). Oncel and Sukan (2014) compared an internal loop airlift and bubble column photobioreactors with respect to their performances during cultivation of *Arthrospira platensis* (*S. platensis*). It was observed that a higher dry biomass weight and chlorophyll-a concentration was obtained in the airlift photobioreactors, yielding a maximum growth rate of 0.45 day⁻¹, while a maximum growth rate of 0.33 day⁻¹ was reached in the bubble column PBR.

Protein and Lipid Content

Spirulina has high quality protein content (59 - 65 %), which is more than other commonly used plant sources such as dry soybeans (35 %), peanuts (25 %) or grains (8 - 10 %) (Habib *et al.*, 2008), but have a low lipid content (6 - 13%). The protein content of *Spirulina* appears to be high also when compared with that of unicellular algae and other cyanobacteria (Vonshak, 1997). *Spirulina* is not rich in lipids (6 - 13%), it has relatively high cell growth rate with easy process control and rapid biomass recovery due to the filamentous cell structure (Ciferri, 1983).

BJHP and BJVP were 34.61% and 40.49%, respectively, while lipid productivity was 20.45% and 18.66%, respectively (Figure 4). Lipid content of *Spirulina* in this study is hgher than the other study, this is because the extraction method used. Reactors BJVP thed to improve the cultivation with higher protein content, and lower lipid content compares to BJHP.

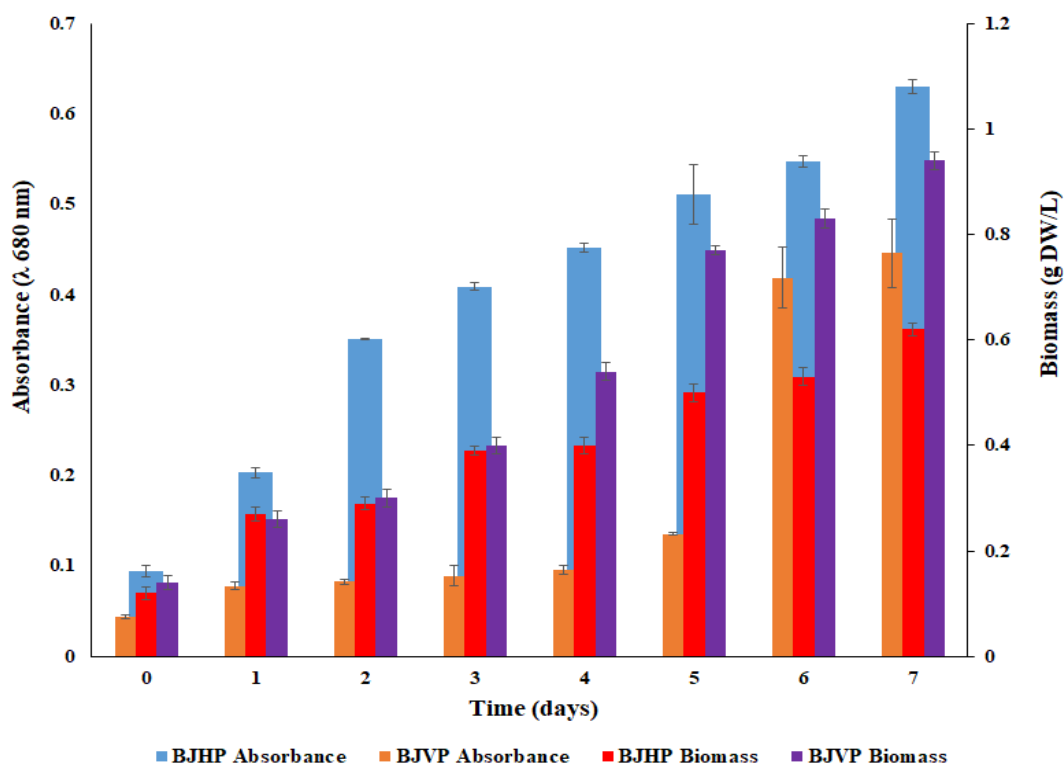


Figure 3. Growth diagram of *Spirulina* during cultivation period

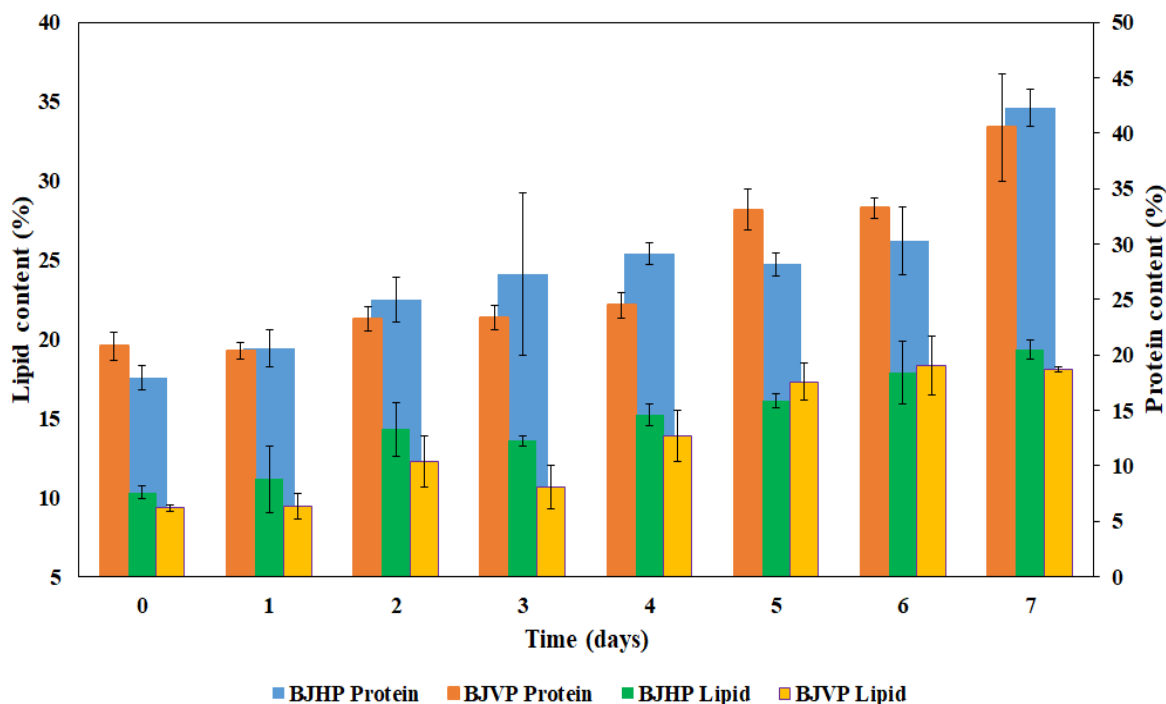


Figure 4. Protein and lipid content of Spirulina during cultivation

Torzillo *et al.* (1986) reported that no significant differences in the protein content between the biomass grown in tubular photobioreactors and open ponds were found (average 50%). Another study reported that *S. platensis* cultivated in bubble column photobioreactor had the highest crude protein content of the biomass obtained was 68.1% (Chang *et al.*, 2013).

Lipid production of microalgae *S. platensis* cultured in tubular and panel photobioreactor and pond in June and September were compared. The highest lipid amounts were obtained as 7.66% and 7.44% in the tubular photobioreactor (Azgin *et al.*, 2014).

Various condition of environmental factors

pH

pH is an essential role in the bioreactor system and as health growth indicator of *Spirulina*. The concentration of hydrogen ions or proton (H^+) in the cell fluid and protoplasm vital for the physiological process. Figure 5 depicting the fluctuation of pH change in the medium. pH fluctuation at the BJHP ranging from 8.55 - 10.86, while the BJVP pH ranging from 8.46 - 10.75. It is suggested pH level of the medium increase during the period of photosynthesis as a consequence of nutrient uptake, such as bicarbonate and nitrate (Ai *et al.*, 2008). In this case, the bicarbonate, $NaHCO_3$ was used to maintain pH at a high level for stabilizing *Spirulina* cultures. *Spirulina* is well known for cyanobacteria, which are requiring high-level pH and osmotic environment (Chen, 2011). The high level of pH in a culture media is related to the amount of bicarbonate in medium,

which then can be used to produce CO_2 to run the photosynthesis process.

S. platensis is a typical alcalophylic organism that apparently has a high dependence of its growth rate on pH level. Optimal growth was observed at pH 9 to 10, and there was minimal growth at pH 7.0. At pH 11.5, a growth rate equivalent to 80% of the maximum was maintained (Belkin & Boussiba, 1991). Effect incompatibility of pH will make lysis cell and can change the growth of pigment (Hariyati, 2008).

Temperature

Temperature is one of the most important conditions for production and the growth of *Spirulina*. Under proper temperature conditions, the algae can grow rapidly. Basically, the increase in temperature will increase the metabolism and cell production of the microalgae. Figure 6 shows the change of the temperature of the culture medium. The average temperature of the cultures (midday) for BJHP was 31.0-35.5°C, while the average temperature was BJVP 30.5-33.9°C, respectively. Optimum temperatures are 30-35°C for *S. platensis*. The species can live at the 18°C minimum and 39°C maximum (Fox, 1996). Generally, all the temperatures measured were optimum for *S. platensis* culture.

The space and light requirements of microalgal farming implies that a commercial cultivation system will most likely be located outdoor and be exposed to a large range of day/night and seasonal temperature changes. Devising cost-effective and reliable temperature control mechanisms is

therefore a significant challenge in photobioreactor design.

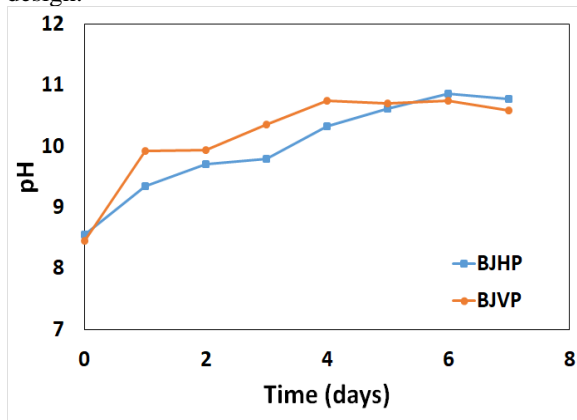


Figure 5. pH observation during cultivation period

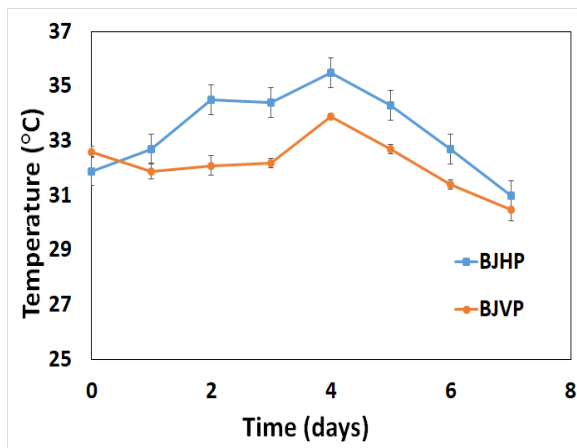


Figure 6. Temperature observation during cultivation

It was demonstrated that, without temperature control, the temperature in a closed photobioreactor could reach a level 10 - 30°C higher than the ambient temperature (Wang *et al.*, 2012).

Light Intensity

In order to enhance microalgal growth in photobioreactors, light penetration is one of the most important parameters to be addressed. The light intensity is a major factor that contributes to the growth of *Spirulina* sp. as all of the microalgae that accomplish the photosynthesis. Lack of light can lead to abnormality of the photosynthesis process that will affect the growth of *Spirulina* sp. Table 1 shows the light intensity performance on BJHP and BJVP during cultivation period.

The results of this study indicate that the light intensity at the BJHP range from 427 - 2001 $\mu\text{mol photon s}^{-1} \text{m}^{-2}$, while the BJVP light intensity ranges between 532 - 2062 $\mu\text{mol photon s}^{-1} \text{m}^{-2}$. *Spirulina* sp. resistant to sunlight intensity in the culture field

scale that ranges 500 - 350.000 lux, with a maximum of 3 hours long lighting (Kabinawa, 2006).

Table 1. Light intensity performance on BJHP and BJVP during cultivation period

Times (day)	Light intensity ($\mu\text{mol photon s}^{-1} \text{m}^{-2}$)	
	BJHP	BJVP
0	1455	823
1	1828	1874
2	1942	1946
3	2001	2062
4	1931	1998
5	1455	1508
6	1489	1661
7	427	532

Salinity

Since the *Spirulina* is derived from marine environment therefore the salinity level is important to be controlled. Salinity is one of the factors that affect aquatic organisms in maintaining osmotic pressure in the protoplasm with water as the environment. Table 2 shows the salinity performance on BJHP and BJVP during cultivation period. The salinity achieved in the BJHP and BJVP were 35 and 33 - 35 per mil level, respectively. *Spirulina* sp. is able to survive at salinity between 20 - 70 ppt (Richmond, 1986).

Salinity affects the water content contained in microalgae cells. When the media salinity is high (above the normal range) and can not be tolerated, the water in the microalgae cell will be absorbed out and it will make the cell died.

Table 2. Salinity performance on BJHP and BJVP during cultivation period

Times (day)	Salinity (per mil level)	
	BJHP	BJVP
0	35	35
1	35	34
2	35	33
3	35	34
4	35	34
5	35	34
6	35	33
7	35	33

CONCLUSIONS

Spirulina is growing faster on the vertical photobioreactor compare to the horizontal photobioreactor systems with the yield of the biomass of approximately 0.94 gDW/L. The fluctuation of environmental factors in BJHP reactors during

cultivation period were appearance on temperature level around 31.0°C to 35.5°C, salinities around 35 per mil level, pH around 8.55 to 10.86, and light intensity around 427 - 2001 $\mu\text{mol photon s}^{-1} \text{m}^{-2}$. On the other hand, the condition of environmental factors in BJVP reactors has averages on the temperature range of 31.4°C to 33.9°C, salinity 33 to 35 per mil level, pH 8.46 to 10.75, and light intensity 532 - 2062 $\mu\text{mol photon s}^{-1} \text{m}^{-2}$. Reactors BJVP tend to improve the cultivation with higher protein content compare to BJHP.

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