THE CULTIVATION OF SPIRULINA PLATENSIS ON VERTICAL AEROPONIC SUBSTRATES

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ABSTRACT

To improve the efficiency of algae production process, most studies have focused on selecting algal strains; controlling nutrient, temperatures and pH as well as biomass processing technologies. However, technical innovations in algae cultivation have remained elusive. For this reason, a new technique in cultivation system was studied to determine if it could produce significant quantities of biomass compared to the traditional hydroponic system. After 8 days at same culture conditions: LED lights on a 24 hours off light cycle, microalgae density (OD$_{560} = 1.4$), 40 mL of the inoculum, luminosity 2000 lux and temperature was maintained between 25–26 °C, the algae was harvested and the effluent was collected for measuring dry weight of algal biomass. The vertical substrate system have a greater productivity on the substrate based system than traditional hydroponic system. There was increased up to 1.7 times for biomass dry weight in substrate-grown system compared to aqueous-grown system after 8 days of growth. This vertical substrate system produced significant areal yield of 0.8 g m$^{-2}$d$^{-1}$ and the areal growth rate of 9.1 g/m$^2$ respectively.

Keywords: Spirulina platensis, aeroponic, the production of algal biomass.

1. INTRODUCTION

Spirulina platensis, is a cyanobacterium, also known as blue-green algae. Spirulina platensis is a source of protein and contains several vitamins, minerals and polyunsaturated fatty acids such as gamma-linolenic acid, hycocyanin [1] and ω-3 and ω-6 polyunsaturated fatty acids [2], which is an important organism for the production of human food supplements, animal feed and pharmaceuticals because of its nutraceutical properties

Actually, algal cultivation systems have been placed on the hydroponic system (Opened Ecosystem-O.E.S and Closed Ecosystem-C.E.S) but to date neither system has been able to manufacture algae biomass in a financially viable manner. The biggest advantage of these O.E.S is very simple, which is easier to construct and operate than most closed systems. The O.E.S are open to the environment so there are major limitations. Major limitations in O.E.S include: poor light utilization by the cells, evaporative losses, diffusion of CO$_2$ to the atmosphere, temperature can be difficult to maintain and contamination from strains of microorganism and other fast
growing heterotrophs. While the C.E.S gives a closed system that minimizes loss from evaporation and contamination, but the closed system requires the use of temperature control and constant maintenance to remove algae agglomeration [3]. Because of the inabilities of OPs and PBRs to manufacture financially viable biomass, the vertical aeroponic substrates are able to increase algal productivity as well as harvest concentration in comparison to O.E.S and C.E.S systems.

The aim of this study is to evaluate the influence of different culture conditions including cultivation time, initial microalgae density and illumination on the growth of *Spirulina platensis* in vertical aeroponic substrates to optimize the conditions for culture.

2. MATERIALS AND METHODS

This system is comprised of many vertical aeroponic substrates which are suspended from a scaffolding system. The substrates are suspended from a scaffolding system using hooks so that the substrates can be easily removed. The substrates would be spaced 5 cm apart from one another and would be placed within a greenhouse. The substrates themselves are composed of cotton and readily retain water. When algae are grown up to an optical density 1.4 (at 560 nm) in a photobioreactor system, the substrates are removed from the scaffolding system and soaked in the inoculum. Following inoculation, the substrates on the scaffolding system are continuously irrigated with a nutrient solution (water and nutrients) and the algae begin to grow on the substrates. When the algae-covered substrates are typically harvested by using gentle wash, the substrates can be removed from the scaffolding system. The substrates be gently washed that removes completely of the algae from the substrates. This entire process can be described and illustrated in four major steps: 1) Stock Solution 2) Inoculate Substrate, 3) Algae Growth on Substrate and 4) Harvest (Figure 1). For the experiments described here Figure 2. *Spirulina platensis* strain was obtained from Research Institute for Aquaculture No.2. The medium used in this study contained (g/L): 16.8 g NaHCO₃, 2.5 g NaNO₃, 0.5 g K₂HPO₄, 1.0 g K₂SO₄, 1.0 NaCl, 0.2 g MgSO₄.7H₂O, 0.04 g CaCl₂.2H₂O, 0.01 g FeSO₄. 7H₂O and micronutrients [8].

![Figure 1](image1.png)

*Figure 1. Aeroponic substrate based cultivation system overview. The process from growing inoculum through harvesting is comprised of four distinct steps: 1) Stock Solution, 2) Inoculate Substrate, 3) Algae Growth on Substrate and 4) Harvest.*
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![Diagram](image)

**Figure 2.** Small aeroponic substrate based system inoculated with *Spirulina platensis*. Each substrate was 15 cm × 24 cm. At the top of each substrate, a small irrigation dripper was installed. The substrates were placed within a closed chamber and illuminated with fluorescent lamps (40 W) at an luminance of 2000 lux and a 24 hour photoperiod at 25 °C.

This medium was also used to prepare the biomass for initial inoculation of each experiment. The medium was optimized by vertical aeroponic substrates for growth on the substrate system. The factors considered in this experiment were cultivation time, microalgae density and luminosity. For the experiment, cultivation time was used at different levels for 1, 2, 4, 6 and 8 days; initial microalgae density levels were 1.6; 1.8; 2.0 (OD<sub>560</sub>) and luminosity was at 2000 and 4000 lux. Biomass of *Spirulina platensis* was monitored using a spectrophotometer (560 nm).

To model vertical aeroponic growth, 40 mL of the inoculum was used to inoculate a aeroponic substrate (15 cm wide, 24 cm long). To inoculate the substrates with algae, they were soaked in 40 mL of the inoculum. Following inoculation, the substrates were harvested by washing gingerly in sterile distilled water. When the algae-covered substrates are typically harvested, each substrate was washed with 800 ml sterile distilled water to remove completely biomass. In parallel to the experiment being inoculated, the control was placed into 200 mL flasks in which 40 mL of inoculum was added to 200 mL of their respective media. *Spirulina platensis* was inoculated at 20 per cent level. The flask were capped with a one waterproof cotton plug and covered with aluminum foil to minimize loss due to evaporation. The temperature was maintained between 25–26 °C. The aeration tubing was placed at the bottom and center of the flasks to mixing. For the experiments, the temperature was maintained between 25–26 °C; luminosity at 2000 lux; LED lights on a 24 h illumination; microalgae density (OD<sub>560</sub> = 1.4). Samples were taken after 8 days of growth for estimation of algal biomass.

The response of the experimental design was achieved by the absorbance read at the end of the experiment, which was converted into biomass (g/L) by curve fitting (Equation 1).

\[
\text{Biomass} = (\text{absorbance reading}) \times 0.8153 + 0.0591 \quad (R^2 = 0.9978) \quad (1)
\]

For the effect of different microalgae densities and light intensities on growth of *Spirulina platensis*, samples were taken after 6 days of growth for estimation of algal biomass. Algal biomass was estimated by the method of Richmond and Gobbelaar (1986) [10].

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3. RESULTS AND DISCUSSION

3.1. The effect of different cultivation time on the biomass productivity

Table 1. Production of *S. platensis* on a vertical aeroponic substrate or in a flask (control) under different cultivation time.

| Cultivation time (day) | Vertical aeroponic substrates system | Control photobioreactor system |
|------------------------|--------------------------------------|--------------------------------|
|                        | Growth rate (dry biomass) (mg/cm²)  | Biomass dry weight (mg/40ml inoculum) | Growth rate (dry biomass) (mg/ml) | Biomass dry weight (mg/40ml inoculum) | Biomass wet weight (mg/40ml inoculum) |
| 1                      | 0.35                                 | 97.2                             | 118.54                           | 0.4                                 | 80                                 | 97.56                           |
| 2                      | 0.47                                 | 129.6                            | 158.05                           | 0.41                                | 82                                 | 100                             |
| 4                      | 0.59                                 | 162                              | 198                              | 0.573                               | 114.6                              | 180.6                           |
| 6                      | 0.76                                 | 211                              | 257.32                           | 0.63                                | 126                                | 153.65                          |
| 8                      | 0.91                                 | 251.1                            | 306.22                           | 0.74                                | 148.08                             | 139.75                          |

*Figure 3*. Growth rate of *S. platensis* under different cultivation time.

*Figure 4*. (a) Substrate cultivating algae after 8 days of growth, (b) Substrate after harvesting the algal substrates.
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Table 1 and Figure 3 shows that production increased with increased time of cultivation. As the biomass concentration increased from day 1 to day 8. Maximum biomass was obtained on the 8 in the 2 systems. Total biomass dry weight was 148.08 mg (0.74 g l⁻¹; 0.05 g l⁻¹d⁻¹ respectively) in control system, which increased to 251.1 mg (9.1 g m⁻²; 0.8 g m⁻²d⁻¹ respectively) on the substrate. Accordingly, there was a 1.7 times increase for biomass dry weight in substrate substrate-grown compared to aqueous-grown. Similar results were reported by Johnson et al. [6] conducted research on a novel aeroponic substrate system for the growth and development of the marine alga and the freshwater alga *Parachlorella kessleri*. The achieved results were there was a high increase (1.5 times for *Parachlorella kessleri*; 4.4 times for *Tetraselmis chuii*) for growth rate in substrate substrate-grown compared to aqueous-grown after 11 days (*T. chuii*) and 34 days (*P. kessleri*) of cultivation. These results demonstrated that there was a highly significant difference between the biomass of substrate and flask biomass. Because of increased oxidative stress, it was originally hypothesized that an aeroponic system would allow for increased biomass productivities. Especially under the new culture method, the growth of algae on the system is also very unique as the substrate based system does not have the traditional log, exponential, stationary and death phase of growth as do OES or CES. With simple harvesting, the moist substrates was removed from the substrates by washing gingerly in sterile distilled water that removes approximately 50 – 70 % of the algae from the substrates and leaves the remaining algae (30 – 50 %) on the substrate for regrowth. So substrate based systems were able to maintain a stationary phase for extended periods of time (> 6 months) without requiring cleaning or maintenance [6].

Thus, the vertical aeroponic substrate was the most logical choice to increase algal productivity as well as harvest concentration in comparison to traditional hydroponic systems. The results (1 g m⁻²d⁻¹ wet biomass, 0.8 g m⁻²d⁻¹ dry biomass) were higher than previous research of Ozkan et al. [7]. The authors indicated that the areal productivity of the biofilm photobioreactor was 0.71 g/m² day cultivating *Botryococcus braunii*.

### 3.2. The effects of light intensity on the growth rates
*Spirulina platensis* cultures were exposed to the light intensities of 2000 and 4000 lux. The results are shown in Table 2.

**Table 2. Growth rate of *S. platensis* under different luminosities at the 0.05 significance level.**

| Cultivation time (day) | Initial microalgae density (OD$_{560}$) | Luminosity (lux) | Growth rate (dry biomass) (mg/cm$^2$) |
|------------------------|----------------------------------------|------------------|--------------------------------------|
| 6                      | 1.4                                    | 2000             | 0.74*                                |
| 6                      | 1.4                                    | 4000             | 0.4b                                 |

*Significant differences at the 5% level are identified on columns by different letters.*

As with all plants, microalgae photosynthesize, they assimilate inorganic carbon for conversion into organic matter. Although light is an essential substrate for photosynthesis, high light intensity causes a decrease of photosynthetic activity. The growth rate of algae is maximal at saturation intensity and decreases with both increase or decrease in light intensity [9]. Light intensity increase above saturating limits causes photoinhibition [4]. This is due to the disruption of the chloroplast lamellae caused by high light intensity [2] and inactivation of enzymes involved in carbon dioxide fixation [5]. According to Gordillo et al, the growth rate of *Dunaliella viridis* decreased to 63% with increasing in light intensity from 700 to 1500 μmol.m$^{-2}$.s$^{-1}$ [4]. In our study, it was shown that at 2000 lux light intensity, the growth rate of *Spirulina* sp. was 0.74 mg/cm$^2$ as compared to 4000 lux (0.4 mg/cm$^2$).

### 3.3. The effect of initial microalgae density on algal growth

The effects of initial microalgae density on the growth rates are shown in Table 3.

**Table 3. Growth rate of *S. platensis* under initial microalgae density at the 0.05 significance level.**

| Cultivation time (day) | Initial microalgae density (OD$_{560}$) | Luminosity (lux) | Growth rate (dry biomass) (mg/cm$^2$) |
|------------------------|----------------------------------------|------------------|--------------------------------------|
| 6                      | 1.6                                    | 2000             | 1.24*                                |
| 6                      | 1.8                                    | 2000             | 0.9*                                 |
| 6                      | 2.0                                    | 2000             | 0.7*                                 |

*Significant differences at the 5% level are identified on columns by different letters.*

*Spirulina* growth was essentially limited by the low intensity of light. The lower the population density, the higher the growth rate will be. This is to be explained for a system that is primarily light-limited because reducing the population density will increase the availability of light to each cell. This explains why the results shown in Table 3, the growth of *S. platensis* decreased with increasing initial microalgae density. The highest specific growth rate was 1.24 mg/cm$^2$ (OD$_{560}$ = 1.6).

### 4. CONCLUSIONS

In this study we have demonstrated that aeroponic system has a high efficiency on the production of *S. platensis* biomass. Growing extremophiles on the system can avoid some of contaminations. This system was at 1.7 times higher than those obtained from traditional...
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hydroponic system after 8 days of growth. The results demonstrated that in the same culture conditions (temperature, nutrient concentration, initial microalgae density, initial algae volume, cultivation time, light intensity, lighting time), the productivity of hydroponic method was only 59% compared to the aeroponic method. Additionally, with simple harvesting method, the algae were removed completely from the substrates. Thus the vertical aeroponic substrate was the most logical choice to increase algal productivity. However the aeroponic system may need a good control of contamination by heterotrophs and other algae since the culture substrates are always surrounded within a high humidity air.

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