Measurement of the cell density of microalgae by an optical method

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Abstract. Microalgae are currently interesting in industry because it can generate many useful products, such as supplementary food, cosmetic and biomass energy. During the cultivation, the growth of microalgae is traditionally followed by cell counting with an optical microscope and a calculation of the cell density. To obtain a precise value of the cell density, the measurement should be performed by ones with good laboratory skills and it takes a long time. We develop a photometer to determine the cell density of microalgae. The photometer consists of a light dependent resistor as the sensor, a light emitting diode as the light source, and a microcontroller which controls all components as well as the cell density calculation using a calibration equation. To test the performance of our device, we cultivate marine microalgae Dunaliella Salina with different aerations and measure the cell density during 18-day cultivation period by using the photometer and the cell counting with an optical microscope. The results show that the cell density values obtained from both measurement methods increase with day of cultivation in a similar manner. Thus, the photometer is a good alternative device to follow the growth of microalgae. Furthermore, it is relatively inexpensive, quick and easy for the operator to use.

1. Introduction

Microalgae are photosynthetic microorganisms that transform sunlight into lipids as an energy reservoir. Lipids can be used as food, feedstock or substrate for biofuel production [1, 2]. Microalgae need a small area for cultivation (0.1–0.2 m²/year/kg biodiesel), grow fast (10 g/m²/day) and accommodate lipid contents (30–70%) higher than food crops [3-5].

Growth of a microalgae population can be divided into several major phases, i.e., lag phase, exponential phase, declining relative growth phase, stationary phase and algal cell death [6]. The phase of microalgae can be specified by considering their growth rate, i.e., the rate of change in the cell density per unit time. In addition, the growth rate is a key quantity considered in a comparison of different cultivation methods as well as to find the proper time for harvesting the microalgae.

Normally, the measurement of the cell density is performed by using a counting chamber under an optical microscope. The counting chamber consists of a glass microscope slide with grid of perpendicular lines. The grid has specific dimensions and the area covered by the lines is known so that it possible to count the number of cells in a specific volume of solution. This method needs good laboratory skills and it takes a long time of about 45 minutes to complete the measurement [7-8].

Recently, the arising of automated cell counting instruments based on image processing has provided the possibility of investigation of a large number of samples in a shorter time. However, they
are constructed for specific cell types and may be not suitable for other types of cells due to technical limitations in their operation [8-12].

In this article, we present an optical method to measure the cell density of microalgae. We constructed a photometer and a calibration equation using the cell density measured by the traditional counting method. To test our photometer using different samples, we measured the cell density of microalgae *Dunaliella salina* which cultivated with four different methods of aeration and compared the results with those measured by counting cells under a microscope.

*Dunaliella salina* is a halophile green microalga and known for high accumulation of the important antioxidant beta-carotene under stress conditions including high light intensity, high NaCl concentration, suitable temperatures, or lack of nitrate [13-14]. Humans and animals can convert beta-carotene into vitamin A which are important for good vision, strong immunity, and general health [15–16].

2. Photometer

![Diagram of photometer](image)

**Figure 1.** (left) Schematic of photometer and (right) the calibration curve of the photometer. Circles depict the cell density *d* measured by counting cells under a microscope vs the difference of digital voltage ∆*v* obtained from the photometer. The dashed line is a linear fit of the data.

The circuit diagram of photometer is shown in figure 1(left). The microcontroller unit (MCU) supplies power to the light emitting diode (LED) which illuminates the sample. The light transmitted from the sample incidents the light dependent resistor (LDR). Then the voltage signals from LDR are converted (A/D) to digital signals with a resolution of 10 bits (1024 digital levels) corresponding to the signals of 0 - 5 volts. The microcontroller calculates the cell density of microalgae using the digital signals and the calibration equation and reports the results via an LCD monitor.

To develop the calibration equation of the cell density, 10 samples with different cell density of microalgae *Dunaliella Salina* were prepared by diluting a concentrated microalgae sample. Then the cells in each sample were counted under an optical microscope. The cell density *d* was in between 2.3 × 10⁶ cells ml⁻¹ and 14.1×10⁶ cells ml⁻¹. The same samples were placed into the photometer and the digitized voltage *v* was measured. To avoid errors originated from the initial light intensity in different measurements, we measured the digitized voltage *v₀* of the cultivation medium (without microalgae) as the blank measurement and used the difference of digital voltage ∆*v* = *v* – *v₀* to construct the calibration equation. As shown in figure 1(right), the difference of digital voltage ∆*v* increases linearly with the cell density *d*. A linear regression results the calibration equation as *d* = 0.2476∆*v* + 1.236 with the R-squared value of 0.99606.

3. Monitoring of microalgae cell density

To test the performance of the photometer, microalgae *Dunaliella Salina* strain KU 11 [17] were cultured with different conditions. As shown in figure 2, the microalgae were cultivated in transparent glass chambers containing 50 litres culture medium with 2 M NaCl prepared as in [13] and illuminated by fluorescent lamps from top all times. From the left to the right of figure 3, different aerations were used: (a) without aeration, (b) circulation by water pump, (c) bubbles by aerator, and (d) both
circulation by water pump and bubbles by aerator. At the beginning (day 1), the samples were very dilute and appeared transparent. Then, the cell density and the opaque of the chambers increased with time (e.g. day 10 and 18).

**Figure 2.** Cultivation of microalgae *Dunaliella Salina*. From left to right chambers, different aeration methods were used: without aeration, circulation by water pump, bubbles by aerator, and both circulation by water pump and bubbles by aerator. Images from top to bottom show the cultures at day 1, 10, and 18, respectively.

In each day of cultivation, we monitored the cell density of microalgae *Dunaliella Salina* in all chambers by using the photometer. The density was also measured by counting under a microscope for comparison. As shown in figure 3, the cell density was initially about \(2 \times 10^6\) cells ml\(^{-1}\) in all chambers. In the course of time, both the cell densities as measured by the photometer and by counting under a microscope in each chamber increased with similar manners. At the last day of observation, the cell densities in chambers from left to right in figure 2 measured by the photometer were \(11.9 \times 10^6\), \(11.6 \times 10^6\), \(8.2 \times 10^6\) and \(5.4 \times 10^6\) cells ml\(^{-1}\), respectively. The results imply that the growth of cells depends on the aeration.

4. **Discussion and Conclusion**
We have demonstrated an optical method to measure the cell density of microalgae. As examples, we cultivated microalgae *Dunaliella Salina* using different aerations. During the first two weeks of cultivation, the cell density monitored by our photometer were almost identical to that measured by counting under a microscope. In later days, the cell density obtained from the photometer were lower than that from the counting method. This difference might result from changes of cell properties such
as color and cell size which affect the optical density as reported for silver nanoparticles [18]. Further studies should be conducted to improve the performance of the photometer.

Since the cell density monitored by our photometer increased in a similar trend to that measured by counting under a microscope, this device is an appropriate tool for observation of the growth of microalgae in a long duration of cultivation. We expect that the photometer will be useful for monitoring microalgae cultivation in either research laboratories or industries since this optical method is fast and low cost and it does not require laboratory skill to operate.

![Figure 3. Cell density of microalgae Dunaliella Salina cultivated with different aeration methods: (a) without aeration, (b) circulation by water pump, (c) bubbles by aerator, and (d) both circulation by water pump and bubbles by aerator. $d_c$ and $d_r$ depict the cell density measured by the photometer and by counting under a microscope, respectively.](image)

**Figure 3.** Cell density of microalgae *Dunaliella Salina* cultivated with different aeration methods: (a) without aeration, (b) circulation by water pump, (c) bubbles by aerator, and (d) both circulation by water pump and bubbles by aerator. $d_c$ and $d_r$ depict the cell density measured by the photometer and by counting under a microscope, respectively.

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