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A portable fluorescent sensor for on-site detection of microalgae

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ABSTRACT

This work reports the development of a portable and low cost fluorescent sensing system with a disposable microfluidic chip for on-site detection of a microalgal sample and its concentration. The sensor system has multiple light emitting diodes (LEDs) for excitation and a photodetector for measuring a fluorescent signal from a microalgal sample. The concentration of a microalgal sample is determined by measuring the fluorescent signal emitted by chlorophyll *a*. A dichroic filter and a color filter are also added to allow only the fluorescent signal from chlorophyll *a* to pass through the photodetector. The microfluidic chip consists of a glass slide and a PDMS channel with a vacuum pump, which collects a small volume of the microalgal sample (<10 μl). The fluorescent sensor was characterized with varying concentration of microalgal samples and demonstrated its capability of measuring microalgal concentrations. The sensor was also tested with microalgal samples mixed with different turbidity water to validate its selectivity. The results show that the fluorescent detection of microalgal concentration is not influenced by the turbidity level of the sample solution. The developed system can be used for on-site screening and monitoring of microalgal population with an integrated excitation and detection circuitry.

1. Introduction

The lipids from microalgae have great potential to be used as a next generation renewable fuel [1–3]. In order to obtain the maximum microalgal production yield from the culturing system, environmental conditions of cultivating system must be optimized and controlled [4,5]. Therefore, an effective monitoring system is necessary to manage the microalgae cultivation system.

There are several techniques available to detect and to quantify microalgae in water. Optical and fluorescent microscopy techniques are manual cell counting methods that require a laboratory setup and laborious efforts [6]. Flow cytometry is a commonly used method to count microalgal cells providing an accurate result with rapid-analysis, however it is expensive and requires a skilled operator to acquire desirable performance [7]. An optical density technique is simple and yet provides an accurate result for the total suspended solids measurement in water [8,9]. However, it cannot differentiate between microalgal cells and debris in water possibly drawing an erroneous result.

Chlorophyll fluorescence offers many advantages over other detection methods in terms of accuracy, measurement time, and portability [10]. In addition, it is capable of differentiating microalgal cells from debris since fluorescence only comes from chlorophyll pigments. Several portable microalgal fluorescent sensors have been developed for the *in situ* monitoring purposes [11–17]. There are largely two types of portable chlorophyll fluorescence sensors—a submersible and a non-submersible type. A submersible sensor can detect and monitor microalgae at underwater environment, but it is not suitable for measuring a small amount of sample. A non-submersible fluorescent sensor is capable of measuring a small amount of sample but it is difficult to integrate into a real-time monitoring system.

Microfluidic technology has enabled the small sample volume requirement, shorter analysis time with a lower cost, and miniaturization. In order to develop a portable sensor device with a microfluidic PDMS chip, simple pumping method without any power source or tool is essential for the system. The passive pumping method of finger powered microfluidic pumping system, and the passive pumping lid system showed a good demonstration of

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power free portable microfluidic device [18,19]. However, these pumping methods require relatively difficult PDMS chip fabrication process, and low design freedom due to an additional apparatus such as a lid. The vacuum pumping method reported by Hosokawa et al. [20] and Liang et al. [21] is a convenient way to deliver a liquid sample without external or on-chip pumping devices. Xu et al. [22] suggested a syringe-assisted point-of-care pumping system to achieve better controllability compared to the vacuum pumping system and to remove the vacuum chamber treatment process. However, our sensor device requires minimum controllability during the sample loading process. Also, for the field application, the testing chip can be packaged in a vacuum sealer that is commonly used for medical devices. Before loading the sample, the sealer will be opened for vacuum pumping [21,23].

Here, we report a microalgal fluorescent sensor that is portable and non-submersible for quantifying the population of microalgal cells in an aliquot sample volume. The main purpose of our sensor is to use a field deployable sensor as well as an in-line sensor integratable to a massive microalgal cultivation system called the Hydraulically Integrated Serial Turbidostat Algal Reactor (HISTAR) system [24,25]. The sensor has an excitation and a detection apparatus in the same plane, which can easily be configured for either purpose. The turbidostat in the HISTAR system is necessary for monitoring the microalgae concentration and the contaminants in the culturing system. However the turbidostat does not differentiate contaminants from microalgae [26]. Thus, our microalgal fluorescent sensor will provide more accurate result of microalgal cell concentration of HISTAR system compared to the turbidostat. The turbidostat can be used only to measure the contaminants in water, since the fluorescent sensor cannot detect the non-fluorescence contaminants.

The working principle of chlorophyll fluorescence is detecting the emitted fluorescent light from chlorophyll molecules when they have absorbed the light. The *Chlorella vulgaris* that is the target species of interest has chlorophyll *a* as its major photosynthetic pigment [27]. To demonstrate the on-site microalgae fluorescent detection, we have utilized the fluorescent property of chlorophyll *a* pigment which has absorption wavelength of 440 nm-peak and emission wavelength of 680 nm-peak [17,28]. We have chosen the 448 nm wavelength LED light as an excitation light and a long pass color filter having cut-off at 645 nm. A dichroic filter has been added to reduce the noise. Since the light source and the detector with filters are mounted below the PDMS microfluidic chamber filled with a sample solution, the sensor can be easily integrated into an in-line monitoring setup (e.g., the HISTAR system) by simply removing the microfluidic chip and top cover and deploy it under the clear pipeline in the system. The microalgal population count value is compared with an optical density sensor measurement value to compare the accuracy and selectivity when debris and dirt are added into the microalgal sample solution.

2. Experimental methods

2.1. Detection principle

Fig. 1 shows the detection concept of the fluorescence sensor. The system consists of three different parts: light emission, detection, and sample handling. Philips Lumileds Rebel color LEDs were used for the light emission part. Philips Lumileds Rebel color LED (Philips, Netherlands) with 448 nm peak wavelength was selected to detect the microalgae which emits 680 nm fluorescent light from chlorophyll *a*. A photodiode (FDS100, Thorlabs, USA) with optical filters was placed below the PDMS microfluidic chamber to detect the fluorescent light signal from the sample. The first optical filter on the top side of the printed circuit board (PCB)

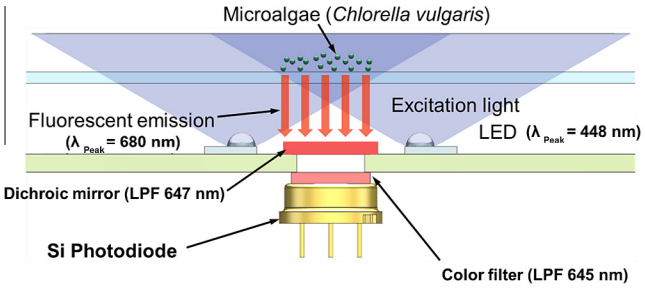

Fig. 1. Schematic illustration of the fluorescent microalgae detection system.

where all LEDs are mounted is a dichroic filter (PIXELTEQ, USA) working as low pass for 647 nm wavelength light. The second optical filter below the PCB is a color filter (Edmund Optics, USA) working as low pass for 650 nm wavelength fluorescent light. Microalgal sample solution is introduced into a disposable PDMS microfluidic chip placed on top of the LEDs.

2.2. System design configuration

The schematic configuration of the sensor system is illustrated in Fig. 2(a). The sensor jig was fabricated with a three dimensional (3-D) printer. Polylactic acid (PLA), a biodegradable thermoplastic material derived from renewable resources, was used as the structural material for 3-D printing. The photodetector and the color filter are mounted in the bottom cover ($90 \times 50 \times 20\ mm^3$) and covered with the PCB. The PCB's aperture was aligned with photodetector's window to receive the fluorescent signal from microalgal sample. Tray guide and the tray with a PDMS microfluidic chip containing the sample were aligned precisely to have maximum overlapping excitation light projected on the microfluidic chamber and to obtain maximum fluorescent light signal. The top cover is to achieve the fluorescence measurements in the dark environment. The top and the bottom cover block ambient light when fully assembled as shown in Fig. 2(a). The actual device is shown in Fig. 2(b).

2.3. PCB design

The PCB comprises six excitation LEDs with 448 (±10) nm peak wavelength. The aperture in the middle of the PCB allows for the fluorescent light from the microalgae to pass through to the photodetector. A dichroic mirror filter was installed on the top side of the PCB and a color filter was installed on the bottom side of the PCB to reduce the noise. Thelen and Chu [29] have demonstrated a portable low current sensing circuit design for a fluorescence optical detection. For a fully integrated system, a nanoampere range current meter for the photocurrent detection can be easily implemented and integrated with our proposed device for the portable detection of the microalgae.

2.4. Microfluidic chip

The microfluidic PDMS chip design is shown in Fig. 3(a). The dimension of the sensing chamber is 5 mm in diameter and 200 μm in thickness. The PDMS chip consists of a vacuum pumping square chamber and the sensing chamber of 10 μl in volume. Since our suggested microfluidic PDMS chip was fabricated with a single SU-8 main mold, the sensing chamber thicknesses of the PDMS chips were supposed to be identical. The thicknesses of ten random PDMS chips have been measured, and the thickness variation was negligible. The vacuum treated PDMS chip pumps up the microalgal sample solution into the sensing chamber as shown in Fig. 3(b).