Optical fiber fluorescence sensor integrated into a photobioreactor

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ABSTRACT

In this work, we integrated, via optical fibers, a simple fluorescence sensor into a small, low-cost photobioreactor. For the proper implementation, plastic optical fibers were used to excite the colloid, while the fluorescence signal was collected by another fiber and guided to a spectrometer. Real-time tests were carried out with cyanobacteria cultures, confirming the usefulness of monitoring these autofluorescence changes in the photobioreactor. This device provides a preliminary approach for the characterization and detection of cyanobacteria, demonstrating the feasibility of developing small, practical sensors through a simple fiber configuration for monitoring aquatic ecosystems.

Keywords: cyanobacteria, phytoplankton, fluorescence, environmental monitoring, plastic optical fibers

1. INTRODUCTION

Phytoplankton consists of photoautotrophic microorganisms that perform photosynthesis using light as their primary energy source. It plays a crucial role in maintaining the health and balance of aquatic ecosystems by acting as a primary producer in food webs and contributing significantly to oxygen production. However, alterations in its dynamics can have negative effects on water quality, aquatic ecosystems, and even human health [1]. Under certain favorable conditions, some phytoplankton species can proliferate massively and cause significant harm to the environment. These events are known as Harmful Algal Blooms (HABs). Their rapid and sudden onset makes them difficult to predict and control, underscoring the importance of their monitoring and identification. Traditional HAB monitoring typically involves periodic field sampling and laboratory analyses, including chlorophyll-a measurement through pigment extraction, morphological analyses, and species counting. These methods require highly specialized expertise and are often time-consuming, labor-intensive, and expensive. Consequently, technologies based on optical measurements have gained prominence in this field, as they provide high resolution, are noninvasive, and offer real-time data [2].

One of the main challenges in applying optical techniques is the high variability of phytoplankton fluorescence, which is closely linked to its physiology. Autofluorescence is modulated by multiple factors, including taxonomic classification, pigment composition, nutritional status, growth phase, photo-adaptation, and other physiological states [3]. Therefore, optical detection and monitoring call for an in-depth understanding of the temporal evolution and spectral characteristics of the signals involved. In this regard, photobioreactors offer an ideal setting to investigate these processes, allowing for controlled culture conditions.

The primary objective of this study is to integrate a fluorescence module into a photobioreactor to monitor the evolution of phytoplankton fluorescence under different environmental conditions. Additionally, this work aims to demonstrate the versatility of optical fibers in the development of devices for fluids or liquids, laying the groundwork for evaluating the feasibility of these techniques in more compact spectroscopic systems. These advances could also lead to significant improvements in the detection, monitoring, and classification of phytoplankton by providing a deeper understanding of how culture conditions influence optical measurements.

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2. MATERIALS AND METHODS

2.1 Phytoplankton fluorescence

Fluorescence is a fundamental tool in the study of phytoplankton and the monitoring of harmful algal blooms (HABs). Its autofluorescence arises from endogenous pigments, whose types and concentrations vary depending on the phytoplankton class. In addition to facilitating phytoplankton identification and detection, autofluorescence can provide valuable insights into the physiological state of these microorganisms [4], [5]. Accordingly, this study presents a device capable of measuring the temporal dynamics of fluorescence in cyanobacterial cultures subjected to different environmental conditions. Because of their importance in forming HABs, cyanobacteria were used as the model organism in this study to evaluate the device. Cyanobacteria are classified within the "blue" spectral group, whose primary fluorophore is phycocyanin. This pigment absorbs light around 620 nm and emits fluorescence at approximately 650 nm [3].

The cyanobacteria employed in this work consisted of pure cultures of *Dolichospermum crassum* UAM 502. Both the cell lines and the sterile BG-11_0 culture medium were provided by the Department of Biology at the Autonomous University of Madrid (UAM). Initial cultures were grown in a temperature-controlled room at 25 ºC under continuous illumination, with a light intensity of 70–130 µmol photons·m^{-2}·s^{-1}. The flasks were periodically agitated to facilitate gas exchange and prevent cell sedimentation.

2.2 Sensor Design

To easily control the culture conditions and establish the desired laboratory environment, we employed an open-source, low-cost photobioreactor known as the Pioreactor [6]. This system consists of a Raspberry Pi microcomputer along with the Pioreactor hardware. The device is compact (approximately 9 × 6 × 12 cm), using a vial with a working volume of about 15 mL as the reactor. It provides the necessary agitation through a 15 mm magnetic stirrer, enabling speeds ranging from 100 to 1000 RPM. A heating pad with an integrated temperature sensor maintains the culture at the desired temperature. The Pioreactor measures optical density every five seconds using a near-infrared LED (around 900 nm) to estimate growth.

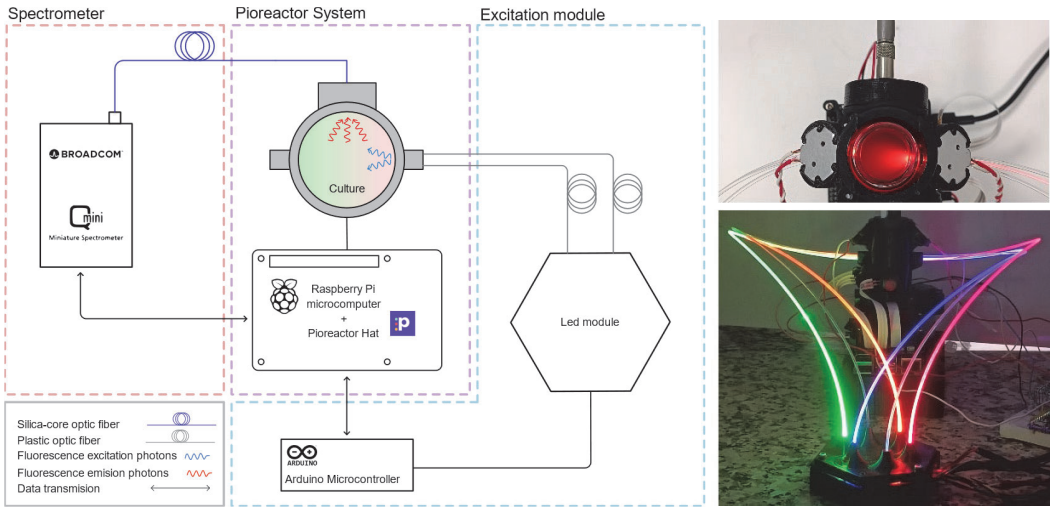

Figure 1. Configuration of the fluorescence sensor integrated into the photobioreactor. On the left, a simplified diagram illustrates the overall configuration. The top-right image shows the reactor vial open from the top, providing an example of how the optical fiber excites the sample. The bottom-right image depicts the LED module, which couples the light into the plastic optical fibers to guide it toward the sample.

The fluorescence module designed for integration into this photobioreactor consists of an excitation unit and a spectrometer (Figure 1). Although not all excitation wavelengths are strictly necessary for cyanobacteria, the design has been generalized to accommodate a broader range of phytoplankton by incorporating six LEDs of different wavelengths covering the visible spectrum (657, 615, 586, 520, 451, and 373 nm). Because of the photobioreactor's limited size, there is insufficient space to adequately incorporate all excitation LEDs, making optical fibers essential for excitation and signal collection. The optical fibers used for light guidance are CK-60 Eska™ High-Performance Plastic Optical Fibers with a 1.5 mm diameter and a high numerical aperture of 0.5, providing enhanced collection and excitation efficiency as well as alignment

tolerance. A custom housing was designed to couple the LEDs to the fibers; this housing accommodates the six LEDs, and the fibers are inserted through apertures on the top to ensure proper alignment. For fluorescence signal collection, a UV/SR-VIS optical fiber (200–1100 nm) with a 600-µm core from Ocean Optics directs the collected signal to the Broadcom Qmini AFBR-S20M2WU spectrometer, which is compact and offers a spectral resolution of 1.5 nm and a range of 225–1000 nm.

To integrate these components, the Pioreactor mount was redesigned and 3D-printed. Excitation is provided via the optical fibers inserted through the wall, pointing toward the center of the vial. The collection fiber is positioned at a 90° angle in the horizontal plane relative to the excitation. As for the control system, all operations are managed by a Raspberry Pi 4 Model B running the Pioreactor software image. The LEDs are driven by an Arduino Nano 33 BLE microcontroller, which, in tandem with the spectrometer, is overseen by the Raspberry Pi and managed through a custom Python plugin.

3. RESULTS

In the following, we present some results obtained from tests conducted with the device. First, we verified its ability to acquire fluorescence spectra from different fluorophores. Next, we monitored the fluorescence in cyanobacterial cultures to validate its integration into the photobioreactor, which allows the generation of various culture conditions.

3.1 Fluorescence spectra measures

Fluorescent compounds were introduced into the vial of the developed device and excited using the LED that best matched their specific excitation spectrum. Measurements were conducted with the spectrometer, applying an appropriate integration time for each compound. Four different compounds were tested, as shown in Figure 2. Furthermore, because our goal is to monitor cyanobacteria, a dense colloid of these organisms was also introduced to characterize their fluorescence. When exciting cyanobacteria at various wavelengths, the most noteworthy response occurred with 615 nm excitation, where a peak associated with the phycocyanin in their phycobilisomes was observed. This peak was relatively weak and appeared close to the excitation wavelength, with its maximum intensity at 657 nm.

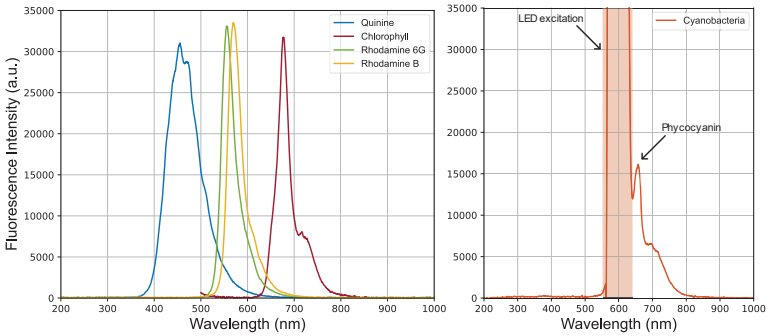

Figure 2. Fluorescence spectra obtained of quinine, rhodamine 6G, rhodamine B, chlorophyll, and phycocyanin in a cyanobacteria culture, excited with 373 nm, 520 nm, 520 nm, 451 nm, and 615 nm LEDs, respectively. The saturated region in the cyanobacteria spectrum corresponds to scattered LED light, while the adjacent peak represents phycocyanin fluorescence.

3.2 Monitoring of cyanobacteria culture

Cyanobacteria require appropriate light-dark cycles and controlled light intensity for optimal growth. In our experiment, a dense culture of *Dolichospermum crassum* UAM 502 was exposed to intense illumination in a small photobioreactor, leading to photoinhibition. Figure 3 a) shows that optical density and fluorescence decreased due to damage to photosynthetic pigments and proteins. During the first few hours, a brief increase in fluorescence was observed, but excessive light ultimately destroyed the pigments, indicating a loss of photosynthetic capacity.

Moreover, fluorescence monitoring can provide additional information beyond optical density. In Figure 3 b), we observe a scenario in which fluorescence declined while optical density increased, suggesting the growth of unwanted microorganisms resulting from culture contamination. Contamination in cyanobacterial cultures compromises viability and can lead to erroneous interpretations; thus, early detection can be highly beneficial.

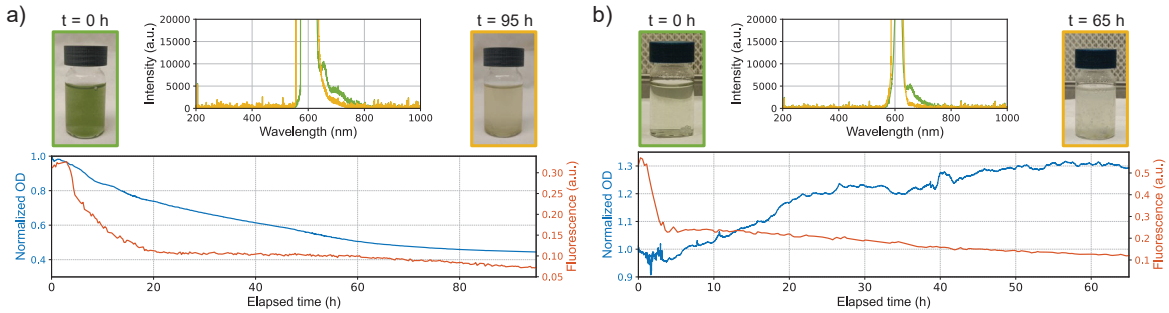

Figure 3. Fluorescence dynamics in a *Dolichospermum crassum* UAM 502 cyanobacteria culture a) subjected to photoinhibition conditions; b) during contamination. The figure displays the normalized optical density, phycocyanin fluorescence, the initial and final spectral measurements, and photographs of the culture vial at the beginning and end of the experiment.

4. CONCLUSIONS

Considering the challenges posed by harmful algal blooms (HABs), we propose integrating a fluorescence sensor into a photobioreactor via optical fibers to measure phytoplankton changes that provide valuable information. The proposed solution offers a fast, versatile, and low-cost method for investigating the evolution of phytoplankton autofluorescence in colloids subjected to different environmental conditions. In our tests with cyanobacteria, we focused on the phycocyanin fluorescence peak, which yielded additional information beyond that provided by optical density measurements. The integrated device features an excitation module with up to six different LEDs that excite the colloid through plastic optical fibers; a silica optical fiber then collects the emitted fluorescence and guides it to the spectrometer for real-time analysis within the photobioreactor system. This configuration demonstrates the versatility of optical fibers for developing fluid or liquid-based devices and serves as a precedent for evaluating the feasibility of this technique in more compact spectroscopic systems, such as lab-on-a-chip devices, which could enable more efficient and accurate real-time monitoring of phytoplankton.

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