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Design of Optical Biological Sensor for Phycocyanin Parameters Measurement using Fluorescence Technique

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Abstract

Remote sensing and measurement are of paramount importance of providing information on the state of water quality in water bodies. The formation and growth of cyanobacteria is of serious concern to in land aquatic life forms and human life. The main cause of water quality deterioration stems from anthropogenic induced eutrophication. The goal of this research to quantify and determine the spatial distribution of cyanobacteria concentration in the water using remote sensing technique. The standard approach to measure water quality based on the direct measurement of the fluorescence of the chlorophyll a in the living algal cells and the same approach used to detect the phycobilin pigments found in blue-green algae (a.k.a. cyanobacteria), phycocyanin and phycoerythrin. This paper propose the emerging sensor design to measure the water quality based on the optical analysis by fluorescence of the phycocyanin pigment. In this research, we developed an method to sense and quantify to derive phycocyanin intensity index for estimating cyanobacteria concentrations. The development of the index was based on the reflectance difference between visible light band 620nm and 665nm. As a result of research this paper presents, an optical biological sensor design information to measure the Phycocyanin parameters in water content.

Key words: *Fluorescence, Phycocyanin, Optical, Biological Sensor, cyanobacteria, hyperspectral remote sensing, spectral reflectance*

1. Introduction

The quality of surface waters around the world is receiving increasing attention because the demand for good quality is increasing and yet in the past surface waters have been badly regulated, leading to severe

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degradation that may preclude their long-term use. Symptoms of these degraded waters include the occurrence of toxin-producing phytoplankton, like cyanobacteria. Cyanobacteria pose a threat to recreation, drinking water supply and aquatic ecosystems because they produce many different toxins.

Monitoring of cyanobacteria and other phytoplankton has received much attention over the years. One important monitoring issue has been the need for rapid detection and quantification techniques. Microscopy, the classical way of quantification, is very labor-intensive and its slow reporting and depends too much on the cell counter.

In vivo fluorometry (IVF) has been by aquatic researchers for several decades. This based on the direct measurement of the fluorescence of the chlorophyll in the living algal cells. The same methodology is used to detect the phycobilin pigments of cyanobacteria in water. The benefits of IVF include ease, speed and the ability to collect large quantities of data. There is no special sample handling or processing required, making IVF ideal for real-time data collection. IVF is the easiest method for collecting large quantities of data but there are variables associated with IVF that result in errors and interference.

This paper present the Fluorescence analysis and Optical biological sensor design for effective Phycocyanin parameters measurement to find the water quality. As all know, fluorescence for a given cell concentration is affected by a number of factors including; the amount of light the cell was exposed to prior to the measurement and variation amongst different species, physiological states and environmental conditions. For the most accurate data, sense data is correlated to quantitative data that can be collected by taking occasional samples to be analyzed for pigment concentration by a technique that is not affected by the conditions of the live sample.

2. Phycocyanin Parameters

Phycocyanin is a pigment-protein complex from the light-harvesting phycobiliprotein family, along with allophycocyanin and phycoerythrin.. It is an accessory pigment to chlorophyll. All phycobiliproteins are water-soluble, so they cannot exist within the membrane like carotenoids can. Instead6, phycobiliproteins aggregate to form clusters that adhere to the membrane called phycobilisomes. The Phycocyanin chemistry bonding structure is shown in figure 1.

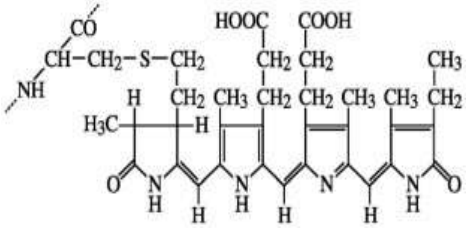


Figure 1. Phycocyanin Structure

Phycocyanin is a characteristic light blue color, absorbing orange and red light, particularly near 620 nm, and emits fluorescence at about 650 nm. Allophycocyanin absorbs and emits at longer wavelengths than phycocyanin C or phycocyanin R. Phycocyanins are found in Cyanobacteria. The absorbance and emission spectra is shown in figure 2.

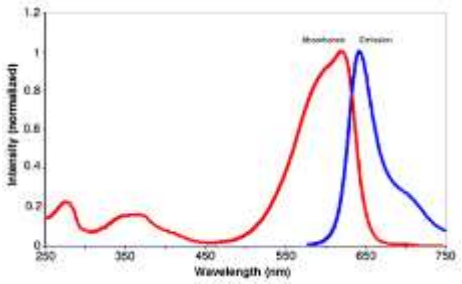


Figure 2. Phycocyanin Absorbance-Emission Spectrum

Phycobiliproteins have fluorescent properties that are used in immunoassay kits. C-phycocyanin is often found in cyanobacteria which thrive around hot springs, as it can be stable up to around 70℃, with identical spectroscopic (light absorbing) behaviors at 20 and 70℃. Thermophiles contain slightly different amino acid sequences making it stable under these higher conditions. Photo-spectral analysis of the protein after 1 min exposure to 65℃ conditions in a purified state demonstrated a 50% loss of tertiary structure.

3. Phycocyanin Fluorescence Analysis

Phycocyanin measurement make use of fluorescence excitation. In fluorescence analysis when chlorophyll molecules absorb light, a fraction of the energy absorbed is reemitted as fluorescence. Chlorophyll a detection supplies data on the total algal biomass in which all photosynthetic organisms contain the chlorophyll a pigment. Phycocyanin is the predominant phycobilin in freshwater environments while phycoerythrin is the predominant pigment in water environments. The graphical presentations of how fluorescence analysis work for the detection of chlorophyll, phycocyanin shown in Figure 3 with different light wavelengths.

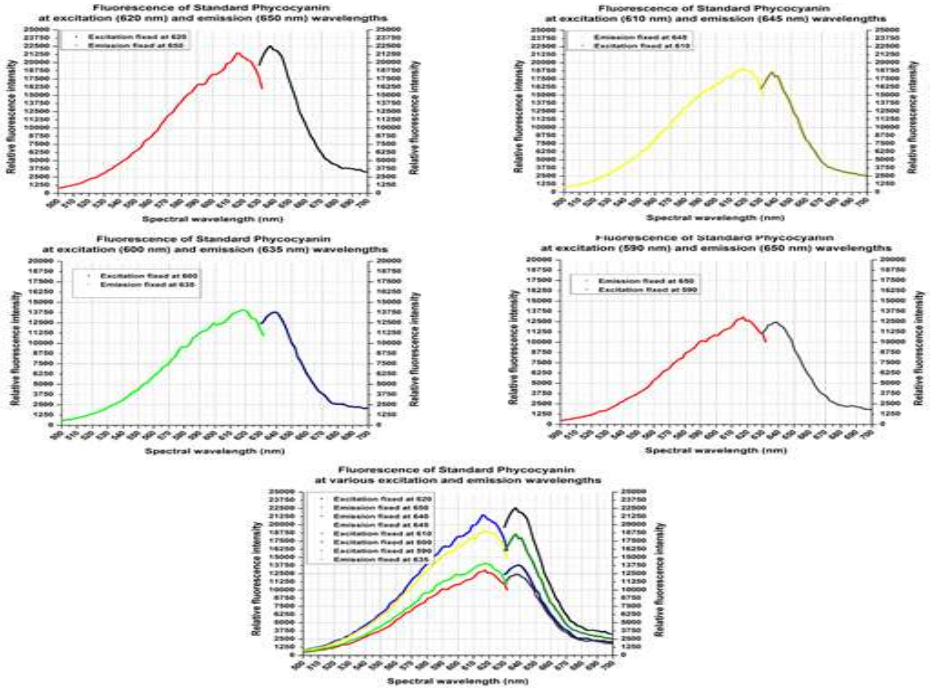


Figure 3. Fluorescence Standard Solution Spectrum

The pigments of these cells absorb the energy of light with a certain efficiency and reemit light of different wavelengths as absorbed. The energy transfer of a cell as fluorescence of a certain wavelength allows for quantification of different phytoplankton groups which usually cyanobacteria, green algae and diatoms present in the sample. The main substances excitation and emission wave bands shown in Table 1.

Table 1. Fluorescence based Excitation and Emission Wavebands for Water Substances

Substance	Excitation (nm)	Emission (nm)
Chlorophyll a	400-450	650-750
Phycoervthrins	545-565	565-585
Phycocyanins	615-650	640-660
CDOM (typical)	360-390	450-470
Hydrocarbons	250-350	300-450
Fluorescein	410-510	500-600
Rhodamine B	490-590	510-690

4. Optical Sensor for Fluorescence Technique

In fluorescence process in which a photon is absorbed by an atom or molecule and re-emitted at a lower energy. The emission of the lower-energy photon generally occurs on a time scale of nano/pico seconds. The quantum yield of fluorescence is defined as the ratio of the numbers of long-wavelength photons emitted to short-wavelength photons absorbed, and this yield can be strongly modified by the chemical environment in which the process occurs. The submersible optical sensor design includes the Fast Repetition Rate and Pulse Amplitude Modulated instruments used to study photosynthesis. The typical Optical Sensor design model shown in Figure 4.

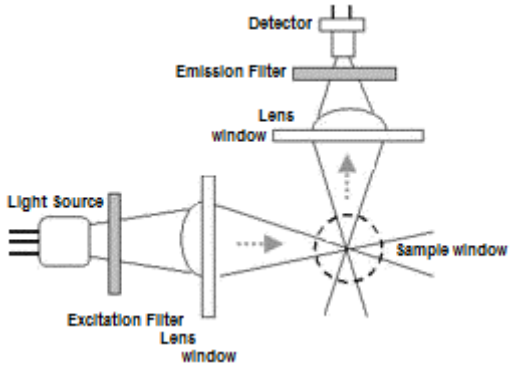


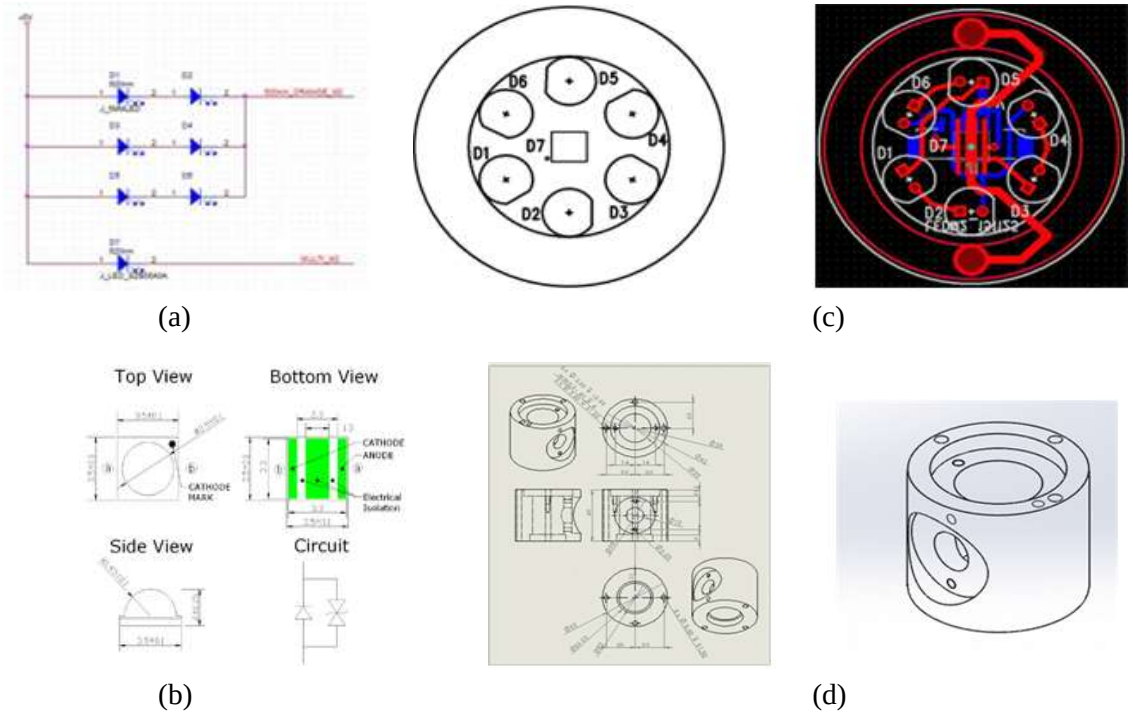
Figure 4. Optical Sensor for Fluorescence Technique

The submersible Fluorescence measurement sensor consisted of 5 main optical components: a light source, a lens system to convey the exciting light to the sample volume, a second lens system to gather the emitted fluorescence, one or more optical filters to separate the excitation and emission wavelengths, and a photo detector.

5. Optical Biological Sensor Design

The main optical problem encountered is the tendency of ambient light, or excitation scattered back from the sample volume, to interfere with the measurement of the relatively weak fluorescence signal. The proposed

submersible optical biological sensor design replaced by light-emitting diodes and silicon detectors for most applications with considerable savings in instrument size and power consumption from the standard optical sensor design. Modern compact design is implemented out of this research and use a wide range of proprietary optical configurations, including coaxial arrangements of the excitation and collection optics or light pipes rather than lenses. The proposed design specification of LED based light module design is shown in Figure 5.

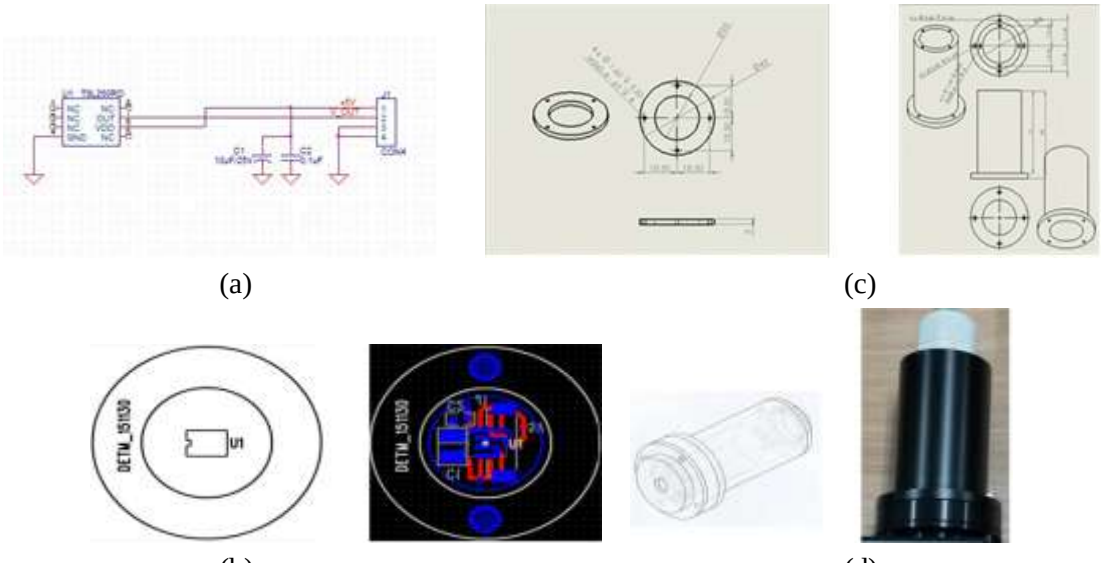


(a) (c)

(b) (d)

Figure 5. (a) LED Circuit (b) LED Design Drawing (c) LED PCB Design (d) LED Module Housing

The proposed design specification of photo detector module design details shown in Figure 6.

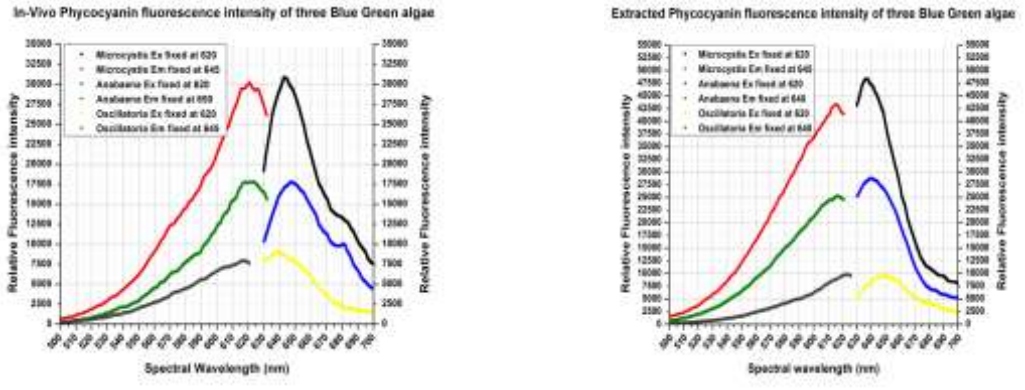


(a) (c)

(b) (d)

Figure 6. (a) Photo Detector Circuit (b) PCB Drawing (C) Mechanical Drawing (d) Photo Detector Housing

The fluorescence detector designed using photo detector module H10722-XX manufacture by Hamamatsu PMT package by metal with low power consumption. The graphical presentations of how fluorescence detection of chlorophyll, phycocyanin of designed module is shown in Figure 7 and the performance compared with In-Vivo approach.



(a) (b)

Figure 7. (a) In-Vivo Phycocyanin Fluorescence Intensity (b) Proposed Approach Phycocyanin Fluorescence Intensity

6. Conclusions

The equipment for online in Phycocyanin monitoring using Fluorescence technique has developed more reliable and easy to use and the cost of the device has decreased. The sensor technology is carefully tested and approved to be reliable, continuous monitoring of an environmental parameter in seconds to hours interval gives more accurate information on short time changes compared to normal monitoring programs were sample interval could be days to months. The water samples and inverted microscopy cell count results however are not in any case totally replaceable by the in customized fluorescence sensors as they can provide accurate quantitative and qualitative information on phytoplankton community structure on a taxonomical level. The automated monitoring stations equipped with fluorescence probe produced reliable and good quality up-to-date data on cyanobacteria abundance.

Acknowledgment

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