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## Low-cost optical sensor to automatically monitor and control biomass concentration in microalgal cultivation



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ARTICLE INFO	A B S T R A C T
<i>Keywords:</i> Biomass density Low-cost in-line sensor Microalgae Turbidostat	Low productivity of microalgal cultures leads to a high cost of the fuel feedstock. Turbidostat operation, which automatically monitors and controls biomass density, is a mean to manage biomass density and internal light intensity, so that biomass productivity can be maximized. Available versions of turbidostat control are expensive and not amenable to large-scale operation. We designed a system that costs less than \$250 and that can be used for any type of microbiological system. It includes an in-line, infrared turbidity sensor connected to an Arduino ATmega microcontroller and auxiliary power replays. The target biomass density is adjustable, and key operating data – such as time stamps, pump status, and set and measured values of biomass density – are available in real time and logged continuously. The sensor's output was linear for OD <sub>730</sub> from 0.5 to 4.5, which brackets the realistic ranges for microalgae culturing. We tested the turbidostat with step-down and step-up experiments with <i>Synechocystis</i> cultures. The turbidostat maintained stable biomass concentrations for all steps. The results of the turbidostat experiments demonstrated how turbidity control leads to systematic management of average internal light intensity, specific growth rate, and biomass production rate. This open-design, low-cost system should promote higher productivity and help make microalgae biomass an affordable fuel feedstock.

#### 1. Introduction

Maintaining microalgal-biomass productivity of at least 25 g dryweight/m<sup>2</sup>-day, averaged over a year, is one of key input for a feasible production of algal biodiesel [1,2]. High productivity requires that the microalgae cannot be nutrient limited, and they also must be exposed to a light intensity that is neither too low nor too high [3,4]. Growing microalgae in a turbidostat is one way to provide a constant supply of nutrients and to control the light intensity at a desired level. A turbidostat maintains the biomass concentration at a set point based on in situ, real-time measurement of the biomass concentration through an optical sensor or photocell that transfers the signal to a controller that gives instructions to the system's pumps and valves. Assaying and controlling biomass concentration continuously makes it possible to maintain the best-possible growth conditions, particularly internal light intensity [5,6].

A chief obstacle to achieving turbidostat operation is the capital investment. Commercial photobioreactors (PBRs) that include a turbidostat option, such those made by Photon Systems Instruments (www.psi.cz), are designed for laboratory research. Such systems are not amenable to expansion or functional upgrading. In principle, commercial turbidity probes (e.g., [7,8]) can be purchased separately and

integrated with an in-house system to control biomass. However, those probes usually are designed for integration only with proprietary parts that are not designed to meet the needs for operation of a large-scale turbidostat. In either case, a system to measure and control biomass density can cost thousands to tens of thousands of dollars per system. (Table S1 provides price information on these systems and components).

The intent to manage phototrophic cultures in turbidostat mode dates back to early 1950s [9], when turbidity was measured by two photovoltaic cells and controlled by galvanometric relays. Since then, different versions of turbidostats were developed either using an oxygen probe as a secondary sensor [10], a laser diode coupled with a photocell [6], or an infrared light emitting diode (LED) with a filter [11,12]. Using infrared wavelengths to measure the turbidity for biomass density appears to be an economical approach, since generic turbidity sensors are routinely used in home appliances, such as dishwashers and washing machines.

Previously, we used a pH probe to create a pH-stat system using pure  $CO_2$  to control pH; it gave us the ability to uncouple pH and inorganic carbon concentration as controlling factors for the growth rate of a cyanobacterial culture [13,14]. Here, we design a simple and inexpensive turbidostat system to monitor and control biomass density.

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Our low-cost turbidostat uses simple and inexpensive components: an infrared turbidity sensor employed in washing machines [15], an active feedback loop based on an Arduino ATmega microcontroller unit (MCU) [16], and pumps that periodically take sample and that add growth medium to maintain a pre-set turbidity. A Linux-based shield stacked on top of the MCU logs the data and provides Internet connectivity to retrieve the data directly or to download to a text file. Our approach offers flexibility for any type of reactor, whether large or small, and it is not limited to reactors for cultivating microalgae. We demonstrate effective turbidity control in experiments conducted in a flat-panel photobioreactor (PBR) in which we cultivate the cyanobacterium *Synechocystis* sp. PCC 6803.

#### 2. Materials and methods

#### 2.1. Turbidity sensor and biomass-control system

We used a TSD-10 turbidity sensor (Amphenol, U.S.) to measure biomass density. This sensor commonly is used in washing machines or dishwashers to measure the turbidity between 0 and 4000 NTU [15]. The sensor is composed of an infrared light-emitting diode in one side and a photo transistor to detect the light intensity passing through the open channel to the opposite side. The light intensity at the detection side is reduced as the turbidity increases. The output signal ( $V_{out}$ ) is read directly by the MCU, or it can be read after amplification with noise reduction, such as by an Operational Amplifier MCP6021 [17].

The conceptual design for using the light sensor is presented in Fig. 1. The desired biomass concentration is set by adjusting a rotary 10 K-ohm variable resistor ( $V_{input}$ ). When the system runs in the turbidostat mode, the value of  $V_{out}$ , produced by the turbidity sensor, is compared with  $V_{input}$ . The signal is represented with 10-bit resolution, which means that any value for  $V_{input}$  or  $V_{out}$  is represented by a number between 0 and 1023. A denser culture (greater turbidity) leads to a lower  $V_{out}$ . A value of  $V_{out}$  close to zero means that almost no light

reaches the detector side, and this represents the upper range of measureable turbidity. A small Light-Emitting Diode (LED) panel displays the two key parameters:  $V_{input}$  and  $V_{out}$ . The display information is programmed with the Arduino Integrated Development Environment (IDE) (https://www.arduino.cc/en/Main/Software).

We used a generic 12-V peristaltic pump for taking samples for turbidity measurement. The sampling pump pulls the sample up from the reactor to the sensor, a strategy that eliminates a headspace and bubbles. The sensor itself is oriented vertically to eliminate cells settling directly on the sensor's walls.

The feeding pump is a dual-channel pump head (Cole-Parmer Masterflex L/S, U.S.). The flow is set up to be counter-current so that the biomass is extracted from the reactor and fresh medium is added to the reactor simultaneously and with the same flow rate.

To control the two pumps, the MCU sends an on/off signal through a set of power-relay modules, shown in the upper left of Fig. 2. The relay either closes or opens the circuit of external power to turn a pump on or off. Two 3-position switches allow an operator to manually turn a pump on or off; alternatively, the pumps are operated automatically by the algorithm stored in the MCU. We used an Arduino board, ATmega2560, that integrates an Atmel MCU to execute the operation sequences. The chipset has an allocated internal memory that is programmable. The operational parameters – time stamp,  $V_{out}$ , and  $V_{input}$  – and the pumps' status (on or off) are stored in a Secured Digital (SD) card attached on the Yun Shield, which is a compatible add-on for the Arduino board that has a Linux-based system for data collection, recording, and communication with another computer via the Internet (wireless or via Ethernet cable).

#### 2.2. Operation sequence

Fig. 3 shows the three-block sequence for operating the turbidostat. Block 1 (in cyan colored elements) is for sampling, which includes turning on the sampling pump, clearing old culture from the previous

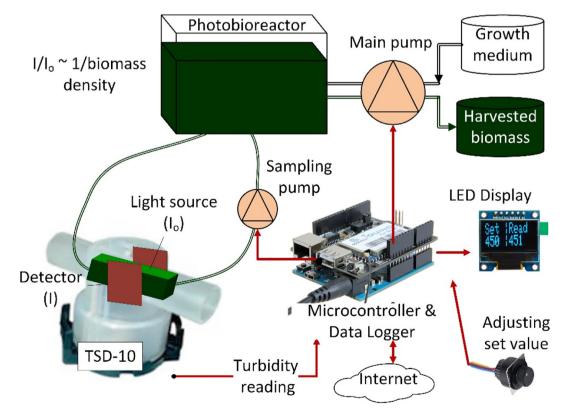


Fig. 1. Conceptual design of the biomass-monitoring and -control system based on a TSD-10 turbidity sensor. A photo of the implementation of the turbidostat with a 2.5-L photobioreactor (PBR) is provided in Fig. S3. Table S1 identifies all components, as well as their prices.

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