Bioprocessing and Biomass Technology

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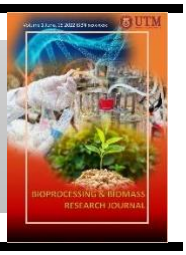

Research Article

Evaluation of a Portable Arduino-based Mini Spectrometer Prototype for Cells Optical Density Measurement

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ARTICLE INFO

Article History:
Received 30 September 2023
Received in revised form 12 December 2023
Accepted 12 December 2023
Available online 31 December 2023

Keywords:
Cells Optical density,
Fermentation,
Arduino,
Microbiology,
Spectrometer

ABSTRACT

Cell optical density (OD) measurement is essential for tracking the progress of microbial fermentation processes. It is typically performed through a spectrophotometer at the wavelength of 600 nm. In this paper, a portable Arduino-based mini spectrometer prototype is presented. In the proposed prototype, a turbidity sensor was utilized to perform the optical density measurement of cells based on the Beer's Lambert law. Differences on the light intensity from the excitation source and the collective point were correlated with the known cell concentration (using *S. cerevisae* yeast with concentration between 1 g/L and 5 g/L). The data attained was used as the standard curve and also to validate the functionality of the device. Fermentation experiments were carried out and the progress of the fermentation were measured using the proposed OD sensing prototype and compared to the standard bench top spectrometer. Both devices produced a calibration curve with R-squared value greater than 0.95 (0.95 for bench spectrometer and 0.97 for the Arduino device) and a comparable fermentation curve attained from both devices highlighted the workability of the proposed prototype. In addition, the device has a small footprint, portable and affordable.

INTRODUCTION

Fermentation is a process where cells are grown in a controlled environment i.e., either using a shake flask platform or a bioreactor system (Abdella et al., 2020). In fermentation experiments, it is essential to monitor the progress of the fermentation process as it allows one to determine the phase of the fermentation processes. This is usually done by measuring the cells optical density (COD) at specific wavelength in which microbiologist and/or bioreactor operators are greatly relaying on spectrometer to perform the OD measurements (Balado Sánchez et al, 2019; Das, 2021; Poh et al., 2021; Stephenson, 2016). In practice, sample containing suspended cells (bacteria) is placed in a cuvette and light source at specific wavelength is excited through the sample. Some of the light is scattered where else some passed through the suspended cells and read by a photo detector, which outputs a value. The concentration of the suspended cells and the transmitted light can indeed be correlated by the application of the Beer-Lambert's law (Laganovska et al, 2022; Zainal Alam et al., 2018). Often a linear relation is obtained over a certain range of cells concentration and this correlation is used to estimate the cells concentration (cells OD) within the measured sample.

Many spectrometers have the capacity to operate at wide range of wavelength i.e. ranging from visible light spectrum between 380 nm and 700 nm to near infrared light spectrum between 750 nm and 1400 nm (Chaianantakul et al, 2018). Nevertheless, for a simple microbiology work such as day-to-day cells OD screening, tracking of cells growth and for a quick identification of fermentation status, normally a single wavelength is selected. Most commonly applied measurement range for cells OD is at 600 nm (Sargazi & Kaykhaii, 2020). This renders such sophisticated

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benchtop spectrometer unnecessary compared to the simplicity of the workload at hand. Even a handheld spectrometer is considered costly to carry out such simple task.

There are numbers of effort made to miniaturize the spectrometer for a quick cells OD measurement purposes. Zainal Alam et al. (2018) developed a simple transmission measurement device for reading cells OD in disposable microfluidic chips. They used a LED with peak emission wavelength at 660 nm and incorporates a photodetector with spectral range between 400 and 1000 nm. A smartphone application was also established to acquire the cells OD data and thus, enable one to perform OD measurement without the use of laptop or computer. The entire device is automated using Arduino. Their device requires only 30mL of sample and able to perform cells OD measurement over the range of 0.25- 15 g/L (R^2>0.98). In a more recent development, Laganovska et al. (2022) built an affordable miniature spectrometer that is also operated using a mobile application and interfaced wirelessly via Bluetooth module for fast cells OD measurements at wavelength of 750 nm. A compact size mini spectrometer was integrated to obtain light spectrum between 340 nm and 850 nm. It is very clear that both setups aimed at creating an affordable and portable spectrometer device that allows for simple and fast cells OD measurement.

In this paper, a mini spectrometer device is presented. The work presented in this paper is motivated by the idea of having one spectrometer per student in any types of microbiology task. The idea has driven us to utilize inexpensive electronic parts in the construction of a mini spectrometer prototype. Turbidity sensor is used as the cells OD measurement tool and reading of output value is carried out automatically using Arduino. The workability of the device is tested on measuring cells OD of yeast culture at various concentration and compared the data attained with cells OD measured using a commercial benchtop spectrometer. In conclusion, the work presented here demonstrated a semi-commercial cells OD measurement prototype that is suitable to be applied by microbiologist for a quick data analysis on cells growth.

MATERIALS AND METHOD

Design and Fabrication of Spectrophotometer Unit

Figure 1 shows the design of the Arduino-based mini spectrometer prototype presented in the work. The automation parts used for the OD measurement are concealed within a plastic electronic box that has a footprint of about 15 cm (length) x 10 cm (width). The box includes ports for the power supply and also the turbidity sensor for the placement of the cuvettes.

Figure 1 Image of the Arduino-based Mini Spectrometer prototype. Inset show the 3D printed cuvette used and the display of the OD readings.

The general schematic diagram of the COD reading setup for the prototype is shown in **Figure 2.** For the cells OD measurement, a turbidity sensor was utilized. The measurement was based on a light transmittance through a sample. The turbidity sensor is comprised of two components: a light-emitting diode (LED) and a phototransistor. The light emittance is from the LED (KODENSHI EL-23G) with a wavelength of 940 nm while the phototransistor (KODENSHI ST-23G) received and converted detected light into an electrical signal. A conditioning circuit was connected to Arduino in order to convert the electrical signal from the turbidity sensor into a direct-current (DC) voltage signal within the voltage range readable by the Arduino UNO board i.e., between 0 and 5 volts. The cuvette is made of Polylactic Acid (PLA) polymer and was fabricated using a three-dimensional (3D) printer (Flashforge). Only 0.5 mL of sample is required for every measurement performed.

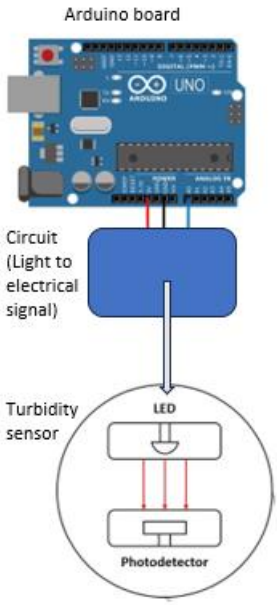


Figure 2 Schematic of the electrical connectivity of the Arduino-based mini spectrometer prototype.

System validation through off-line analysis

In order to validate the functionality of the mini spectrometer prototype, off-line cells OD measurements were performed using yeast cells suspended in distilled water. Suspended yeast culture was prepared using a commercial dry yeast obtained from a local store. Yeast culture sample solutions with different cell concentrations ranging between 1 g/L and 5g/L was prepared for the off-line OD analysis. In each sample measurement, raw OD readings were attained as voltage values (volts).

Beer-Lambert law was applied to calculate the actual OD values, which is the ratio (in log) between the intensity of light, I and the reference signal, I_o. I is the raw OD values (in volts) that passed through the sample and I_o is the OD values (in volts) attained using water as blank. The Beer-Lambert law equation is as follows:

$$\text{Optical Density} = -log_{10}\left(\frac{I}{I_0}\right)$$

A correlation between OD values and the sample concentration was constructed as the standard curve for the OD measurements. Similar curve was built using the commercial benchtop spectrometer (Jenway 7305) for comparison. Triplicates were made for every OD measurement.

Validation through fermentation experiments in a 2L Bioreactor

Two different types of fermentation experiments were carried in a 2-L Bioreactor (Sartorius Stedim Biostat® B). The first experiment was the cultivation of *Saccharomyces cerevisiae* culture. The strain was grown on a 1 L medium containing 10 g/L of Dextrose and 5 g/L (and 10 g/L) of yeast extract. The inoculum (10%v/v) for this fermentation process was prepared using 2 g of wet yeast (attained from local market) and incubated overnight at 37 °C for 15 hours. In the second experiment, *E. coli* strain was grown in 5 g/L (and 7.5 g/L) of nutrient broth medium. The working volume was similar to the first experiment and the inoculum (also 10%v/v) was grown for 15 hours at 37°C.

In each fermentation experiments, fermentation was carried out aerobically until the process came to stationary phase. All the fluidic ports and tubing of the bioreactor were tightly sealed to avoid contamination. The progress of both fermentation processes was tracked based on the cell's OD measurement. The OD measurements were taken every hour using the mini spectrometer prototype and the commercial benchtop spectrometer setup. Each experiment was repeated twice to check for data reliability.

RESULTS AND DISCUSSION

Validation: Off-line cells OD measurement

The Arduino-based mini spectrometer prototype could only output a raw DC voltage value in each measurements made. Beer Lambert law equation must be utilized in order to compute cells OD of the sample. This is imperative and calibration is probably required for different bacteria strain – different strain may differ in terms of size and shape and may affects the light transmittance across the optical pathlength of the 3D printed cuvette used. **Figure 3** shows the calibration curve for yeast culture optical density produced for different sample concentration ranging between 1 g·L^{-1} and 5 g·L^{-1}.

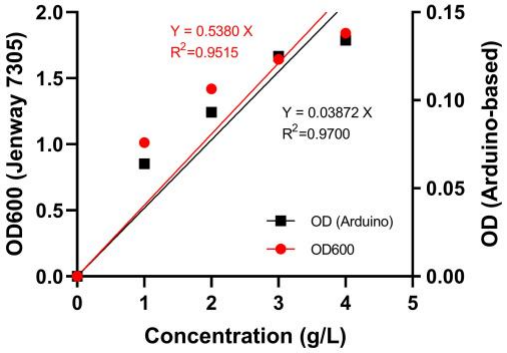


Figure 3 Comparison of standard curves attained for yeast culture between cells concentration of 1 g/L and 5 g/L.

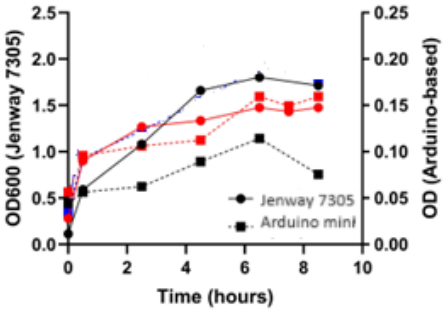
As shown in **Figure 3**, the experimental data followed a common trend of a Beer–Lambert law relation where the cells OD readings proportionally increased with the increasing of sample concentration. Despite some data non-linearity across the range of the sample concentration measured, a reasonable fit to the Beer–Lambert equation was achieved as indicated by a high regression coefficient, R^2 values ($R^2 \geqslant 0.95$). This indicated that the measurement performed is highly reproducible and accurate especially considering that measurements were repeated using triplicate samples. Moreover, despite the difference on the volume of sample used for the OD measurements, the data attained from the mini spectrometer prototype is comparable to the calibration curve obtained using the commercial benchtop spectrometer (Jenway 7305). The results also showed that the linearity of the curve has begun to compromise at yeast concentration closer to 4 g/L. It can be presumed that as the cell's concentration increases greater than 4 g/L, the space occupying the optical pathlength is highly concentrated with cells and little light passed through the cuvette for OD measurements. Sample dilution is perhaps needed to provide a better measurement accuracy if cells are too concentrated or if raw voltage values attained are close to 0 (for Arduino spectrometer) and if OD readings are close to 1 (for commercial spectrometer). Despite the differences in the excitation wavelength, our measurement data is like the results attained by Laganovska et al. (2022) and Zainal Alam et al. (2018) where OD attained for cells concentration range between 0 and 4 g/L is within OD values of 0 and 0.15. This signified that for as long as measurement is based on light transmittance (predominantly attenuate light through scattering), comparable OD range is attainable regardless of the source of LED excitation wavelength used (Benner et al, 2020).

On-site diagnosis of fermentation experiments

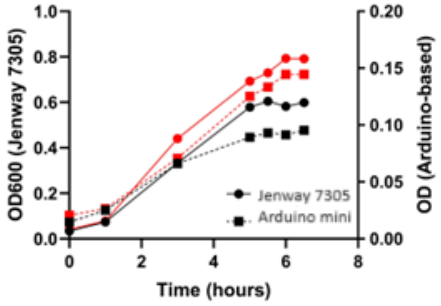
In common practice, small portion of the reactor content are sampled out periodically (typically between 1 and 2 hours of operation) during the fermentation experiment to track the progress of the cell growth over time. Cells growth is analysed based on the cells OD and often off-line measurements are carried out to measure the cells OD. In our fermentation experiments, comparison of the OD values attained for the fermentation processes (yeast and *E. coli* culture) were made using the Arduino mini spectrometer prototype and the commercial benchtop spectrometer

(Jenway 7305). The growth curve attained from the cells OD measurements is illustrated in **Figure 4**.

Based on the results, growth curves attained from both instruments are almost identical to one another. In the yeast fermentation experiment (**Figure 4a**), there is no significant lag phase is seen and the cell yield increases as the yeast extract concentration increases from 5 g/L to 10 g/L. Cell grew exponentially at growth rate of about 0.16 hr^{-1} before reaching steady-state after 8 hours of fermentation. As for the *E. coli* fermentation (**Figure 4b**), nearly an hour of lag phase was observed before cell growth accelerate into the exponential phase (μ = 0.15 hr^{-1}). *E. coli* culture came to stationary phase after 6 hours of fermentation.



(a)



(b)

Figure 4 Growth curve of fermentation experiments of (a) yeast culture and (b) *E. coli* strain.

Clearly, the Arduino-based mini spectrometer is just as good as the commercial bench top spectrometer for on-site analysis of cells OD. The prototype however has a much smaller footprint and therefore, rendering it a suitable tool for on-site diagnosis of fermentation processes. Moreover, due to its low cost, multiple units can be utilized to facilitate studies by different group of students. This could realize the idea of one group one spectrometer learning environment. Another added advantage of the proposed prototype is that the configuration of the 3D printed cuvettes can easily be altered to cater on-line cells OD measurement. This of course requires the use of pump to transport the fermentation broth through the cuvette and back to the reactor without the need for sampling. Indeed, a useful feature as it avoids contamination and more precise cells OD measurement can be attained at higher sampling rate. In current practice, one is required to take out sample every 1-2 hours and some data may not be available for sampling due to restriction on working in the lab after normal working hours. The setup can also be further modified to include Bluetooth trans-receiver to facilitate wireless communication data transfer using a specific application. This way data can be analysed using a mobile phone and could even established Internet-of-things (IoT) feature for data sharing in cloud server.

CONCLUSION

An Arduino based mini spectrometer prototype was designed and fabricated. The device built utilized a turbidity sensor for cells OD measurement as alternative to the commercial bench top spectrometer. The functionality of the prototype was evaluated through series of off line analysis OD measurements using yeast culture and *E.coli* strain. Data attained from the signify the potential of the device as a reliable instrument for cell OD analysis. Several advantages and future modifications of the devices were also discussed to highlight its usefulness as a simple measurement tool for tracking the progress of fermentation experiments or for any microbiology work that requires the need for cells OD measurement.

Acknowledgement

The authors acknowledge the support and technical assistance provided by the staff of Bioprocess Engineering Laboratory, Faculty of Chemical and Energy Engineering, Universiti Teknologi Malaysia.

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