OPTICAL BIOSENSOR READER FOR DETECTING AMMONIUM

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RINGKASAN: *Satu alat pengimbas biosensor mudah alih tanpa wayar telah dibangunkan untuk mengesan dan mengukur kandungan ammonium di dalam kolam akuakultur. Pengimbas ini dibangunkan menggunakan kaedah tindakbalas enzim glutamate dehydrogenase (GLDH) dan diaphorase (Dph) yang dapat memberi respon kepada kehadiran ammonium menggunakan NADH sebagai pengantara (mediator), apabila terdedah kepada LED lampu hijau pada panjang gelombang (wavelength) 565 nm. Kepekatan ammonia yang diukur adalah berkadar terus dengan pengurangan kepekatan NADH di dalam larutan. Pengimbas biosensor ini akan mengukur keamatan cahaya (I_o & I_s), memproses signal dan seterusnya mempamerkan keputusan kepekatan ammonium pada telefon pintar melalui komunikasi Bluetooth. Perbandingan graf penentukuran linear di antara pengimbas biosensor dengan kaedah makmal mengunakan spektrofotometer dalam pengesanan ammonia menunjukkan bacaan ketepatan dan R^2 pada pengimbas biosensor adalah lebih baik berbanding dengan kaedah spektrofotometer.*

ABSTRACT: A portable and wireless optical biosensor reader was developed to detect and measure the ammonium concentration on site. The biosensor reader uses glutamate dehydrogenase (GLDH) and diaphorase (Dph) enzyme as biosensors that respond to the presence of ammonium upon exposure to green light at the wavelength of 565 nm. The absorption of light depends on the concentration of the ammonium compounds. The reader measures light intensity *(I_o & I_s)*, performs signal processing and displays the concentration result on any android based smartphone via Bluetooth communication. With various calibration curve established for every test, a new linear model is developed. The R^2 and accuracy of the device is measured and compared with that of a standard spectrophotometer. It is found that the device has better performance and accuracy.

KEYWORDS: Optical biosensor reader, smartphone, ammonium, glutamate dehydrogenase, diaphorase, Internet of Everything (IoEs).

INTRODUCTION

The content of ammonia and ammonium ions (NH_4^+) in water serves as an indicator of water pollution. Ammonium ions in water are the result of municipal, agricultural and industrial pollution (Environmental Analysis, 2015). It is known to be toxic to various organisms (U.S. Environmental Protection Agency,

1987; Kwan, R.C.H., 2005). Excess ammonium in the ponds can cause direct fish mortalities, a decrease in production and increased incidence of related cases. Therefore, ammonium is an important parameter in the assessment of water quality specifically used in aquaculture. The development of innovative analytical device for detecting ammonium has increased due to the increasing demand for high precision, sensitive and rapid detection in meeting the regulations on environment pollution control. In Malaysia, the maximum concentration limit of ammonium recommended by the Department of Environment (DOE) for the protection of public health is in the range of 0.1 to 2.7 mg/L (National Water Quality Standards for Malaysia, 2015). Various methods such as traditional analytical methods (chromatographic and spectroscopic) and optical biosensors are used. Traditional methods are accurate and sensitive, but require sophisticated and expensive instrumentation, expert personnel and complicated sample preparation (Long, F., 2013). Optical biosensors, although highly sensitive, portable and cost effective (Frances, S., 2008; Dey, D., 2011), require the samples to be taken to a laboratory for accurate measurement. Detection using optical biosensors is done through a standard Ultraviolet-visible (UV-Vi) spectrophotometer by measuring the absorbance, reflectance or fluorescence emissions that occur in the ultraviolet (UV), visible or near-infrared (NIR) spectral regions (Martins, T. D., 2013). The equipment is necessary for analytical purpose but not practical and cost effective if used for ammonium measurement only. Therefore, a portable reader is essential to give rapid detection and instantaneous measurement result of ammonium concentration on site.

This paper describes the development of a portable optical reader in detecting the ammonium. The reader is developed based on the optical biosensor developed by Azmi, N. (2009; 2012) using dual enzymes, i.e. glutamate dehydrogenase (GLDH) and diaphorase (Dph) that are immobilized on a glass slide. These enzymes respond to the presence of ammonium upon exposure to light where the absorption of light depends on the concentration of ammonium compounds. A calibration curve of the absorbance versus ammonium concentration shows that as the ammonium concentration increases, the absorbance decreases. By using a UV-Vi spectrophotometer, the optical biosensor was found to have its maximum absorbance at

the wavelength of 565 nm. The developed portable biosensor reader uses a Light Emitting Diode (LED) at this wavelength to detect and measure ammonium. A new model is predicted based on various calibration curve established in every test. The correlation coefficient (R^2) and the accuracy of the device is measured and compared with that of a standard spectrophotometer. It is found that the average accuracy of the developed reader is higher than the spectrophotometer.

DESIGN AND METHODS

DESIGN

The biosensor reader system consists of a portable device and android based smartphone as shown in Figure 1. An optical biosensor probe is inserted into an insertion unit (IU) of the device. The IU consists of a green LED which emits light to the biosensor probe and a photo detector that absorbs the amount of light and converts it to current signal. The green LED was chosen due to its wavelength (565 nm) which satisfies the absorbance spectrum's peak of the biosensor at various ammonium concentrations as shown in the Figure 2. An 8-bit micro controller (PIC18F452) is configured to convert the analog current signal into digital and send it to the smartphone via Bluetooth communication. A smartphone application software to calculate the absorbance was developed using Android Studio with SQLite as mobile database. The reader is battery operated and has its own charging circuitry. The block diagram of the reader system is shown in Figure 3.

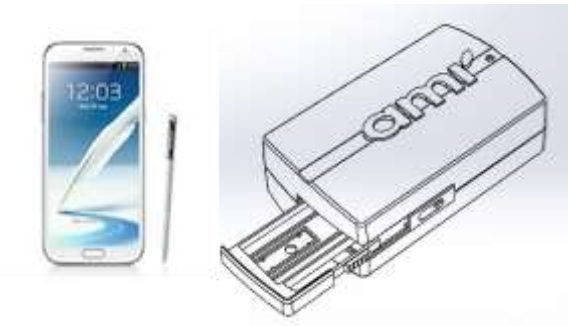


Figure 1. Biosensor Reader system

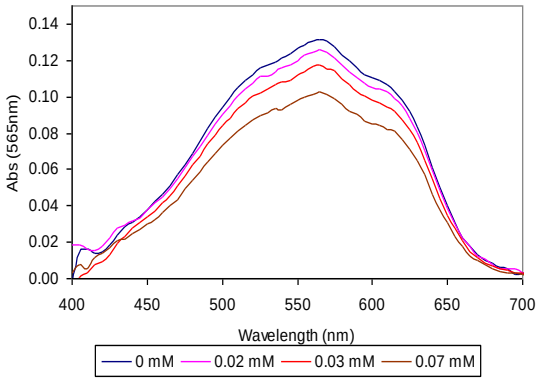


Figure 2. Biosensor absorbance spectrum with various ammonium concentration

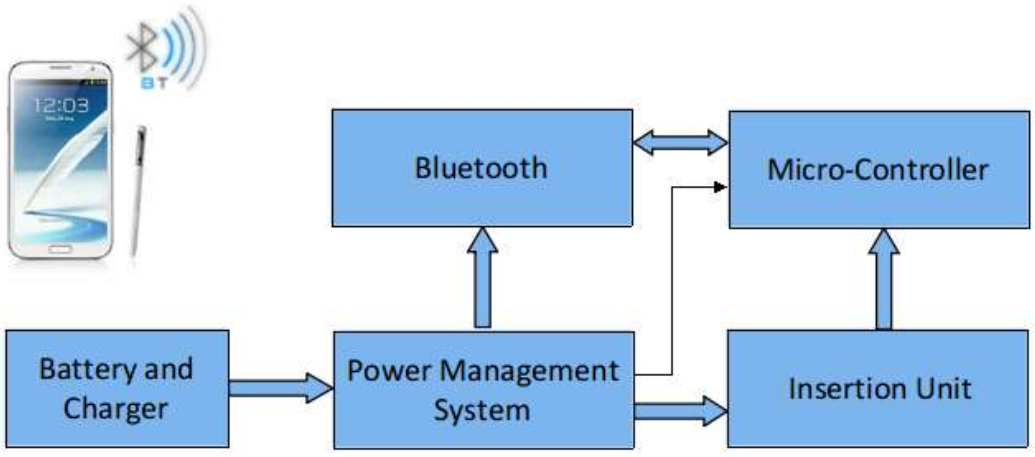


Figure 3. Block diagram of the biosensor reader system

Two biosensor probes are used to measure the light absorption. The first reading is taken from a controlled probe, I_s, with no solutions involved whilst the second reading is taken from the probe that is dipped into thiazolyl blue tetrazolium bomide (MTT) and ammonium solution. This probe is denoted as I_o.

The absorption is determined by the following formula:

$$\text{Absorption} = -\log (I_o/I_s) \quad (1)$$

The apps calculates the absorption and refers the result with the calibration curve for ammonium concentration value. Accordingly, the value is compared to DOE (Department of Environment) standard values for aquaculture system. The result will be displayed in green color if it is less than 2.7 ppm, and in red colour with high pitching sound if the result is more than 2.7 ppm.

Figure 4. Application on smartphone

METHODS

Six sets of tests were performed to establish the calibration curve. Each set consists of more than 40 probes tested on seven (7) ammonium concentrations ranging from 0-10.7 ppm. The controlled probe was first measured with the standard spectrophotometer and then the biosensor reader; then followed with the probes dipped into various concentrations of ammonium.

A total of 12 calibration curves were established from the spectrophotometer and biosensor reader. All the curve shows that the absorbance is linearly related with the ammonium concentration. In estimating a new model, the average of the gradient and constant of the curves are calculated. The absorbance is recalculated using the equation of the new model, based upon the concentration range. This value is set as the threshold for the calculation of the number of tests that are true positive and true negative. True positive (TP) is

positive test result when ammonium is present, while true negative (TN) is negative test result when ammonium is absent. Table 1 is used to calculate sensitivity, specificity and accuracy of both spectrophotometer and portable biosensor reader, using the following equations:

$$\text{Sensitivity} = TP / (TP+FN) \quad (2)$$

$$\text{Specificity} = TN / (TN + FP) \quad (3)$$

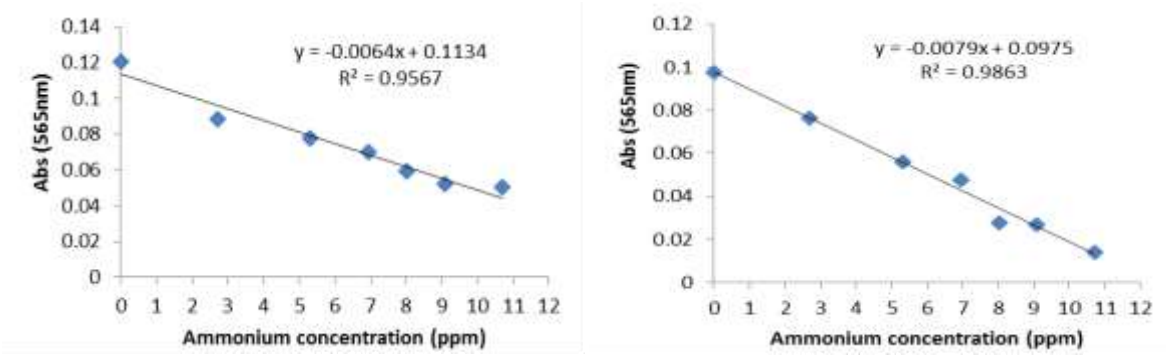
$$\text{Accuracy} = (TP + TN) / (TP + FN + FP + TN) \quad (4)$$

Table 1. Diagnostic Test Accuracy Table

		Ammonium present	Ammonium absent
		TRUE	**FALSE**
Test Outcome	Positive	True Positive (TP)	False Positive (FP)
	Negative	False negative (FN)	True Negative (TN)
		↓	↓
		sensitivity	spesificity

RESULTS AND DISCUSSION

For each set of tests, a calibration curve is plotted and comparisons are made between the spectrophotometer and developed biosensor reader. Figure 5 shows calibration curve of the first test set measured from both devices. Both curves have inverse relationship between absorbance and ammonium concentration with linear proportion. It is observed that the data taken from the developed reader has better fit with R^2 of 98.6 %.



(i) (ii)

Figure 5. Calibration curve of (i) spectrophotometer, (ii) developed biosensor reader

A new model is predicted by taking the average of these linear relationships. Table 2 shows the summary of the gradient, constant and R^2 value of each test set.

Table 2. Summary of the gradient, constant and R^2 value of each test set.

	m		c		R^2	
Test No.	Spectro meter	Developed reader	Spectro meter	Developed reader	Spectro meter	Developed reader
t1	-0.0064	-0.0079	0.1134	0.0975	0.9567	0.9863
t2	-0.0165	-0.0127	0.2721	0.1836	0.8054	0.731
t3	-0.0137	-0.0101	0.1819	0.144	0.9738	0.9825
t4	-0.0155	-0.0129	0.1814	0.1553	0.6708	0.8154
t5	-0.0051	-0.0055	0.1069	0.0827	0.9798	0.9778
t6	-0.0139	-0.0077	0.1778	0.1068	0.9165	0.9402
average	-0.01185	-0.00947	0.17225	0.12832	0.88383	0.90553

Out of six sets of tests, both devices have R^2 values of more than 90 % for four (4) sets of tests. The highest R^2 is at t5 for spectrophotometer (97.98 %) and at t1 for the developed reader (98.63 %). Average value of R^2 for the spectrophotometer and developed reader is 88.4 % and 90.6 % respectively.

The predicted models for both devices are:

$$y_{spectro} = -0.01185x + 0.17225 \text{ for spectrophotometer} \quad (5)$$

$$y_{amr} = -0.00947x + 0.12832 \text{ for the developed reader} \quad (6)$$

The predicted model is calculated for each concentration. At concentration of 2.7 ppm (0.05 mM), it was found that the value of threshold absorbance for the spectrophotometer and developed reader is 0.14 and 0.1 respectively. If the test sets have the absorbance greater than this threshold value, the sample is considered ammonium absent and if lesser, the sample is considered ammonium present. Table 3 calculates the sensitivity, specificity and accuracy of the devices when applying the predicted model to all test sets.

Table 3. Calculation of the accuracy of the predicted model for both devices

		t1	t2	t3	t4	t5	t6
DEVELOPED READER	sensitivity	100	86.667	80	90	100	100
	specificity	0	83.333	83.333	58.333	0	33.333
	accuracy	72%	86%	81%	81%	71%	81%
SPECTRO PHOTOMETER	sensitivity	100	50	90	100	100	83.333
	specificity	14.286	91.667	41.667	50.000	0	33.333
	accuracy	77%	62%	76%	86%	71%	69%

Both developed biosensor reader and spectrophotometer has three (3) test sets of 100 % sensitivity (true positive). For specificity (true negative), spectrophotometer has one test set (t2) of 91.7 % while developed reader has two test sets (t2, t3) of 83.3 %. The average accuracy of the spectrophotometer and developed reader is 73.5 % and 78.7 %, respectively.

CONCLUSION

In this study, we have developed a portable biosensor reader for measuring the ammonium based on the optical biosensor of dual enzyme system. We have also demonstrated a simple model to predict the

absorbance by taking the average of the calibration curves obtained from both the spectrophotometer and developed reader. The developed biosensor reader exhibits better performance in determining R^2 and it is found to be more accurate than the spectrophotometer. It also provides an alternative method in in-situ measurement of ammonium at any site.

FUTURE WORK

The study continues to perform tests of the real samples for further improvement of the accuracy result and validation of the predicted model.

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