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A BOD Sensor Using Immobilized Microbial Consortium in Alginate-Based Matrix for Rapid Detection of River Water Pollution

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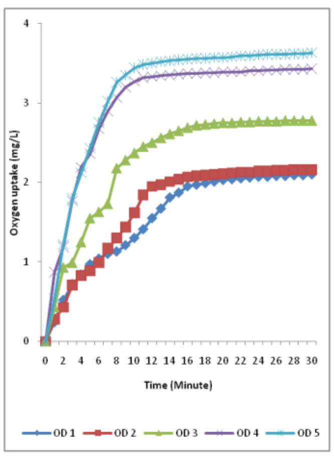
Graphical abstract



Abstract

The development of a rapid sensor for Biochemical Oxygen Demand (BOD) is important for rapid determination of the degree of pollution in river water. A short-term BOD (BOD_{st}) has been developed using a consortium of locally isolated bacteria as the sensing element. YSI 52 Dissolved Oxygen Meter has been used to measure the oxygen uptake in the test solutions. Of the three alginate-based matrices, namely calcium alginate, calcium alginate-gluteraldehyde and polyvinyl alcohol-sodium alginate (PVA-SA), calcium alginate-gluteraldehyde was found to be the best microbial immobilization method. Typical response time of the developed BOD_{st} sensor was 8 minutes, and the immobilized microorganisms were suitable for single use. BOD response was observed at 37°C and pH7.0. For river waters, the BOD values estimated by the BOD_{st} biosensor correlated well with those determined by conventional BOD_5 test using artificial wastewater (AWW) as the calibration solution.

Keywords: Biochemical oxygen demand; dissolved oxygen; BOD_{st} sensor; pollution; river water

Abstrak

Pembangunan kaedah penentuan keperluan oksigen biokimia (BOD) yang pantas adalah penting untuk menentukan kadar pencemaran air sungai. Satu kaedah menentukan keperluan oksigen biokimia jangka pendek (BOD_{st}) telah dibangunkan dengan menggunakan konsortium bakteria tempatan sebagai elemen pengesan. Meter oksigen YSI 52 telah digunakan untuk mengukur tahap penggunaan oksigen di dalam larutan yang diuji. Daripada tiga jenis matriks berasaskan alginat yang diuji; iaitu kalsium alginat, kalsium alginat-gluteraldehid dan polyvinyl alkohol-sodium alginat (PVA-SA), kalsium alginat-gluteraldehid merupakan teknik pemerangkapan bakteria yang terbaik. Tindak balas penderiaan BOD_{st} adalah 8 minit dan mikroorganisma tersekatgerak hanya sesuai digunakan sekali sahaja. Tindak balas BOD ini diuji pada suhu 37°C dan pH7.0. Untuk air sungai, nilai BOD dianggarkan menggunakan biopenderiaan BOD_{st} adalah berkadaran baik dengan nilai yang diukur menggunakan teknik konvensional BOD_5 dengan menggunakan air sisa tiruan (AWW) sebagai larutan kalibrasi.

Kata kunci: Keperluan oksigen biokimia; oksigen terlarut; penderiaan BOD_{st}; pencemaran; air sungai

1.0 INTRODUCTION

Biochemical oxygen demand (BOD) is an important parameter for environmental monitoring used to measure the amount of dissolved oxygen consumed during biodegradation of organic compounds in wastewater. The American standard method for BOD analysis is BOD_5, which requires a 5 day incubation period at 20°C (APHA, 1992). The major drawbacks of the conventional BOD test are that it is time consuming and labour intensive. To overcome the problem, rapid determination of BOD can be achieved by applying a biosensor-based method.

Many researchers have reported on the development of BOD biosensors which are a combination of immobilized microorganism, either as single or mixed cultures, with the Clark type dissolved oxygen electrode (Karube *et al.*, 1977; Riedel *et al.*, 1988; Karube *et al.*, 1990; Galindo *et al.*, 1992; Li *et al.*, 1994; Sangeetha *et al*. 1996; Chan *et al.*, 1999; Liu *et al.*, 2000; Liu and Mattiasson, 2002; Rastogi *et al.*, 2003). In most of the above reported BOD biosensors, the immobilization of microorganism(s) was achieved via adsorption on a synthetic membrane. This microbial membrane was then sandwiched between a dialysis membrane and a gas-permeable membrane of the oxygen electrode.
Liu *et al.* (2004) reported that a common feature of whole-cell based BOD biosensor is the presence of a microbial layer immobilized between a porous cellulose membrane and a gas permeable membrane as a bio-receptor that oxidizes the organic substrate to be quantified. A good sensor performance was

achieved regarding a wide detection linearity and give a good agreement between BOD values estimated by the biosensor and the conventional BOD_5 test. The performance factors being in the control range of ±7.5% for reusability and ±7.3% for reproducibility.

The objectives of this study were to select and characterize suitable locally isolated bacteria for their ability to oxidize biodegradable organics, select the best alginate-based matrix to immobilize the selected bacteria as a mix culture onto an oxygen electrode for BOD_{st} sensing, the working performance factors of the developed BOD_{st} biosensor and to apply the optimized biosensor for rapid determination of BOD in river water.

2.0 EXPERIMENTAL

2.1 Bacterial Cultures

Bacterial strains previously isolated from multiple sources were used in the microbial consortium.

2.2 Test Solution

All the experiments were done using either sterile glucose-glutamic acid (GGA) (APHA, 1992) or artificial wastewater (AWW) (Chee *et al.*, 1999) solution as standard for the calibration of BOD_{st} biosensor. All test solutions were aerated for 1 hour in sterile condition before use.

2.3 Cells Immobilization and Attachment to Oxygen Electrode

A series of microbial consortium absorbance ranging from OD_{600}=1 to OD_{600}=5 were tested to determine the optimum absorbance of microbial cells required for in the BOD_{st} biosensor. The optical density for each microorganism was adjusted to the required value at 600 nm using a 100 VIS Spectrophotometer (BUCK Scientific). The tests were carried out in GGA (150 mg/L). Microbial consortium suspension was immobilized using calcium alginate, calcium alginate-gluteraldehyde and polyvinyl alcohol-sodium alginate (PVA-SA).

For calcium alginate immobilization technique, the microbial consortium suspension (10 µL) was added to 30 µL sodium alginate (5.0%, w/v). The mixture (40 µL) was then vortexed and pipetted onto a dialysis membrane (2 cm x 2 cm). The dialysis membrane was then immersed into calcium chloride solution (0.1 M) for 30 seconds to solidify the bacterial disc. For calcium alginate-gluteraldehyde immobilization technique, the microbial consortium suspension (10 µL) was added to 30 µL sodium alginate (3.0% w/v). The mixture (40 µL) was then vortexed and pipetted onto a dialysis membrane (2 cm x 2 cm). The dialysis membrane was then immersed into calcium chloride solution (0.1 M) for 30 seconds to solidify the bacterial disc. The disc was then washed with phosphate buffer (pH 7) and immersed in gluteraldehyde solution (0.5% v/v) for 1 hour. For PVA-SA immobilization technique, the microbial consortium suspension (10 µL) was added to 30 µL PVA-SA solution. The mixture (40 µL) was then vortexed and pipetted onto a dialysis membrane (2 cm x 2 cm). The dialysis membrane was then immersed into calcium chloride solution (4% w/v) for 30 seconds to solidify the bacterial disc.

All the immobilized cells (bacterial disc) was then washed with phosphate buffer (pH 7) and attached to the top of an oxygen electrode probe secured by a rubber "O"-ring. The microbial disc was soaked in phosphate buffer for 10 minutes as a pretreatment process to stabilize the cells.

2.4 Measurement Of Oxygen Uptake

The bacterial incorporated oxygen electrode was connected to a YSI 52 Dissolved Oxygen Meter. The aerated test solution was placed in a thermostat that controls the temperature at 37°C and stirred throughout the test. The constructed biosensor was immersed into the test solution and the first reading was taken when the dissolved oxygen concentration stabilized. The dissolved oxygen concentration (mg/L) remaining in solution was recorded at every one minute interval for 30 minutes. The oxygen uptake (oxygen consumption) by the immobilized cells was calculated as follows:

$$BOD_{st} = \text{Oxygen uptake} = [DO]_{Initial} - [DO]_{Final}$$

The oxygen consumed by the cells is correlated to the concentration of organics present in a sample.

3.0 RESULTS AND DISCUSSION

3.1 Microbial Consortium Absorbance Optimization

Figure 1 shows the oxygen uptake by the immobilized bacteria in the BOD_{st} biosensor for each microbial consortium absorbance. The oxygen uptake is proportional to the microbial consortium absorbance, as the increased quantity of cells in the bacterial disc increases the oxygen consumed to degrade organic compounds in the test solution. 10 µL of OD_{600}=5 is equivalent to 0.0238 mg of dry weight cell biomass. It was found that 10 µL of immobilized cells to be the best concentration with a higher oxygen uptake and a shorter response time (9 minutes).

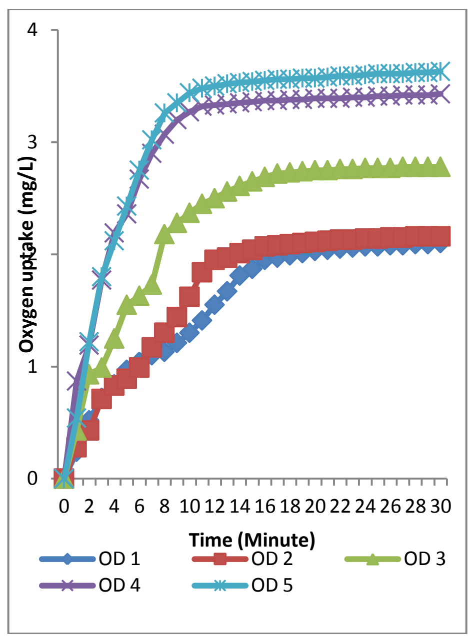


Figure 1 Oxygen uptake by the BOD_{st} biosensor over a series of microbial consortium absorbance

3.2 Sensitivity of the Immobilized Cells in Each Immobilization Matrix

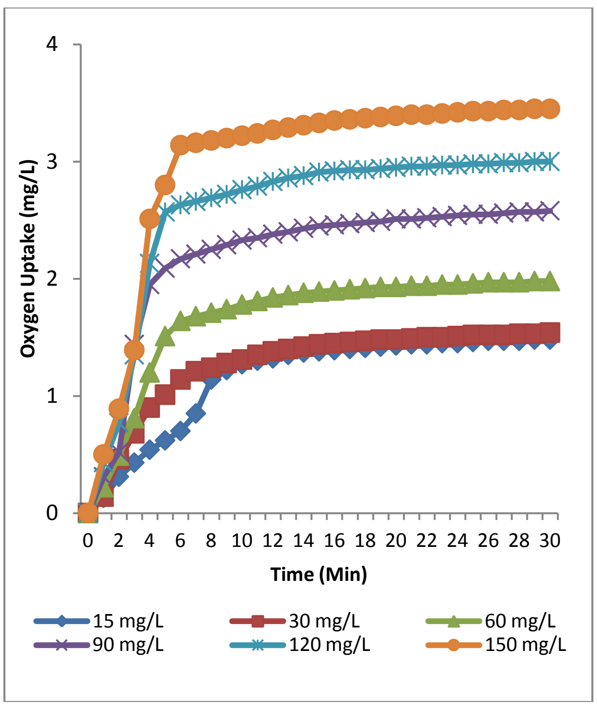
Table 1 shows the slope, R^2 value and steady-time of each immobilization matrix after sensitivity test was done. It can be seen that calcium alginate-gluteraldehyde gives the best result compared to the other 2 immobilization matrices and therefore it was chosen to be used for the next optimization processes. Earlier studies have shown reduced cell leakage by cross-linking of immobilization materials with gluteraldehyde (Oluoch *et al.*, 2006). Gluteraldehyde forms a network of bridging between functional groups on outer membrane of the cells and the immobilization matrix thus reducing the loss of cells (Lei *et al.*, 2006).

Table 1 Slope, R^2 value and steady-state of each immobilization matrix after sensitivity screening

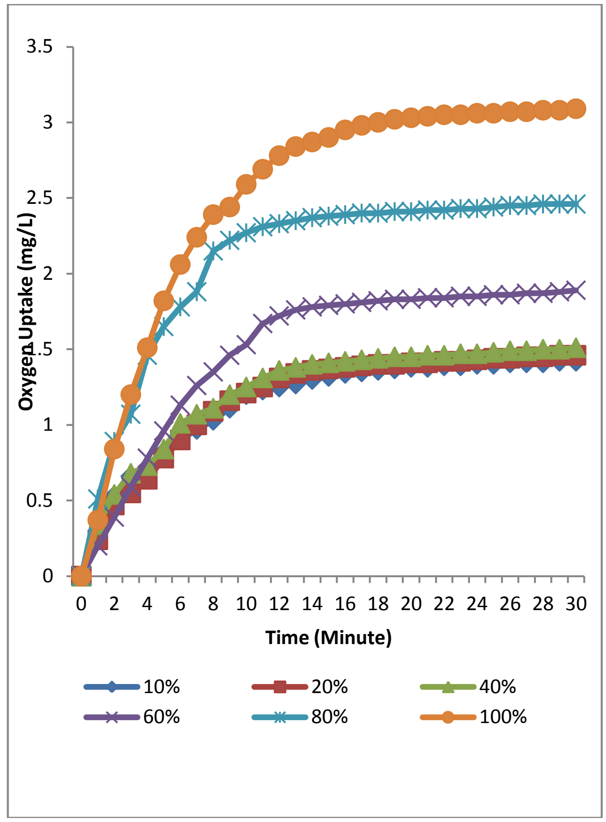
Matrix	*Slope value*	*R^2 value*	*Steady-state (Minutes)*
Calcium alginate	0.005	0.955	10
Calcium alginate-gluteraldehyde	0.015	0.996	8
Polyvinyl alcohol-sodium alginate	0.006	0.793	10

3.3 Response of the BODst Biosensor

The sensitivity of the BOD_{st} biosensor was assessed by determining the amount of oxygen uptake by immobilized cells in different concentrations of organics. Figure 2 shows that as the concentration of GGA and AWW increased the oxygen uptake by the immobilized cells increased rapidly and then reached a steady-state within 8 and 11 minutes, respectively.



(A)



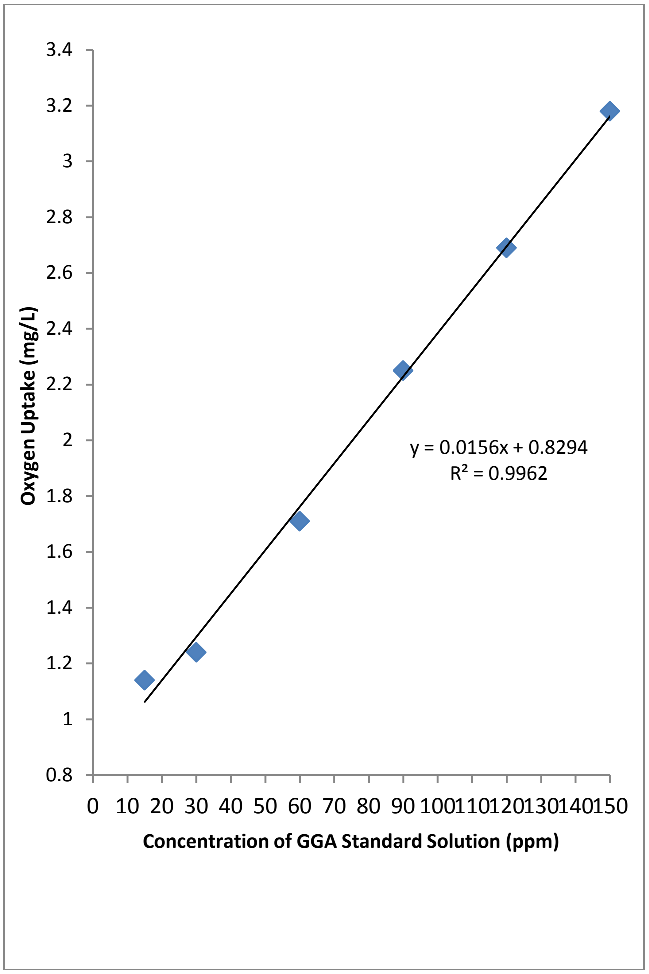
(B)

Figure 2 Oxygen uptake by calcium alginate-gluteraldehyde immobilized cells in a series of diluted GGA (A) and AWW (B) standard solution at 37°C

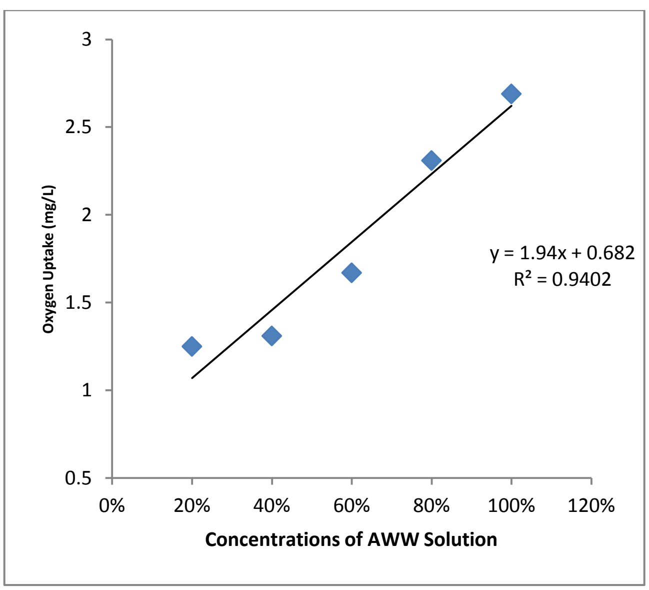
The relationship between sensor response (oxygen uptake in mg/L) and concentration of GGA standard solutions was also found to be fairly linear in the range of 15-150 mg/L GGA concentrations with slope value of 0.015 and a regression coefficient (R^2) of 0.996 (Figure 3A). As for AWW, the sensor response was linear in the concentration range of 20-100% (v/v) (slope = 1.94, R^2 = 0.940).

3.4 Reusability and Reproducibility of the Biosensor

Reusability refers to the ability of a biosensor to produce reproducible results when repeatedly applied to a sample (Liu and Mattiassion, 2002). It was found that repeated use of the microbial disc resulted in a decline in sensor response. When using GGA and AWW as standard solutions, the sensor response during the third measurement declined by more than 30% compared to the first. This may be due to leakage or lyses of cells as the disc is repeatedly used, leading to the reduction in oxygen uptake (Liu *et al.*, 2000).



(A)



(B)

Figure 3 Calibration curve of the BOD_{st} biosensor over a series of diluted GGA (A) and AWW (B) standard solution

Reproducibility was defined as the standard deviation of a series of tested sensor by more than one operator under different conditions (Liu and Mattiasson, 2002). Liu *et al.*, (2000) reported that a sensor based on microbial consortium had reproducibility varying from ±9.3% to ±15.0% depending on the applied wastewater samples. A good reproducibility of the oxygen uptakes after renewal of the microbial disc was obtained with average oxygen uptake of 3.27±0.061 mg/L (1.8%) for GGA and 2.66±0.317 mg/L (12%) for AWW. These results suggest that the microbial disc employed in the BOD_{st} biosensor is suited for single use only.

3.5 Application of the BODst Biosensor to River Water

The BOD_{st} biosensor was applied to determine the BOD of river water and the values were found to be comparable to the conventional 5-day BOD when using artificial wastewater (AWW) as standard calibration solution with the ratio of BOD_5:BOD_{st} between 0.60-0.91 (Table 2). When using GGA as the standard solution, the BOD values were overestimated. AWW has been reported to be the preferred standard solution for determining BOD load of samples that contain less biodegradable organics such as river water (Chee *et. al.*, 1999).

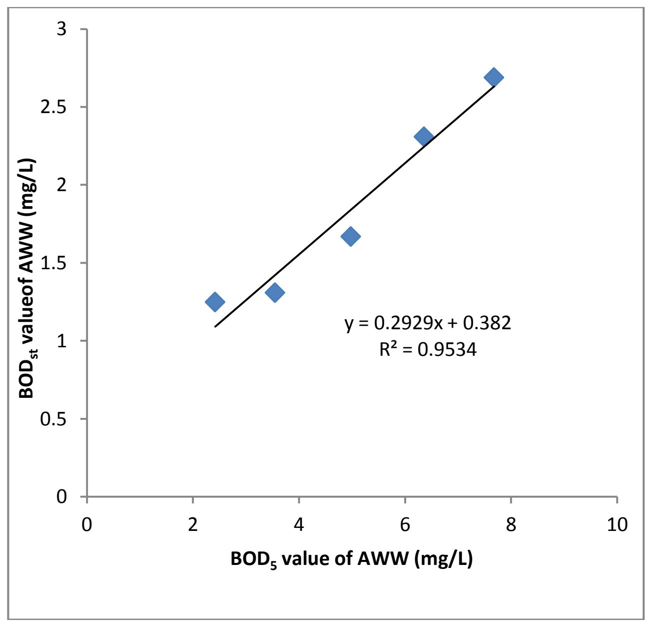


Figure 4 Calibration curve depicting the correlation of sensor BOD with conventional BOD (BOD_5) using AWW as calibration solution

4.0 CONCLUSION

The present study has demonstrated the potential of using calcium alginate-gluteraldehyde immobilized cells as sensing material for rapid estimation of BOD within 8 minutes response time. The immobilized cells comprised of a defined mixture of microorganisms isolated from various sources in Malaysia. Bacterial concentration of OD_{600}=5 (10 µL, dry weight = 0.0238 mg) was determined as the best microbial concentration to be employed in the BOD_{st} biosensor. GGA and AWW organic standard solutions were used as the calibration solution for the developed BOD_{st} biosensor. The developed BOD_{st} biosensor's bacterial discs were proven not to be reusable as the performance decreased by more than 30% after repeated used. However the discs produced fairly reproducible result for each BOD_{st} test with an average oxygen uptake of 3.27±0.061 mg/L (1.8%) for GGA and 2.66±0.317 mg/L (12%) for AWW, respectively. AWW was found to be the suitable standard calibration solution for river water samples as good correlation between the BOD_{st} biosensor values and that of the conventional BOD_5 test was achieved. A short response time is the major advantage of using the BOD_{st} biosensor and it is applicable for rapid detection of BOD in river waters.

Table 2 Comparison of BOD_{st} values estimated by the sensor with those determined by the 5-day method (BOD) for various river water

River water	*BOD_5 value (mg/L)	BOD_{st} biosensor value (mg/L) using AWW as standard solution	BOD_{st} biosensor value (mg/L) using GGA as standard solution	BOD_5:BOD_{st} ratio in AWW
RIVER 1	19.61±1.17	12.94	197.06	0.60
RIVER 2	4.32±0.26	6.35	95.06	0.60
RIVER 3	5.78±0.85	4.06	59.73	0.70
RIVER 4	10.42±1.92	6.95	104.40	0.66
RIVER 5	5.60±0.38	5.10	75.73	0.91

*BOD_5 values determined via the standard conventional method (APHA, 1992)

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