December 2009 Volume 4 Issue 6

Trade Science Inc.

Environmental Science
An Indian Journal

Current Research Papers

ESAIJ, 4(6), 2009 [520-525]

Microbial biosensor for BOD analysis

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Received: 24th August, 2009 ; Accepted: 3rd August, 2009

ABSTRACT

Microbial biosensors can be used to overcome the limitations of conventional BOD analysis. In the present study a biosensor was designed based on amperometric oxygen sensor, an easily replaceable biomembrane of cellulose acetate and immobilised cells of different microorganisms. Immobilised cells of *Bacillus subtilis* and *Pseudomonas sp.* were used in the study. Biosensor output signal depends on the concentration of substrate that indicates the organic pollution of waters. Biosensor output signal was analysed classically according to the steady-state method. The calibration of biosensor was based on the change of biosensor response between an initial value and the stable end-point of the signal. Studied biosensor can be used for the measurements of biochemical oxygen demand more effectively in the concentration range of 30-80 mg/L for *bacillus subtilis* sp. and 5-45 mg/L for *pseudomonas* sp.

KEYWORDS

Biosensor;
BOD;
Calibration curve;
Regression.

INTRODUCTION

The BOD test has its widest application in measuring waste water loading in treatment plants and in evaluating the BOD removal efficiency of such treatment systems. Traditional analysis of biochemical oxygen demand (BOD) is very much time consuming that it involves 5 to 7 days incubation period and is thus ineffective for operation of activated sludge processes in wastewater treatment plants or determination of the concentration of biodegradable organic pollutants in polluted waters. Because of the need for express method of BOD determination, biochemical oxygen demand (BOD) biosensors have been developed.

A biosensor is an analytical device that combines a biological sensing element with a transducer to produce a signal proportional to the analyte concentration. [ ]This signal can result from a change in protons concentration, release or uptake of gases, light emission, absorption and so forth, brought about by the metabolism of the target compound by the biological recognition element. The transducer converts this biological signal into a measurable response such as current, potential or absorption of light through electrochemical or optical means, which can be further amplified, processed and stored for later analysis.[ ]

In the present study a microbial biosensor for BOD measurement was fabricated. The sensor consists of a DO probe, an easily removable biomembrane and immobilised cells of microorganisms. Microorganisms used are *Bacillus subtilis* and *Pseudomonas sp*. Optimization of the sensor was done and after that calibration of the sensor with the suitable range of BOD was done.

OBJECTIVES OF THE STUDY

The objectives of the present study are the following:

- Set up a microbial biosensor (MBS) for BOD analysis of waste water sample
- Optimise the performance characteristics of the BOD biosensor
- Correlate the results obtained with the conventional analysis result
- Check the sensor performance with *Bacillus subtilis* and *Pseudomonas sp.*

MATERIALS AND METHODS

A microbial biosensor consisting of DO probe and sensor tip was fabricated. The biosensor was connected to a micro ammeter in order to convert the sensor signal in the form of potential difference.

BOD sensor consists of DO probe, easily removable biomembrane and immobilised bacterial cells have designed. DO probe was constructed using a test tube opened at both ends. One end was covered with gas permeable Teflon membrane and through the other end electrolyte was introduced. The opened end can be closed with a glass stopper. Lead was used as anode whereas cathode was comprised of Platinum. Cathode was placed such that the space between the Teflon membrane and cathode should be of minimum. The electrolyte used was 0.1M KCl solution. The DO probe was connected to a micro ammeter using copper wires as well as to the sensor tip.(Figure 1)

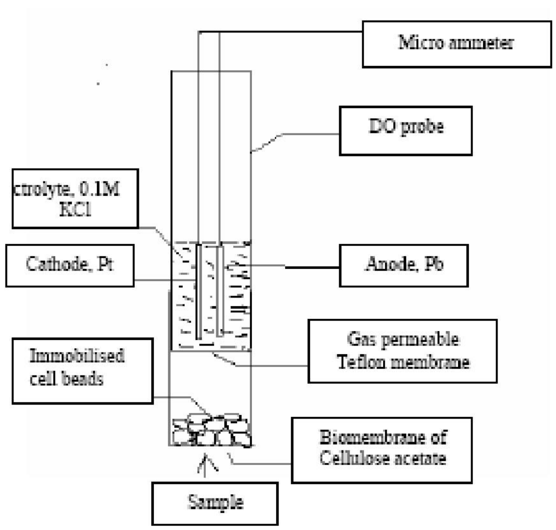

Figure 1 : Schematic presentation of a BOD biosensor

The primary study include optimization of biosensor based on 1) repeatability of cell beads, 2) repeatability of filter membrane, 3) pH of the sample and 4) concentration of substrate. The output current is directly taken as the sensor response. After optimization of biosensor, the range of BOD_5 that the sensor can respond was found out. By knowing the effective range of BOD_5 by doing regression analysis, we can draw the calibration curve. A calibration curve was plot using sensor signal in µA as abscissa and BOD_5 as ordinate. Calibration curve is different for different species of microorganisms. Now the sensor is ready for the BOD5 measurement of real water samples.

The measuring technique taken for BOD sensor response analysis is the steady-state (also entitled as endpoint, dynamic) method. We get sensor current when we immerse sensor tip to the sample. This current is deducted from the current produced for phosphate buffer solution having 0 mg/L of BOD_5. This current difference is taken as the sensor signal. Extent the sensor signal (µA) at abscissa to the calibration curve and find out the corresponding BOD_5 value in mg/L from the ordinate. Thus we get the BOD_5 value of sample in a short time.

Calibration curve for BOD measurement

The BOD_5 of samples varied from 8mg/L to 120mg/L. Sensor tip was immersed in the phosphate buffer solution until we get a steady state current. The time taken and the steady-state current were noted. After that the sensor tip was immersed in the sample until the current reduced to reach a steady state. Difference in the two steady state currents was directly proportional to the easily assimable organic matter present in the sample. Plot the currents against the known BOD_5 values and find the reliable range of BOD_5 that the sensor can measure.

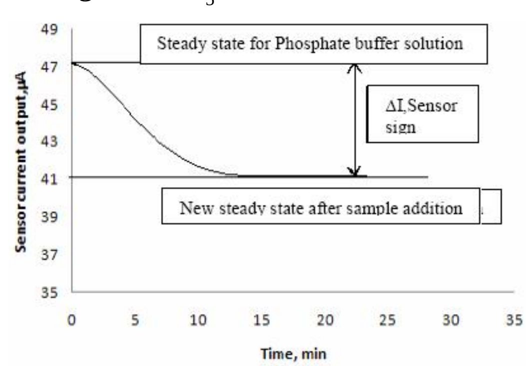

Figure 2 : Typical response curve from BOD biosensor (Steady state method)

$$\text{Sensor signal} = \text{Steady state current for phosphate buffer solution} - \text{Steady state current after sample addition}$$

The calibration curve was drawn for the effective range of BOD_5. Find the difference in current produced for both phosphate buffer solution and waste water sample. Extend the current difference generated (sensor signal- µA) to the calibration curve and find the corresponding BOD_5 in mg/L.

RESULTS AND DISCUSSIONS

Optimisation calibration of BOD sensor for bacillus subtilis

Repeatability of cell beads

Microorganisms immobilised on inert tile pieces were used as the biological recognition elements. Ten pieces of tiles were used at a time as a set for the experiment. First time when fresh set of cell beads and membrane are new, the currents was as high as 47.2µA. At the third time, the current suddenly dropped to 25 µA that the efficiency of the cells reduced to 52.96%. Consistency of the result was checked using another set of cell beads. Therefore each sample testing need a fresh set of cell beads. The efficiency reduction may be due to the death of some bacterial cells. The results are graphically shown in Figure 3.

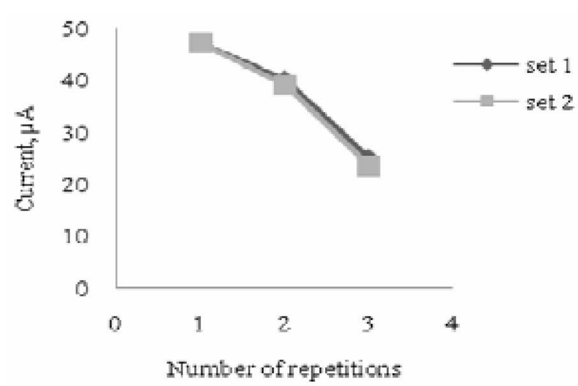

Figure 3 : Repeatability of immobilised cell beads

Repeatability of filter membrane

Cellulose acetate of 0.2µ pore size was used as the filter membrane. First time when both cell beads and membrane are new, the currents may be as high as 47.2µA and. For the following two times new cell beads were used but the same filter membrane was used. At the third time, the current suddenly dropped to 3.6µA. The efficiency reduction may be due to clogging and the corresponding reduction in pore size. The two sets of readings show a similar pattern in current production. Therefore each sample testing need a fresh cellulose acetate membrane. The results are graphically shown in Figure 4.

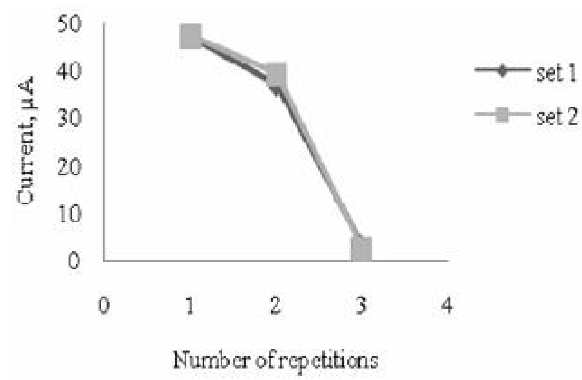

Figure 4 : Repeatability of filter membrane

Sensor response with pH

This test was conducted to study the effect of pH of the sample on the sensor behaviour. The Figure 5 shows low sensor current variations at the extreme acidic and alkaline regions.

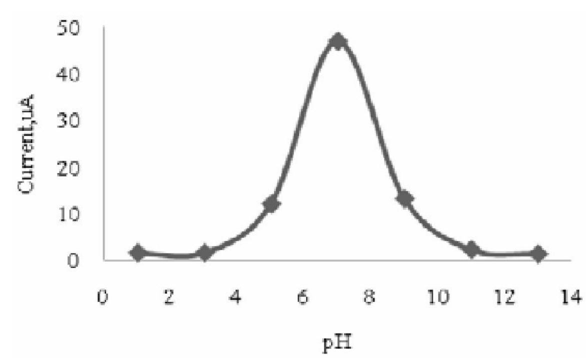

Figure 5 : Variation in current production as pH of the sample varies.

Sensor response with concentration of substrate

This test was conducted to study the effect of substrate concentration of the sample on the sensor behaviour. The current produced for each sample decreases as the substrate concentration increases. Above 800mg/L of substrate concentration, the sensor did not respond. The current produced by the oxygen electrode is reduced as the substrate concentration increases. This is because the oxygen present in the sample is utilised by immobilised microbial cells for the organic matter depletion.

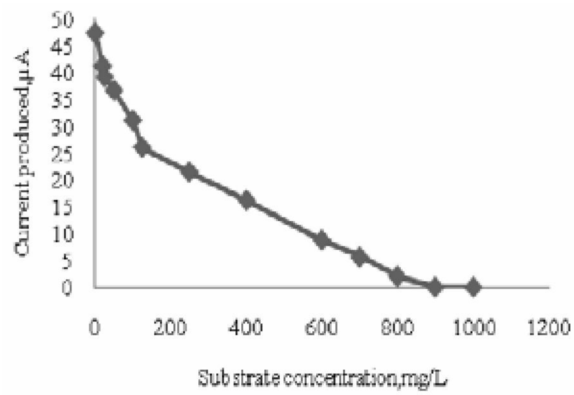

Figure 6 : Sensor response with concentration of substrate

Calibration curve for BOD_5 measurement

To find the effective range of BOD_5 that Bacillus subtilis can identify, the sensor signal Response with Conventional BOD_5 was studied. Sensor signal here mentioned is the current differences between phosphate buffer solution and sample solution.

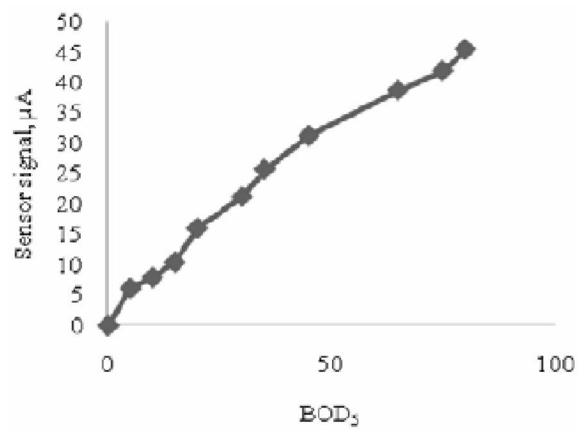

Figure 7 : Signal response with BOD_5

Under the optimum conditions determined above, a calibration curve was obtained using standard starch solution. The results showed a linear relationship from 30 to 80mg/L of BOD_5. The regression analysis of the

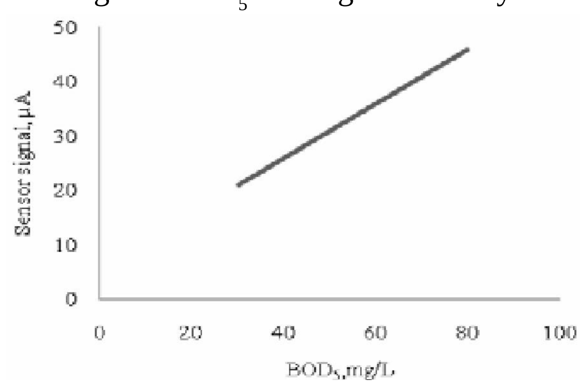

Figure 8 : Calibration curve -bacillus subtilis as biological recognition element

sensor signal with BOD_5 values showed a good correlation of 0.9987. The calibration curve when Bacillus subtilis was used as the biological recognition element is shown in Figure 8

Optimisation and calibration of BOD sensor for pseudomonas sp.

Repeatability of cell beads

Microorganisms immobilised on inert tile pieces were used as the biological recognition elements. They utilise the dissolved oxygen present in the wastewater and start to respirate. When the cells beads used repeatedly the current producing efficiency of the sensor also get reduced. The two sets of readings show a similar pattern in current production. The efficiency reduction may be due to the death of some bacterial cells. Therefore of cell beads can be taken as three. The results are graphically shown in Figure 9.

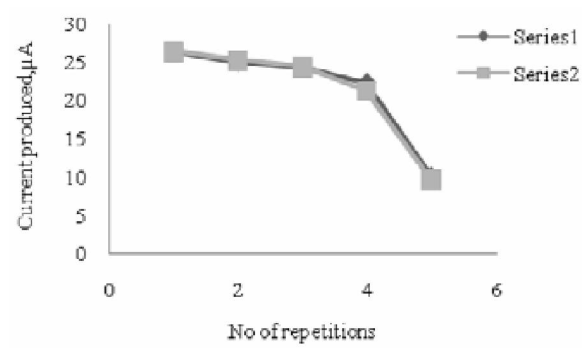

Figure 9 : Repeatability of immobilised pseudomonas sp. cell beads

Repeatability of filter membrane

Cellulose acetate of 0.2µ pore size was used as the filter membrane. first time when both cell beads and membrane are new, the currents may be as high as 26.3µa. The two sets of readings show a similar pattern in current production. Therefore each sample testing

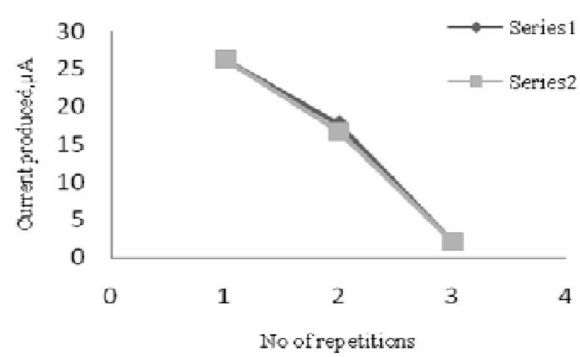

Figure 10 : Repeatability of filter membrane

needs a fresh cellulose acetate membrane. The results are graphically shown in Figure10.

Sensor response with pH

This test was conducted to study the effect of pH of the sample on the sensor behaviour. The results are graphically showed in Figure 11. From the figure it is clear that the test condition preferable for microbial analysis is neutral pH of the sample.

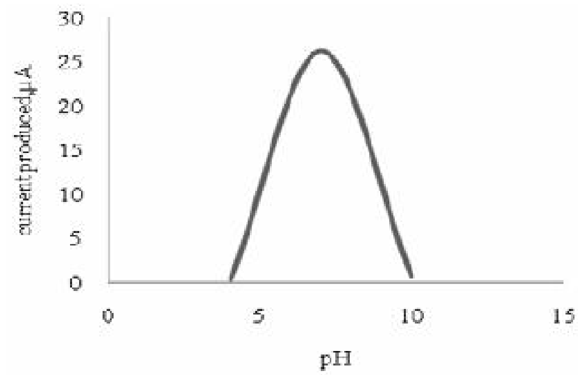

Figure 5 : Variation in current production as pH of the sample varies.

Sensor response with concentration of substrate

This test was conducted to study the effect of substrate concentration of the sample on the sensor behaviour. The current produced for each sample decreases as the substrate concentration increases. Above 600mg/L of substrate concentration, the sensor did not respond.

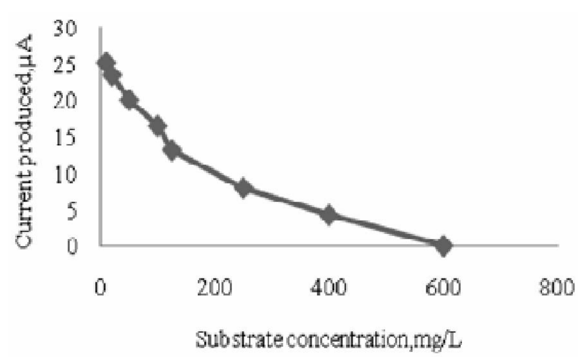

Figure 11 : Sensor response with concentration of substrate

Calibration curve for bod measurement

To find the effective range of BOD that the biocatalyst Pseudomonas sp. can identify, the sensor signal Response with Conventional BOD was studied. Sensor signal here mentioned is the current differences between phosphate buffer solution and sample solution.

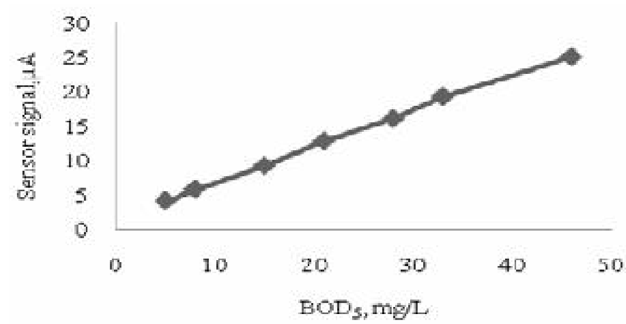

Figure 12 : Signal response with BOD_5

Under the optimum conditions determined above, a calibration curve was obtained using synthetic wastewater. Synthetic wastewater was prepared by adding starch to distilled water and adjusts BOD as required. The results showed a linear relationship from 5 to 40mg/L of BOD. The regression analysis of the sensor signal with BOD_5 values showed a good correlation of 0.99825. The calibration curve when Pseudomonas sp. is used as the biological recognition element is shown below.

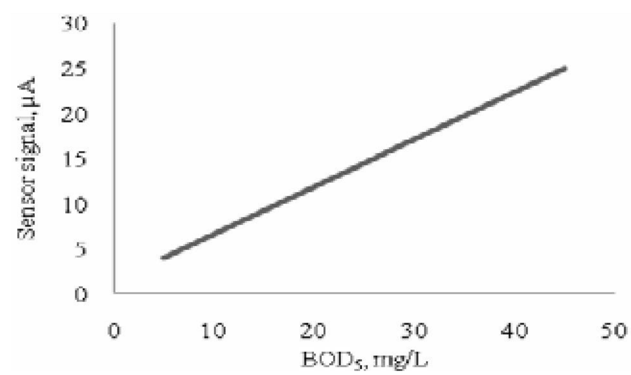

Figure 13 : Calibration curve –*Pseudomonas sp*. as biological recognition element

CONCLUSION

The following conclusions can be drawn from the above study .

Optimisation of biosensor was done based on repeatability of cell beads, repeatability of cellulose acetate membrane, pH of the sample and substrate concentration. The Sensor signal shows a linear variation with specific range of BOD_5 values. The regression analysis of the sensor signal with BOD_5 values showed a good correlation of 0.9915 for *Bacillus subtilis* and 0.9982 for *Pseudomonas* sp.

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