Instant and reliable estimation of BOD by developing a mixed culture based BOD biosensor

R. Kumar*, P. Dhall and A. Kumar
Institute of Genomics and Integrative Biology, Delhi, India (rita@igib.res.in)

Introduction

Amongst all the parameters for which the waste-water is to be monitored, BOD is the most important and widely used parameter to indicate the strength of water pollution. In an era where speedy, sensitive, small, portable, instant and real time measuring devices have replaced the conventional analytical method, BOD is still being carried out using the classical method which requires a long incubation period and considerable skill rendering it tedious for direct process control. Thus, it is necessary to develop a method that could circumvent the weaknesses of the conventional method. BOD biosensors are one such category of analytical device as described by (Karube 1977) and BOD beads sensor offers practical alternative to all such problems. Present study deals with the development of microbial biosensor based on a synergistic consortium of nine bacteria immobilized in an appropriate immobilizing agent resulting in the formation of beads and the beads are used for instant BOD estimation using an electronic device. BOD of a wide range of industrial wastewaters samples could be assessed within a short time period (5-10 minutes).

Material and Methods

Inoculum preparation

The inoculum (mother culture) was prepared by inoculating one loopful of all the nine individual bacterial isolates in 25ml of sterilized nutrient broth having 0.01% Tween-80. The inoculated broths were incubated in a shaker at 37^0C for 16-24 hours so as to obtain actively growing mother cultures.

Preparation of the microbial consortium

The above mentioned actively growing individual bacterial cultures were inoculated separately in 100ml of sterilized nutrient broth and incubated at 37^0C, 150 rpm for 16-24 hours. Finally, these bacterial cultures were mixed in equal proportions on the basis of their optical density values at 625nm. This microbial mixture was centrifuged at 7000 rpm for 20 min at 4^0C. The cell pellet was washed twice with 50mM sodium phosphate buffer and suspended in small volume of the same buffer. The resultant cell slurry was kept at 4^0C till further use.

Immobilization in agarose solution

The agarose was boiled to get homogeneous solution and was kept in water bath maintained at 50^0 C. In the mean time, the temperature of mustard oil was maintained by keeping at 4^0 C . The prepared cell slurry was properly mixed with the help of glass rod to get uniform suspension. Simultaneously, the beaker containing oil was kept in an ice box to avoid the temperature change. A syringe (10ml) capacity was filled with the suspension and poured drop by drop in mustard oil to get the beads.

BOD_5 test

The 5-day BOD (BOD_5) of samples was determined using a range of dilutions of the standard GGA solution and industrial waste water (APHA, 1998).

Measurement of the response

The response of the developed BOD beads sensor was measured by adding a range of 2-12 beads in 100ml sodium phosphate buffer .A Clark type probe for dissolved oxygen was used as the physical transducer, which consisted of a platinum cathode as the working electrode and a silver anode as the reference electrode. In order to stabilize the BOD beads sensor system, the sensor was immersed in 100ml of sodium phosphate buffer system under constant moderate stirring. The electrodes of the sensor were connected to a potentiostat and the output current was recorded through a multimeter, which amplified the response to nA. An applied potential of -650mV versus Ag/AgCl was delivered to Pt-working electrode throughout all the measurements. Aliquots of stock synthetic/industrial samples were added after stable current was attained for 30min (initial steady current). The current change (decrease) was observed after addition of the samples until a final steady state reached. The response was calculated on the basis of current difference between the initial steady state current and the final steady state current.

Results and Discussion

A number of beads were tried to optimize the response in terms of current change. The prepared beads were stored at 4°C in phosphate buffer of pH 6.8. A range of 1 to 25 beads were taken and the optimum number was found to be twelve beads.

GGA was used as a reference standard for all BOD measurements and for the calibration of the sensor as well. Stock solution of GGA having a concentration of 12,000 mg/l was prepared. Different aliquots of GGA were added in the measuring cell of the BOD beads sensor system, to achieve desired GGA concentrations of 30-300 mg/l. The response of the sensor with different GGA concentrations was observed and recorded. The readings were plotted and on the same excel sheet, a second mantissa was drawn showing the conventional BOD (BOD_5) values against the same GGA concentrations used for the developed BOD sensor. The linearity and calibration curve corresponding to the developed BOD beads sensor system is shown in figs 1 and 2.

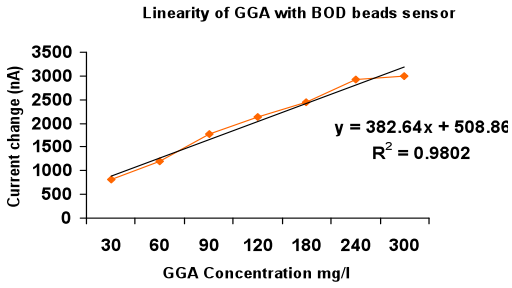

Figure1. Linearity of GGA with BOD Beads Sensor

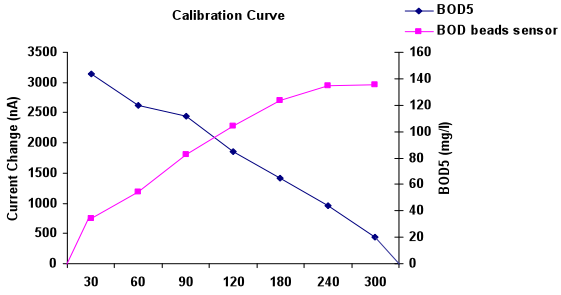

Figure 2. Calibration curve

The developed BOD bead sensor was used to estimate the BOD value of different percentages of industrial wastewater from different industries. In parallel, the same percentages of the sample were analyzed using the conventional BOD method. The results showed that BOD values of wastewater as determined by BOD beads sensor were comparable to BOD_5 as performed by conventional method. The deviation in the results lay well within limits.

Table 1 depicts the BOD values of inlet of beverage waste water as determined by the beads sensor. The BOD load of the waste water was also determined by the conventional method and the results when compared to the beads sensor showed a variation of -0.01 to -2.5 percent which lies very much within the permissible limits (In conventional method, a variation of +_ 15% is permissible).

S. No.	Percentage Used	BOD beads sensor (mg/l)	BOD_5 (mg/l)	Percentage Deviation w.r.t BOD_5
1	8.0	615	600	-2.5
2	7.5	682	674	-1.1
3	6.4	756	748	-1.0
4	5.8	946	952	0.6
5	6.2	640	632	-0.01

Table 1. BOD Values of beverage waste water (Inlet) as determined by BOD beads sensor & comparison with BOD_5

BOD load is measured conventionally by BOD analysis using Wrinkler's method. So, the BOD values determined by developed sensor need to be compared with BOD values determined by the conventional method for sensor performance check. The GGA solution check is the only reference point for evaluation of dilution water quality, seed effectiveness, and analytical technique. However, the BOD biosensors reported so far, when applied to analyze real wastewaters have not shown good correlation with the conventional BOD values. The variation in the ratio of BOD_{sensor}: BOD_5 in case of real wastewater is because of variable composition of wastewater over a period of time (Qian & Tan 1999; Riedel 1998; Tag 2000). These authors have corroborated this to the different measuring principles involved in both the cases as well as due to variability in composition of wastewaters. However, it has been predicted by Liu and Mattiasson (Liu, 2002) that it is possible to minimize the problems by selecting suitable microbial strains as biological recognition elements.

Rastogi et al. (Rastogi 2003) reported that a BOD biosensor based on a pre-tested, synergistic formulated microbial consortia was capable to sense the BOD load of a wide variety of industrial waste waters having low-moderate-high biodegradability. It has been emphasized that a pre-selection of microorganisms for their capability to degrade a range of pollutants can serve as an ideal BOD biosensor. Such a biosensor can cater to a variety of industrial effluents in determining the BOD load. Earlier, BOD biosensors have been tried for various synthetic substrates but when applied to real wastewaters did not give good correlation with BOD_5 values (Kim and Park 2001). An equation has been derived (patent filed), with the help of which, it is possible to calculate % of waste water to be used for BOD load with the sensor irrespective of the BOD load of waste water at any time. This variability could be overcome by developing BOD biosensor with pre-tested consortia. Even when dealing with an individual industry, where there are variations in composition over a period of time, comparable results with varying loads of BOD (300 mg/litre to 2500 mg/l) in a beverage wastewater could be determined. After conducting COD, one can directly go for measuring BOD with the sensor using few calculations.

Conclusions

The developed BOD beads sensor has been found to determine BOD load of real waste waters in in few minutes as against conventional BOD method which requires 5 days.

The BOD beads sensor was found to be able to determine the BOD load of a range of industrial waste waters i.e. low, moderate and high biodegradable waste water. However, when the waste water arrives in the laboratory, it becomes difficult to decide upon the concentration of wastewater of any industry. So an attempt was made to prepare the micro-organisms immobilized beads to be used to determine the pollution load of BOD in real wastewaters. The beads were prepared for use in BOD beads sensor and optimized the reproducibility and repeatability of BOD beads sensor was confirmed. BOD beads were used to study the linearity of a standard reference solution i.e. glucose glutamic acid used in conventional BOD analysis. During the experiments conducted in the laboratory, it was observed that GGA exhibited good linearity ($r^2 = 0.9921$).

Further, the developed BOD beads sensor was studied with respect to real waste water obtained from different industries (both inlet as well as outlet) over a period of time. It was observed that BOD load of waste waters, as determined with the developed BOD beads sensor had conformity with the conventional BOD analysis.

References

1. Karube et al. (1977) *Microbial electrode BOD sensors*. Biotechnology and Bioengineering 19 pp. 1535–1547.

2. APHA (1998) *Standard methods for the examination of waters and watse waters*, 20th ed., American public health association, Washington , D.C.

3. Riedel et al. (1998*) Arxula Adeninivorans based sensor for the estimation of BOD.* Analytical letters 31(1) pp. 1-12.

4. Qian & Tan (1999) *BOD measurement in the presence of heavy metal ions using a thermally-killed-Bacillus subtilis biosensor.* Water Research 33 (13) pp. 2923-2928

5. Tag et al. (2000) *Measurementof biodegradable substances with a mycelia-sensor based on the salt tolerant yeast Arxula adeninivorans LS3*. Sensors Actuators B 67 pp. 142-8.

6. Liu et al. (2002) *Microbial BOD sensors for wastewater analysis*. Water Research 36 (15) pp. 3786-3802.

7. Rastogi et al. (2003) *Development of characterization of a novel immobilized microbial membrane for rapid determination of biochemical oxygen demand load n industrial waste waters.* Biosensor & Bioelectronics 18 pp. 23-29.

8. Kim and Park (2001) *Klebsiella BOD sensor.* Sensors & Actuators B 80 pp. 9-14.