J. Indian Chem. Soc.,
Vol. 73, September 1996, pp. 472-475

On the Estimation of Biological Oxygen Demand of collected Water Samples with Special Reference to that of the Ganges Water

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Manuscript received 30 November 1994, accepted 2 February 1995

A procedure for measuring BOD of collected water samples using a Karube type microbial BOD sensor is described. BOD of water samples collected from the Ganges at Calcutta, decreases on standing. This phenomenon has been attributed to consumption of the organic matter present in the collected sample due to microbial growth and cut-off the source of the organic matter from the sample. The water samples should therefore be sterilised at collection point so that BOD can be measured later on in test laboratories either by the 5-day BOD or by the 30-minute sensor method.

There has been great concern the world over for the ever increasing pollution levels of ponds, lakes, streams, rivers, etc. with increasing industrial and agricultural growth. The level of organic pollution in water bodies is reflected in the value of Biological (Biochemical) Oxygen Demand (BOD) which is a quantitave measure of the amount of O_2 in mg required to oxidise the organic matter present in 1 dm^3 of water by microbes through their metabolic processes. The lower the BOD value of a water body, lower is its pollution level and greater is the amount of dissolved O_2 in it. However, a zero BOD is neither attainable nor desirable for any natural water-body. The pollution is at a tolerable level when the BOD is 3 mg dm^{-3} or below. When it exceeds the value 5 mg dm^{-3} the water is of doubtful purity but when the value exceeds 10 mg dm^{-3} the water is bad indeed[1].

In India a cleaning programme for the Ganges named 'Ganga Action Plan' has been initiated about seven years ago. Any improvement of the quality of the Ganges water arising out of the programme should result in a lower BOD than it was before. BOD should gradually lower with the progress of the plan. The target should be to attain and maintain a BOD value of 3 mg dm^{-3} or less. The BOD is normally measured by the so called 5-day chemical analysis method (BOD_5), which is a time consuming process. However, a significant development occurred about 15 years ago when Karube[2] developed a microbial sensor. The sensor reduced the measuring time of BOD to 30 min. In our laboratory, we have fabricated a sensor following Karube and measured the BOD of various samples including water from the Ganges at Calcutta. During the course of our investigation, we noticed that BOD of the collected Ganges water decreases on standing. A substantial decrease takes place within a couple of hours after collection. To our knowledge, no such reports exist in the literature. Here we describe a procedure for measuring BOD of collected water samples using a BOD sensor, report the results of our analysis of water from various sources, offer an explanation for the phenomenon of BOD decrease of the Ganges water on standing and suggest ways to deal with the problem so as to get a true measure of BOD when on-line measurement facility is not provided.

Results and Discussion

For the measurement of BOD using the sensor, it is necessary to keep the ionic strength and the pH of all solutions same. This ensures reproducible functioning of the outer membrane of the sensor in various samples. The pH was maintained at 7 (0.01 *M* phosphate buffer) following the method of Karube[2].

In the sensor described by Karube *et al.*[2], the sample and the buffer are introduced into the measuring cell through separate channels using two peristaltic pumps. This configuration is essential for on-line measurement as would be evident from the discussion later.

Analysis of collected samples can however be done using one peristaltic pump which pumps the buffered analyte through the flow (measuring) cell of the sensor. But the samples needs to be sterilised before buffering with the phosphate buffer since the BOD of many unsterilised samples decreases at a significant rate on buffering with phosphate as would be evident from the results that follow.

Fig. 1 shows the recorder trace from our sensor for the BOD measurement of a sample of pond water under different conditions. When the pond water was sterilised, buffered and used for measurement (sample I) a signal of 65 recorder division was obtained. When the pond water so treated and kept standing for 3 h before analysis (sampel II) the signal remains unchanged proving thereby that the BOD of the sterilised pond water does not decrease with time on buffering. However, when the water was not sterilised but buffered (sample III) and fed into the measuring cell the signal strength becomes 45% of that given by the sterilised sample (samples I and II). The signal

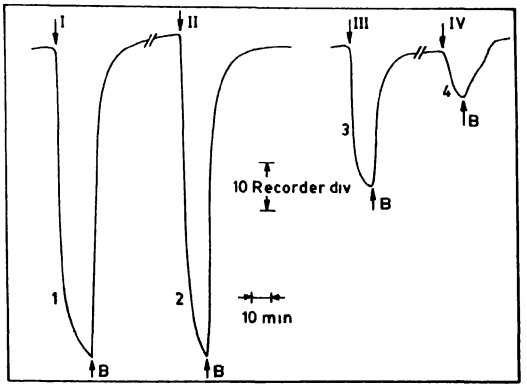

Fig 1 BOD response for pond waters which were variously treated (I) sterilised pond water, (II) sterilised pond water keeping under buffered condition for 3 h, (III) unsterilised pond water, (IV) unsterilised pond water keeping under buffered condition for 3 h and (B) 0 01 *M* phosphate buffer

decreases still further and becomes 15% of the sample when the unsterilised sample was kept buffered for 3 h before being fed into the measuring cell. These results thus show that the BOD of the sample of unsterilised pond water decreases significantly on buffering with phosphate. The BOD lowering is presumably due to the rapid growth of microbes present in the unsterilised pond water in presence of the phosphate ion. The microbes consume dissolved organic matter and hence lower BOD. For this reason in this work on collected water samples, we sterilised every sample either using an autoclave or a domestic pressure cooker before buffering, unless otherwise stated. In the method followed by Karube *et al.*[2], the mixing of the buffer and the analyte was done inside the measuring cell by pumping the two into the measuring cell separately with the help of two peristaltic pumps. Hence no sterilisation of the analyte was required in their method.

The recorder traces from our sensor for various samples are shown in Fig. 2. The switching of samples or buffers to the measuring cell is indicated in the figure by arrows outside the trace. Each thumb-like trace refers to the movement of the recorder pen when a sample or the calibrant is introduced into the measuring cell, and after the recorder reading reaches a steady level, the measuring cell inlet is switched to the 0.01*M* phosphate buffer solution. The satisfactory working of the sensor is evident from two aspects. First, the response for the standard sample (calibrant) remains the same when the standard was repeated, the time gap between the two runs being 220 min. In between, several other samples were tested. Second, the response for the samples marked I, II and III, of Fig. 2 when plotted against the relative concentrations gives a straight line as shown in Fig. 3. Sample I was water from a local pond, the BOD of which was lowered to different levels by dilution with distilled water (samples II and III). The depth of the

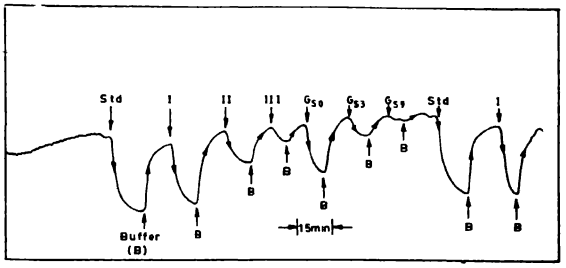

Fig 2 Representative recoder response for BOD measurement using various water samples (Std) Standard nutrient solution, (B) 0 01 *N* phosphate buffer, (I) local pond water, (II) pond water 1/2 strength, (III) pond water 1/4 strength, (G_{S0}) Ganges water sterilised at collection point, (G_{S3}) Ganges water sterilised 3 h after collection and (G_{S9}) Ganges water 9 h after collection

signal remains same when the same sample was analysed again as shown in Fig. 2 for sample I after a time gap of 160 min.

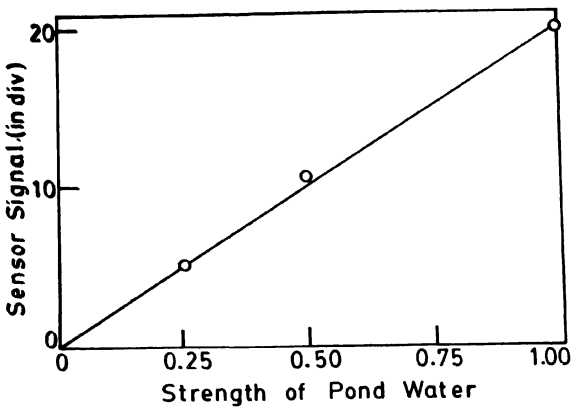

Fig 3 Linearity test response vs BOD of a pond water diluted to various extents

BOD of the Ganges water : The response of samples of water from the Ganges at Calcutta which were variously treated before being fed into the measuring cell of the sensor, are also shown in Fig. 2. Samples GS_0, GS_3, GS_9 were those of the Ganges water sterilised respectively, at 0, 3 and 9 h after collection. It is evident from the recorder trace that the BOD of the Ganges water progressively decreases as the time interval between collection and sterilisation of the sample is increased. In other words BOD of the collected Ganges water decreases on standing. It therefore follows that for BOD measurement the collected samples of Ganges water should be sterilised at collection point and then brought to the laboratory for analysis either by the sensor method as described here or by the 5-day BOD method. The comparison of the performance of the BOD values measured using the sensor with the BOD_5 values

determined for waters collected from various sources are shown in Table 1. The agreement between the two is of similar order as were found by Karube *et al.*[2] and Li and Chu[3].

The behaviour of water collected from ponds was different from that of the Ganges as far as BOD decrease with time of collected samples is concerned. The BOD of the pond water does not decrease noticibly within 6 h of collection if not buffered with phosphate (as was evident from Fig. 1).

In order to find an explanation for the BOD decrease of the Ganges water on standing, we measured the phosphate content of the latter. The phosphate level of the Ganges ranged between 800 and 1600 μg of phosphate ion per litre of water whereas that of the pond water was immeasurably low. This difference in phosphate level may explain the difference in behaviour of water from the Ganges and that of the pond as far as BOD decrease on standing is concerned. It is well known that the microbes multiply greatly in the presence of phosphate and consume the organic matter present in the water and thus lower BOD values are obtained for the Ganges water when greater times are allowed for the microbes to act before analysis is performed. The analysis data of the BOD of the Ganges at Calcutta as recorded in Table 1, show that the BOD is above the desired value which is 3 mg dm^{-3}.

In conclusion, this study shows that when on-line measurement facility using a sensor is not available, it is necessary to sterilise the water to be analysed for BOD at the collection point and then carry out the analysis either by the usual BOD_5 or by the sensor method as described in the present work. This is specially true for the measurement of BOD of the Ganges water.

Experimental

Malt extract (Difco Laboratories), yeast extract, peptone, and agar (Oxoid Laboratories) and dextrose (B.D.H.) were used as received.

Culture of microorganisms : *T. beigelli* MTCC-255 (obtained from the Institute of Microbial Technology, Chandigarh, India) was cultured on 6 ml of a suitable medium (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 1% dextrose and 1.3% agar) in a test tube (1.5 × 16 cm) at 25° for 2 days.

Entrapment of microbes in membrane : The microorganisms (0.3 g wet weight) cultured as above were suspended in sterilised saline (5 ml; 0.9% w/v NaCl solution). This suspension (3 ml) was dropped onto a membrane composed of biologically inert mixtures of cellulose acetate and cellulose nitrate (hereinafter referred to as mixed ester membrane), (Millipore type, HA, 0.45μ pore size, 47 mm dia) placed over a Buchner funnel and gently sucked using a water aspirator. The preparation was then washed with 0.9% sterile saline under gentle suction and stored at 4°. The preparation would be referred to as microbial mat.

Configuration of BOD sensor : The BOD sensor was fabricated in our laboratory following the procedure of Karube[2]. A schematic diagram of the sensor and the measurement set-up is shown in Figs. 4 and 5 respectively. The oxygen electrode of the sensor was purchased from Elico. The microbial mat was layered over the O_2 permeable membrane of the O_2 electrode. The mat was then covered with a protective membrane filter that was used to entrap the microbe (Fig. 4). The whole assembly was then inserted into a flow cell of 2 to 3 cm^3 capacity (Fig. 5). The

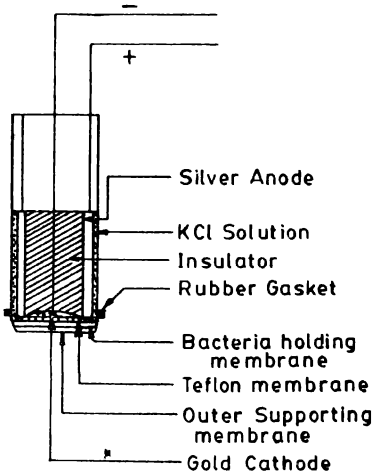

Fig. 4. Schematic design for microbial BOD sensor.

flow cell had two inlet tubes, one of which was used to pass air through the cell at the rate of 575 cm^3 min^{-1} while the other used to pump liquid through the cell at the rate of 1.5 cm^3 min^{-1} using a peristaltic pump.

Measurement procedure : For BOD measurement, phosphate buffer (0.01 *M*; pH 7) was pumped through the measuring cell by connecting one arm of the T-inlet to the buffer reservior through the peristaltic pump and air-flow was turned on through the other arm of the T (Fig. 5). When the recorder trace became steady the peristaltic pump connection was shifted to the reservior of the nutrient solution consisting of 6 mg dm^{-3} each of glucose and glutamic acid and buffered to pH 7 by phosphate by turning the knob of the multiport valve (Fig. 5). The air-flow was maintained. The reading for the recorder pen position changed as a result and became steady after about 15 min. The measuring cell was then switched back to buffer whereupon the recorder pen returned to the base line after about 15 min or so. The recorder trace over the 30 min period had the shape of the thumb. The difference in the recorder reading (from the base line to the top of the thumb shaped trace) is proportional to the BOD of the nutrient which was used as the calibrant also. The analyte

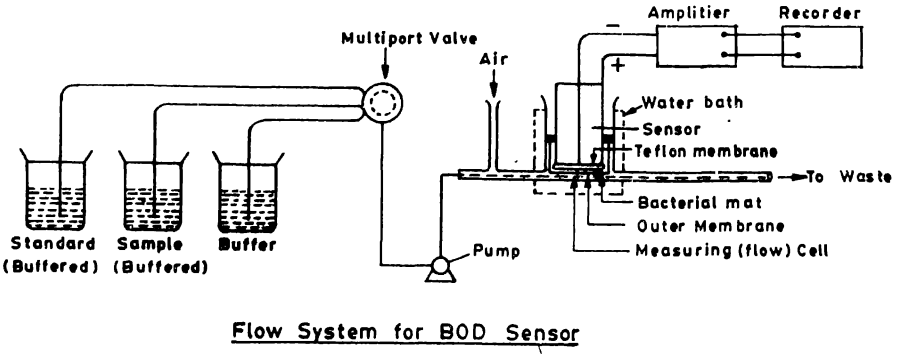

Fig 5 Experimental set up for BOD measurement using the BOD sensor.

which was appropriately diluted, sterilised and mixed with the phosphate concentration at 0.01 *M*, was then introduced into the cell using the peristaltic pump and the measurement was done as above. The response of the analyte was then compared with that of the calibrant the BOD of which was known and thus the BOD of the analyte was determined. It was however necessary to check up the linearity range of the sensor before a measurement. For this purpose the sterilised analyte was diluted to various extents by means of distilled water and the response of the sensor for such diluted sample was measured. The diluted samples which gave responses proportional to the concentration gave the linear working region of the sensor. The response of the calibrant should also be in the linearity range.

For the analysis of samples with high BOD (e.g. the waste from breweries, paper mill, or other industries) using the sensor the wastes need to be appropriately diluted with distilled water so that the BOD of the diluted sample falls within the linearity range of the sensor.

TABLE 1–COMPARISON OF BOD DETERMINED BY 5-DAY CHEMICAL METHOD (BOD_5) WITH THE BOD DETERMINED BY THE SENSOR

Water source	BOD_5	BOD_{sensor}
	mg dm^{-3}	mg dm^{-3}
Ganges at Calcutta	4 9[a]	4.6
Brewery waste	103[a], 120[b]	122
Paper Mill Effluent	990[a], 900[b]	1 100
Pharmaceutical Effluent	12 200[a], 12 800[b]	10 200

[a]Ganges water used as seed. [b]Seed prepared from the Ganges water and the sample

5-Day chemical method of BOD determination : BOD_5 was determined in the usual way[1]. For the measurement of BOD_5 of the Ganges water sample, the latter was sterilised using a domestic pressure cooker for 15 min at collection point and unsterilised water from the Ganges was used as seed. Phosphate estimation was made following standard method[4].

For measurement of BOD of the various waste waters, two types of seeds were used. The Ganges was the receiving water body for all the wastes studied here. In one set of analysis, water from the Ganges 5 to 10 miles downstream the discharge point of the wastes was used as the seed. In the other set, the seed was prepared by feeding the Ganges water (200 cm^3) with waste water (2 cm^3) and fresh Ganges water (50 cm^3) daily for 3 days and aerating the mixture which was incubated at 20°. The incubation was continued for few days ranging from 3 to 7 days depending on the waste. The BOD of the seed was monitored using our sensor from the 4th day and the seed was used when the BOD became negligibly small. The BOD_5 of the two industrial wastes measured by using these two types of seed are presented in Table 1.

Acknowledgement

The authors' grateful thanks are due to Department of Science and Technology, New Delhi, for financial support.

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