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COMMENT

Comment on "Response characteristics of a dead-cell BOD sensor" by Z. Qian and T. C. Tan, *Wat. Res.* **32**(3) 801–807

This is a good paper on an important topic. The authors have made a very thorough study of the performance of their dead-cell biosensors on individual organic solutes, known mixtures of these solutes, and wastewaters from several sources. As dead-cell biosensors have several advantages of storability and durability over live-cell units, and since rapid BOD measurement (Iranpour *et al.*, 1997a, 1997b; Tan *et al.*, 1993) is becoming more and more important, instruments using these sensors are likely to be on the market in a few years. The following questions and comments are intended as ways of clarifying our own understanding and perhaps that of other readers.

1. The authors' Table 1 shows a large range of strengths of response to the organic solutes tested. A comparable table was presented by Harita *et al.* (1985) showing the response of the biosensor electrode that uses *Trichosporon cutaneum* yeast. Although there are many similarities between the results (e.g., ethyl alcohol produces an especially large response from both the dead cell biosensor and the yeast biosensor, while the responses to glucose and glutamic acid are smaller, and not significantly different for either sensor), there are also some potentially significant differences. For example, the response of the dead-cell sensor to lactic acid is nearly twice that of the yeast sensor, but the yeast sensor responds approximately twice as strongly as the dead-cell sensor to glycerol and sucrose. Also, although the response of the dead-cell sensor is usually consistent with the response of the authors' live-cell sensor using *Bacillus subtilis* and *Bacillus licheniformis 7B*, the live-cell sensor responds only half as strongly to ethyl alcohol and glycerol as the dead-cell sensor, but more than twice as strongly to formic acid.

These results and others are summarized in the following table, which is compiled from the authors' Table 1 and Table 1 from Harita *et al.* The BOD5 column is from Bond and Straub (1980), as quoted by the authors, but these values agree so closely with the BOD5 values in the other table that we presume that Harita *et al.* also derived their BOD5 values from Bond and Straub, so that any discrepancies are typographical errors. NL indicates that the dead-cell sensor produced a nonlinear response for these solutes.

This combination of results provides additional perspective on the problem of standardization. We agree with the authors that their data are strong evidence that a glucose–glutamic acid mixture is not sufficient as a standard for predicting the response of a sensor system to wastewaters with a wide range of substrate compositions, but perhaps it should be emphasized even more strongly that a combination of these widely differing responses to substrates, heterogeneity of microbial populations and variability of wastewater compositions provides a plausible explanation for the well-known 10 or 15% uncertainty in the results of the standard BOD5 test.

2. Since the standard BOD5 test has such variable results, the discrepancies of a few percent between the dead cell biosensor and the APHA method shown in Table 2 (for all samples except the intentionally aberrant M1 and M2) would not be significant individually. However, the systematic underestimation shows that a genuine discrepancy exists. It appears small enough to be compensated by simply multiplying the dead-cell result by a correction factor in cases where the discrepancy cannot be ignored. Do the authors agree that this discrepancy is probably a consequence of the standardization difficulties and response variations discussed in item 1?

3. Although they do not mention it, using dead cells would provide a possibly significant advantage in safety. *Trichosporon cutaneum*, which is now used in the BOD-2000 biosensor (Iranpour *et al.*, 1997a), is infectious, and it is claimed that the Lange ARAS SensorBOD instrument (Riedel, 1985) is safer because the *Issatchenkia orientalis* and *Rhodococcus erythropolis* organisms used in its biosensor are not infectious. Also, as dead bacteria cannot cause infection, perhaps this point should be emphasized more in discussing the potential advantages of this type of biosensor.

4. Do the authors expect to have support to investigate similar sensors using more than one type of dead cell, such as the *Bacillus licheniformis 7B* used in their previous work? We understand that studying one type of dead cell is the necessary starting point for this kind of development, but the authors' Fig. 4 is strong evidence that the responses of this kind of preparation are almost perfectly additive, so using a

Table 1. Combined solute response results

	(gr BOD/gr Solute)			
	Dead cell	Live cell	Yeast	BOD5
Solute				
Ethyl alcohol	2.46	0.92	2.90	0.93–1.67
Lactic acid	1.13	1.52	0.72	0.63–0.88
Glucose	0.69	0.67	0.72	0.50–0.74
Glutamic acid	0.65	0.63	0.70	0.63
Fructose	0.68	0.73	0.54	0.71
Formic acid	0.42	1.07	–	0.02–0.27
Glycerol	0.26	0.14	0.51	0.64–0.83
Sucrose	0.16	0.19	0.36	0.49–0.76
Citric acid	–	–	0.17	0.4
Acetic acid	NL	0.12	1.77	0.34–0.88
Glycine	NL	0.51	0.45	0.52–0.55

wider range of bacteria, or even nonbacterial cells, might relieve some of the standardization difficulties observed here.

Let us close by repeating that this is a good work, and we hope to see it extended further.

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AUTHORS' REPLY

The response of a microbial BOD sensor depends significantly on the type of microbial system and also on the molecular size of the solute relative to the pore size of the membranes including the medium and the method for the immobilization of the microbes used for the fabrication of the biosensor. Unlike the conventional APHA measurement, which commonly used activated sludge for seeding the water sample, the microbial system used in preparation of the BOD biosensors is usually single microorganism and the species varies. The medium and the methods of immobilization are generally different. Membranes of different types and pore size are used. The basis of measurement by the APHA method and by a BOD biosensor is also different. The APHA method is based on the actual consumption of the dissolved oxygen during the 5-day incubation, while the biosensor measurement is essentially based on the correlation between the concentration gradient of the dissolved oxygen across the biofilm-membrane composite against the 5-day BOD equivalence of the glucose–glutamic acid BOD check solutions. As such, one would expect to see greater difference in the BOD measurements by the different microbial biosensors than by the conventional APHA method. However, we do expect the response of the thermally-killed cells BOD sensor for the same solutes or wastewater sample would be less than or at best equal to that of the living cells of the same microbes. This was true in most of the solutes and particularly the wastewater samples analyzed. The larger response shown by the thermally-killed cell sensor in the case of ethyl alcohol and glycerol was rather unexpected and the results have been confirmed by repeated experiments. A plausible reason for this anomaly could be the adverse effect of these solutes on the living cells, which would affect their assimilability for these solutes, while the effect of the solutes on the enzymic oxidation in the dead cells is relatively negligible.

The conventional BOD measurement based on the APHA method is carried out in a free suspension of the microbial system, usually activated sludge. The living microbial cells are in direct contact with the solutes in the test solution. The difference between any two laboratory results would only result from a difference in the characteristics of the activated sludge, or some similar microbial system used for seeding the water sample, and on the expertise of the analyst. This difference would probably be buffered by the complexity of the activated sludge in regards to the large number of different microbial species and population. Standardization of these measurements with respect to simple glucose–glutamic acid mixtures within the range of deviation specified by APHA has been shown and accepted to be reasonable in view of the nature of the system. The 5 day incubation permits even the solutes with very slow assimilation rate by the microbes to contribute significantly to the total oxygen consumption during the incubation period. However, in the case of BOD biosensors, the microbial system is immobilized usually on a liquid permeable membrane. The method of immobilization and the type and properties of the liquid permeable membranes used vary. Since the BOD measurement by a biosensor basically depends on the oxygen concentration gradient across the biofilm-membrane composite, the response would necessarily depend not only on the bio-oxidation rate of the solutes but also on the mass transfer characteristics of these solutes through the membranes and biofilm. The significance of mass transfer and diffusion of the solutes and oxygen through the biofilm-membrane composite and oxygen into the dissolved oxygen probe on the response of a biosensor is described in many mathematical models. We have also developed and experimentally verified mathematical models in connection with the transient and the pseudo steady-state sensing of a single solute using a bio-oxidation related sensor (Chen and Tan, 1995, 1996) and with pseudo steady-state multicomponent biosensing with application to BOD measurement (Qian and Tan, 1998). The biosensor measurement is usually in the order of minutes or hours and solutes of low diffusivity or enzymic oxidation rate could not effectively contribute to the overall response during the short time taken for a measurement. This has been verified experimentally for starch as reported in some detail in our paper. This explains the usually lower BOD values and greater difference among biosensor measurements compared with the conventional BOD measurements by the APHA method. Also, for the biosensors, calibration and standardization are effectively one procedure using BOD check solutions of glucose–glutamic acids mixtures. Standardization with respect to simple glucose–glutamic acid mixtures is found to be inadequate to resolve the different mass transfer characteristics of the different biosensors with respect to the solutes of a wide

range of molecular sizes and diffusional characteristics. The inadequacy is further accentuated by the more diversified microbial systems, usually single species, used in biosensors. In this respect, the problem of standardization, in particular with respect to the simple glucose–glutamic acid mixtures, is different in immobilized microbial systems in biosensors compared with the free microbial suspension in APHA conventional measurement. This is what we have illustrated in our paper to substantiate our basis of advocating the use of a more complex BOD check solution, which should preferably contain solutes, with different molecular sizes and structures, commonly found in wastewaters. A complex matrix would represent more closely the test samples and reflect the effect of mass transfer and bio-oxidation rates on the sensor response better than a simple solution of small molecules of glucose and glutamic acid.

We were looking for a method of killing the microbial cells with negligible adverse effects on the enzyme–cofactor system in the cells for various reasons, particularly the ease of preparation and commercial fabrication of biosensors for both dedicated single solute and multicomponent sensing (BOD), storage and safety. Safety is imperative for *in vivo* or *in situ* monitoring and control, a singularly important application for which biosensors are being developed. Safety is also important for *in vitro* (laboratory) analysis using microbial systems. Iranpour *et al.* have rightly pointed out the danger of using infectious microbes. However even with non-infectious microbial systems, they are still unsafe in some ways for the environment and human handling. In some countries mandatory regulations concerning the safe handling of microorganisms, in particular their disposal, have been established. Dead cells prepared by the short-time exposure to high temperature could satisfy these objectives. It is, however, best to note that the danger of enzymic activity is still an important consideration for *in vivo* sensing applications.

The thermally-killed cells have also been applied successfully to dedicated single solute sensing apart from BOD sensing. A paper is currently being prepared describing the sensing characteristics of a thermally-killed microorganism for dopamine. We have also successfully prepared the thermally-killed multispecies microbial system such as that found in activated sludge and commercially available BOD seed in the form of a capsule. Good BOD sensing characteristics were observed with these thermally-killed cells of such a mixed cell population. We have reported these findings in a paper currently under review. We are presently working with synthetic mixtures of different microorganisms thermally killed by short-time exposure to high temperature. Iranpour *et al.* have correctly concluded from our paper on the logical and rational extension of this work from single species to multi-species systems.

We appreciate the very kind and encouraging comments of Iranpour *et al.* Their understanding of our objectives and their anticipation of our continuing work in this area are most encouraging and rewarding. We thank them for their interest.

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