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Rapid Estimation of Biochemical Oxygen Demand

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Abstract

The feasibility of using columnar reactors containing immobilized microorganisms for the rapid estimation of BOD was demonstrated in this study. Dilutions of three types of industrial effluents were tested by the BOD_5 test and by this experimental system. A high degree of correlation ($r = 0.98$) was observed between results of the two tests. The mean standard error of estimation of the experimental system was 11%.

Index Entries: Biochemical oxygen demand, rapid estimation of; BOD, rapid estimation of; oxygen demand, biological; immobilized microorganisms, BOD estimation with; sewage, rapid BOD estimation for.

Introduction

Determination of biochemical oxygen demand (BOD) provides a measure of organic pollution in water. This determination currently requires a 5-day, 20°C incubation period. Furthermore, this determination is subject to wide intralaboratory variability (*1*), and it is affected by toxic substances (*2, 3*), making it inapplicable for analyzing certain wastes. In spite of these limitations, the 5-day BOD test is widely used because it is employed by many regulatory agencies to measure the

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pollution potential of industrial and municipal effluents (*4*). Numerous alternative approaches for the rapid determination of the 5-day BOD results have been published (*5–10*). However, many of these approaches require special equipment and do not shorten the incubation time sufficiently to be cost-effective or of practical operational value (*6*). As a result, a need exists for a simple and inexpensive biological method for rapidly evaluating plant operations and for measuring levels of biodegradable organic pollution.

Recently, increasing attention has focused on the use of immobilized microbial cells for a variety of purposes, including BOD determination wth a microbial electrode (*10–12*). The purpose of this study was to explore the feasibility of using immobilized microorganisms in a columnar reactor for rapid BOD determination. The present paper describes the properties of this system, shows the correlation between the standard and rapid BOD determinations, and demonstrates the use of the system for rapidly determining the BOD of several industrial effluents.

Materials and Methods

Bioreactor

The bioreactor consisted of a 152 mm long × 8 mm id glass tube packed with 6–7 g of an inorganic, porous support material (cordierite, 47% porous, 1–20 μm pore diameter range, 3 μm median pore diameter) in the form of cylindrical rods, 7 mm long × 2 mm od. The tube was sealed at both ends with caps designed to allow attachment to Tygon tubing.

Microbial Seed

The source of microorganism was influent municipal sewage (Corning Sewage Treatment Facility, Corning, NY). Fifty to sixty grams of cordierite rods were placed in 300 mL of undiluted sewage and stored at room temperature for at least 2 h to allow adsorption of microorganisms onto the surface of the rods. Approximately 6–7 g of these rods were then packed into a column. A glucose–glutamic acid mixture (described below) was then passed through the reactor before use.

Activity of a reactor was maintained by flushing all test solution from the reactor at the end of each day, refilling it with freshly prepared glucose–glutamic acid solution, and storing the reactor in this static condition at room temperature for no more than 3 days before changing the glucose–glumatic acid solution.

Enumeration and Visualization of Immobilized Microorganisms

Immobilized microorganisms were enumerated by measuring, in a luminescence biometer (760 Biometer, Dupont Instruments, Wilmington, DE), the cellular ATP extracted from 1.0 g of cordierite treated with dimethylsulfoxide. An ATP extraction procedure without filtration (*13, 14*) was used.

Cordierite rods from an active reactor were fixed for 1 h each in 4% glutaraldehyde in 0.1*M* cacodylate buffer, pH 7.3, and in 1% osmium tetroxide. Samples were washed with 0.2*M* cacodylate buffer, pH 7.3, between and after fixation peri-

ods. After fixation, samples were dehydrated through a graded acetone series and dried by critical point drying using CO_2. Samples were then coated with gold to a thickness of approximately 300 Å, and were examined by scanning electron microscopy (AMR 1400D, Advanced Metals Research Corp., Bedford, MA).

Solutions

Dilution water containing various inorganic compounds and a synthetic waste solution containing glucose and glutamic acid (GGA) were prepared as described in Standard Methods (*1*). Waste samples from a pumpkin processing factory, a pulp and paper factory, and a municipal sewage treatment facility were diluted and analyzed by the standard BOD_5 method and by the rapid BOD system.

Rapid BOD System

Dissolved oxygen electrodes (Berkeley Controls, Irvine, CA) were housed in specially designed lucite flow cells. A small magnetic stirring bar (3 mm × 10 mm), controlled by a rotating magnet outside the flow cell, was located within the flow cell and stirred the fluid within it. Both the influent and the effluent of the bioreactor were monitored for dissolved oxygen in a continuous flow system (Fig. 1). Each electrode was connected to a dissolved oxygen meter (Critikon Division, McNeil, Inc., Irvine, CA) that made discrete measurements of dissolved oxygen. A dual-channel chart recorder (Linear Instruments Corp., Irvine, CA) was used to record the responses observed on the meter graphically. Samples were drawn through the system at a flow rate of 2 mL/min by a piston pump (Fluid Metering, Inc., Oyster Bay, NY).

BOD_5 Test

The BOD_5 procedure, as outlined in Standard Methods (*1*), was used in these studies.

Rapid BOD (rBOD) Estimation

The difference (ΔO_2) between the influent and effluent dissolved oxygen measurements of various dilutions of the GGA solution were plotted against the BOD_5 test results of the same GGA dilutions to establish a standard curve for rBOD estimations. The ΔO_2 measurement of each diluted waste sample was then used to estimate, from the standard curve, the BOD_5 of that diluted sample. This BOD result was then multiplied by the appropriate dilution factor to yield the actual BOD concentration of the sample.

Results

Electron micrographs revealed many bacilli attached to the surface of the microbial support material (Figs. 2a,b). Microorganisms were also observed within the pores of the support. Microbial concentration, as determined by the ATP assay, was approximately 10^7 cells/g of carrier.

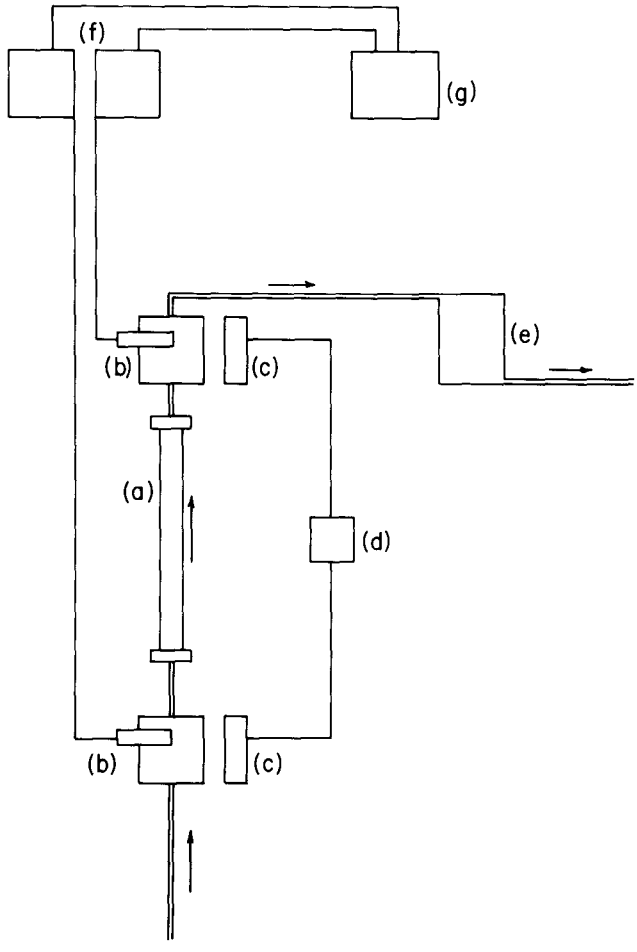

Fig. 1. A schematic outline of the rapid BOD estimation system. Major components include: (a) bioreactor, (b) dissolved oxygen electrode, (c) stirring magnet assembly, (d) transformer, (e) pump, (f) oxygen meter, and (g) chart recorder.

Response characteristics of a bioreactor are shown in Fig. 3. The time to reach a stabilization of oxygen consumption was dependent on the BOD of the sample being analyzed; stabilization response time increased as substrate concentration increased. Generally, oxygen utilization endpoints were obtained after 30 min at room temperature (24°C). The relative variation of oxygen utilization endpoints determined by repeatedly analyzing a GGA solution (5 mg/L of glucose and of glutamic acid) on two bioreactor systems was approximately 5%.

Oxygen utilization in a solution free of organic materials returned to a steady level within 30 min.

These baseline levels, caused by microbial endogenous respiration, were determined by measurement of oxygen utilization in dilution water only. These respiration levels (seed correction) were found to be unstable during periods of testing samples of varying BOD concentration (Fig. 4). Therefore, oxygen utilization in dilution water was measured after each sample (Table 1). The procedure compen-

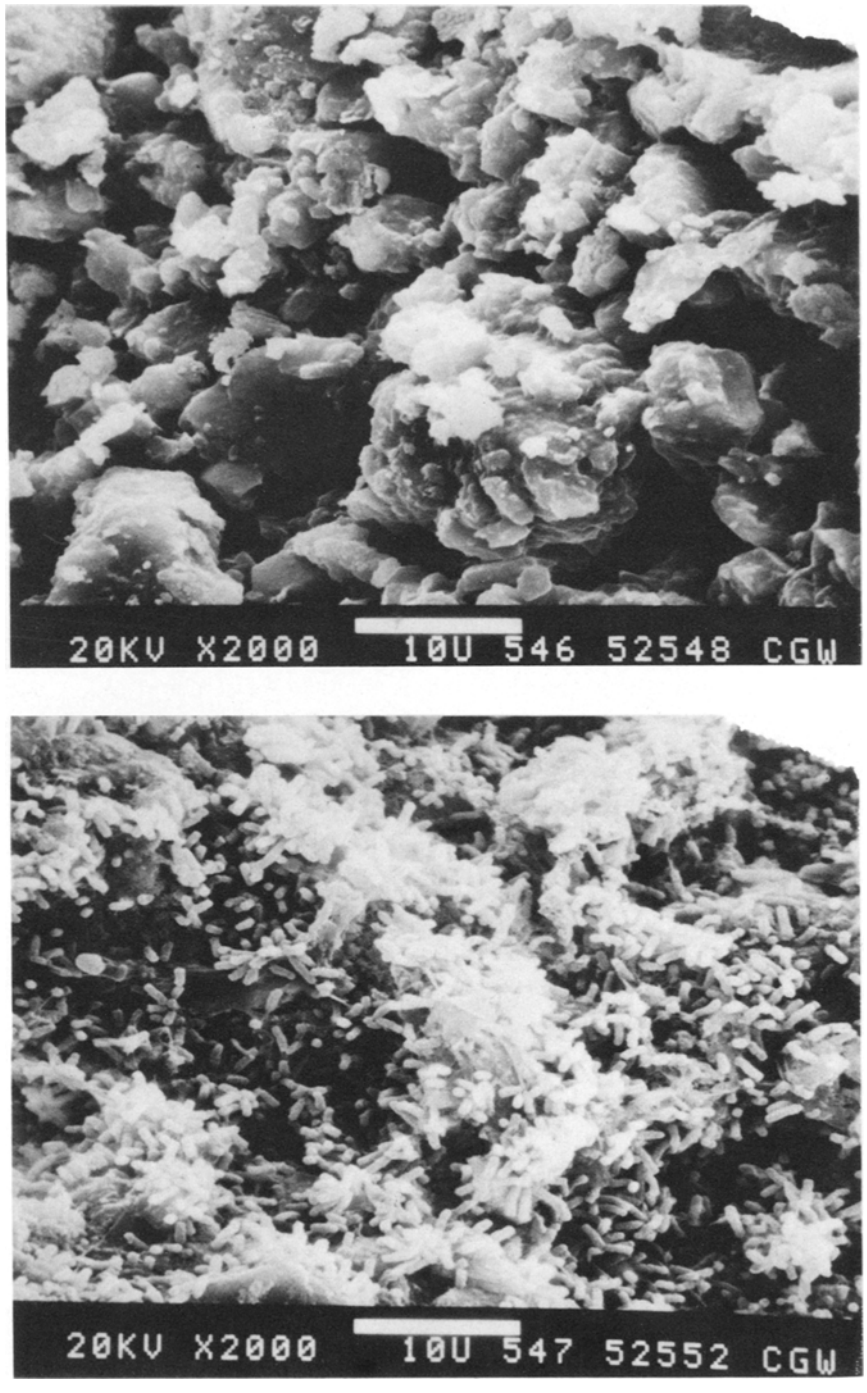

Fig. 2. (a) Scanning electron micrograph of cordierite rod surface before use in bioreactor. No microorganisms were observed (×2000). (b) Scanning electron micrograph of cordierite rod surface after use in bioreactor. Numerous microorganisms on the surface were observed (×2000).

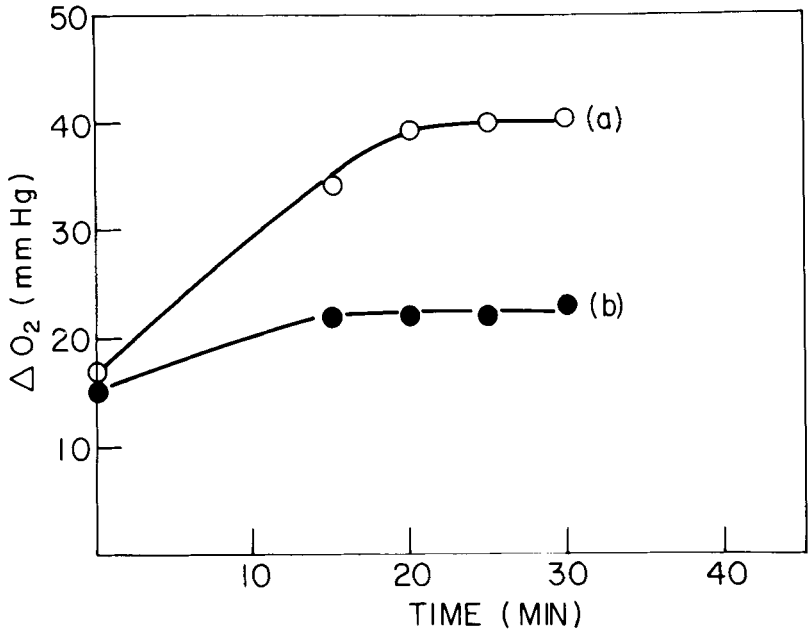

Fig. 3. Relationship between bioreactor oxygen consumption (ΔO_2) and time using solutions containing (a) 5 mg/L glucose and glutamic acid and (b) 1.25 mg/L glucose and glutamic acid. Response time was 25–30 min for (a) and 15–20 min for (b).

sated each result for seed correction levels and contributed toward the reproducibility and accuracy of data. The tota l determination time was approximately 60 min (30 min with the diluted sample and 30 min with the dilution water).

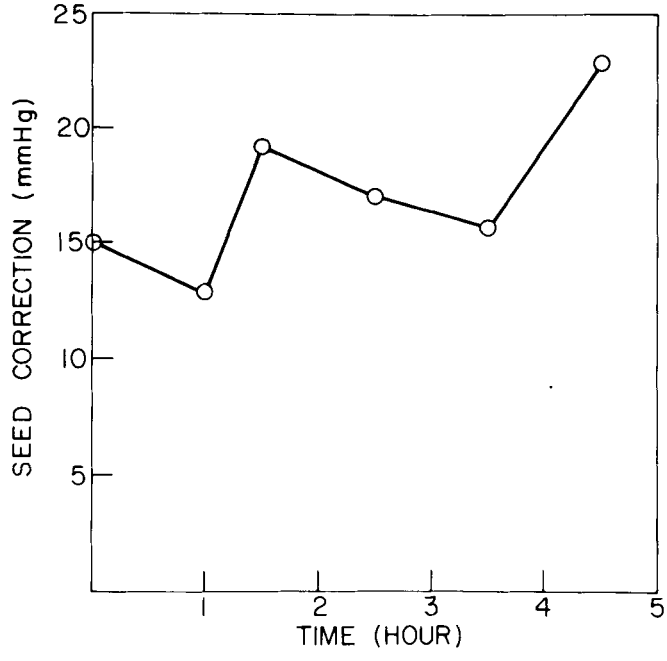

Fig. 4. Instability of microbial endogenous respiration levels (seed correction) in a bioreactor over a 5-h period.

TABLE 1
Determination of Seed Correction

Sample sequence	Type of sample	ΔO_2 concentration, mm Hg[c]		Net O_2
		Test Sample	SC[d]	Concentration, mmHg[e]
1.	GGA (1/25 dil)[a]	43	—	
2.	Dil. H_2O[b]	—	13	30
3.	GGA (1/50 dil)	28	—	
4.	Dil. H_2O	—	10	18
5.	GGA (1/25 dil)	51	—	
6.	Dil. H_2O	—	21	30
7.	GGA (1/50 dil)	37	—	
8.	Dil. H_2O	—	20	17

[a]GGA = glucose–glutamic acid solution.
[b]Dil. H_2O = dilution water.
[c]ΔO_2 concentration = (initial O_2 concentration) − (final O_2 concentration) at 2/mL/min.
[d]SC = seed correction.
[e]Net O_2 concentration of test sample − O_2 concentration SC.

The relationship between oxygen consumption in the reactor and BOD was then examined by testing various dilutions of the GGA solution. A linear relationship was found between oxygen consumption and BODs between 1 and 7 mg/L (Fig. 5). The relationship was non-linear above BOD's of 7 mg/L.

Subsequently, effluents from three types of industries were diluted and were an-

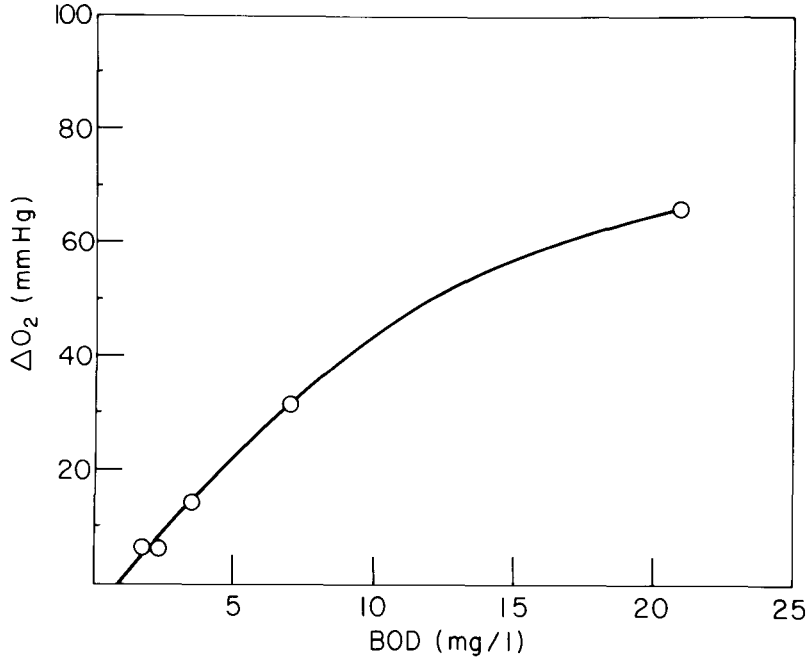

Fig. 5. Relationship between oxygen utilization (ΔO_2) in the bioreactor and the BOD of the standard glucose–glutamic acid solution.

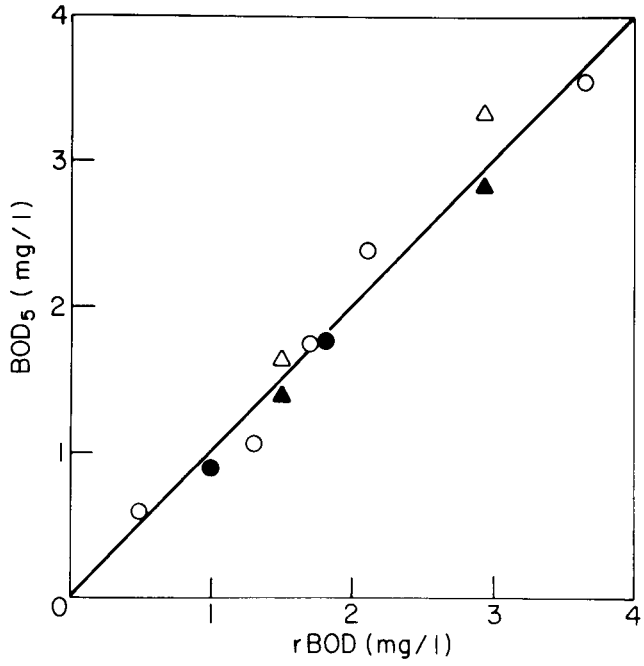

Fig. 6. Relationship between BOD_5 and rBOD test results of a glucose–glutamic acid solution (○), of pulp and paper factory effluent (●), of municipal sewage treatment facility effluent (△), and of pumpkin processing factory effluent (▲).

alyzed by the standard BOD_5 procedure and by the rBOD procedure. A high degree of correlation ($r = 0.98$) was obtained between the results of the two procedures (Fig. 6). Comparison of actual BOD results is shown in Table 2. The mean standard error of rBOD estimation was approximately 11%, and the rapid estimations were not biased in either direction.

Discussion

One of the most common analytical methods for measuring the oxygen requirements of industrial and municipal effluents is the biochemical oxygen demand test. This basic test, however, requires a 5-day incubation period, resulting in an undesirable time delay. In this paper, we have described a system that eliminates this delay by providing BOD results in approximately 60 min.

The bioreactor used in this system was seeded simply with municipal sewage, and a very high density of immobilized microorganisms was observed in the reactor. This high concentration of microorganisms contributed to the rapid oxidation of organic material in sample solutions. In contrast, the standard BOD_5 procedure starts initially with a low concentration of microorganisms and measures the level of biochemical oxidation after 5 days incubation at 20°C.

Results were generated by this system in approximately 60 min, which represents a major time advantage over the standard BOD_5 test. These rapid results would be more relevant to on-going plant operations and may also lead to the

TABLE 2
Comparison Of rBod Estimations
With Bod_5 Results[a]

Solution	Dilution	BOD, mg/L rBOD	BOD, mg/L BOD_5	Difference, [b]%
GGA[c]	0.004	123	147	−16.33
	0.0075	175	143	+22.38
	0.0125	137	141	−2.84
	0.01667	134	144	−6.94
	0.025	146	143	+2.10
Sewage	0.002	755	825	−8.48
	0.004	735	835	−11.98
Pumpkin	0.00167	906	846	+7.09
	0.00333	882	852	+3.52
Pulp/paper	0.0075	133	119	+11.76
	0.0150	121	118	+2.54

[a]Standard error of estimation (%) = 10.66.
[b]Difference, $\% = \frac{[(BOD_5\text{-rBOD})}{BOD_5]} \times 100.$
[c]CGA = Glucose–glutamic acid.

avoidance of fines or penalties for excessive BOD loads in plant effluents for at least 5 days.

At first, it may appear that the linear range between oxygen consumption in the reactor and BOD may require excessive sample dilutions. However, all samples are diluted to within this approximate range for the BOD_5 test currently. Therefore, it is not an unreasonable requirement to fulfill before analyzing the sample in this rapid system. Furthermore, since the response time with the sample is only 30 min, it would not be a serious problem to redilute and retest a sample if the original sample had not been diluted to the correct range.

Results of these studies also indicated that a single reactor seeded with municipal sewage could be used to estimate BODs of at least three different types of effluents. The range of effluent types that can be analyzed with a single reactor is not yet known. It may be difficult to analyze effluents containing organic compounds that cannot be degraded by sewage microorganisms. However, reactors seeded with organisms adapted from these effluents could be used to estimate the BOD of that specific effluent.

In summary, these studies have demonstrated the feasibility and potential of a rapid BOD system involving a columnar reactor containing immobilized microorganisms. This system was simple to prepare and easy to operate; it displayed a linear relationship between oxygen consumption in the reactor and BOD_5s of 1–7 mg/L; it provided results in 60 min, and it used one reactor for analyzing three effluent types.

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