Continuous Monitoring of Oxygen Concentrations in Air-Purged Shake-Flask Fermentations

PHILLIP H. HODSON
Monsanto Company, Inorganic Chemicals Division, Research and Development Department, St. Louis, Missouri 63166

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Continuous monitoring of the oxygen concentrations in the gas and liquid phases in air-purged shake-flask fermentations was accomplished by means of steam-sterilizable oxygen probes.

Many methods have been employed to measure the dissolved oxygen concentration in fermentations (1, 6–9). A review of mass transfer in gas-liquid systems is reported by Sideman et al. (10). Several workers have demonstrated that cotton plugs adversely affect the diffusion of oxygen into a shake-flask culture (3–5, 11). However, the measurement of the oxygen level in the gas phase in shake-flask cultures has received little attention.

This paper presents results obtained by using a specially equipped air-purged culture flask in which oxygen probes allow continuous monitoring of the oxygen concentrations in the liquid and gas phases during incubation on a rotary shaker.

A drawing (which is not to scale) of the special culture flask is in Fig. 1. The four baffles are 1 cm deep and 10.5 cm long. To prevent the liquid from splashing onto the cotton plug during incubation on the shaker [model G-25, (New Brunswick Scientific Co., New Brunswick, N.J.), 37 C, 310 rev/min], a glass pipe (3.2 by 19 cm) was fitted with a rubber stopper and inserted into the flask. Approximately 3 cm of the glass pipe was allowed to protrude through the stopper above the flask and closed with a cotton plug. The stopper and glass pipe were taped to the flask to insure a tight enclosure during the incubation. The oxygen probes were constructed essentially as described by Borkowski and Johnson (2), with the exception that special care was taken to insure a snug fit of the lead helix anode. The Teflon membrane was held in place by a silicone rubber sleeve. The probes were used many times and a total of 350 hr of shaking time has been logged before they malfunctioned.

After the probes had been attached, the flask was charged with 500 ml of Trypticase soy broth (Fisher Scientific Co., Pittsburgh, Pa.) containing 2% glucose and sterilized at 121 C for 15 min. The flask was cooled, the wire leads of the oxygen probes were attached to recorders, and the system was equilibrated at 37 C. The medium was seeded with 5% inoculum of an 18-hr-old Trypticase soy broth culture of Bacillus subtilis NRRL B3411. The flask was purged with 500 ml of sterile air per min. The air purge system was composed of a Universal air regulator part no. 100-A (Perfecting Service Co., Charlotte, N.C.) and a Brooks Mite no. 2001-V 0.2 to 4.5 SCFH rotameter (Brooks Instrument Division, Hatfield, Pa.). The air purge was purposely interrupted several times during the incubation period, and the oxygen concentrations were plotted hourly (Fig. 2). The air purge was interrupted for the first time at the 20th hr, at which time the dissolved oxygen level in the culture was below 10% of air saturation. As a result of the interruption, the oxygen level rapidly dropped to below 10% of PO2 in the air phase. The second
The interruption of the air purge was made at the 26th hour, and the prior results were essentially repeated. The incubation of the culture was allowed to proceed until the oxygen demand had lessened and the dissolved oxygen had stabilized at about 60% of air saturation, whereupon the air purge was again interrupted. This time the oxygen depletion in the gas phase was about 50% slower than

![Graph of Oxygen Content](image)

**Fig. 2.** Oxygen content in a 2-liter shake-flask culture of Bacillus subtilis NRRL B3411 growing in 500 ml of Trypticase soy broth containing 2% glucose at 37 C with air-purge interruptions at the times indicated.

![Graph of Dissolved Oxygen Depletion](image)

**Fig. 3.** Actual tracing of dissolved oxygen depletion after air-purge interruption to a shake-flask culture of Bacillus subtilis NRRL B3411. For comparison the gas-phase levels were plotted.
FIG. 4. Dissolved oxygen concentrations after addition of HgCl to a growing culture of Bacillus subtilis NRRL B3411 in 500 ml of Trypticase soy broth containing 2% glucose.

The two earlier air purge interruptions. After the oxygen levels reached less than 10% in each phase, the cotton plug was removed for 1 hr. During this time, the dissolved oxygen increased from 1.6 to 2.0%, and the oxygen content in the gas phase increased from 10 to 21% of PO2. When the air purge was again introduced, there was almost an immediate response in both phases.

When the glucose concentration in the Trypticase soy broth was reduced to 0.5% and inoculated, the amount of dissolved oxygen was reduced to less than 10% of air saturation in 5 hr and increased to 78% by the 21st hr. At this time, the air purge was terminated (Fig. 3). (Because the chart speed of the gas phase oxygen probe recorder was too fast to show the actual recording, the gas-phase oxygen was calculated and drawn on the actual recording of the dissolved oxygen.) When the air purge was terminated, the rate of oxygen disappearance in both the gas and the liquid phases was essentially a straight-line function over the entire curve.

When the dissolved oxygen was low, 1 g of HgCl was added to a culture which was being air-purged (Fig. 4). As the respiration of the microorganisms was interrupted by the poison, there was an increase in the dissolved oxygen. At 25 min after the addition of HgCl, the dissolved oxygen level began to rise and attained 97% of air saturation in 54 min, at which time the air purge was stopped. This time, there was essentially no decrease in the oxygen levels in either the gas or liquid phases as observed previously.

These experiments demonstrated that oxygen probes can be constructed which allow monitoring of the oxygen level in both the liquid and gas phases during culture incubation on the rotary shaker. Addition of an air purge to shake-flask fermentations allows the rate-limiting step to be the diffusion of the oxygen into the liquid rather than diffusion through the flask closure. By means of this modification, conditions can be created in shake flasks which more closely resemble those in air-sparged fermentors. Flasks of this construction could be used advantageously in studies on aeration, product formation, inhibitor effects, and other parameters.

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