Polarographic Measurement of Dissolved Oxygen in Yeast Fermentations

J. Strohm, R. F. Dale, and H. J. Peppler

Red Star Yeast and Products Company, Milwaukee, Wisconsin

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Millions of cubic feet of air are sparged daily into a commercial baker's yeast fermentation to fulfill the oxygen demands of the organism. The costs for air compression are sizeable and, in some cases, the capacity of the air supply system may impose restrictions on the number or types of fermentations which can be scheduled.

The rate of air supply required for each type of yeast fermentation has generally been established by a process of trial and error and is taken as the least amount of air needed for maximal yield. This approach is rather drawn-out and insensitive because the yields are affected by other variables which cannot be completely eliminated or appraised. Air requirements vary in different periods of a fermentation depending on the feed rate, yeast population, and so forth, and it is impractical to carry this empirical approach for air regulation far enough to completely prevent overaeration and attain maximal economy. Furthermore, this approach does little to minimize the risks of low yield or poor yeast quality resulting from improper aeration in fermentations following major operational or equipment changes.

The objective of this work, therefore, was to investigate and develop techniques which could be employed to obtain more direct and accurate indications of the aeration conditions prevalent in active fermentations.

Experimental Methods

Sulfite-oxidation tests. The aeration efficiency of several sizes of geometrically dissimilar agitated and nonagitated yeast fermentors was determined using the well known sulfite-oxidation method of Cooper *et al.* (1944). The liquid level and temperature for these tests were essentially the same as those during an actual yeast fermentation. The oxygen absorption rates (mM O_2 per L per hr) in the fermentors were measured at several air flow rates and at more than one impeller speed.

A series of experimental yeast fermentations were then made in this equipment at several aeration conditions of known sulfite-oxidation value. All fermentations were made with continuous molasses feed and incremental ammonia additions for pH regulation and as a nitrogen source to meet growth demands. Figure 1 presents a comparison of the fermentation yields obtained with the sulfite-oxidation values measured at the corresponding operating conditions. Two fermentations were made in 5-gal agitated vessels under conditions referred to as a "flooded" impeller; the air rate used in these fermentations exceeded that which the agitator blades could effectively disperse.

Except for the two fermentations made with flooded impeller, the data of figure 1 indicate that fairly good correlation exists between the yield and equivalent sulfite-oxidation value for a specific yeast fermentation made in various type and size propagators. This degree of correlation is somewhat surprising in view of the more than 10-fold range of values which would be obtained if the air rate for any specific yield were expressed in other commonly used terms, such as lineal air velocity or volumes of air per volume of solution. The sulfite-oxidation method, therefore, can be useful in predicting the required air flow rate for new or modified fermentors, in addition to being a useful tool for scale-up and control purposes. The desired air flow rates obtained from sulfite-yield correlations, however, only represent minimal air requirements in highly fed portions of the fermentation which approach the condition where air is limiting, at other times the fermentation may be overaerated. The shape and position of the yield-sulfite curve shown above is, of course, specific for one particular fermentation and may vary with changes in the patterns used for feed rate, yeast population, and so forth.

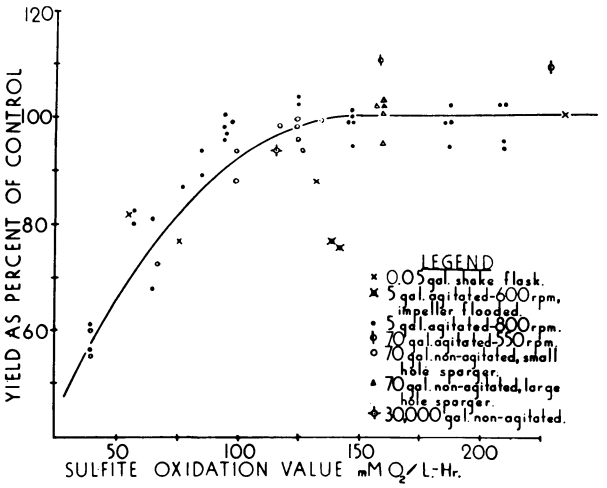

Figure 1. Comparison of fermentation yield with sulfite-oxidation values at comparable aeration conditions.

Air regulation with polarographic measurements. The most direct method of air regulation seemed to be to measure the dissolved oxygen concentration actually present in the medium during the active fermentation. After a number of determinations, it should be possible to establish the critical oxygen concentration required by a particular culture for maximal yield and final quality. Once established, optimal performance could be accomplished by adjusting the air supply to maintain this critical oxygen concentration. Logically, modifications in the fermentation equipment or feed schedule will have no effect on this method of control. To test this reasoning, a polarographic method for the measurement of dissolved oxygen was chosen because of its high sensitivity, speed, and reported use in other biological systems (Petering *et al.*, 1939; Bartholomew *et al.*, 1950; Hixon and Galen, 1950; Wise, 1951).

Polarographic apparatus. Several conditions were found to be present in a commercial baker's yeast fermentation which complicated the use of conventional polarographic techniques, namely:

1. The low pH of the medium (often below pH 5) and the presence of other electroreducible materials caused a large residual current to flow even when no soluble oxygen was present.

2. The composition of the medium changed throughout the fermentation, so the magnitude of the residual current was not constant. Although the residual current bore a general inverse relationship to pH, it could not be predicted with the required accuracy from pH records or from one similar fermentation to another. It was necessary, therefore, to measure the no-oxygen reading (residual current) every time a dissolved oxygen concentration measurement was taken.

In view of the above conditions, the apparatus shown in figure 2 was devised to measure the dissolved oxygen concentration present in metabolically active yeast propagations.

The glass housing for the dropping mercury electrode was mounted as close as possible to the outside wall of the fermentor under test. An unshielded 2 in. length of 6 to 12 sec capillary tubing was used for the dropping mercury electrode. A saturated calomel (SCE) reference electrode with a surface area of about 50 cm^2 was connected to the system electrically through a salt bridge containing a 4 per cent agar-saturated KCl gel. A Sargent-Heyrovsky[1] model 12 polarograph was used to apply a -0.5 v *vs.* SCE to the cathode for current measurement. A platinum cathode was used instead of the dropping mercury electrode during several fermentations. The platinum surface occasionally blackened and low, erroneous currents were observed; cleaning the platinum surface usually restored the current to the normal value.

Dissolved oxygen measurements were taken in the following manner:

1. With valves *A* and *B* open and valve *C* closed, a turbulent mixture of gas and liquor was pumped from the fermentor, past the electrodes for the total current reading, and returned to the fermentor. Less than 1 sec was required for the liquor to travel from the fermentor to the electrodes.

[1] E. H. Sargent & Co., Chicago, Illinois.

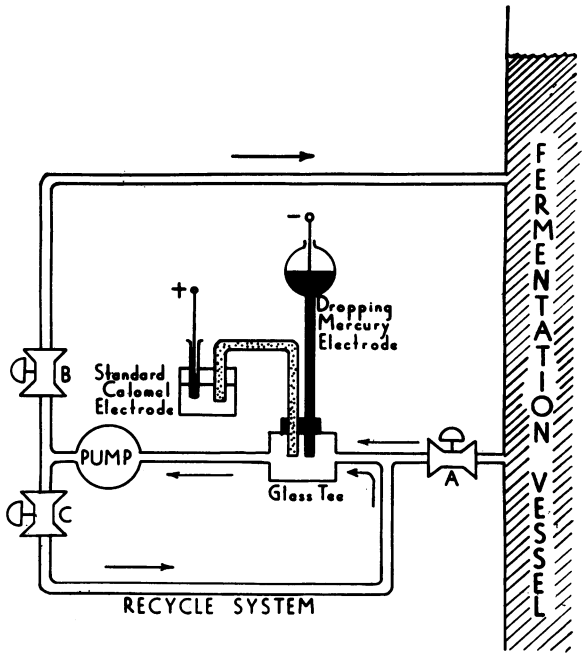

Figure 2. External polarographic assembly for the measurement of dissolved oxygen in fermentations.

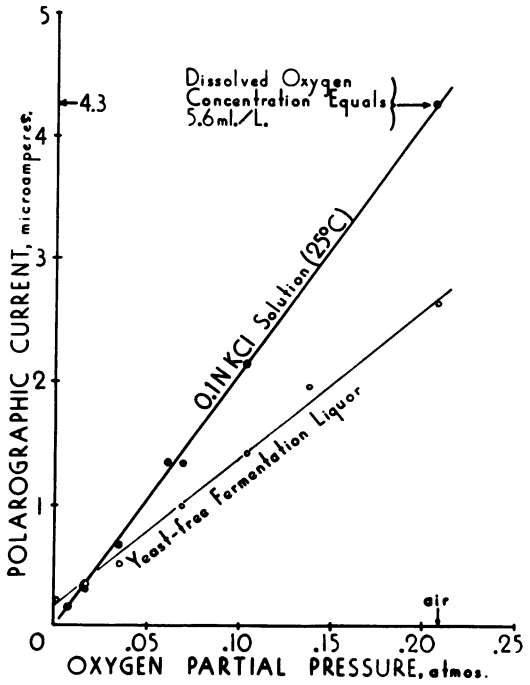

Figure 3. Polarographic calibration curve; identified reference point from Seidell (1940).

2. With valve *C* open and valves *A* and *B* closed, liquor was recycled past the electrodes in a closed system. After several min of recirculation, the yeast contained in the liquor had consumed all of the oxygen present and the residual current reading was noted.

3. The oxygen diffusion current was then taken as the difference between the total and residual currents.

This apparatus was calibrated by measuring the dissolved oxygen content of 0.1 N KCl solution and yeast-free fermentor liquor which had been saturated with various known mixtures of air and nitrogen. Figure 3 shows that the polarographic current attributed to oxygen was found to be directly proportional to the dissolved oxygen content of the solution.

Dissolved oxygen measurements were made with this apparatus in yeast fermentations in several sizes of propagators. It was found that:

1. Very low dissolved oxygen concentrations were detected during highly active portions of the fermentation (less than 0.2 ppm). In most cases, the residual current was several times larger than the current flow due to oxygen alone.

2. The respiration rate in an active fermentation was found to be extremely rapid. Tests made with pure oxygen showed that less than 10 sec were required for the yeast to consume a quantity of dissolved oxygen equivalent to that which would be present if the fermentor liquor were saturated with air.

Due to the combined effect of these points, this system measured erroneously low dissolved oxygen concentrations in the growing phase of the fermentations. Therefore work with this assembly was discontinued. However, the unsuccessful application of this apparatus in a yeast fermentation does not preclude its satisfactory use in other less active biological systems.

Since it was found practical to use a dropping mercury electrode in turbulent gas-liquid mixtures, all subsequent oxygen measurements were obtained by placing the unshielded dropping mercury electrode directly into the test fermentor. The saturated calomel electrode used previously was again connected to the system with an agar-salt bridge; the same polarograph and voltage were used. The residual current readings were taken after flushing the fermentor with high-purity nitrogen for several min. Although practical in small equipment, nitrogen flushing was not feasible for production-sized vessels. The presence of mercury and the nitrogen flushes repeated at hourly intervals had no effect on the yield or the quality of the experimental baker's yeast produced. The effect of the location of the electrodes in the fermentor (5 gal, agitated) was investigated and found to have no influence on the current readings. Current measurements were recorded photographically with the model 12 polarograph. Sample oxygen measurements taken during periods of high and low dissolved oxygen concentrations are shown in figure 4.

The precision of this method is limited by the fluctuations in the current which result from variations in the mercury dropping rate. As shown in figure 4, oxygen measurements obtained by this method are sensitive to about 0.1 ppm.

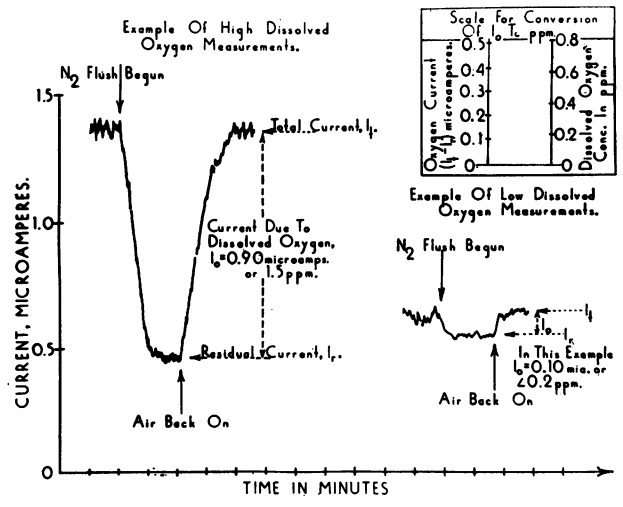

Figure 4. Sample photographs of dissolved oxygen measurements with electrodes directly in the fermentor.

Polarographic Results

Electrodes inside fermentor. Four fermentations of the type previously reported in the sulfite-yield study

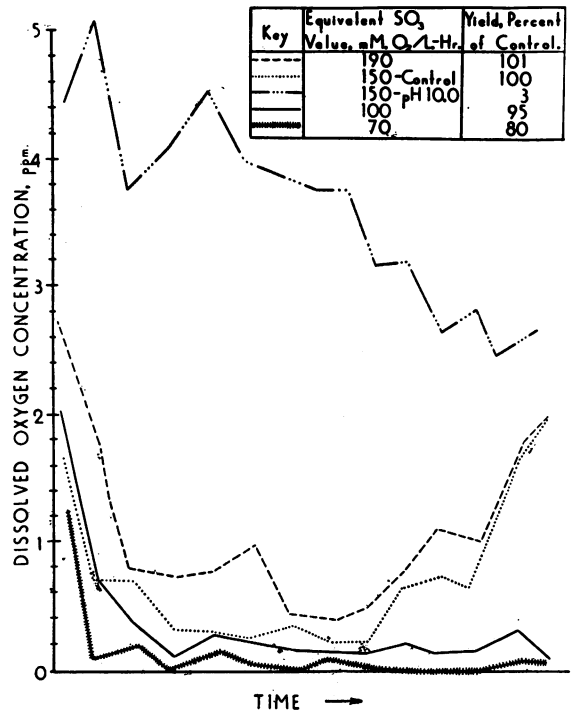

Figure 5. Dissolved oxygen concentrations measured at approximately hourly intervals using the nitrogen-flushing technique.

were made in 5-gal agitated fermentors using different applied air rates. A constant air rate was applied throughout each fermentation, but at levels which ranged from above to below that known to be required for maximal yield. A fifth fermentation was made in which the initial pH of the medium was erroneously adjusted to 10.0; this high pH greatly retarded the respiration rate of the yeast and permitted the dissolved oxygen concentration of the medium to approach saturation.

The dissolved oxygen concentrations present during these five fermentations were measured at approximately hourly intervals using the nitrogen-flushing technique previously described. Current measurements were also taken periodically in a sixth fermentation vessel containing aerated 0.1 N KCl solution. Constant current readings were obtained, which indicated the equipment was functioning properly and served as a reference point of known oxygen concentration. A single set of electrodes was used for current measurement so that all readings would be made on a comparable basis. The results of these measurements are contained in figure 5.

The feed schedule followed in all fermentations was approximately the inverse of the oxygen concentration curve shown for the control fermentation. Several conclusions are indicated by the data of figure 5. (a) Different concentrations of dissolved oxygen were detectable polarographically in a group of yeast fermentations aerated at levels above and below that required for maximal yield; (b) the yields obtained were related to the average oxygen concentration present during fermentations of normal pH; (c) the average oxygen concentrations were proportional to the aeration efficiency as determined by the sulfite oxidation method; and (d) the critical concentration of dissolved oxygen required by a baker's type yeast culture for an adequate yield is about $\frac{1}{25}$ that at saturation or 0.2 ppm.

The gradual decrease in the oxygen concentrations found in the pH 10.0 fermentation was largely due to the decreased solubility of oxygen in the medium as the feed continued and higher sugar concentrations were reached.

ACKNOWLEDGMENTS

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SUMMARY

The sulfite-oxidation technique and polarographic methods were applied to yeast fermentations to obtain more effective systems for air regulation. Yield was found to be a function of the sulfite-oxidation value in a group of geometrically dissimilar fermentation vessels.

An exterior polarographic apparatus was devised to measure the dissolved oxygen content of the medium during fermentation. Because of the high oxygen uptake rate in the fraction of a second required for liquor to reach the electrodes, oxygen measurements were erroneously low in growing phases of fermentation. Although inaccurate in portions of a yeast fermentation, this apparatus may be useful in other less active biological systems.

The polarographic measurement of dissolved oxygen was achieved in pilot plant equipment using an unshielded dropping mercury electrode directly inside the fermentors. A dissolved oxygen concentration of about 0.2 ppm was found to be required for adequate aeration during the production of baker's yeast. The technique employed was not applicable to large fermentors because of the need to nitrogen flush the vessels to determine the residual currents.

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