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# Whey Utilization

## II. Oxygen Requirements of *Saccharomyces fragilis* Growing in Whey Medium

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Availability of oxygen is one of the most important factors controlling the growth of microorganisms. The oxygen demand of the microorganism is also an important consideration. Oxygen demand may be affected by the age and physiological state of the cells, the carbon source, the storage of reserve food material, and by the enzymic complement of the organism. Thus, for the successful economical propagation of microorganisms, oxygen requirements under the particular growth conditions must be known.

The peak oxygen demand of a culture describes the minimal quantity of oxygen that must be supplied to the organisms at the time of greatest activity. Insufficient oxygen may result in lower yields or longer propagation periods. However, few reports in the literature present peak oxygen demands of microorganisms or supply the proper data to allow the calculation of this value. Finn (1954), in his review on aeration and agitation, lists several studies from which such determinations were made. For bacteria and molds, the peak demands ranged from 0.017 to approximately 0.83 mM $O_2$ per L medium per min (Eckenfelder, 1952; Shu, 1953). Yeast oxygen demand appears to be more variable. Maxon and Johnson (1953) and Hixon and Gaden (1950) reported peak oxygen demands of 0.17 to 0.25 mM per L per min in yeast grown in poor media or under limiting conditions. Maxon and Johnson (1953), however, showed that in a rich medium with adequate aeration, the peak oxygen demand was 4.8 to 5.7 mM $O_2$ per L per min. From the data in a recent paper (Strohm *et al.*, 1959), the peak oxygen demand is calculated to be a little less than 2.5 mM $O_2$ per L per min for bakers yeast.

This paper reports the determination of the peak oxygen demand of *Saccharomyces fragilis* growing in whey medium.

### MATERIALS AND METHODS

The yeast was grown as described previously (Wasserman *et al.*, 1958) in a medium of raw whey (cottage cheese), 0.5 per cent ammonium sulfate, 0.5 per cent dipotassium phosphate, and 0.1 per cent yeast extract. The addition of these salts raised the pH to 5.6, the value at which best growth occurred. The temperature was maintained at 32 ± 1 C. The propagator was an all glass column 3½ in. in diameter and 12 in. high with a total capacity of 1.5 L. Agitation was effected by a 2-in. diameter, 6-vaned disc rotating at 3,000 rpm. Metered air, entering an opening centered under the agitator, supplied oxygen to the 500 ml of medium used in the study. The effluent air was collected at a vent in the aluminum cover plate and led to a Beckman Oxygen Analyzer model C.[2] The oxygen content of the effluent air was determined from the following equation:

$$\text{ml } O_2 = \frac{P_{air}}{P_{Bar} - P_{wv}} (V_{air})$$

$P_{air}$ = $O_2$ partial pressure from the oxygen analyzer, mm

$P_{Bar}$ = Barometric pressure, mm

$P_{wv}$ = Water vapor pressure at the temperature of the propagator, mm

$V_{air}$ = Volume of air through propagator, ml

The difference between the volume of oxygen supplied to the propagator and the volume of oxygen in the effluent air at any time is the quantity of oxygen dissolved in the medium to replace the oxygen removed by the growing yeast.

No recorder was available for this model of the oxygen analyzer, so observations were made visually. The graphed values refer to the oxygen consumption at the time of observations.

### RESULTS AND DISCUSSION

The oxygen consumption pattern of a representative culture of *S. fragilis* in 500 ml whey medium aerated at 2 L per min is shown in figure 1. Initially, the oxygen consumption was low, but as yeast growth and sugar utilization continued, the volume of oxygen consumed increased rapidly. Peak consumption was reached with the uptake of 50 ml $O_2$ per min, then declined rapidly.

Variations in the course of oxygen consumption do occur. Peak oxygen demand varies from 50 to 65 ml per

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[2] Beckman Instruments Inc., Fullerton, California. It is not implied that the U. S. Department of Agriculture recommends the above company or its product to the possible exclusion of others in the same business.

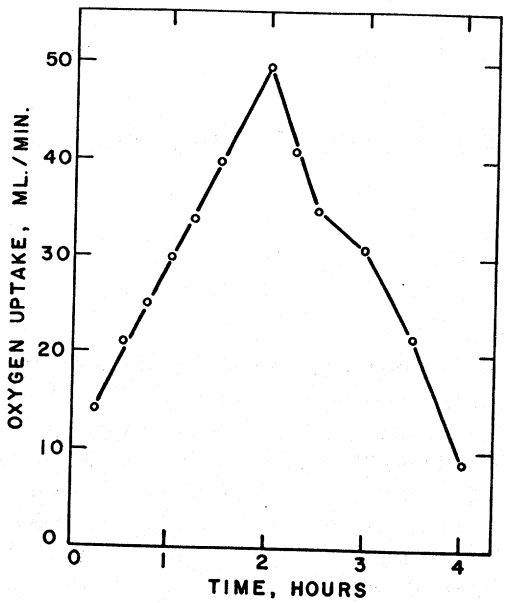


*Figure 1.* Oxygen consumption of *Saccharomyces fragilis* growing in 500 ml whey medium and aerated at 2 L per min.

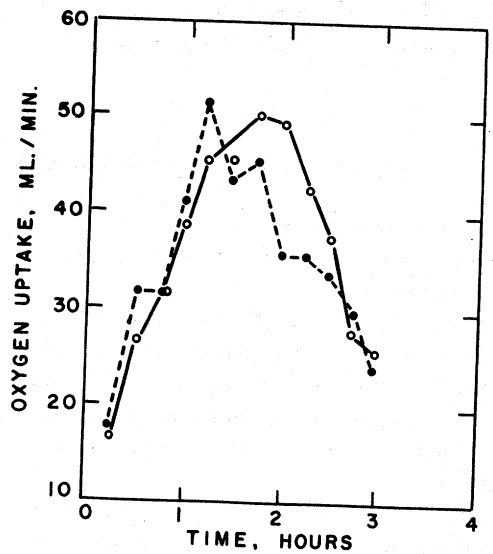


*Figure 2.* Oxygen consumption of *Saccharomyces fragilis* growing in 500 ml whey medium and aerated at 2 L per min (●-----●) and 1 L per min (○——○).

min and occurs between 90 and 180 min. These variations are due to many factors: the size and age of the seed, the physiological condition of the yeast, or the conditions of growth in the propagator. Also, the conditions and source of whey must be considered.

The total volume of oxygen consumed by the yeast during the course of growth was estimated from calculations of the average hourly rate. Between 6 and 7 L of oxygen (12 to 14 L $O_2$ per L medium) were utilized by the cultures regardless of the demand pattern.

*Saccharomyces fragilis*, oxidizing lactose in the Warburg respirometer, has been found to take up only 35 per cent of the total oxygen necessary to convert lactose completely to $CO_2$ and water (Wasserman *et al.*, 1958). On this basis, the 40 g of lactose contained in 1 L of

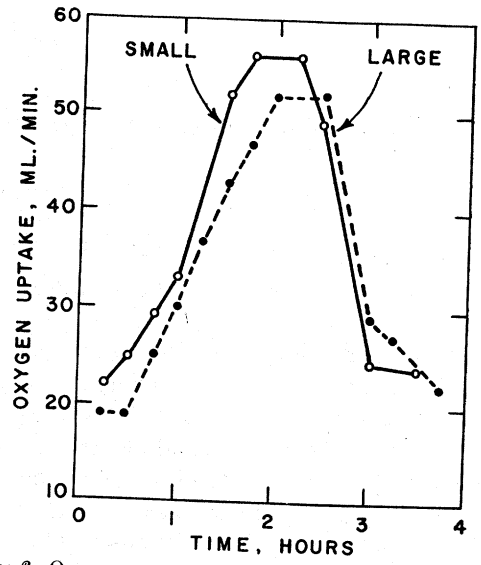


*Figure 3.* Oxygen consumption of *Saccharomyces fragilis* growing in 500 ml whey medium in a small propagator and in 15 L medium in a large propagator.

whey require an uptake of 10 L of oxygen. This is an absolute value, indicating the oxygen requirement for the utilization of a quantity of lactose based on the metabolic pattern of the organism without regard to time limits or other factors. Thus, as 12 to 14 L of $O_2$ per L medium were consumed during the actual yeast propagations, the excess 2 to 4 L of oxygen are presumably due to the endogenous respiration of the yeast and to the oxygen utilized to dissimilate the small amounts of lactic acid and other organic compounds also found in whey.

The above experiments were carried out with the assumption that oxygen was not a limiting factor and the values obtained were actually the maximal oxygen requirements of the yeast. To confirm this assumption, yeast propagations aerated with 1.0 L air per min were conducted in parallel with propagations aerated with 2.0 L air per min. The results in figure 2 indicate that, although aeration was reduced 50 per cent, the yeast still utilized the same quantities of oxygen and metabolized under these conditions at maximal efficiency.

A similar result could be expected if the agitation-aeration system had attained its maximal $O_2$-dissolving ability at a level of aeration less than 1.0 L per min. Then, even at 2 L per min aeration, the same amount of $O_2$ would be dissolved and the yeast would effectively be limited to using this quantity of dissolved $O_2$. However, in a study of the oxygen dissolving rate of the propagator used, this was not the case (Wasserman and Hampson, 1960). Increasing the rate of aeration from 1.0 to 2.0 L per min increased the available dissolved oxygen, but did not affect the oxygen demand of the yeast.

Although these experiments were carried out with 500 ml of medium, it was anticipated that the results

could be scaled-up to apply to larger operations. Fifteen L of yeast-whey medium in a 40 L propagator were aerated at 30 L per min under conditions that assured an adequate supply of dissolved oxygen (Wasserman and Hampson, 1960). The oxygen consumption by the yeast was determined with the Beckman Oxygen Analyzer. To permit direct comparison with the values obtained in the smaller propagator, the oxygen data were divided by 30 (the ratio between the medium volumes of the two propagators). Figure 3 shows the similarity of the oxygen consumption patterns between the two propagations.

The peak oxygen demand of *S. fragilis* growing in a whey medium, approximately 110 ml $O_2$ per L medium per min, is equivalent to 5 mM $O_2$ per L per min. This compares very well with the data of Maxon and Johnson describing a peak demand of 4.8 to 5.7 mM $O_2$ per L per min for *Saccharomyces cereviseae* growing in a rich glucose medium in a continuous propagation.

## Summary

The oxygen demand pattern of *Saccharomyces fragilis* growing in a whey medium is described. Peak oxygen demand is approximately 110 ml $O_2$ per L medium per min (300 mM per L per hr).

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