Effects of Liquid Physical Properties on Oxygen Transfer in Penicillin Fermentation

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The success of fermentation processes often depends upon the maintenance of an environment sufficient in oxygen concentration so as not to limit or impair normal respiratory activity. Maintenance of such an environment is, in turn, largely dependent on the adequacy of oxygen transfer from air to the liquid medium. Of the possible pathways for oxygen transport, absorption into the medium and subsequent diffusion to the cells is undoubtedly the main one in submerged fermentation.

Bartholomew *et al.* (1950b), Hixson and Gaden (1950), and Wise (1951) were the first to consider the mechanisms of oxygen transfer in fermentation systems, and they developed essentially identical methods for the quantitative expression of absorption rates. The absorption of oxygen into aqueous solutions, especially fermentation media, is generally considered as a liquid-film controlled mass-transfer operation. As such it should be expected that the mass-transfer rate, specifically the "oxygen absorption" rate for this case, is a function of those physical properties of the broth which affect interfacial area, turbulence, and boundary (film) conditions. Two such properties which undergo considerable change during many fermentations are surface tension and viscosity. For an excellent treatise on the subject of oxygen transfer in fermentations, the reader is referred to the recent review by Finn (1954).

Apparently, no reports have been published of the changes in the rate of oxygen transfer which are due to physical property alterations of the broth as the fermentation progresses. Many fungal fermentations produce thick non-Newtonian[3] broths as the mycelium concentrations rise, often as high as 25 to 30 g of dry tissue per liter during the fermentation. Metabolic activities, particularly hydrolysis of complex protein structures, utilization of carbohydrates, and the depositing of end-products in the broth, would be expected to change the surface tension considerably. The investigation reported here was undertaken in order to obtain information on the extent of changes in surface tension and viscosity during a penicillin fermentation and the degree to which they influence the rate of oxygen transfer from air to fermentation broth.

Experimental Methods

Penicillin fermentations with *Penicillium chrysogenum* Q-176 in a corn steep liquor-lactose medium were carried out in 2-liter flasks (containing 800 ml of medium) on a rotary shaker. The contents of several flasks were combined at various time intervals in order to permit study of the broth under mechanical agitation (stirring) and aeration in a laboratory 5-liter fermentor similar to one described by Bartholomew *et al.* (1950a). The experimental fermentation medium used was of the type described by Anderson *et al.* (1953): lactose 4.0 per cent, corn steep liquor 4.0 per cent, sodium nitrate 0.3 per cent, potassium dihydrogen phosphate 0.05 per cent, magnesium sulfate heptahydrate 0.025 per cent, and calcium carbonate 0.4 per cent.

Oxygen absorption rates were determined by the nonsteady-state polarographic method described by Bartholomew *et al.* (1950b), but with the following modification: samples were withdrawn periodically and measured in a polarographic cell external to the agitation vessel in order to avoid installation of an internal electrode. For these experiments, respiratory activity of the cells was first inhibited by the addition of sodium azide to a concentration of 1×10^{-3}M per liter in the broth.

Since the viscosity behavior of the broth was non-Newtonian, shear diagrams were constructed from

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[3] Fluids can be broadly classified as either Newtonian or non-Newtonian. Newtonian fluids are characterized by a constant viscosity, independent of the shear imposed on the fluid. In figure 4, a Newtonian fluid would be represented by a straight line curve passing through the origin, the slope of which would be directly proportional to the viscosity of the fluid. Thus, only one point on a shear diagram is needed to characterize completely a Newtonian fluid.

Any fluid in which the viscosity changes as shear conditions are changed is considered non-Newtonian. Non-Newtonian fluids are classified according to their dependence on shear into various general groups, one of which is Bingham plastics. This type of fluid exhibits a definite yield stress which must be overcome before the fluid will flow. The curves in figure 4 are those of Bingham plastics. The slopes, in this case, are proportional to the rigidity of the fluid, a property somewhat analogous to viscosity in a Newtonian fluid. The y-axis intercepts are proportional to the yield stress.

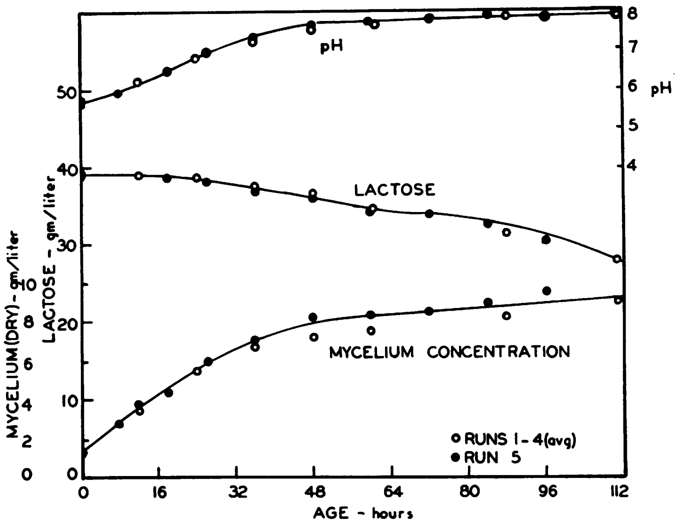

FIG. 1. Penicillin fermentation characteristics

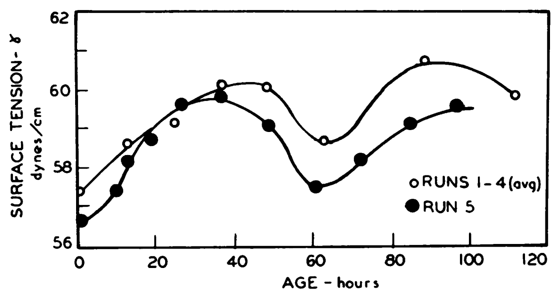

FIG. 2. Surface tension of filtered fermentation broth

MacMichael Viscosimeter readings. Surface-tension measurements were made of the filtered broth with a Traube stalagmometer (Findlay, 1945).

Results

All experimental fermentations exhibited the characteristic pattern expected for *P. chrysogenum* (figure 1) under the fermentation conditions employed. Lactose utilization is slow, and only 10.5 of the initial 38 g were used in the case shown. This low rate of sugar consumption is, however, expected, since aeration in 2-liter shaken flasks containing an 800-ml charge, under the shaker conditions used, is rather poor.

Surface Tension of Filtered Broth During Fermentation

Surface-tension changes occurring "normally" during the fermentation follow a characteristic pattern, illustrated by the two typical runs shown in figure 2. By "normal" changes are meant those occurring in the cell-free (filtered) broth in the absence of such added surface-active materials as antifoam agents. In each case, the lowest surface tension was noted at the beginning of the fermentation. Maximum variations were but 5 to 6 per cent (note that the scale of figure 2 is greatly amplified). Apparently, the metabolic activities of the organism do not bring about very great changes in surface tension under the conditions employed.

TABLE 1. *Absorption coefficient of filtered broth during fermentation*

Fermentation Time	Absorption Coefficient $k_d \times 10^4$
hours	*g mols/ml × hr × atm*
0	0.89
12	0.88
24	0.87
36	0.85
48	0.82
62	0.84
86	0.88
111	0.88

Absorption Rates in Filtered Broth

The oxygen absorption rate for filtered, cell-free broth was also found to vary but slightly during the fermentation (table 1). The maximum change observed was a decrease of about 8 per cent about midway in the fermentation, after which the absorption rate rose slowly to a final value close to that at the start. Viscosity measurements made simultaneously on the filtered broth showed changes in this property of less than 5 per cent. Apparently, the effects of biochemically produced changes on the oxygen-transfer characteristics of the cell-free medium are inconsequential, and, in the absence of added materials, the liquid-phase resistance to oxygen absorption is fairly stable.

Effect of an Antifoam Agent on Surface Tension

Foaming conditions encountered during fermentation often require the addition of an antifoam agent for collapsing the foam. The effect of one such agent, the commonly used mixture of 3 per cent Alkaterge C[4] in lard oil, was observed when it was added at various concentrations to 62-hour-old filtered broth. As shown in figure 3, this agent readily lowered the surface tension of the filtered broth, rapidly at low concentrations, until an apparent saturation was reached at higher levels. At a concentration of 0.25 per cent, the agent caused a 13 per cent reduction in surface tension. Such external factors as the addition of this antifoaming agent obviously have much greater effects on surface tension than the metabolic activity of the microorganism.

Effect of an Antifoam Agent on Oxygen Transfer

The accompanying effect of 3 per cent Alkaterge C in lard oil on oxygen absorption is also illustrated in

[4] Commercial Solvents Corporation, Terre Haute, Indiana.

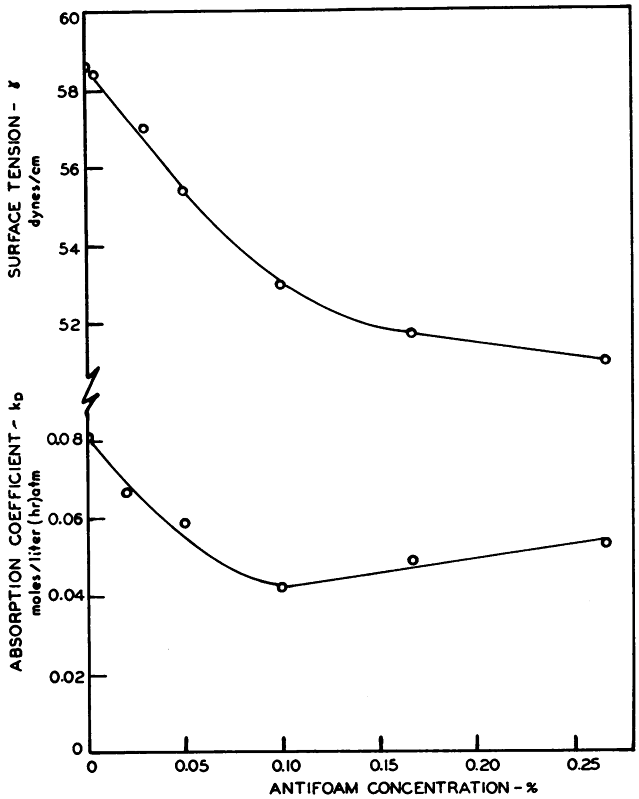

FIG. 3. Effect of antifoam concentration on surface tension and absorption coefficient of 62-hr filtered broth.

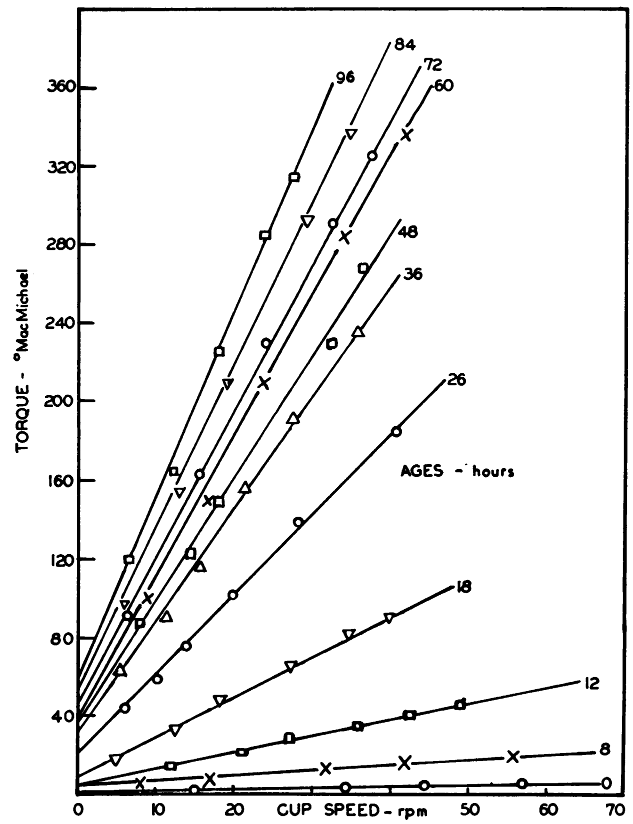

FIG. 4. Shear diagrams for penicillin broth during fermentation

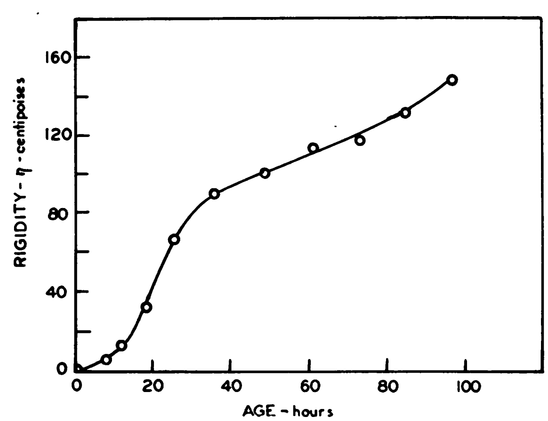

FIG. 5. Rigidity of whole broth during fermentation

figure 3. A decrease in the absorption coefficient of almost 50 per cent is caused by the addition of very little agent (0.10 per cent). The fact that the coefficient is so severely reduced, despite the lower surface tension, and hence greater interfacial area, suggests that an added film resistance is formed as a result of congregation of the agent at the air bubble-liquid interface. Oldshue (1953) reported similar effects when antifoam agents were used during oxygen-transfer measurements in a sulfite-sulfate system. Of course, the addition of an antifoam agent will, by collapsing the foam, also reduce the air hold-up volume, and hence the residence time. In the experiments reported here, there was, however, never any appreciable foam accumulation; so the idea of an additional interfacial transfer resistance seems most likely.

Fluid Behavior of Whole Broth During Fermentation

Cell weight (mycelium) developed to a concentration of about 8.5 g of tissue (dry) per liter during the fermentation. Shear diagrams (figure 4) made to characterize the whole broth indicated typical Bingham-plastic behavior, thus increasing in yield stress and rigidity with increasing mycelial concentration (the parameter for the curves in figure 4 is actually "fermentation" time, but this is related to tissue concentration in the manner shown in figure 1). In figure 5, rigidity values for the broth obtained from the slopes of the shear diagrams are plotted against mycelial concentration. A rigidity as high as 148 centipoises developed.

Since the completion of this work, Brown and Petsiavas (1954) presented data on the mixing characteristics of a mycelial-type broth. Their results greatly extended the observations on viscosity behavior noted above. Oxygen absorption was not considered.

Effect of Mycelial Concentration on Fluid Behavior

For the purpose of observing the effect of mycelial concentration on the absorption coefficient, various quantities of 40-hour-old mycelium, poisoned with

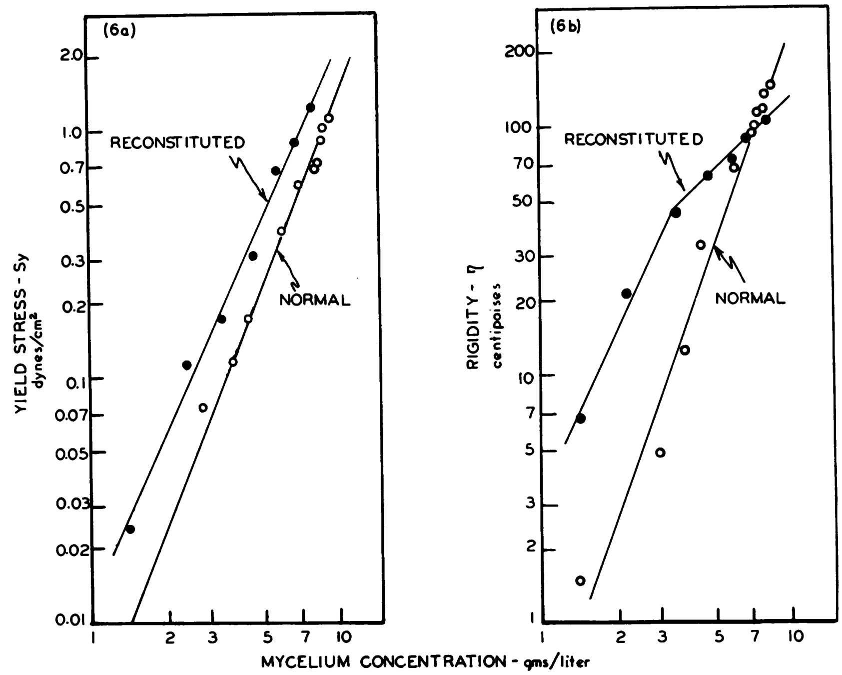

FIG. 6. Effect of mycelium concentration on yield stress and rigidity

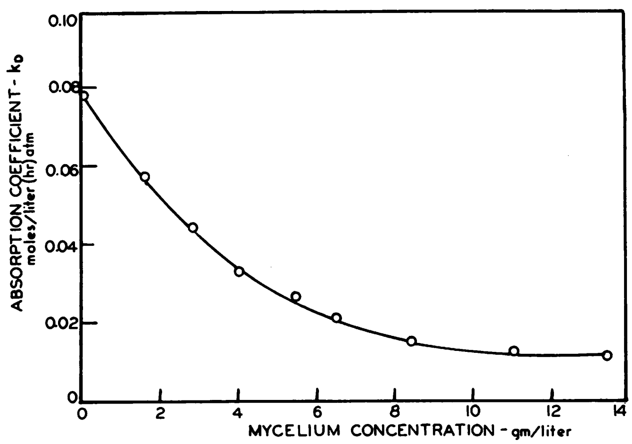

FIG. 7. Effect of mycelium concentration on absorption coefficient.

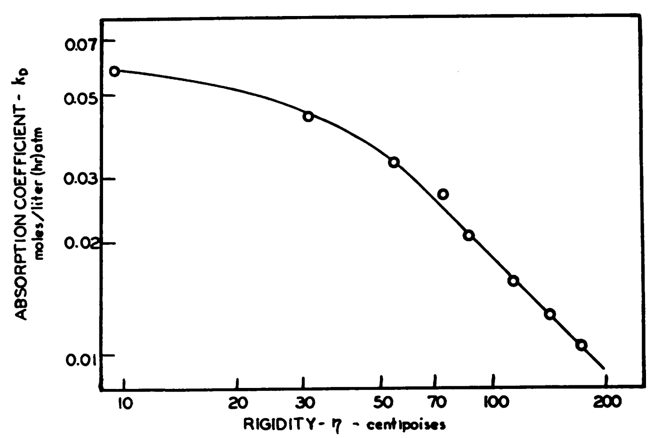

FIG. 8. Effect of rigidity on absorption coefficient

sodium azide in order to inhibit respiration, were used for reconstituting filtered broth. Shear diagrams of the reconstituted broth showed a behavior essentially similar during the fermentation to that of normal broth (figure 6*a*, *b*). Slight differences do exist, and are expected, since viscosity behavior is a function not only of mycelial concentration, but also of the mycelial structure and extent of physical interlacing of hyphal networks. It is difficult, in filtering and resuspending, not to disturb the original nature of the mycelium in these respects.

Effect of Mycelial Concentration on Absorption Rate

In figure 7, the oxygen-absorption rates observed at various mycelial concentrations (reconstituted broth) are shown. Because of the changes in broth consistency noted above, a rapid decrease in the absorption rate occurs as mycelial concentration is increased. At a mycelial concentration of 13.4 g tissue (dry) per liter, the rate was reduced by nearly 85 per cent.

Relationship Between Rigidity and Absorption Rate

The effect of various mycelial concentrations on the absorption rate of reconstituted medium is best interpreted through the fluid characteristics of these broths. Rigidity values at the mycelial concentrations used were obtained from figure 6*b* for reconstituted broth. Absorption rates were then plotted against these rigidities in figure 8. The absorption rate decreases rapidly with increasing rigidity. This is consistent with the concept of liquid-film controlled mass-transfer operations, in which liquid turbulence or "film-thickness" is decidedly dependent on viscosity characteristics.

Summary

Fluid behavior was found to be typically Bingham-plastic, the broth increasing in rigidity 100-fold during the fermentation. Rigidities as high as 148 centipoises developed. An 85 per cent reduction in absorption rate was caused by a mycelial concentration of 13.4 g dry tissue per liter. Oxygen absorption rates were related to broth rigidity, the rates decreasing markedly with increasing mycelial concentrations.

Changes in surface properties brought about by metabolic activity of the microorganism apparently did not influence oxygen absorption in fermentation media to any appreciable degree. Maximum changes in the absorption rate for cell-free medium amounted to less than 8 per cent during the course of a typical penicillin fermentation.

Changes in surface characteristics caused by the introduction of a mixture of 3 per cent Alkaterge C in lard oil, a common antifoam agent, were considerable. At a concentration of 0.25 per cent antifoam agent, the absorption coefficient was reduced almost 50 per cent, despite the greater interfacial area resulting from an associated 13 per cent reduction in surface tension. The existence of an added film resistance established by accumulation of the agent at the gas-liquid interface is suggested.

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