Agitation-Aeration in Submerged Fermentation

II. Effect of Solid Disperse Phase on Oxygen Absorption in a Fermentor

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For the successful operation of aerobic fermentations it is essential to supply the microorganisms with sufficient oxygen to meet their requirements at any stage in the process. Failure to supply enough oxygen may lead to undesirable changes in enzymatic makeup (Rollinson, 1952) or death of the organisms (Hromatka et al., 1951), with a subsequent lower yield of the desired product. In general, the supply of oxygen to bacterial cultures is more easily maintained than is the supply to submerged cultures of filamentous fungi. The extremely rigid (non-Newtonian) broth produced by filamentous fungi promote the coalescence of bubbles with consequent reduction in transfer area between the gas and liquid phases and alteration of turbulence and liquid-film conditions. For example, Chinn and Guandalini (1954) observed about 57 per cent reduction in the oxygen solution rate in the presence of 1.35 per cent dry weight of mycelium of Penicillium chrysogenum. Deindorfer and Gaden (1955) reported a 100-fold increase in the rigidity of penicillin broth during the course of a fermentation in which about 1 per cent dry weight of mycelium was produced; a mycellial concentration of 1.34 per cent reduced the oxygen absorption rate by 85 per cent. Also, the addition of 0.25 per cent antifoam agent (3 per cent Alkaterge C in lard oil) reduced the oxygen absorption coefficient by 50 per cent. In studies using paper pulp to simulate mold mycelium, Bowers (1955) found that the addition of 2 per cent pulp reduced the oxygen solution rate given by a sintered aerator by a factor of 20; this was altered to a factor of 2 by high speed agitation (vaned-disc impeller) over an open-pipe sparger.

Although the physical effects of mold mycelium on the oxygen absorption rate in submerged fermentations have been recognized, a study of the effects of mycelium concentration and morphology on the correlations between oxygen absorption coefficients and operating variables (air flow rate, agitator speed) seemed desirable. Hence studies were made on the effects of filamentous mycelium, paper pulp, and sago pellets (the latter to simulate smooth mold pellets) on the rate of solution of oxygen in a fermentor.

Materials and Methods

Fermentor and accessories. Fermentor design (2.5-L operating capacity) and the polarographic system used for the determination of oxygen absorption coefficients (Kₐ values) have been described previously (Steel and Brierley, 1959). Briefly, the electrode system consisted of a rotating "radial" platinum cathode and a silver/silver chloride reference anode. The applied potential was −0.90 V. Oxygen absorption-coefficients were determined by the "gassing-out" procedure of Bartholomew et al. (1950) and Wise (1951).

Cultivation of a filamentous mold. A strain of Aspergillus niger, N1E, was grown in a Wadden fermentor (8-L operating volume) in a medium containing sucrose, salts, and corn steep liquor. At 5 days, the mycelium was harvested by vacuum filtration through nylon, washed 3 times by resuspension in distilled water, and finally washed with sodium azide solution to poison respiratory enzymes. The bulk of the water was removed from the mycelium by drying on a vacuum filter. The mycelium was used in experiments as soon as possible although storage at 2°C for 7 days as a wet pad did not seem to affect its physical properties. Dry weight determinations were made on all samples of mold by drying to constant weight in a vacuum oven (60°C).

Preparation of paper pulp. The desired amount of finely shredded filter paper was soaked in the potassium chloride solution (0.5 M) in the fermentor for 30 min prior to experimental work.

Preparation of sago pellets. A weighed amount of dry sago was added to boiling water and stirred until swelling was complete. The pellets were washed several times with cold distilled water by decantation and excess water was removed by vacuum filtration.

Scope of the work. Preliminary studies were made to determine the optimum positions of sparger and impeller within the fermentor since it was believed that these might be affected by the presence of mycelium. In subsequent work, oxygen absorption coefficients were measured in the presence of various amounts of filamentous mold mycelium, paper pulp, or sago pellets.

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pellets at agitator speeds from 500 to 1500 rpm and air flow rates from 0.5 to 5.5 L per min (0.2 to 2.2 volumes of air per volume of medium per min). Data obtained for a two-phase (gas-liquid) system, over a similar range of operating variables, have been given previously (Steel and Brierley, 1959) and some of these are reproduced in this paper for purposes of comparison.

RESULTS

Operation of the electrode system. In the presence of filamentous mold or sago pellets the variability of oxygen absorption coefficients within an experiment, under constant operating conditions, was similar to that found previously (Steel and Brierley, 1959) for the gas-liquid system (coefficient of variation less than 2 per cent). Further, using sago pellets the variability between experiments carried out on different days was of a similar order. However, in the case of filamentous

TABLE 1
Effect of various positions of sparger and impeller on oxygen absorption in the absence and presence of mycelium (0.15 per cent dry weight)*

<table>
<thead>
<tr>
<th>Distance from base of fermentor, in.</th>
<th>Impeller</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>3½</td>
</tr>
<tr>
<td>7½</td>
<td>315†</td>
</tr>
<tr>
<td>13½</td>
<td>310</td>
</tr>
<tr>
<td>23½</td>
<td>315</td>
</tr>
</tbody>
</table>

* Operating conditions; agitator speed, 800 rpm; air flow rate, 2.5 L per min.
† Mold absent.
‡ Mold present.
§ Not measured.

Figure 1. Effect of filamentous mycelium on the rate of oxygen absorption. Operating conditions, agitator speed, 800 rpm; air flow, 2.5 L per min.

Figure 2. Effect of paper pulp concentration on the rate of oxygen absorption. Operating conditions, agitator speed, 800 rpm; air flow, 2.5 L per min.

Figure 3. Effect of agitator speed on oxygen absorption rate in the presence of different amounts of mold. Logarithmic plot; air flow, 2.5 L per min; the value of Y is given for the relationship $K_{La} = K N^r$ where $N$ is agitator speed, rpm.
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mold, the variability between experiments was larger (up to 10 per cent) than in the absence of mold (about 6 per cent); this larger variation may be attributed to differences in the physical relationship of the hyphae because of alterations in the degree of physical interlacing of hyphal networks when resuspending the mycelium in potassium chloride solution in the fermentor (see Deindoerfer and Gaden, 1955). In the presence of paper pulp the maximum galvanometer deflection (given by an oxygen saturated solution) oscillated over about 4 scale divisions (for example, 78 to 82 mm); also, the scale deflection on aeration and agitation of a solution previously gassed-out with nitrogen was not as regular as it was with the gas-liquid system. Accordingly, more replication (3 to 4 runs) was necessary in experiments using paper pulp.

Relative position of sparger and impeller. The positions of sparger and impeller tested are given along with the results in table 1. The relative position of the two members made no significant difference in the oxygen solution rate either in the presence or absence of mold. In subsequent work, the impeller was situated one impeller diameter (2 in.) from the base of the fermentor and the sparger orifice was located $\frac{3}{8}$ in. below the impeller.

Experiments with mold and with paper pulp. Measurement of oxygen absorption coefficients in the presence of various amounts of mold or paper pulp were made

Figure 4. Effect of agitator speed on oxygen absorption rate with air flow and paper pulp concentration as parameters. Logarithmic plot; the value of $Y$ is given for the relationship $K_{La} = KN^2$ where $N$ is agitator speed, rpm.

Figure 5. Effect of air flow on oxygen absorption rate in the presence of different amounts of mold. Logarithmic plot; agitator speed, 800 rpm; the value of $X$ is given for the relationship $K_{La} = KG^x$ where $G$ is air flow, L per min.

Figure 6. Effect of air flow on oxygen absorption with agitator speed and paper pulp concentration as parameters. Logarithmic plot; the value of $X$ is given for the relationship $K_{La} = KG^x$ where $G$ is air flow, L per min; open markers = zero pulp; closed markers = 1 per cent pulp.
under constant operating conditions (agitator speed, 800 rpm; air flow rate, 2.5 L per min). Upon the addition of mold, the absorption coefficient was reduced markedly (figure 1). The largest decrease in absorption coefficient per unit weight of mold was brought about by the first small additions. Reductions of 67 and 85 per cent in the absorption coefficients were obtained by the addition of 1 and 2 per cent, respectively, of paper pulp (figure 2). Low concentrations of pulp (below 0.5 per cent) did not reduce the oxygen solution rate as markedly as the addition of a similar weight of mold. Visual observations of bubble dispersion indicated a smaller bubble area in the presence of the added solid phase (mold or pulp) because large bubbles broke at the liquid surface. At the highest concentration of solid phase tested, movement (mixing) at the liquid surface was very poor.

The effect of mold concentration on the correlation between absorption coefficient \(K_{La}\) and agitator speed \(N\) is shown by the logarithmic plot in figure 3. The reduction in \(K_{La}\) was less at high agitator speeds than at low agitator speeds, hence the exponent in the relation \(K_{La} = KN^r\) increased with increase in mold concentration. Essentially, similar results were obtained with paper pulp (figure 4) except that the change in the exponent on \(N\) was larger than it was with the addition of a similar weight of mold.

The correlation between absorption coefficient \(K_{La}\) and air flow rate \(G\) at constant agitator speed (800 rpm) decreased with increase in mold concentration (figure 5). The exponent on \(G\) in the relation \(K_{La} = KG^x\) was reduced from 0.53 in the absence of mold to 0.11 in the presence of 1.73 per cent dry weight of mycelium. Essentially, similar results were obtained with paper pulp, the correlation being unaffected by agitator speeds from 700 to 1500 rpm (figure 6).

Experiments with sago pellets. Under constant conditions of equipment operation (agitator speed, 800 rpm; air flow rate, 2.5 L per min) sago pellets made no significant difference to the rate of oxygen transfer since the reduction in \(K_{La}\) in the presence of 3 per cent sago was only 8.1 per cent (figure 7). No marked differences in bubble dispersion were detected visually as the sago concentration was increased from 0 to 3 per cent.

**Discussion**

It is clear from the foregoing results that the morphology of the solid phase largely determines its physical effect upon the oxygen transfer rate (figures 1, 2, and 7). Additions of filamentous mold or paper pulp markedly reduced the oxygen transfer rate because of their effect on bubble size and liquid turbulence, increasing the former and decreasing the latter. Although a high degree of shear, which is desirable for high rates of oxygen transfer, occurs at the impeller tip, the shear decreases markedly as the distance from the impeller tip increases. It was noted visually that relatively little movement (mixing) occurred at the upper surface of broth containing about 2 per cent mold. As noted previously by Bowers (1955), the reduction in oxygen transfer rate upon the addition of solid phase can be overcome to a certain extent by increasing the agitator speed (figure 3).

Smooth pellets which do not tangle together, but which are easily fluidized, give a system with oxygen transfer characteristics similar to a gas-liquid system (figure 7); thus a mold fermentation utilizing smooth pellets (see Martin and Waters, 1952) seems to offer a decided advantage as far as oxygen supply is concerned.

What do the results of the present work imply with reference to the submerged growth of fungi? First, since the correlation between oxygen absorption rate and air flow rate decreased markedly as the mold concentration was increased (figure 5), the practice of increasing the air flow rate to increase the rate of oxygen supply to a mold culture becomes almost valueless. Secondly, if the oxygen solution rate into broth is 100 mmoles per L per hr, the addition of the necessary antifoam agent would reduce this to about 50 mmoles per L per hr (from Deindoerfer and Gaden, 1955) and the production of 2 per cent dry weight of mycelium would further reduce the oxygen supply rate to about 5 mmoles per L per hr (from figure 1). For the culture to receive sufficient oxygen its demand rate must not exceed 5 mmoles per L per hr, an exceedingly low level. From another viewpoint, in the present equipment operated at agitator speed 800 rpm and air flow rate 2.5 L per min, the oxygen solution rate was 62 mmoles per L per hr at zero mold concentration (figure 3). Accordingly, a culture with a maximum demand rate of 30 mmoles per L per hr would grow only to a dry weight of 0.3 per cent before the oxygen supply rate became limiting (from figure 1).

It is apparent from the above examples that further work is desirable to find better methods of cultivating molds. It might be possible with other arrangements
of baffles and impellers to introduce more shear into the broth. Alternatively, studies on submerged mold growths to obtain cultures showing Newtonian rather than non-Newtonian characteristics are perhaps worthy of consideration.

**Acknowledgments**

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**Summary**

Studies were made on the effects of sparger and impeller positions, filamentous mold mycelium, paper pulp, and sago pellets (the latter simulating smooth mold pellets) on the rate of oxygen absorption in a fermentor. Within the range of conditions tested, the relative positions of sparger and impeller did not affect the oxygen solution rate significantly either in the presence or absence of mold. Marked reduction in oxygen solution rate were brought about by the addition of filamentous mold (90 per cent reduction by 2 per cent dry weight of mycelium) and paper pulp (85 per cent reduction by 2 per cent dry weight), whereas the addition of 3 per cent dry weight of sago pellets made no significant difference. The correlations between oxygen absorption coefficients and operating variables (agitator speed, air flow rate) were altered substantially by the addition of mold or paper pulp to the fermentor; in fact, the practice of increasing the air flow rate to increase the rate of oxygen absorption was found to be almost valueless in the presence of mold or pulp. From the viewpoint of oxygen supply rate, the results of the present work indicate the importance of mold morphology in submerged fermentations.

**References**


