APPLIED MICROBIOLOGY, Jan., 1965 Vol. 13, No. 1
 Printed in U.S.A.

Effect of Oxygen-Supply Rates on Growth of *Escherichia coli*

II. Comparison of Results in Shake Flasks and 50-Liter Fermentor

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Received for publication 10 September 1964

ABSTRACT

McDANIEL, L. E. (Rutgers, The State University, New Brunswick, N.J.), E. G. BAILEY, AND A. ZIMMERLI. Effect of oxygen-supply rates on growth of *Escherichia coli*. II. Comparison of results in shake flasks and 50-liter fermentor. Appl. Microbiol. **13**: 115–119. 1965.—Growth of *Escherichia coli* and chemical changes in the medium were very similar in highly baffled flasks and in a 50-liter fermentor run under the same oxygen-supply conditions, based on sulfite-oxidation rates. Flasks with stainless-steel baffles (Biotech) gave growth patterns and rates of glucose and NH_4-N utilization almost identical to those of the fermentor; results with Bellco 598 flasks (with 6 to 7 mm deep indentations) were quite similar. Unbaffled and Bellco 600 flasks (3 to 4 mm indentations) were similar to the fermentor at very high and very low oxygen-transfer rates, but gave much less growth than the fermentor at intermediate levels. Maximal oxygen-uptake rates occurred in the fermentor at the end of the logarithmic-growth phase when growth was 40 to 75% of maximum. In the fermentor, both sulfite-oxidation rates and rates of oxygen uptake correlated reasonably well with the total amount of growth produced.

One of the problems in fermentation development is that of obtaining the same results in shake flasks and pilot-plant fermentors. The scale-up factor which has been most investigated is that of oxygen supply. Jensen, Schultz, and Shu (1961) reported fairly good scale-up of chlortetracycline yields from shake flasks to 100-gal fermentors by duplicating oxygen-uptake patterns. Good correlations in yeast growth have been reported when results were compared on oxygen-transfer capacity of the equipment (Olson and Johnson, 1947; Strohm, Dale, and Peppler, 1959). However, Lockhart and Squires (1963) stated that different pieces of fermentation equipment, including shake flasks, with similar oxygen-transfer rates may give entirely different results. Roxburgh, Spencer, and Sallans (1954) reported very different requirements for equal ustilagic acid production by *Ustilago zeae* in shake flasks and fermentors, on the basis of sulfite-oxidation rates.

A wide range of oxygen-transfer rates can be obtained in commercially available shake flasks (Gaden, 1962; McDaniel, Bailey, and Zimmerli, 1965). It was shown in the latter paper that total yields of *Escherichia coli* cells depend on oxygen-supply rates and on the type of flask used. In the work reported here, we studied the growth and fermentation characteristics of *E. coli* B in a 50-liter fermentor, as compared with different types of shake flasks under conditions of high, intermediate, and low oxygen-supply rates. We wished, first, to find out which type of shake flask compared most closely with the fermentor, and, second, to gain some insight into factors influencing microbial behavior in shake flasks and fermentors which would permit good duplication of results between the two.

MATERIALS AND METHODS

Culture, media, and shake-flask procedures. The strain of *E. coli* B, the inoculum and growth media, the types of shake flasks, and shaking conditions were all as described by McDaniel et al. (1965). The shake-flask tests were run by removing medium from the fermentor after inoculation and dispensing it aseptically into sterile flasks in volumes to give the desired sulfite oxygen-absorption rates (OAR). One flask of each type was removed at each sampling time.

Inoculum development. Quantities of 1 liter of inoculum were grown in 2-liter Erlenmeyer flasks. The cultures were incubated at 37 C on a shaker running at 220 rev/min; 10% inoculum was used, and the fermentor volumes were calculated so that the batch size would be 25 liters after removal of medium for shake flasks.

Fermentor and accessories. The 50-liter fer-

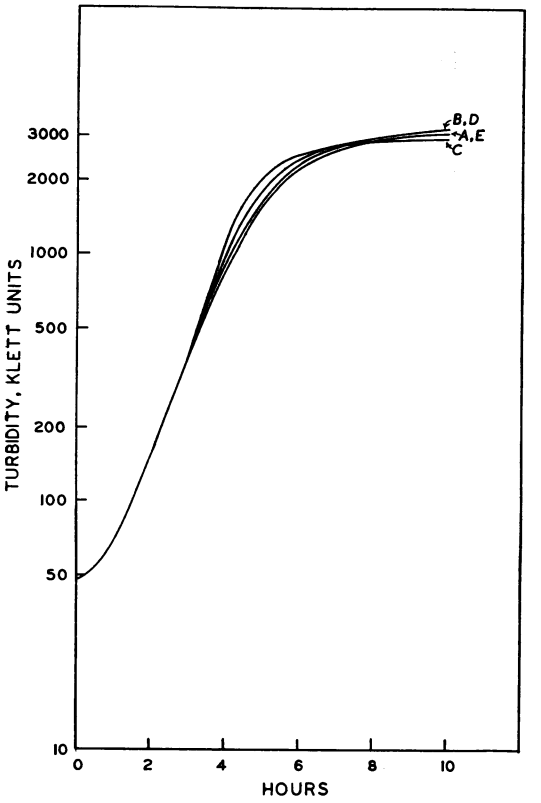

FIG. 1. *Fermentor and shake-flask growth curves at OAR 2.5. (A) Unbaffled flasks. (B) Bellco 600. (C) Bellco 598. (D) Biotech baffles. (E) 50-liter fermentor.*

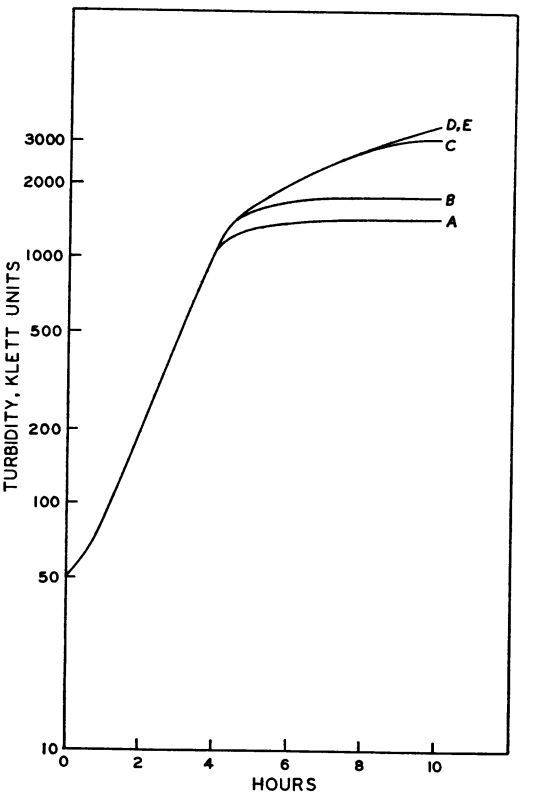

FIG. 2. *Fermentor and shake-flask growth curves at OAR 1.0. Symbols same as Fig. 1.*

mentor was a Fermacell model F-50 (New Brunswick Scientific Co., New Brunswick, N.J.). The agitation system consisted of two 5-in. diameter four-bladed impellers. A model F3 continuous oxygen analyzer (Beckman Instrument Co., Fullerton, Calif.) was used for measuring oxygen depletion in the vent gas.

Analytical procedures. The methods used for turbidity measurements, OAR measurements, and for glucose and NH_4-N determinations were those listed in the preceding paper.

RESULTS AND DISCUSSION

Figures 1 through 4 give *E. coli* growth curves for the 50-liter fermentor and for the corresponding shake-flask controls run at 2.5, 1.0, 0.65, and 0.16 mmoles per liter per min, as measured by sulfite-oxidation rates (OAR). The times of the shake-flask curves were adjusted for the 15- to 20-min delay in getting the shake flasks started.

The corresponding glucose-utilization, NH_4-N-utilization, and *p*H curves are given in Fig. 5 through 8.

The flasks with Biotech baffles gave growth and chemical changes most like those of the fermentor over the 0.65 to 2.5 OAR range. These flasks could not be run as low as the 0.16 level. Bellco 598 flasks were quite similar to the fermentor, but had slightly lower maximal growth at the higher OAR levels. Unbaffled and Bellco 600 flasks compared reasonably well with the fermentor at high and low OAR levels, but were different in the intermediate range.

For the culture and medium used in these studies, we would choose flasks with Biotech baffles or Bellco 598 flasks for shake-culture work since they gave results most like the fermentor. High OAR levels and maximal *E. coli* growth can be obtained in both at good working volumes.

Oxygen-uptake data are difficult to obtain in shake flasks, but can be measured readily in fermentors. In a fermentor, maximal uptake occurred very near the end of the logarithmic-growth phase (Table 1). Increasing the oxygen-supply rates from 0.28 to 2.5 extended the logarithmic-growth phase about 1 hr. Oxygen consumption leveled off as the growth rate diminished, and oxygen used per unit weight of

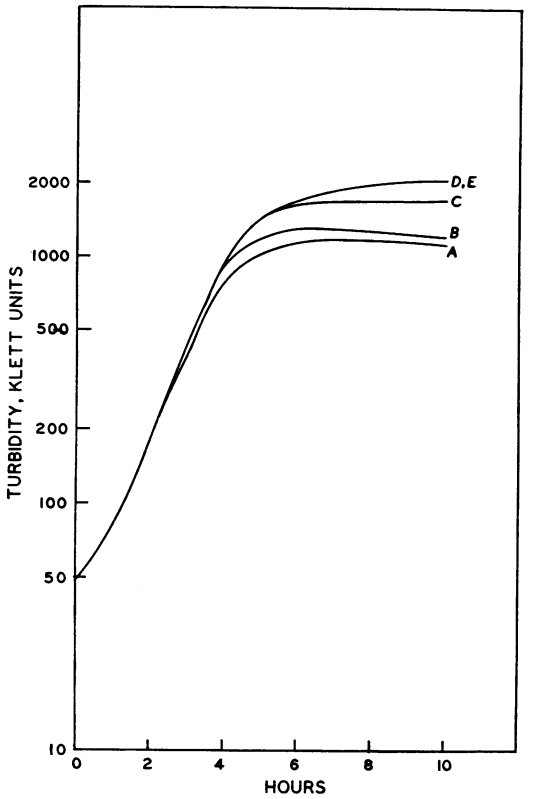

FIG. 3. *Fermentor and shake-flask growth curves at OAR 0.65. Symbols same as Fig. 1.*

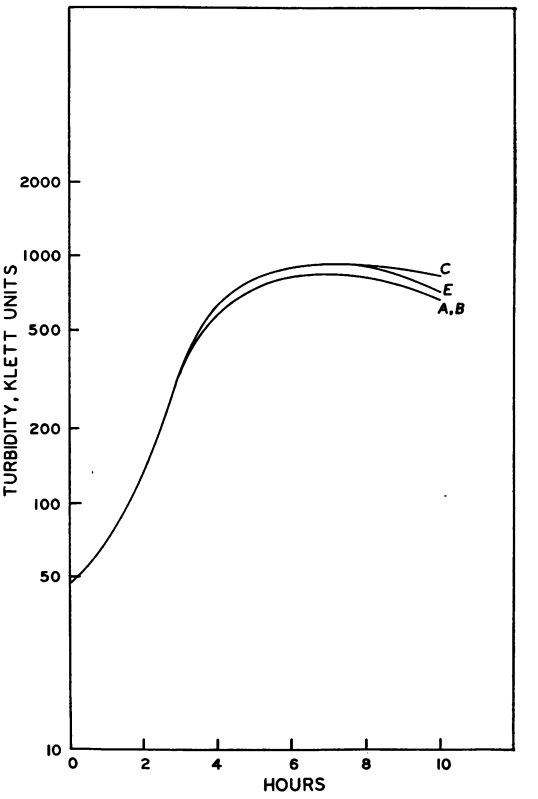

FIG. 4. *Fermentor and shake-flask growth curves at OAR 0.16. Symbols same as Fig. 1.*

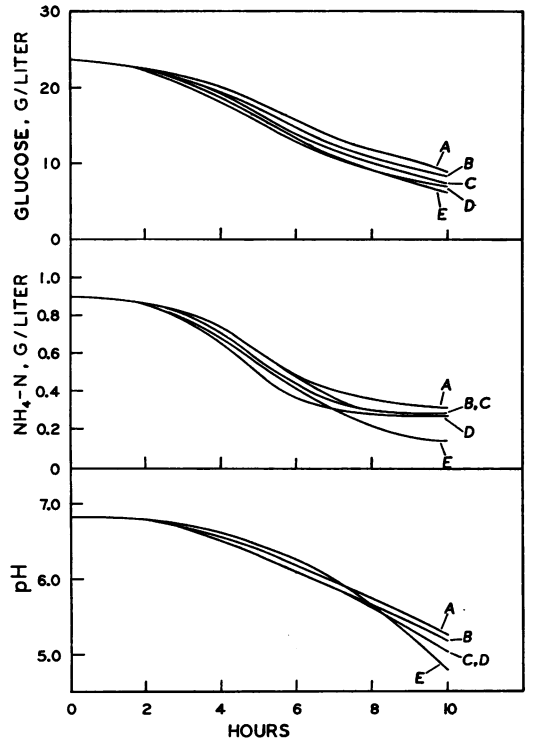

FIG. 5. *Glucose utilization, NH_4-N utilization, and pH curves at OAR 2.5. Symbols same as Fig. 1.*

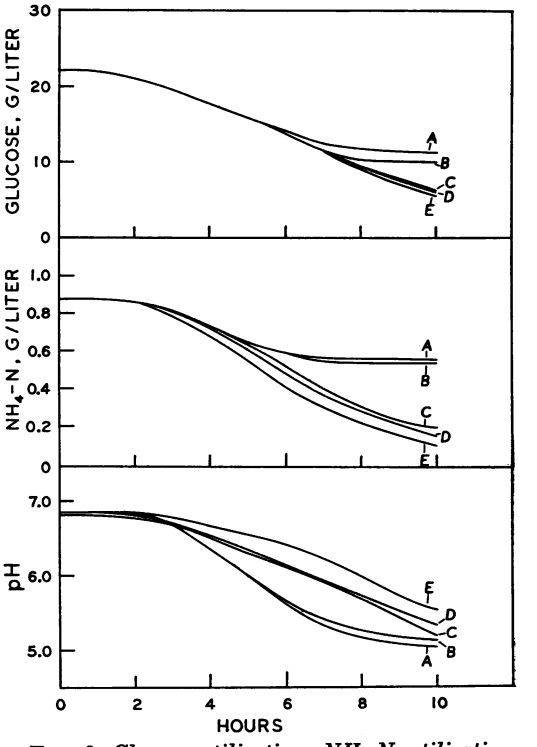

FIG. 6. *Glucose utilization, NH_4-N utilization, and pH curves at OAR 1.0. Symbols same as Fig. 1.*

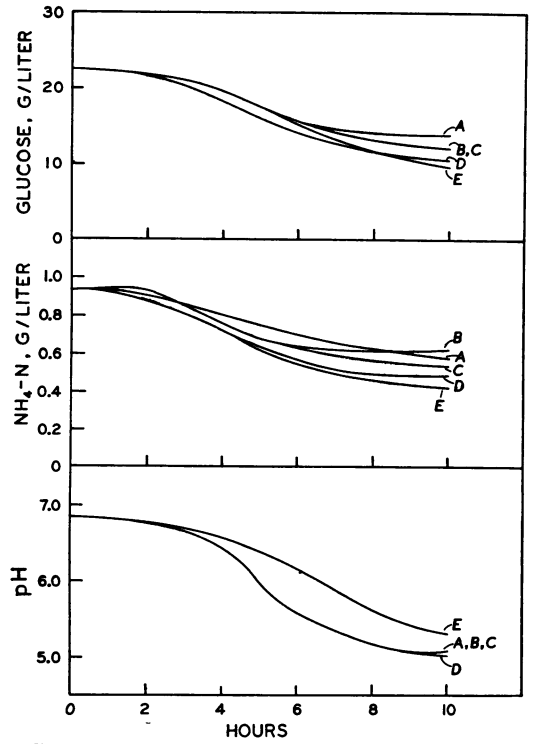

FIG. 7. *Glucose utilization, NH_4-N utilization, and pH curves at OAR 0.65. Symbols same as Fig. 1.*

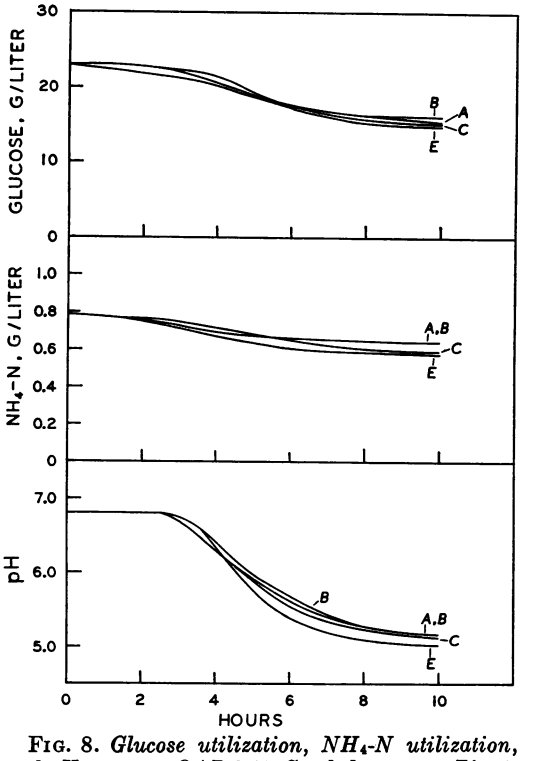

FIG. 8. *Glucose utilization, NH_4-N utilization, and pH curves at OAR 0.16. Symbols same as Fig. 1.*

TABLE 1. *Oxygen uptake in 50-liter fermentor at different oxygen-supply rates (average of four batches at each OAR)*

OAR	Age* at		O_2-uptake rates at maximum	
	End of log phase	Maximal O_2 uptake	Amt per liter per min	Amt per g per hr
	hr	*hr*	*mmole*	*mmole*
2.5	5	4.75	0.86	27.5
1.0	4.5	4.75	0.87	26
0.65	4.25	4.25	0.53	27
0.28	4	3.5	0.34	26

* Average to nearest 0.25 hr.

TABLE 2. *Oxygen-transfer rates (millimoles per liter per minute) in 50-liter fermentor measured by sulfite-oxidation procedure and by O_2 uptake by Escherichia coli*

Rev/min*	Sulfite-oxidation rate		O_2 uptake by culture†	
	In aqueous solution	In killed culture	At maximal demand	With excess O_2 demand
390	2.5	1.93	0.86	—
260	1.0	0.67	0.87	0.72
230	0.65	0.40	0.53	0.55
130	0.28	0.28	0.34	0.28

* Air-flow rates were 2 volumes per volume per min.

† Each value is the average of four batches.

cells decreased from this time on. Maximal uptake occurred when growth was only 40 to 60% of maximum at OAR levels of 1.0 and 2.5, 45 to 65% at 0.65, and 50 to 75% at 0.16. There was no abrupt termination of growth such as Ecker and Lockhart (1961) reported with *E. coli* K-12 grown at an OAR of about 0.1.

Increasing the OAR from 0.28 to 2.5 resulted in more than a twofold increase in total oxygen uptake (Table 1), due entirely to increase in growth. Oxygen uptake per gram of cells was 26 to 27.5 mmoles per g per hr, and did not change with change in OAR.

Table 2 gives comparisons of sulfite values and measured rates of oxygen uptake by cells. Sulfite values were determined by the usual procedure as well as in the presence of phenol-killed cultures. Oxygen-uptake rates were measured by two procedures. The first was that used for Table 1. In the second, cultures were grown at 600 rev/min with nutrients added in increments, and with the *p*H controlled at 6.6. This gave maximal oxygen-uptake rates of 1.1 to 1.3 mmoles per liter per min. At the maximal uptake point, the agitation speed was reduced successively to 390 260, 230, and 130 rev/min to determine the

oxygen-transfer capacity at each speed in the presence of a known excess oxygen demand. At 390 rev/min, there was no drop in oxygen-uptake rate, indicating that at this speed there was not an excess oxygen demand. At lower speeds, there was fairly good agreement between these uptake values and those obtained by the first procedure verifying that, under reduced agitation conditions, oxygen-uptake rates (and growth) increase fully to the limit imposed by the oxygen-transfer capacity of the physical system employed. Similar limitations no doubt apply in shake flasks.

Neither set of sulfite-oxidation rate values coincided with culture oxygen-uptake measurements. However, both sulfite values and oxygen-uptake measurements correlated reasonably well with the relative amounts of growth produced.

ACKNOWLEDGMENTS

This investigation was supported in part by Public Health Service research grants AI 04744 and AI 04745.

LITERATURE CITED

ECKER, R. E., AND W. R. LOCKHART. 1961. Specific effect of limiting nutrient on physiological events during culture growth. J. Bacteriol. **82**:511–516.

GADEN, E. L., JR. 1962. Improved shaken flask performance. Biotech. Bioeng. **4**:99–103.

JENSEN, A. L., J. S. SCHULTZ, AND P. SHU. 1961. Scale-up of an antibiotic fermentation by control of oxygen utilization. Abstr. 140th Meeting Amer. Chem. Soc., 5P.

LOCKHART, W. R., AND R. W. SQUIRES. 1963. Aeration in the laboratory. Advances Appl. Microbiol. **5**:157–187.

MCDANIEL, L. E., E. G. BAILEY, AND A. ZIMMERLI. 1965. Effect of oxygen-supply rates on growth of *Escherichia coli*. I. Studies in unbaffled and baffled shake flasks. Appl. Microbiol. **13**:109–114.

OLSON, B. H., AND M. J. JOHNSON. 1947. Factors producing high yeast yields in synthetic media. J. Bacteriol. **57**:235–246.

ROXBURGH, J. M., J. F. T. SPENCER, AND H. R. SALLANS. 1954. Factors affecting production of ustilagic acid by *Ustilago zeae*. J. Agr. Food Chem. **2**:1121–1124.

STROHM, J., R. F. DALE, AND H. J. PEPPLER. 1959. Polarographic measurement of dissolved oxygen in yeast fermentation. Appl. Microbiol. **7**:235–238.