$$\text{var } (a) = s^2\left[\frac{1}{n} + \frac{\bar{X}^2}{\sum X^2 - n\bar{X}^2}\right]$$

$$= 2.09\left[\frac{1}{20} + \frac{25.40}{530.97 - 20(25.40)}\right]$$

$$\text{var } (a) = 2.42$$

$$\text{cov } (ab) = -\frac{\bar{X}}{\sum X^2 - n\bar{X}^2}\, s^2$$

$$= -\frac{5.04}{530.97 - 20(25.40)}\, 2.09$$

$$\text{cov } (ab) = -0.461$$

In the same manner are found

$$\text{var}(a') = 0.0643$$

$$\text{var}(b') = 0.0007$$

$$\text{cov}(a'b') = -0.0065$$

Then substituting

$$\text{Var } (\theta) = (6.19)^2\left[\frac{2.42 + 0.0643}{(15.04 - 1.42)^2} + \frac{0.09 + 0.0007}{(-2.17 - 0.03)^2} + 2\,\frac{-0.461 - 0.0065}{(15.04 - 1.42)(0.032 + 2.17)}\right]$$

$$\text{Var } (\theta) = 0.046$$

Therefore the 95 per cent confidence interval for θ is

$$\pm 2\sqrt{.046}$$

$$\pm 0.42$$

An Air-Lift Laboratory Fermentor

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Since the advent of the technique of submerged fermentation, a number of fermentors have been described based upon one of the following principles: (a) Forced aeration without mechanical agitation; (b) forced aeration with propeller-type agitation; (c) forced aeration in a rotary drum; and (d) an air-lift pump which circulates the culture medium in a closed system (Scholler and Seidel, 1940). It is apparent that to carry out submerged fermentation with forced aeration alone usually is less effective than when combined with other means of agitation (Finn, 1954). However, the cost of mechanically agitated laboratory fermentors often may be prohibitive.

The laboratory-size fermentor described herein, a modification of Scholler and Seidel's plant-size apparatus (also described by Saeman *et al.*, 1945), was constructed to overcome some of the limitations with which the teacher or researcher is confronted when a conventional means of mechanical agitation is not available or is too costly. In Scholler and Seidel's fermentor, the tubes in which the culture medium was circulated were located outside of the main unit. It has been found more advantageous to construct the circulation tubes within the main unit; this simplifies construction, facilitates handling and sterilization, and is more efficient for small-scale operation.

Apparatus

Fermentation vessel. The laboratory fermentor consists of an inverted, wide-mouth, Pyrex solution bottle with accessory equipment (figure 1). Solution bottles of 1- to 5-gal. capacity have been used successfully. The following tube-lengths are for a 2½-gal. fermentor. The mouth of the bottle is fitted with a rubber stopper (size 12) containing four holes; the stopper is secured in the mouth by a simple metal clamp. Entering the fermentor through the stopper are four Pyrex glass tubes, as follows: (1) A 23-in. tube, 18-mm outside diameter, extends to within about 1 in. of the top of the bottle. Near the end of this tube outside the fermentor is attached, through a side arm, a ⅜-in. o.d. "Y" tube. (2) An 8-in. tube, 18-mm o.d., extends only as far as the inner side of the stopper. The two tubes (nos. 1 and 2) entering the fermentor are connected at their external ends by means of a heavy neoprene rubber tube (20-in. length, ¾-in. bore, ⅛-in. wall). This rubber tube allows free circulation of the culture medium, lessens the breakage problem and offers a means of breaking up (by squeezing the tube) masses of microbial growth which may retard circulation within the fermentor. Heavy twine encircling the rubber tubing ensures a smooth U-shaped bend. All hose-to-glass connections are made secure by tying with copper wire or by clamps. (3) A 23-in. tube, 8-mm o.d., with a 2-in. U-bend at the internal end to which a small funnel is attached, extends to within 2 in. of the top of the bottle, and serves as the air-outlet tube. The funnel deflects the falling spray of culture medium, thereby minimizing the amount of medium sucked out with the air exhaust. The level of the culture medium should be at

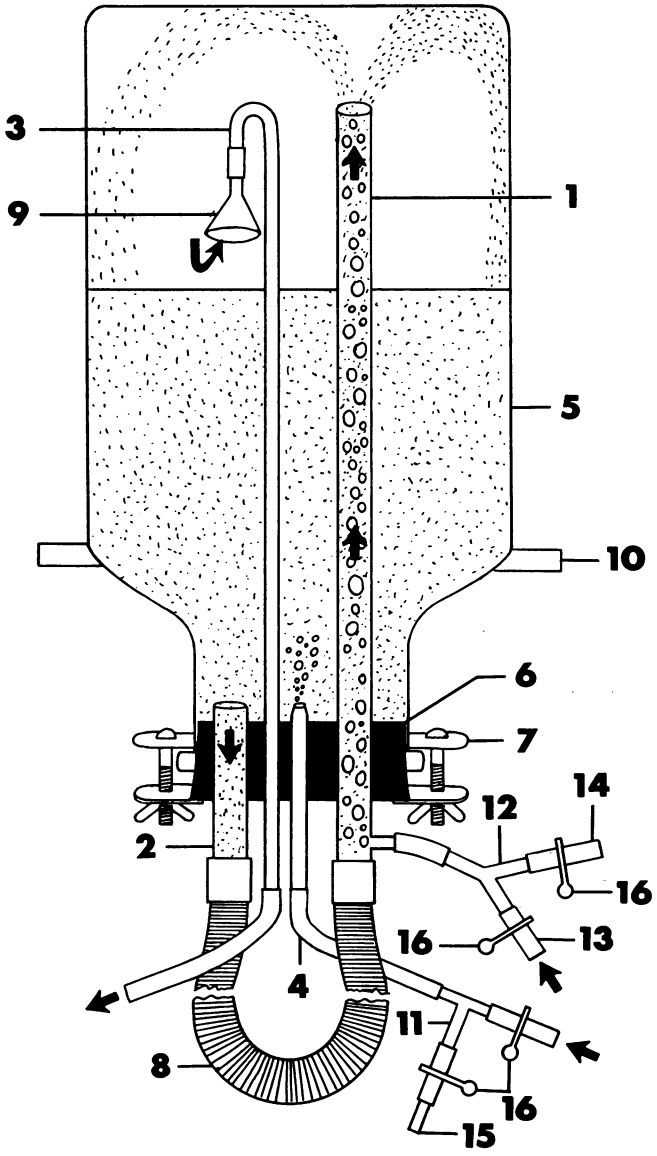

FIG. 1. Functional diagram of air-lift fermentor

1. Liquid lift tube
2. Circulation tube
3. Air outlet tube
4. Secondary air inlet tube
5. 2½-Gallon solution bottle
6. Rubber stopper
7. Stopper clamp
8. String-wound rubber tubing
9. Funnel (baffle)
10. Bottle support
11. T tube
12. Y tube
13. Primary air inlet tube
14. Inoculation port
15. Sampling port
16. Hose clamp

least two inches below the funnel. (4) A 5-in. tube, 8-mm o.d., which extends just above the stopper, serves as a secondary source of aeration and also prevents masses of growth from accumulating in the mouth of the fermentor. The tip of this tube may be constricted somewhat to produce smaller air bubbles. At the end of this tube outside of the fermentor is attached a ⅜-in. o.d. "T" tube.

Agitation and aeration. Sterile humidified air under pressure continuously forces the culture medium up tube No. 1; the air enters the column through the "Y" tube inlet. The forcible impingement of the liquid culture on the bottom of the bottle results in both mechanical agitation and aeration. A secondary source of air enters through tube No. 4. Compressed air, reduced to 5 to 15 lb per sq. in., is supplied by a conventional compressor. The air is first cleaned by passing it through a Koby air purifier, then sterilized by passing through sterile Kelly bottles containing glass wool, and finally humidified by passing through a sterile Selas bacteriological filter (porosity No. 10) into water. Such treatment prevents both contamination and evaporation of the culture medium. An aeration rate of 1.5 to 3.0 liters of air per minute per liter of medium has been found effective.

Sterilization. The fermentor, with its accessory parts such as air filter, humidifiers, and so on, is sterilized as a unit. All openings are plugged with cotton and capped prior to sterilization. The fermentation substrate ordinarily is sterilized within the fermentor; specific substances which require separate sterilization are treated individually, and can be added aseptically through the inoculation port. The size of the solution bottles may prevent sterilization in small-size autoclaves; in this laboratory it was found convenient to sterilize large units in an inexpensive vertical-type laboratory retort.

Inoculum. The inoculum is supplied to the fermentor from a 500-ml Erlenmeyer flask by means of a rubber hose connection through either the "Y" tube or the secondary air-supply tube (No. 4). The inoculum usually is added to the culture medium before the solution bottle is inverted. However, in cases where it is necessary to add the inoculum only after optimum culture conditions have been attained, inoculations are made, without discontinuing the operation, through the secondary air-supply tube (No. 4).

Operation. The air-lift type fermentor can be used for continuous, semicontinuous or batch fermentations. By proper construction and manipulation of the inlet and outlet hose lines, the substrate can be continuously added during the fermentation, or added at intervals. The same is true of substrate withdrawals.

TABLE 1. *Yields of microorganisms in 2½-gal. fermentor*

Organism	Cell Yields* 96 hours, 28 C (grams dry weight)
Ashbya gossypii	31.9
Penicillium chrysogenum	71.6
Fomes subroseus	25.6†
Saccharomyces cerevisiae	31.6
Chlorella vulgaris	16.7

* From 6 liters of medium seeded with 1 per cent inoculum.
† Incubated for 7 days.

Propagation Experiments

The following experiments illustrate the use of the fermentor for growing microorganisms; maximum yields were not the primary objective.

Propagation experiments were run with the following test organisms: *Ashbya gossypii*, *Penicillium chrysogenum*, *Fomes subroseus*, *Saccharomyces cerevisiae* and *Chlorella vulgaris*. All fermentations, except with *F. subroseus* (7-day incubation), were run for 96 hours at 28 C in a 2½-gal. fermentor containing 6 liters of culture medium. A nonsynthetic medium was used for the propagation of *A. gossypii*, *P. chrysogenum*, and *S. cerevisiae*, and contained the following components: Glucose, 4 per cent; corn steep liquor, 1 per cent; and proteose peptone (Difco), 0.5 per cent. The medium prior to inoculation was adjusted to pH 6.5, 5.5 and 4.5, respectively, for three organisms. *C. vulgaris* was grown in a synthetic medium containing $Ca(NO_3)_2 \cdot 4H_2O$, 1 per cent; KNO_3, 0.025 per cent; NaCl, 0.005 per cent; KH_2PO_4, 1 per cent; $MgSO_4 \cdot 7H_2O$, 0.05 per cent; $FeSO_4 \cdot 7H_2O$, 0.002 per cent; $Na_3C_6H_5O_7$, 0.05 per cent; and glucose, 2 per cent. The pH of this medium after autoclaving was 6.7. *F. subroseus* was grown in a synthetic medium containing glucose, 4 per cent; glutamic acid, 1.0 per cent; KH_2PO_4, 0.7 per cent; $MgSO_4 \cdot 7H_2O$, 0.25 per cent; plus the addition of common trace elements (Jennison *et al.*, 1955). The pH of this synthetic medium after sterilization was 5.2. One ml of Vegifat Y[1] per liter of culture medium was added to retard foaming.

The test organisms were grown in shake culture, in the same medium used for the final fermentation, to produce cells for inoculating the larger culture. A 1 per cent inoculum was used in each case. Blended pellets of mycelium were used as the inoculum with *P. chrysogenum* and *F. subroseus*. Cells were harvested by centrifugation, then dried at 60 C in a vacuum oven for dry-weight determination.

Table 1 shows the dry-weight yield (in grams) of cells obtained in the above media.

Discussion

Expensive fermentation equipment has previously been needed for basic experiments, studying pilot plant operation and fermentation scale-up in the teaching laboratory. Many institutions find it difficult to equip fermentation laboratories with enough apparatus for an average-size class to explore and solve problems common to the fermentation field. A simple and inexpensive apparatus is described that operates on the principle of an air-lift pump which circulates, agitates and aerates the culture medium in a closed system. The cost of this fermentor is approximately twenty dollars; a mechanically agitated fermentor of similar size is approximately two hundred and fifty dollars. The biological efficiency of an air-lift fermentor may, in many instances, be less than that of a mechanical-type fermentor, but its simplicity and low cost justify its consideration as an aid to teaching and research.

Data on typical propagation experiments with five organisms showed the cell yields to be relatively high. These compare favorably with yields in mechanically agitated fermentors. In the teaching laboratory, the air-lift fermentor has been used successfully to produce antibiotics, vitamins, and enzymes, and to obtain suitable amounts of microbial cells for chemical analysis.

The simple construction of the fermentor makes it applicable to semicontinuous as well as continuous fermentations. Temperature of the fermentor is readily controlled by operation in a temperature-controlled incubator, or, for higher temperatures, by applying a heating tape externally to the solution bottle. Propagation of obligate thermophilic bacteria at 55 C has been accomplished. The effects of various gas mixtures on microbial growth can readily be studied.

[1] Nopco Chemical Co., Harrison, N. J.

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Summary

A fermentor, operating on the principle of an air-lift pump which circulates the culture medium in a closed system, is described. It is simple in design, versatile, inexpensive, and can be made in various capacities. A variety of culture conditions can readily be controlled.

Typical experiments showing the usefulness of the fermentor for mass cultivation of microorganisms are reported. High yields were obtained with five test organisms.

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