AN IMPROVED LABORATORY-SCALE FERMENTOR FOR
SUBMERGED CULTURE INVESTIGATIONS

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A small laboratory fermentor which employs mechanical agitation for the dis-
\begin{flushleft}
\textit{persion}\end{flushleft} of air introduced under pressure has previously been described by Feustel
and Humfeld (1946). This fermentor has an operating capacity of 500 to 2,000
ml and has been found very useful for small-scale, yeast-culturing investigations,
as well as for studies on the production of subtilin activity by \textit{Bacillus subtilis}
(1947).

Certain limitations, however, principally those of capacity and effectiveness
of foam breaking, have led to the development of an improved fermentor having
a larger capacity and a more effective mechanical foam breaker. This fermentor
also has a stirring device, which is so designed that air for the aeration of the
culture liquid can be drawn in from the atmosphere by suction created behind the
stirring blades, as compared with air introduced under pressure. This paper
describes the improved fermentor and some of the preliminary results obtained.

DESCRIPTION OF THE FERMENTOR

The fermentor vessel consists of a standard pyrex glass jar, 12 inches in di-
ameter and 24 inches high. This jar is fitted with a gasketed, stainless-steel
cover. The stainless-steel agitation-aeration assembly shown in figure 1 is
suspended from the cover and is inserted in the pyrex jar.

Agitation and aeration are accomplished by means of a special air-dispersing
device, mounted at the lower end of the stirring shaft near the bottom of the
fermentor. Two adjustable truncated cones 3½ inches in diameter are mounted
on the shaft above the agitation-aeration device. By inverting these cones the
stirring characteristics may be changed. Four metal struts are attached at
right angles to the cover at equidistant points around the periphery of the cover,
approximately 1 inch from the edge. A metal web fastened to the lower end of
these struts furnishes rigidity and support for the stirring shaft.

The agitation-aeration device consists of a short, central, hollow cylinder, to
which four sets of tubes are fastened. These tubes are bent in the form of arcs at
right angles to the cylinder. A small vane is attached to each alternate set of
tubes. This device rotates between two plates. The upper plate is attached
to the supporting web described above. The lower plate is attached, and kept
parallel with, the upper plate by studs. The lower plate has a large circular
hole, through which the culture liquid enters. An air-intake pipe extends from
the cover to a point directly below the hollow, central core of the agitation-

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aeration device. As the turbine device rotates, the suction created behind the vanes at the end of the tubes draws air through the air-intake pipe into the central, hollow cylinder, from which it is conducted into the culture liquid by the radiating tubes. The height of the intake pipe is adjustable at the cover, so that the amount of air drawn in can be regulated. The maximum amount of air is drawn in with the lower opening of the intake pipe raised as high as possible without touching the revolving hollow cylinder of the turbine. Lowering the intake pipe decreases the air flow.

The foam-breaking device consists essentially of a disk mounted on the shaft just below a larger fixed cone. The disk, about 8 inches in diameter, is provided with an interior set of vanes and an exterior set of vanes; half of the vanes of each set are turned up and half are turned down. The outer edge of the cone extends just beyond the interior set of vanes. The inner set of vanes scoops up the foam as it rises to the height of the disk; the centrifugal force created by the rotation throws the foam against the inner surface of the cone, which, in turn, forces it out onto the upper surface of the disk, whose outer set of vanes dis-
tributes the liquid phase against the sides of the fermentor vessel. During this operation the foam is broken effectively, and the liquid is returned down the inside wall of the vessel to the culture liquid.

The fermentor is equipped with a set of pH meter electrodes, the leads of which pass through removable waterproof tubes to the pH meter; hence, the pH of the culture may be determined at any instant. A thermometer inserted through a small well provides for the reading of the temperature of the culture at any time. The fermentor is also provided with a sampling tube, by means of which a sample may be drawn periodically for analyses. The power for operation of the fermentor is furnished by a ½-hp ball-bearing, variable-speed, electric motor, mounted vertically at the center of the cover and fastened to the stirring shaft by a self-aligning coupling.

The entire fermentor assembly may be taken apart readily for cleaning and replacement and adjustment of parts. It may be assembled without the motor for sterilization. The operating capacity of the fermentor ranges from 10 to 18 liters of culture medium.\footnote{Detailed engineering drawings for the construction of similar units may be obtained from the Western Regional Research Laboratory, Albany, California.}

**OPERATION OF THE FERMENTOR**

The fermentor has been found suitable for the submerged culturing of aerobic microorganisms. It has been employed in the propagation of yeast, in which case sterilization of the media and of the equipment is not essential, as well as for the production of antibiotics. For the latter purpose the equipment and the culture medium are sterilized, since the maintenance of pure culture is usually essential. Except for the details of sterilization and care necessary for keeping the culture free of contamination, the technique of operation in all cases is essentially identical. The air drawn in is sterilized by passage through a previously sterilized tube loosely packed with glass wool.

The inoculated medium is transferred to the fermentor, the motor is placed in position on the cover, and the motor shaft and stirrer shaft are connected by means of the self-aligning coupling. The motor is started and the amount of air drawn in is regulated by adjusting the height of the air-inlet pipe by means of the adjusting screw on the fermentor cover. The air flow also may be controlled by adjusting the speed of the motor, which, of course, simultaneously changes the rate of stirring. For most operations a ratio of one volume of air per minute per volume of culture medium is satisfactory. By reducing the distance between the lower tip of the inlet tube and the hollow bore of the agitation-aeration unit, volumes of air as high as two and one-half times the volume of the culture medium may be drawn in per minute. As the volume of growth in the medium increases during the fermentation, the viscosity of the culture suspension gradually increases; hence, if it is desired to maintain a uniform rate of air flow, it is necessary to readjust the distance between the core and the inlet pipe. A record of the pH of the medium and its temperature is kept, and if it is desired to control the pH, a suitable amount of base or acid solution is added as may be required.
Since the fermentation usually generates considerable heat, it has been found desirable to place the fermentor in a suitable water bath and to hold the water in the bath at such a temperature as will maintain that desired in the fermentor. By means of the sampling tube samples are withdrawn periodically for determinations of cell volume, nutrient concentrations, and, in the case of the production of antibiotics, for the bioassay purposes. The fermentor in operation is shown in figure 2.

**FIG. 2. LARGE LABORATORY FERMENTOR FOR SUBMERGED CULTURE INVESTIGATIONS IN OPERATION**

**FERMENTATIONS**

_Yeast production._ An example of the use of the fermentor for yeast production is presented here. The yeast used in this run was _Torulopsis utilis_ (NRRL Y-900). The medium was made from pear juice concentrate from cannery pear waste. One liter of the concentrate, which contained 26 per cent sugar, was diluted initially with 12 liters of water. The mineral salts required—15.4 grams of 85 per cent phosphoric acid, 3.6 grams of potassium sulfate, and 1.0 gram of magnesium sulfate—were added.

The inoculum was prepared by transferring a 10-ml suspension from the growth of a stock culture slant to the surface of a shallow layer of wort agar in two Fernbach flasks. The flasks were incubated for 24 hours at 30 C. The growth on the agar was suspended in a small amount of the pear juice medium and, with the rest of the medium, transferred to the fermentor. The motor was operated at
1,160 rpm, and an air volume of 16 liters per minute was used for aeration at the start of the fermentation. Ammonium hydroxide was added to bring the pH to 6.4, and from time to time, as the pH dropped, more ammonia was added.

After the sugar in the pear juice medium at the start of the fermentation had been utilized, additional full-strength concentrate was added from time to time. The details of operation and the yield of yeast obtained are given in table 1.

Production of antibiotics. The feasibility of using the fermentor for the production of antibiotics was tested with a culture of B. subtilis. The volume of cells and the subtilin activity produced were measured in samples taken periodically during the fermentation run. The fermentor was assembled and sterilized in the autoclave for 1 hour at 15 pounds' steam pressure. The procedure was identical with that described by Stubbs et al. (1947) for the production of subtilin in small fermentors. The medium was made by diluting 1,400 grams of an asparagus juice concentrate (70 per cent total solids) to 14 liters. This medium was distributed in 3.5-liter portions in 4-liter bottles and sterilized for 30 minutes at 100 C in the autoclave. When cool the reaction of the medium was adjusted aseptically from about pH 5.5 to pH 7 by addition of the required amount of 10 N NaOH, then transferred aseptically to the fermentor, and the inoculum added.

The inoculum was made by suspending the growth from an agar slant in a small amount of medium and adding this suspension to 500-ml portions of the asparagus juice medium in each of two Fernbach flasks. These flasks were incubated

<table>
<thead>
<tr>
<th>TIME</th>
<th>pH</th>
<th>YEAST VOLUME</th>
<th>VOLUME IN FERMENTOR</th>
<th>PEAK JUICE CONCENTRATE ADDED</th>
<th>NH4OH ADDED</th>
<th>AIR</th>
<th>WEIGHT OF YEAST PRODUCED (DRY BASIS)</th>
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<tr>
<td>hr</td>
<td></td>
<td>%</td>
<td>liters</td>
<td>12 H2O</td>
<td>ml</td>
<td>liters/min</td>
<td>g</td>
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<tr>
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<td>13</td>
<td>16.5</td>
<td>14.3</td>
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<tr>
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<td>1.00</td>
<td>13</td>
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<tr>
<td>7 ½</td>
<td>3.3</td>
<td>3.00</td>
<td>13</td>
<td>16.5</td>
<td>94.4</td>
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<td>9</td>
<td>5.1</td>
<td>5.8</td>
<td>13</td>
<td>16.5</td>
<td>166</td>
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</tr>
<tr>
<td>9 ½</td>
<td>4.2</td>
<td>6.5</td>
<td>13</td>
<td>16.5</td>
<td>186</td>
<td></td>
<td></td>
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<tr>
<td>10 ½</td>
<td>3.5</td>
<td>7.5</td>
<td>14</td>
<td>16.5</td>
<td>231</td>
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<tr>
<td>11 ½</td>
<td>3.9</td>
<td>10.5</td>
<td>14</td>
<td>16.5</td>
<td>323</td>
<td></td>
<td></td>
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<tr>
<td>12 ½</td>
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<td>11.2</td>
<td>15</td>
<td>16.5</td>
<td>370</td>
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<tr>
<td>13 ½</td>
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<td>13.0</td>
<td>15</td>
<td>16.5</td>
<td>429</td>
<td></td>
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</table>

Total sugar supplied .......................................................... 780 g
Dry yeast produced ............................................................. 429 g
Yield of yeast (based on sugar supplied) ................................ 55%
Increase of yeast over inoculum ............................................ 148X
Average generation time ...................................................... 112 min
Number of generations ........................................................ 7.23
for 24 hours at 35 C; then the contents were transferred to a sterile Waring "blendor," and the bacterial pellicle was thoroughly broken up and suspended in the medium. This suspension was added to the sterile medium in the fermentor.

The initial motor speed was 1,300 rpm, and the aeration was 14 liters of air per minute. As the bacterial cells in the medium multiplied, the medium became more viscous and had a tendency to decrease the rate of aeration. The rpm were gradually increased until 1,600 rpm were attained; thus the rate of aeration was kept uniform. The pH at the start was 6.9 and gradually dropped to 6.15 in 5 hours, after which it gradually increased to 7.45 at 8 hours, when maximum cell volume had been obtained. The culture medium was harvested after 8½ hours' incubation at 35 C.

The bioassay for subtilin activity was made according to the method described by Lewis et al. (1947). The volume of the centrifuged cells calculated as percentage of the culture medium and the subtilin activity calculated as milligrams of subtilin per liter are shown in figure 3.

**DISCUSSION**

The evidence presented indicates that this type of fermentor is well suited for yeast production. The size is intermediate between that of the small laboratory...
fermentor described by Feustel and Humfeld (1946) and equipment such as might be used in a pilot plant. It is large enough to be used in the investigation of the effects of variations in stirring and aeration, and in foam-breaking devices. Its operation should yield certain information useful in the development of larger, or pilot-plant-scale, fermentors.

The fermentor described in this paper possesses another advantage over the one previously described by Feustel and Humfeld (1946) in that a source of compressed air for aeration is unnecessary. The agitator draws in the air required and disperses it simultaneously. However, the extent to which this manner of aeration might be applicable to larger units is not known.

This fermentor should prove particularly useful in building up inocula in sufficient amounts for pilot plant or commercial operation, as well as for testing variations in the culture medium.

For yeast production it is possible to maintain a high rate of production and a large volume of yeast in the fermentor by periodically withdrawing a portion of the culture whenever the available nutrients have been utilized and then adding fresh medium to replace the amount harvested. In this manner the effect of changing the composition of the medium, rates of agitation and aeration, and other variables may be studied under comparable conditions. Favorable or adverse effects will be reflected in cell volume, rate of growth, or both.

Yields of T. utilis that compare favorably with similar results reported in the literature have been obtained. For instance, Feustel and Humfeld (1946) found that in small fermentors the yield of yeast based on sugar supplied was 51.8 per cent, while in the larger fermentor it was 55 per cent. In the small fermentor the increase of yeast over inoculum was 32.8X in 10 hours, while in the larger fermentor it was 148X in 13½ hours. When calculated as average generation time, the figures were 104 and 112 minutes, respectively.

Results obtained in growing B. subtilis for subtilin production compare favorably with those obtained by Stubbs et al. (1947) in small fermentors. It was found that the growth rate was more rapid in the larger fermentor and resulted in a cell volume of 40 per cent, as compared with a cell volume of about 27 per cent in 8 hours in the small fermentor. The rate of subtilin production, however, was slower in the larger fermentor, especially during the period of 6- to 8-hours' incubation. Since it was not the objective in this study to determine the optimum conditions for maximum subtilin yields, the factors involved were not investigated.

It is evident that the fermentor also lends itself to investigations in pure-culture fermentation, such as the production of antibiotics. In most cases the quantity of available medium will make it possible to obtain data on actual yields of purified material.

ACKNOWLEDGMENTS

The author is indebted to Mrs. E. M. Humphreys for making the subtilin assay. Special recognition is accorded Mr. Ernest Aeschlimann and Mr. John R. Hoffman for valuable assistance in the mechanical development and construction of the fermentor.
SUMMARY AND CONCLUSIONS

A fermentor with an improved agitation-aeration device and a specially designed mechanical foam breaker is described. The agitation-aeration device is so designed as to pull in air for aeration at atmospheric pressure, thus eliminating the use of air under pressure. The foam breaker utilizes a specially designed disk and cone rotated at relatively high speed, which causes the foam to break under the centrifugal force generated under these conditions.

Data are presented which give results of a yeast \textit{(Torulopsis utilis)} propagation run and which indicate a method by which the fermentor may be utilized in continuous operation processes after a constant yeast volume has been attained.

The adaptability of the fermentor for the cultivation of \textit{Bacillus subtilis} for subtilin production has been indicated.

REFERENCES

