Laboratory Scale Activated Sludge Unit

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Recently, many investigators have turned attention to devices for continuous culture of microorganisms. In the basic sciences, the externally controlled “chemostat” of Novick and Szilard (1950) and Monod’s “bactogen” (1950) have found considerable usefulness in studies where fairly large amounts of cells were required and where it was desirable to maintain the population at a specific growth rate. Continuous culturing devices also find useful application in the sanitary engineering field. Garrett and Sawyer (1952), Schulze (1956), Garrett (1958), and Busch (1959) have made use of continuous flow bench scale activated sludge units.

The basic advantages and requirements for these units for use in sanitary engineering studies have been discussed by Coe (1952), and Hatfield and Strong (1954) and Busch (1959). The advantages lie primarily in similarity of operation to the prototype as compared with the dissimilarity of batch units. The requirements as set forth by Busch embody positive control of air flow, aeration solids, and solids wasting.

Regardless of the degree of control, extrapolation of bench scale data for the prediction of prototype operation is precarious. A more important need for such control is to insured that the sludge used for experimentation retains some degree of constancy throughout the investigation. Ideally, the most useful experimentation requires that the system remain constant as one variable is studied. This is one of the valid arguments for working with pure cultures. However, in applying biochemistry and microbiology to the treatment of organic wastes, a heterogeneous population should be used, since under natural conditions, such systems would evolve subject to the intrinsic selectivity embodied in the enrichment culture principle. To control the constancy of a heterogeneous population is a far more difficult problem than that presented by a pure culture since selection in a pure culture depends on random mutation which under normal conditions is a comparatively rare occurrence. Therefore, before confidently applying the chemostat principle to a heterogeneous population, it is necessary to obtain some assurance that system constancy can be at least roughly maintained or controlled.

A bench scale unit which is to be used for research on the activated sludge process must, in addition to maintaining sludge constancy, have a high degree of flexibility since the many modifications now in use render it no longer possible to deal with the activated sludge process per se. Although the biochemical principles of all successful biological treatment systems are similar, the various environmental conditions imposed by each modification make each unique in operating characteristics. It is not felt that continuous flow units should replace batch systems for sanitary engineering research, but it seems ideal that they be used to provide a source of biological solids for experimentation. Such experimentation may be carried out in batch or in continuous flow depending on the particular requirements of the research.

The present interest in continuous culturing devices arose during an investigation into the biochemical effects of a rapid change in the chemical structure of a waste during waste treatment by the activated sludge process. The term “qualitative shock load” has been adopted to distinguish this type of shock load from phenomena usually implied by the term “shock load.” To study these effects it was necessary to gain some assurance that, throughout the investigation, the population used for study would remain fairly constant in metabolic characteristics. It was proposed, therefore, to design and operate a continuous culturing device from which cells could be harvested for subsequent subjugation, in batch study, to a qualitative shock load.

The purposes of the present paper are to review briefly the principles upon which externally controlled continuous flow units are based, to describe the unit designed, and to present operational data and observations which may be useful to others interested in the use of such units for experimental purposes.

Fundamental Concepts

Kinetic treatment of continuous flow units has been developed in essentially three fields. Denbigh (1947), in considering the kinetics of steady state polymerization, points out that the mathematical simplicity of completely mixed continuous flow apparatuses makes the study of steady state systems more attractive than
discontinuous or semidiscontinuous systems. Also in
the field of physical chemistry, Branson et al. (1949)
used a similar kinetic development for continuous
crystallization operations and verified the results of
steady state systems using batch studies to control
and correlate operational variables.
In the biological field, Novick and Szilard (1950)
and Monod (1950) used identical kinetic developments
to describe the steady state kinetics of continuous
culturing devices.
In the sanitary engineering field, Garrett and Sawyer
(1952) and Schulze (1956) showed identical kinetics
for control of laboratory scale activated sludge plants.
Later, Garrett (1958) recommended use of these kinetic
principles as a means of "hydraulic control" for the
operation of a full scale activated sludge plant.
In relation to Garrett's recommendation, it should
be noted that the kinetic development of continuous
flow systems requires complete mixing in the aerator.
Although most systems tend toward this condition, it
does not rigidly hold for long rectangular aeration tanks.
Also, the new steady state which may be set up in
response to external changes such as feed concentra-
tion, rate of flow, and temperature may not always be
the most desired one. In addition, the chemical com-
position of the waste stream may change, which may
affect the predominance of bacterial species and the
rate of sludge growth. Therefore, the principle may not
be applicable to activated sludge plants. However, it
may be applied to industrial fermentations where these
variables may be subject to close control.
Concerning the activated sludge process, the principle
is of value in providing the experimenter with a theo-
retically sound laboratory apparatus which can be
subject to controls not easily attained with the full
scale plant. The development of system kinetics is the
same for both the chemostat and the laboratory scale
activated sludge plant.
However, there is a difference in the apparatus, that
is, the incorporation of a sludge return line for recycling
thickened sludge to the aerator, which is a salient fea-
ture of the activated sludge process. This does not af-
tect the theoretical operation but does have an effect
on the over-all rate of sludge growth. If the rate of
sludge return is constant, the effect on the growth rate
is constant and does not directly enter into the
considerations.
Novick and Szilard (1954), Spicer (1955), and Moser
(1958) have dealt with the development of the kinetic
theory of continuous flow units in considerable detail.
The brief presentation below may serve to bring into
focus the essential identity of the kinetic fundamentals
employed in the three fields previously cited.
In an undiluted (batch) biological system, the general
kinetic expression for bacterial growth is:
\[ \frac{dN}{dt} = KN \]  
that is, growth follows first order kinetics wherein the
change in population is proportional to the amount
present at any time. In a diluted (continuous flow)
system this equation does not hold since the over-all
change in solids production is affected by the continu-
ous dilution provided by the incoming flow of substrate.
In a completely mixed system, biological solids are
carried out of the tank at a rate equal to the inflow rate,
Q, thus diluting the solids concentration by the factor
\( Q/V \), that is, the ratio of the inflow rate to the fixed
volume of the tank. Under these conditions, system
kinetics may be expressed as:
\[ \frac{dN}{dt} = KN - \frac{Q}{V}N \]  
(2)
In the steady state \( \frac{dN}{dt} \) is zero. Therefore, trans-
posing and cancelling like terms, it is seen that the
proportionality factor \( K \), the growth rate constant) is
wholly a function of the hydraulic rate of flow \( Q \), that
is,
\[ K = \frac{Q}{V} \]  
(3)
which states that \( K \) is equal to the reciprocal of the
detention time, \( T \) (\( T = V/Q \); mean residence time).
Internally controlled devices usually employ some
method of measuring cell density in the aerator. A
photoelectric cell is commonly used; it responds
to changes in cell density and can be made to control the
rate of feed flow, thus controlling \( K \). However, it is the
externally controlled unit which applies more directly
to the activated sludge model. More precisely, it is the
fact that a continuous culturing device can be ex-
ternally controlled which makes its application to the
activated sludge process possible.
Under conditions of external control \( Q/V \) is held at
any desired value below \( K_{max} \). This is essential since if
\( Q/V \) were greater than \( K \), the solids would be diluted
out and if it were equal to \( K \), the system could not
respond to an increase in organic feed concentration.
This aspect is particularly important concerning ef-
fluent control in the activated sludge system. If the ef-
fluent biochemical oxygen demand (BOD) is to be
subject to hydraulic control, the system must be poised
to respond successfully to any possible increase in in-
fluent BOD concentration. One such response is an
increase in the number of organisms metabolizing the
waste. This can be accomplished artificially by returning
more sludge to the aerator or naturally, according
to equation (2), by increasing the growth rate. If the
growth rate is already at its maximum, that is, \( K_{max} =
Q/V \), the natural response is impossible.
\( K \) can be externally controlled through the design
of the substrate. All essential nutrients except one can
be provided at nonlimiting concentrations. The limit-
ing or controlling constituent, depending on the system
studied, may be an amino acid, a nitrogen source, phos-
phorus, sulfur or, as in the case most applicable to the
study of the activated sludge process, the energy source, which may be expressed as the pollutional strength or BOD of the waste.

Under these conditions $K$ becomes a function of $C_n$ the concentration of the controlling nutrient and equation (3) is modified to:

$$K(C_n) = \frac{Q}{V}$$

(4)

The rate of sludge growth is thus controlled by the rate of flow, the physical boundaries of the aerator, and the substrate composition. With a constant sludge recycling rate, the cell density, $N$, is accordingly fixed at any specific steady state condition. Under defined experimental conditions, the factors affecting such a steady state condition can be varied and studied in a controlled manner. Conversely, if all controlling variables are held constant, it would seem possible that the sludge produced should maintain some degree of reproducibility or constancy in its metabolic activity.

Although there may be valid objections to the applied use of this principle as a field control device for full scale activated sludge processes, it should find extensive and valid use in controlled laboratory experimentation aimed at delineating fundamental phenomena and effects of changes in operational variables, both of which will increase understanding of the biological treatment processes.

**Experimental Methods**

*Description.* The experimental unit is shown in figure 1. Organic and inorganic constituents are fed separately from 4-L and 11-L reservoirs. Both tanks are petcock-controlled. A constant hydrostatic head is maintained through the use of the air inlet tube shown in the diagram. Successful operation requires an air-tight seal. To insure that air bubbles are not drawn into the siphon, the air inlet tube should be at least $\frac{1}{4}$ in. above the siphon inlet. This device has been recommended by Hoover (1951) for maintaining a constant feed rate at minute flows. It was found to be wholly satisfactory at the low flow rate used during the present experimentation.

The aerator is made from a 4-L Erlenmeyer flask cut as shown and fitted with $\frac{3}{4}$ in. diameter outflow

![Figure 1. Continuous feed activated sludge unit](http://aem.asm.org/)

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pipe set to provide a 2-L volume of aerated liquor. Aeration is provided by compressed air introduced through two carborundum diffusers.

The sludge return-settling tank and accompanying sludge return air lift were included to incorporate the characteristics of the activated sludge process. If the unit is to operate as a true chemostat, these components are not needed. However, since the return of thickened sludge will exercise a pronounced effect on activated sludge systems, it is ideally included in a laboratory unit designed for such research.

The sludge return-settling tank consists of a 4-L Erlenmeyer flask, cut as shown and fitted with outlet and inlet devices. The operating capacity of the tank is 2 L. The tank bottom is rubber-stoppered and fitted with an air inlet tube and sludge draw-off tube. This unit serves to partially thicken the sludge and provides a means of daily wasting a fixed volume of thickened underflow. It also provides a reservoir of thickened mixed liquor for return to the aerator.

The sludge return air lift serves to reaerate the thickened mixed liquor and to return a portion of it to the aerator. The sludge reaeration line was incorporated into the design to insure proper control of the return flow and the hydraulic head in the sludge distribution hopper. It also provides reaeration and recirculation of the flocculated cells. All compressed air enters the aerator and sludge return line through the liquid and dry traps shown.

Standard waste. The basic unit was designed to provide an external and known control on system kinetics. The operational techniques and selection of the standard waste were designed to enhance this control, to provide an experimental population which would remain fairly constant through the investigation, and to simulate the heterogeneous population in an activated sludge aerator.

The continuous flow unit was seeded with 200 ml of settled domestic sewage and diluted to 2 L with organic and inorganic feeds. Microscopic examination revealed a heterogeneous population consisting of many morphologically different bacteria and a moderately large and varied protozoan population. Filamentous forms were present but not abundant. No algae or diatoms were observed. The system was batch operated for 1 week while the solids built up and thereafter as a continuous flow unit.

In designing the organic feed, consideration was given to the carbon source which consisted solely of glucose, fed at a concentration of 1,000 mg per L. This concentration was chosen since it falls well within the range of concentrations treatable with the activated sludge process and because it is low enough to provide an external control for the unit. Glucose was chosen in order to develop a population which might be expected in activated sludge and since it was desirable to use a compound readily used by all aerobic organotrophic bacteria.

It should be recognized that any waste treatment system will function as an enrichment culture for the organism(s) best suited to the particular environment created by the physical conditions of the process and the chemical composition of the waste (Gaudy, 1955). An aerobic system fed glucose, an inorganic nitrogen and phosphorous source, and inorganic constituents in proper concentration, will foster the predominance of aerobic organotrophs, in particular the pseudomonads. The inorganic constituents and their concentrations, shown in table 1, were based on cultural requirements found to be optimum in the bacteriological literature and were in general present in excess concentration. This was necessary to insure that the external control would be the carbon source only. The BOD/N and BOD/P ratios found in the sanitary literature were not maintained because these are minimal requirements empirically determined on the basis of economical BOD removal. Although they can be defended on a theoretical basis, it cannot be unequivocally stated that they will not limit a biological system.

**Loading parameters.** The activity of an aerobic culture may be measured on the basis of oxygen used per unit cell mass per unit of time. This is often expressed as

$$Q_{O_2} = \mu \text{liters } O_2 \text{ uptake/mg dry weight of cells/hr}$$

This same parameter is also used to express the loading of a biological treatment process

**Loading** = 1 lb 5 day BOD/1000 lb suspended solids/hr

Although different interpretations may be placed on these two expressions, they are nevertheless fundamentally similar. Use was made of the latter expression in checking the efficiency of the model activated sludge system. The former expression was used to check the activity of the sludge in the unit.

**Loading chart for various solids concentrations.** The system was operated at a constant volumetric loading of 2 L per day. However, due to periodic withdrawals...
for various experiments, it was expected that the solids concentration would vary from time to time.

It was desirable therefore to construct a loading chart for the plant so that its efficiency could be readily measured at any time a sample was taken.

The basis for calculations is as follows:

Theoretical BOD = (1000) (192/180)(0.7) = 750 mg/L
where 0.7 = fraction of total BOD exerted in 5 days at 20°C (k taken as = 0.1).

**BOD loading.**

\[ 2 \text{ L at } 750 \text{ mg/L} = 1.5 \text{ g 5-day BOD/day} \]
\[ 1.5 \times 1/453.6 = 0.00332 \text{ lb BOD/day} \]

**Design volume.**

(a) Aerator volume = 2 L
(b) Settling-return tank volume\(^3\) = 2 L total

A table of loadings at various values of aeration solids and a sample calculation are shown in table 2. The information is shown graphically in figure 2. System efficiency can be easily computed from BOD or chemical oxygen demand (COD) data and compared with generalized or average findings readily available in the literature for municipal activated sludge plants (Imhoff and Fair, 1956). It should be noted that the efficiency of substrate removal was measured using COD results and that the effluent tested was "membrane filter effluent" obtained in conjunction with biological solids measurement using the membrane filter technique (Engelbrecht and McKinney, 1956). Therefore, the system was tested as to its biochemical efficiency rather than over-all efficiency which includes settleability of the aeration solids. This was desirable because of the nature of subsequent experiments for which the sludge was developed (not herein reported). However, in applying the principles involved in hydraulically controlled continuous flow units, the more standard methods of measurement may be employed with equal facility.

**RESULTS**

**System efficiency.** Operational results for 3 months are shown in table 3. The observed efficiencies are some-

![Figure 2. System loading at various solids concentrations](http://aem.asm.org/)

**TABLE 3**

<table>
<thead>
<tr>
<th>Date</th>
<th>Biological Solids (mg/L)</th>
<th>COD Remaining (mg/L)</th>
<th>Theoretical BOD Remaining (mg/L)</th>
<th>Loading*</th>
<th>System Efficiency</th>
<th>Expected Efficiency</th>
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<td>126</td>
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</tbody>
</table>

* May be obtained from figure 2; pounds 5 day BOD/1000 lb suspended aeration solids/hr.

† Approximate efficiency to be expected at the stated loading (Imhoff and Fair, 1956).

**TABLE 2**

**System loading at various solids concentrations**

<table>
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<tr>
<th>Aeration Solids (mg/L)</th>
<th>Calculation</th>
<th>Loading*</th>
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<tr>
<td>250</td>
<td>lb BOD/day</td>
<td>62.8</td>
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<tr>
<td>500</td>
<td>31.4</td>
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<tr>
<td>750</td>
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</tr>
<tr>
<td>2000</td>
<td>7.9</td>
<td></td>
</tr>
</tbody>
</table>

* Pounds 5 day BOD/1000 lb of suspended aeration solids/hr.
what lower than those predicted for domestic treatment plants. Had the calculations been based on a 2-L volume under air (the aerator volume), these values would have coincided more closely. The point to be emphasized is that the organic removal remained fairly constant throughout (80 to 87 per cent removal) which in some degree substantiates the conclusions of Garrett concerning hydraulic control of BOD removal.

**Sludge activity.** The measurement of system efficiency is in some respects a measure of sludge activity. However, it is a measure of sludge activity in a limited system controlled by the hydraulic rate of a feed used in such concentration that the maximal growth rate is not attained. The \(Q_{O_2}\) measurement is made in an undiluted batch system under respiring conditions at a sufficiently low cell concentration or high substrate concentration to insure that substrate is not limiting. With sufficient agitation, the manometric measurement of \(Q_{O_2}\) should yield that uptake attributable to the intrinsic nature of the cells rather than to the system in which they are grown. The absence of an extracellular nitrogen source other than that of the atmosphere also tends to remove the complicating factor of cell replication during the test.

The \(Q_{O_2}\) values obtained are shown in figure 3. The values are fairly closely grouped but are by no means identical. The most probable reason for this is discussed below.

**Operational observations.** While the constant-head tank and petcock arrangement provided an excellent feed control, it was found that the organic feed orifice required close surveillance since the aerosol produced in the aerator provided ample seed for growth at the orifice thereby altering its hydraulic characteristics and thus its flow. Flow rates were checked for both feed lines three to five times daily during the first month of operation and daily thereafter. The effluent volume was also measured as a check. Early in the work, losses varied from 20 to 40 ml per day. During the more humid periods prevalent in the latter part of the investigation, an evaporative loss was not detectable.

During the first few weeks of operation, it was found that if the pH were allowed to stay below 6 for 24 hr the flocculating characteristics of the system were decreased and the color of the aerator culture turned from greenish-yellow characteristic of the pseudomonads to a more gray appearance, indicating a change in predominant species. Microscopic examination did not reveal any readily discernable morphological differences in the species present, but there was considerably less aggregation of the cells. After pH adjustment, 48 hr were required to restore the system to its usual appearance. It should be noted, however, that during a period when pH was purposely held low there was no apparent difference in the substrate removal efficiency of the system. It would seem entirely possible that a slight change in the pH could cause a large change in species predominance but might not affect the biochemical efficiency of the system. In substantiation of this view, Sawyer et al. (1955) have reported very little difference in BOD removal efficiency at widely differing pH values. However, since a varying pH might be expected to effect some changes in biochemical response in subsequent experiments with the sludge, the pH was adjusted to 7 whenever it approached 6.7.

One aspect of operation that militated against successful application of external control principles was the proliferous amount of growth found adhering to the aerator walls. True hydraulic control requires that the culture be wholly suspended. Some investigators have employed a "windshield wiper" arrangement to prevent accumulation of side growth (Novick, 1955). Having observed the operational characteristics of the unit for some time, it is felt that one of the prime causes for side growth in this unit involves the relatively small amount of agitation near the wall of the tank. Vigorous aeration was maintained at all times, but the shape of the aerator militated against vigorous agitation at the bottom and lower portion of the walls of the tank. Although this was ideal in assisting to simulate the flocculated growth prevalent in the activated sludge process, there was not sufficient agitation to slough the side growth. The effects of side growth, although very important in the control of continuous flow bench scale units, are probably insignificant in a full scale plant since the ratio of tank surface to liquid volume is decreased. However, in applying the principle of hydraulic control to the activated sludge process, the settling tank must be considered and a specific amount of sludge wasted. Therefore, the development of sludge pockets in the settling tank, which is essentially identical in effect with side growths, must be avoided.

The above comment illustrates what is believed to be a rather important point concerning the reconciliation of phenomena observed in small scale laboratory units as compared to large scale pilot plants and full scale plants. Although some of the physical peculiarities

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**Figure 3.** Variability in metabolic activity of sludge produced in activated sludge unit.
of each may be different, they may have formally analogous counterparts which if recognized can assist in analyzing data obtained using either approach to sanitary engineering research.

Settling characteristics of the sludge were extremely variable, ranging from 74 to 90 per cent compaction in 30 min. Settling tests were not run regularly on the unit since this data was of no immediate concern to the investigation. The effluent was always observed to be turbid. It was noted, however, that this turbidity could be sharply decreased by gentle stirring of the effluent followed by 1 hr quiescent settling. Microscopic observations of the culture were made approximately twice weekly. It is rather surprising that although the original sewage seed contained a moderate amount of filamentous forms, these forms were never observed except in trace amounts in the experimental system. The system appeared to consist wholly of flocculated and individual bacteria with varying numbers of free swimming and attached protozoa. No attempt was made to control temperature; however, it was measured on the average of 2 times daily and found to vary from 19 to 23 C.

**Summary**

It is concluded that the unit described provides a fairly constant biological system based on organic removal efficiency and sludge activity. Analysis of the results of the response studies for which these cells were used also substantiated this conclusion (Gaudy, 1959). The unit is not identical with the “chemostat” or “bactogen” after which it was modeled because the return of sludge, which was necessary to simulate an activated sludge treatment process, provided a partial internal control of growth rate. The return of sludge allows a higher population to exist than the energy source would permit without recirculation. This situation is characteristic of most activated sludge systems. However, with a constant amount of wasted sludge and effluent, the over-all characteristics and the mathematical validity of the concept are identical for the units described in the biological literature, the one used here, and that described by Garrett (1958) in the sanitary literature. The unit recently described by Busch (1959) also functions in the same way.

If the unit, or a modification of it, is used for the various studies for which it may usefully serve, as much automation as possible should be incorporated into its design. The incorporation of a wall scraping device would considerably increase the cost of the unit; however, the elimination of side growths would assure a more constant sludge activity since it prevents retention of older cells. The continuous flow model plant, operating on the chemostat principle, is considered to be sufficiently validated for use in sanitary engineering research to warrant more elaborate construction and inclusion of such automation as positive and continuous removal of side growth and continuous pH and temperature control.

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