AN ABSTRACT OF THE THESIS OF

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Title: The Effect of Dissolved Oxygen Concentration on the Activated

Sludge Process

Abstract approved: **Redacted for privacy**
Kenneth J. Williamson

Since the introduction of pure oxygen activated sludge systems in the late 1960's, proponents have claimed several advantages resulting from the elevated dissolved oxygen (DO) levels maintained. However, numerous comparative studies have failed to produce a consensus of professional opinion regarding the validity of these claimed advantages.

The purpose of this study was to evaluate fundamental theories that might explain the effects of elevated DO levels, to assess the validity of claimed advantages for pure oxygen systems, and to provide guidance regarding appropriate DO levels in activated sludge systems.

A literature review revealed that dispersed bacterial cells respond to DO, as measured by respiration rate or intracellular concentrations of metabolites, only at very low concentrations (less than about 0.1 mg/l). Consequently, the occurrence of oxygen diffusional limitations in large bacterial floc and the development of an anoxic core was preliminarily identified as the only reasonable theory for explaining effects of high DO levels.

The first phase of this study involved the development of

dispersed bacterial cultures in chemostats. Results indicated no significant difference in yield, kinetic coefficients, or specific ATP content between chemostats operated at a low DO level (2.0 mg/l) and a high DO level (15 mg/l). These results confirmed that differences in activated sludge characteristics that apparently result from the maintenance of high DO levels can only be attributed to diffusional limitations.

The second phase of this research utilized laboratory-scale activated sludge systems. A simple respirometric procedure was utilized to evaluate the response of respiration rate to DO concentration for these flocculant cultures and plots of specific respiration rate versus DO concentration were developed. These curves were subsequently described in terms of the DO at which the respiration rate was 50 percent of the maximum ($C_o^{0.5}$) and the DO at which the respiration rate first reached the maximum (C_o^c). Values for $C_o^{0.5}$ ranged up to about 1.0 mg/l and for C_o^c up to about 10 mg/l for culture samples with maximum floc diameters of 1200 microns and respiration rates up to 200 mg O_2/g TSS-hr.

The final phase of this research involved respirometric tests on mixed liquor samples obtained from three full-scale activated sludge plants. Results indicated that the critical DO (C_o^c) was typically less than 1.5 mg/l, even at higher respiration rates resulting from the treatment of high-strength industrial wastewaters. Floc size measurements indicated that flocs in full-scale plants rarely exceed 300 microns in diameter. It was concluded that oxygen diffusional limitations typically do not occur at full-scale activated sludge plants if aeration basin DO concentrations are maintained above about

1.0 mg/1.

The Effect of Dissolved Oxygen Concentration
on the Activated Sludge Process

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THE EFFECT OF DISSOLVED OXYGEN CONCENTRATION

ON THE ACTIVATED SLUDGE PROCESS

CHAPTER I

INTRODUCTION

Wastewater treatment commonly utilizes biological processes in
which microorganisms degrade and assimilate dissolved and suspended
organic material. The activated sludge process, the most common bio-
logical process, is involved in the treatment of a major portion of
domestic and industrial wastewaters. Basically, this process involves
the development of a concentrated, suspended, mixed culture of micro-
organisms that is added to the incoming wastewater, aerated and re-
moved from the wastewater by settling. Since its inception over 50
years ago, however, the effect of various system variables have been
largely unclear.

One primary system variable is the dissolved oxygen concentration
(DO) maintained in the aeration basin. Considerable research has been
directed at the effects of DO without reaching any consensus on its
significance. Within the past decade, the application of pure oxygen
to the activated sludge process has fueled a controversy over the ef-
fects of DO. Pure oxygen activated sludge systems maintain a higher DO
(typically 5-10 mg/ℓ) than air systems (typically 1-2 mg/ℓ). Advocates
of pure oxygen systems claim several advantages, including:

  1)   increased metabolism rates,

  2)   decreased sludge yields, and

  3)   a higher degree of settleability.

These advantages to the use of pure oxygen have not been confirmed by

most laboratory studies, but seem to be supported, particularly with respect to the claim of improved settleability, by the now larger compilation of data from full-scale plants. Furthermore, recent studies at Oregon State University have shown a significant difference in the microbial viability as indicated by ATP content between laboratory activated sludge systems operated at different DO levels.

The purpose of this research was to delineate the effects of DO on the activated sludge process and to help resolve this controversy. The specific objectives of this research were originally formulated as:

1) To delineate those conditions under which DO has an effect on activated sludge operational characteristics and process performance.

2) To determine the fundamental mechanism by which increased ATP viability may occur in activated sludge under elevated dissolved oxygen concentrations.

3) If appropriate, to utilize the findings from the above two objectives to modify existing steady-state mathematical models of the activated sludge process so that effects of DO may be predicted.

4) To evaluate the effect of maintaining various dissolved oxygen levels in typical full-scale activated sludge plants, to determine the minimum DO needed to prevent oxygen limitations, and to evaluate the advantages, if any, of maintaining the higher DO levels typical of pure oxygen activated sludge systems.

The results of this study were anticipated to have important implications in two areas. First, it was anticipated that these results

would enable the wastewater treatment plant designer to make a rational choice between air and pure oxygen activated sludge processes in the treatment of a given wastewater. Second, it was anticipated that this study would provide the activated sludge plant operators with a rational basis for setting the dissolved oxygen concentration in aeration basins so that oxygen limitations are prevented and maximum aeration efficiecies are maintained.

CHAPTER II

LITERATURE REVIEW

THE EFFECTS OF DO ON MICROBIAL METABOLISM

The effects of dissolved oxygen concentration (DO) on microbial metabolism has been studied by engineers and scientists in environmental engineering, biotechnology, microbiology and biochemistry. Applicable research from these fields is discussed below. The studies discussed are presented in approximate chronological order to provide a historical perspective as well as the existing state of knowledge.

Microbiological/Biochemical Research

Beginning in the 1920's, under the initiation of Warburg, several studies into the effects of DO on the respiration rate of various pure culture bacteria were undertaken. Most notable among these studies were those of Warburg and Kubowitz (1929), Meyerhof and Schulz (1932), Shoup (1929), and Cook (1930). Manometric techniques were used to measure oxygen consumption and, thereby, the specific respiration rate. The generally agreed upon conclusion of these studies was described by Shoup (1929):

> "In general, organisms such as protozoa or bacteria are
> small enough to allow complete diffusion of gases dis-
> solved in the medium and consume oxygen at a constant
> rate independently of oxygen pressure until the oxygen
> concentration falls to a point [the critical oxygen
> tension] allowing incomplete activity of the respira-
> tory mechanism within the cell. Only then does the rate
> of oxygen consumption diminish, and in many forms this
> limiting value for adequate respiration will be very
> small indeed . . .".

Due in part to limitations of the respirometric technique, however,

accurate measurements of the critical DO were not possible. Values

reported for various microorganisms ranged from 0.0025 to 0.5 mg/ℓ.

(See Harrison, et al., 1969, and Harrison, 1972 for reviews.)

Longmuir (1954) attempted to refine the results of these early

studies by utilizing a polarographic oxygen probe for measurement of

the DO. The respiration rate versus DO curves for nine species of bac-

teria grown in batch culture on 1% glucose were found to follow

Michaelis-Menten kinetics. The half-velocity coefficient for oxygen,

K_O, was found to be related to bacterial size, varying from 0.001 mg/ℓ

for Aerobacter aerogenes with a diameter of 0.6 microns to 0.10 mg/ℓ

for Bacillus megatherium with a diameter of 4.0 microns. It was con-

cluded that there was a diffusion limitation in the larger organisms,

resulting in a larger concentration gradient between the bulk liquid

and the location of the respiratory system. More importantly, however,

the half-velocity coefficient for oxygen was shown to be very small for

a wide range of bacterial species.

Within the last decade, it has been increasingly recognized that

the effects of DO on microorganisms can only be appreciated with an

understanding of the roles that oxygen serves in the chemical metabo-

lism of microbial cells. Harrison (1972), in an excellent review ar-

ticle, listed the functional roles of oxygen as: 1) electron acceptor,

2) inhibitor, 3) substrate for oxygenases, and 4) enzyme regulator.

The acceptance of electrons from a terminal oxidase to generate

utilizable energy in the form of ATP is the primary and long-recognized

role of oxygen in microbial metabolism. Considering only its role as

an electron acceptor, the response of microorganisms to DO has tradi-

tionally been modeled by Michaelis-Menten kinetics (Longmuir, 1954).

The K_o value for oxygen has been found to be so low, however, that accurate measurements are extremely difficult, even with sensitive dissolved oxygen probes.

As an inhibitor, oxygen is toxic in even small concentrations to most strict anaerobes. However, toxicity to aerobic microorganisms has been demonstrated reproducibly only at hyperbaric oxygen partial pressures (Harrison, 1972).

The incorporation of oxygen directly into organic substrates by oxygenase enzymes is an important mechanism in the aerobic metabolism of aromatic compounds, polyunsaturated fatty acids and aliphatic hydrocarbons. An example of this role in bacteria is the pathway by which the aromatic ring of phenol is broken:

$$\text{Phenol} + NADPH + H^+ + O_2 \xrightarrow[\text{Hydroxylase}]{\text{Phenol}} \text{Catechol} + NADP^+ + H_2O$$

$$\text{Catechol} + O_2 \xrightarrow{\text{Pyrocatechase}} \text{cis, cis Muconic Acid}$$
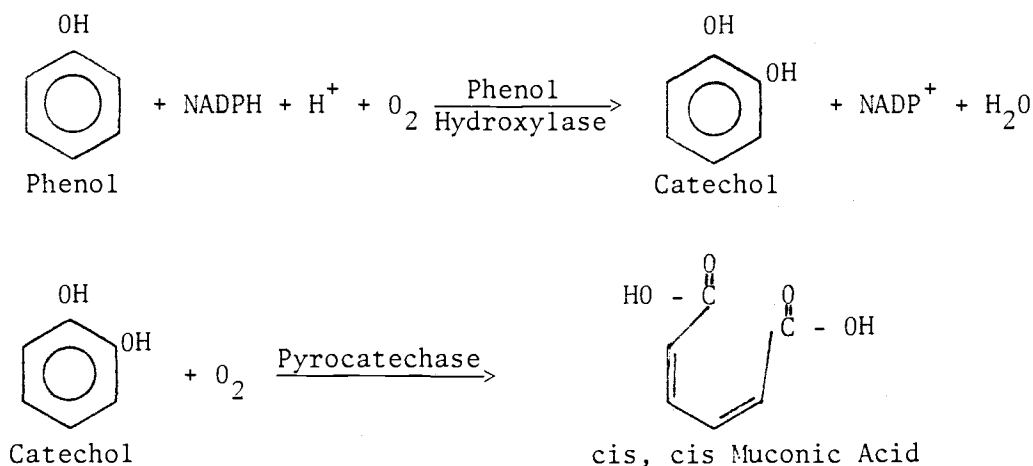
The affinity of oxygenase enzymes for oxygen varies and may be substantially less than that of the terminal oxidase (higher K_o). For example, the K_o for pyrocatechase was reported as 20 μM or 0.64 mg/ℓ for bacteria in the Pseudomonas genus (Hayaishi, et al., 1975).

Klein, et al. (1979) reported that the respiration rate in phenol degradation by free suspended cells of the yeast Candida tropicalis was

oxygen limited below a DO of 5 mg/ℓ. The data suggested a half-velocity coefficient for oxygen, K_o, of approximately 0.7 mg/ℓ, which is very similar to the value reported for the free enzyme and suggested that the pyrocatechase reaction may be the rate-limiting reaction in phenol degradation by microbial cells. In contrast, Englande and Eckenfelder (1973) reported a critical DO for phenol degradation by a mixed, flocculant bacterial culture as less than 0.5 mg/ℓ.

Using extremely accurate DO probes, recent microbiological research has focused upon the effects of very low DO levels (less than 0.1 mg/ℓ), and the changeover from aerobic to anaerobic metabolism in facultative bacteria. It is in this range that enzyme induction and repression effects have been observed, particularly of the cytochromes. Changes in metabolic pattern have been noted among facultative bacteria, resulting in the use of different fermentative pathways and production of different metabolic products. Harrison (1972) summarized much of the data on changes in cytochrome contents with changes in DO near or below the critical level. The result of these changes in the cytochrome respiratory chain of bacteria appears to be an increase in the affinity for oxygen (lower K_o), allowing aerobic metabolism to continue at very low DO levels. Changes in cytochrome contents at critically low DO levels appear to be common among facultative bacteria, but the type of change, degree of change, and effects associated with the change are not consistent between species. For example, respiration rates have been reported to both increase and to decrease as the DO is brought below the critical level. Also, there is disagreement over whether the efficiency of ATP generation is affected (Rice and Hempfling, 1978)(Meyer and Jones, 1978).

Research in Environmental Engineering

Early microbiological research into the effect of DO on micro-organisms indicated that the DO had no effect on the respiration rate of bacteria as long as it was maintained above a very low, critical level (less than 0.1 mg/ℓ). In the environmental engineering community, these results were generally interpreted as indicating that the DO maintained in the aeration basin of an activated sludge system had no effect on substrate utilization or overall process performance as long as the system was maintained in an aerobic state. Consequently, operation of activated sludge systems generally did not include consideration of the DO maintained as an important process variable.

Pasveer (1954) was the first to suggest that diffusion of oxygen into larger flocs (aggregates of bacterial cells) may limit the rate of biochemical reaction. He suggested that higher mixing intensities would reduce floc sizes and provide for a higher DO at the water/floc interface, thereby insuring total oxygen penetration into the floc and allowing the maximum rate of biochemical oxidation.

Wuhrmann (1963) addressed the problem of oxygen supply into micro-bial flocs and applied the theoretical analysis of Gerard (1931) for passive diffusion of nutrients into spherical particles. The simple mathematical model developed is described below:

1. Consider an activated sludge floc as a spherical par-
    ticle with radius R and with a zero-order substrate
    utilization rate per unit volume U_v. The concentra-
    tion of the limiting substrate is C_b in the bulk solu-
    tion (assumed equal to the concentration at the floc
    surface) and C_c at the center of the sphere (see
    Figure 2-1).
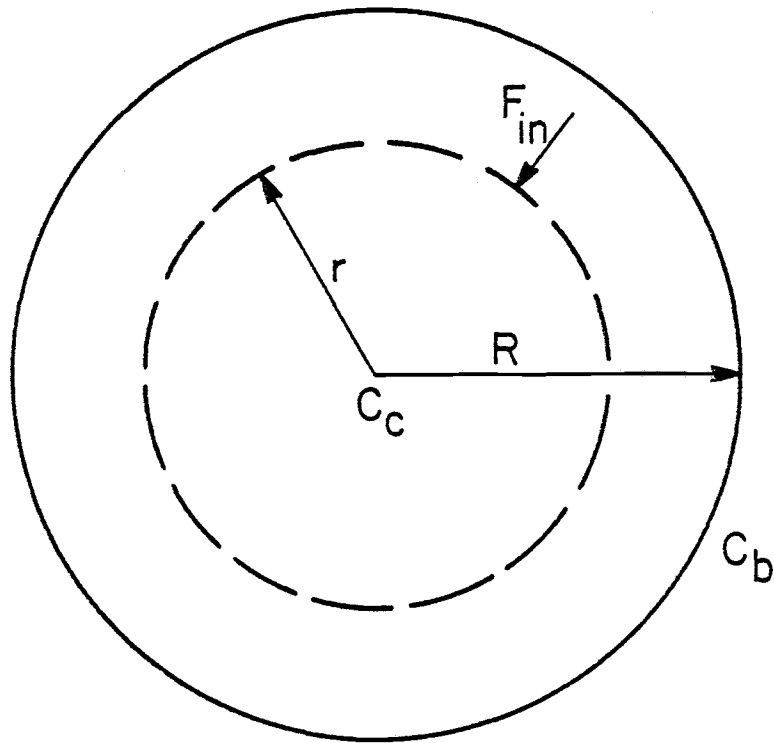
Figure 2-1. Spherical Particle Prepresentation of an
Activated Sludge Floc for the Model of
Wuhrmann (1963).

2.   At steady state, the flux of the limiting substrate (either the organic substrate or oxygen) into the spherical particle at any radius r (F_{in}) is then equal to the utilization rate of that substrate in the volume enclosed, V_r.

$$F_{in} = U_v \, V_r \tag{2-1}$$

3.   The flux inward can be described by Fick's law:

$$F_{in} = DA \, \frac{dC}{dr} \tag{2-2}$$

where D is the diffusion coefficient for the limiting substrate within the floc, A is the particle surface area at radius r, and C is the concentration of the limiting substrate at radius r.

4.   Combining Equations 2-1 and 2-2 and substituting with the appropriate expressions for the volume and surface area of a sphere:

$$D(4\pi r^2) \, \frac{dC}{dr} = 4/3\pi r^3 \, U_v \tag{2-3}$$

5.   Integrating Equation 2-3 between r=0, where $C=C_c$, and r=R, where $C=C_b$, then:

$$C_b - C_c = \Delta C = \frac{U_v \cdot R^2}{6D} \tag{2-4}$$

Equation 2-4 defines the drop in concentration (ΔC) between the bulk solution and the center of a spherical floc.

To estimate the largest floc size that can be kept aerobic, oxygen is defined as the limiting substrate and the concentration of oxygen at the center of the spherical particle, $C_{c,o}$, is set equal to zero. Equation 2-4 is then rearranged to yield:

$$R_{max}^2 = \frac{6D_o}{U_{v,o}} \, C_{b,o} \tag{2-5}$$

where D_o is the diffusion coefficient for oxygen, $U_{v,o}$ is the constant

oxygen utilization rate per unit of floc volume, $C_{b,o}$ is the bulk DO,

and R_{max} the maximum sized, fully aerobic floc particle.

Wuhrmann estimated the diffusion coefficient for oxygen in biologi-

cal flocs as 5×10^{-6} cm^2/s and the uniform oxygen utilization rate as

a minimum of 10^{-4} mg O_2/cm^3-s and a maximum of 5×10^{-3} mg O_2/cm^3-s for

domestic sewage. The bacterial density was approximated by assuming

that each cell in the floc was a sphere with a diameter of one micron

surrounded by a slime layer one micron thick. Based on these approxi-

mations and an average bulk DO of 2.0 mg/ℓ, it was calculated that

flocs of 100 μm and 500 μm in diameter could be adequately supplied

with oxygen at the maximum and minimum assumed utilization rates, re-

spectively.

The mathematical model, Equation 2-4, was also used to address the

question of whether substrate or oxygen typically limits the utiliza-

tion rate in large flocs. Rearranging Equation 2-4 and comparing the

result in terms of both substrate and oxygen utilization:

$$R^2 = \frac{6D_s}{U_{v,s}} \cdot \Delta C_s = \frac{6D_o}{U_{v,o}} \cdot \Delta C_o \qquad (2\text{-}6)$$

where the subscripts s and o refer to substrate and oxygen, respec-

tively. Rearranging Equation 2-6:

$$\Delta C_s = \Delta C_o \cdot \frac{D_o}{D_s} \cdot \frac{U_{v,s}}{U_{v,o}} \qquad (2\text{-}7)$$

The ratio of $U_{v,s}$ to $U_{v,o}$ is simply the overall stoichiometric ratio

between substrate and oxygen consumption. Equation 2-7 relates the

drops in substrate and dissolved oxygen concentrations between the bulk

solution and the center of the spherical particle. Using Equation 2-7

and approximate values of the coefficients for glucose metabolism, it was calculated that ΔC_s was approximately six times ΔC_o. This means that the glucose concentration in the suspending medium would have to be approximately six times the oxygen concentration (both in mg/ℓ) in order for oxygen to be the diffusion-limiting nutrient (to be utilized to zero concentration within the floc first).

In view of the generally low concentrations of substrates in sewage, it was concluded that an adequate oxygen supply into the bacterial flocs could be maintained even at low DO levels. Increased oxidation rates observed at DO levels above 1.0 mg/ℓ were attributed to changes in the species composition of the sludge, with more aerobic organisms replacing facultative bacteria as the DO was increased.

Mueller, et al. (1966a) recognized the importance of floc size and density measurements in evaluating oxygen transfer into microbial flocs. After reviewing various procedures for evaluating floc size and density, a procedure was developed that provided for measurement of the dry density, wet density, percentage water, and the nominal diameter of the floc. The dry density was evaluated by measuring the difference in specific gravity between samples containing a known high concentration of microbial floc and samples containing culture media alone. To measure the wet density, filtered floc were rolled into spheres and their tendency to settle in salt solutions of varying specific gravity was determined. From the measured wet and dry densities, the percentage of water in the floc was calculated. After enumerating microscopically the number of floc per unit volume, the average volume per floc was calculated and, assuming a spherical particle, the volume-averaged or nominal diameter was evaluated.

Activated sludge samples from eight different municipal plants were found to have an average wet density of 1.07 g/cm^3, an average of 80 percent water within the floc, and nominal diameters ranging from 21 to 115 μm using this method. Variations in reported values for the critical DO in activated sludge systems were attributed to differences in floc size.

Mueller, et al. (1966b and 1968) conducted the most extensive experimental investigation to date concerning oxygen transfer into bacterial flocs. A general mathematical model of nutrient diffusion into bacterial flocs was developed as described below:

1. Consider a bacterial floc as a spherical particle with radius R, with a constant bacterial density ρ (dry weight per unit volume), and with a substrate utilization rate per unit volume U_v.

2. Consider a differential element of thickness dr in the spherical particle. The difference between the flux of substrate into this differential element and the flux out must equal the utilization rate within the enclosed volume, V_e, at steady state (see Figure 2-2). In mathematical terms:

$$F_{in} - F_{out} = U_v \cdot V_e \qquad (2-8)$$

3. The flux of nutrient can be described by Fick's Law and the difference between the flux in and the flux out can be described as the differential change in the flux over the distance dr. Equation 2-8 then becomes:

$$\frac{d(DA \frac{dC}{dr})}{dr} \cdot dr = U_v \cdot V_e \qquad (2-9)$$

where A is the surface area at radius r, D is the diffusion coefficient for the nutrient in the bacterial floc (assumed to be constant throughout the particle), and C is the concentration of the nutrient at radius r.
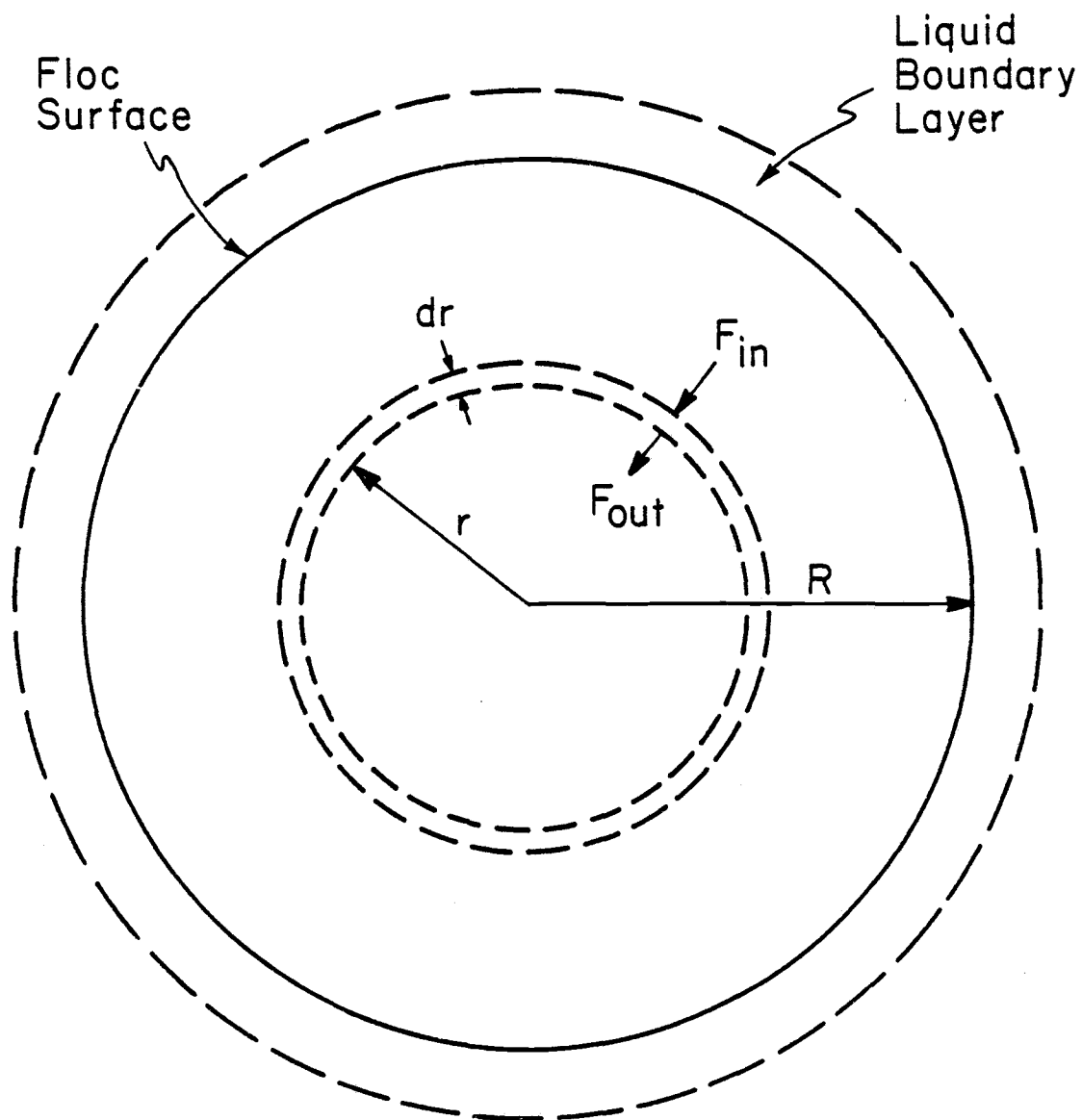
Figure 2-2. Spherical Particle Representation of an
Activated Sludge for the General Model
of Mueller, et al. (1966b).

4. Substituting into Equation 2-9 with the expression for the volume and surface area of a spherical element:

$$\frac{d(D \cdot 4\pi r^2 \frac{dC}{dr})}{dr} \cdot dr = U_v \cdot 4/3\pi[(r + dr)^3 - r^3]$$

and solving the above:

$$D(\frac{d^2C}{dr^2} + \frac{2}{r}\frac{dC}{dr}) = U_v \qquad (2\text{-}10)$$

This is the general equation for diffusion and reaction within a spherical particle. The utilization rate per unit volume may also be described as the bacterial density times the substrate utilization rate per unit of bacterial mass:

$$U_v = \rho U \qquad (2\text{-}11)$$

5. Equation 2-10 does not consider the effect of any external mass transfer resistance. This type of limitation can be modeled by assuming that the spherical floc particle is surrounded by a stagnant liquid boundary layer (see Figure 2-2). Mass transfer from the bulk liquid to the surface of the microbial floc is then described by:

$$F_a = k_L(C_b - C_s) \qquad (2\text{-}12)$$

in which F_a is the mass flux rate per unit area across the liquid boundary layer, k_L is the mass transfer coefficient, and C_b and C_s are the nutrient concentrations in the bulk liquid and at the surface of the particle.

The mass transfer coefficient, k_L, can be evaluated using the equation below for flow past spheres:

$$k_L\, d/D = 2.0 + 0.6\ (v_f d/\nu)^{1/2}(\nu/D)^{1/3} \qquad (2\text{-}13)$$

where d is the particle diameter, D is the diffusion coefficient, v_f is relative velocity between the particle and the liquid, and ν is the kinematic viscosity of the fluid.

Using the above equations, Mueller demonstrated that
external mass transfer resistance was not significant
compared to that of the floc itself under mixing con-
ditions typical of activated sludge and, therefore,
that the substrate concentration at the surface of
the floc can be reasonably assumed to be equal to the
concentration in the bulk liquid.

6.   Because external mass transfer resistance can be
     neglected, the boundary conditions for Equation 2-10
     are:

     1) at r = R, C = C_b (2-14)

     2) at r = 0, $\frac{dC}{dr} = 0$ (2-15)

A specific mathematical model was also developed for the case where
oxygen is completely utilized before it diffuses to the center of the
floc particle, but the organic substrate (S) penetrates to the center
(the anoxic core model). Figure 2-3 illustrates the anoxic core model
for a spherical floc. Development of the mathematical model was very
similar to that of Wuhrmann (1963) and is described briefly below:

1.   Consider the spherical floc model (Figure 2-3) with
     constant bacterial density ρ, constant diffusion co-
     efficient for oxygen D_O, and with a constant oxygen
     utilization rate per unit volume $U'_{v,o}$ in the aerobic
     portion. The bacteria within the anoxic core are not
     capable of respiring and their oxygen utilization
     rate is zero.

2.   At any radius r outside the anoxic core, the flux of
     oxygen inward must equal the oxygen utilization rate
     per unit volume times the aerobic volume enclosed:

     $F_o = U'_{v,o} V_{aerobic}$ (2-16)

3.   The flux of oxygen inward can be described by Fick's
     Law (Equation 2-2) and the aerobic volume enclosed
     as the difference between the total volume enclosed
     and the anoxic core volume:

     $D_o (4\pi r^2) \frac{dC_o}{dr} = 4/3\pi (r^3 - r_a^3) U'_{v,o}$ (2-17)
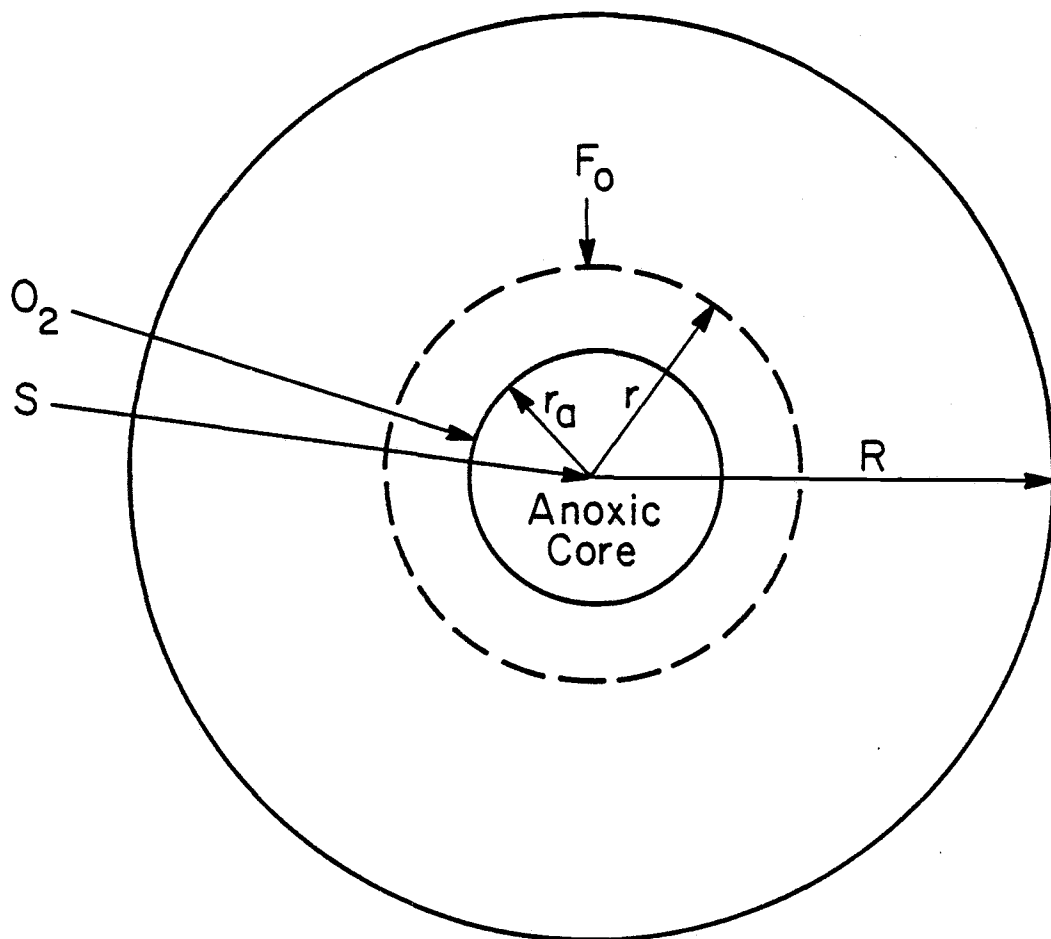
Figure 2-3. Anoxic Core Model of an Activated
            Sludge Floc for the Specific Model
            of Mueller, et al. (1966b).

where r_a is the radius of the anoxic core, C_o is the dissolved oxygen concentration at radius r, and D_o is the diffusion coefficient for oxygen. Rearranging,

$$\frac{dC_o}{dr} = \frac{U'_{v,o}}{3D_o} (r - \frac{r_a^3}{r^2})$$

(2-18)

4.   Integrating the above expression between $r = r_a$ (where $C_o = 0$) and $r = R$ (where $C_o = C_{b,o}$) and cancelling negligible terms:

$$\frac{6 D_o C_{b,o}}{U'_{v,o} R^2} = 2(\frac{r_a}{R})^3 - 3(\frac{r_a}{R})^2 + 1$$

(2-19)

The above equation relates the extent of the anoxic core that will develop at a given bulk DO with the characteristics of the bacterial floc, including the radius, volumetric oxygen utilization rate, and diffusion coefficient. The volumetric oxygen utilization rate, $U_{v,o}$, is more conveniently expressed as the product of the bacterial density on a dry weight basis (ρ) and the oxygen utilization rate per unit of dry bacterial mass (U_o):

$$U_{v,o} = \rho U_o$$

(2-20)

5.   The reduction in the apparent oxygen utilization rate for a bacterial floc caused by the development of an anoxic core can be directly related to the percentage of floc volume that is anoxic by the following:

$$U_o = U'_o [1 - (\frac{r_a}{R})^3]$$

(2-21)

in which U'_o is the oxygen utilization rate per unit of dry bacterial mass for aerobic portions of the floc and U_o is the apparent oxygen utilization rate based on the total floc mass, both the aerobic and anoxic portions.

The anoxic core model was tested by growing pure cultures of the highly flocculant bacteria Zoogloea Ramigera in batch culture and measuring their oxygen utilization rate as a function of the DO. The

cultures were grown on a high concentration of glucose (5000 mg/ℓ), thereby insuring that the maximum substrate utilization rate of the bacteria would be attained and that oxygen would be the diffusion-limiting substrate. The cultures were grown at varying mixing intensities to provide a range of floc sizes and transferred to a mixed, unaerated reactor vessel, where the depletion or dissolved oxygen over time was measured. The slope of the DO versus time curve yielded the oxygen utilization rate as a function of DO. The bacterial flocs were also blended to break up the flocs and the oxygen utilization rate measurements repeated.

A typical example of the results obtained is shown in Figure 2-4 as a plot of oxygen utilization rate (U_o) versus the bulk DO. The blended samples exhibited a very low critical DO, most frequently about 0.1 mg/ℓ, while that for flocs ranged from 0.6 to 2.5 mg/ℓ, depending on floc size. Blending did not result in complete and permanent dispersion of the floc so that the critical DO for blended samples included some effects of diffusional limitations.

Parameters necessary for fitting the data obtained to the anoxic core model, including the floc dry density and the diffusional radius, were obtained by the methods described previously (Mueller, et al., 1966a). The diffusional radius was also estimated as a ratio of floc cross-sectional area to circumference (the specific surface) from photomicrographs of the flocs. The diffusivity parameter, D_o, was estimated as the value that resulted in the best-fitting curve to the data. This curve-fitting procedure yielded an average diffusivity value for oxygen at 20°C of 1.8×10^{-6} cm^2/s and a standard deviation of 0.73×10^{-6} cm^2/s using the specific surface estimation of the diffusional radius,
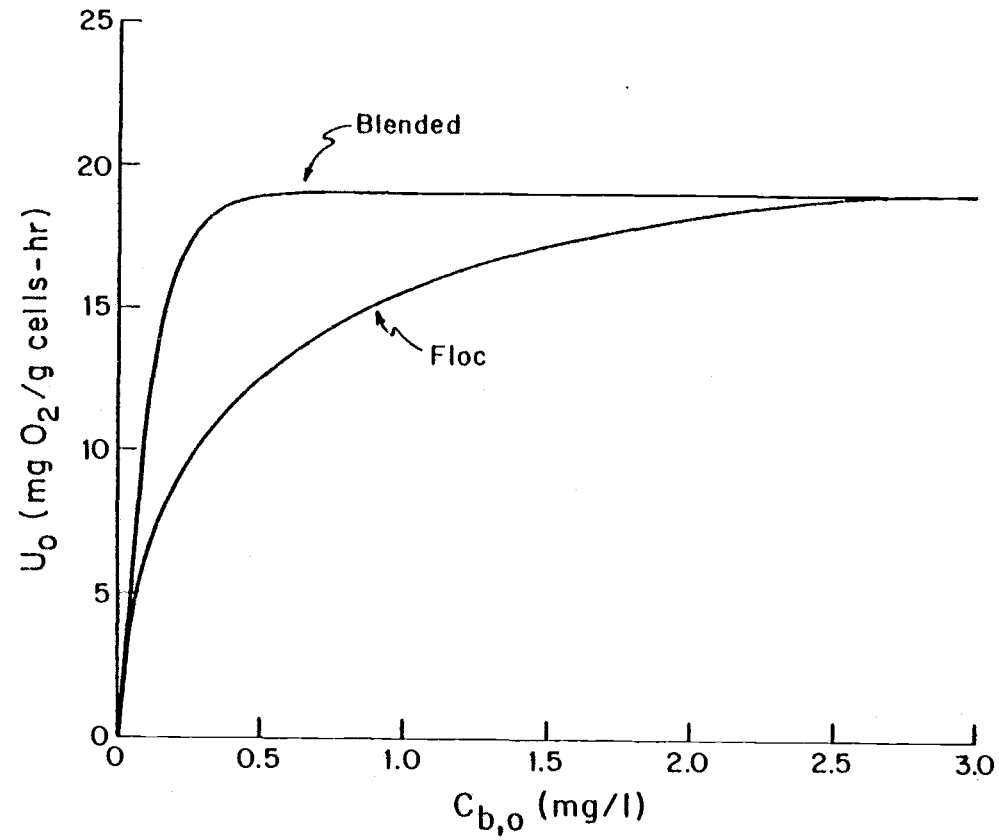
Figure 2-4. Specific Oxygen Uptake Rate vs. Bulk DO for Floc and Blended Samples of Zoogloea Ramigera (after Mueller, et al., 1966b).

while the average based on nominal diameter was 2.05×10^{-5} cm^2/s with a standard deviation of 0.84×10^{-5} cm^2/s. The specific surface ratio was proposed as the better indication of the diffusional radius.

Various shapes in addition to the sphere were tested for their fit to the respiration rate data, including the triangular prism, the flat plate, and the cylinder. It was found that the shape assumed for the floc had only a small impact upon the derived value for the diffusivity compared to the method used to measure floc size.

Krul (1977) investigated the growth and respiration rate of Zoo-gloea ramigera cells grown as natural flocs and as dispersed cells by enzymatic treatment with cellulase. For oxygen respiration rate exper-iments, batch stationary phase cultures with an average floc diameter of approximately 2 mm were harvested, supplied with excess glucose (10 g/ℓ), and their oxygen uptake versus time measured in a respiro-meter. The oxygen uptake of dispersed cells was shown to be a zero-order reaction at DO levels higher than approximately 0.1 mg/ℓ, whereas the oxygen uptake of cells in flocs was zero-order at DO levels above 1.5 mg/ℓ (see Figure 2-5).

Research in Biotechnology

Research in biotechnology has become concerned with oxygen diffu-sional limitations for fungi that naturally form large pellets.

Miura, et al. (1970) studied oxygen transfer into naturally formed spherical fungal pellets grown on glucose. Oxygen uptake rates for pellets with diameters from 1.3 to 5.2 mm were correlated with pellet diameter and mixing intensity. Half-velocity concentrations of dis-solved oxygen ranged from 1.0 to 6.0 mg/ℓ, indicating severe diffusional
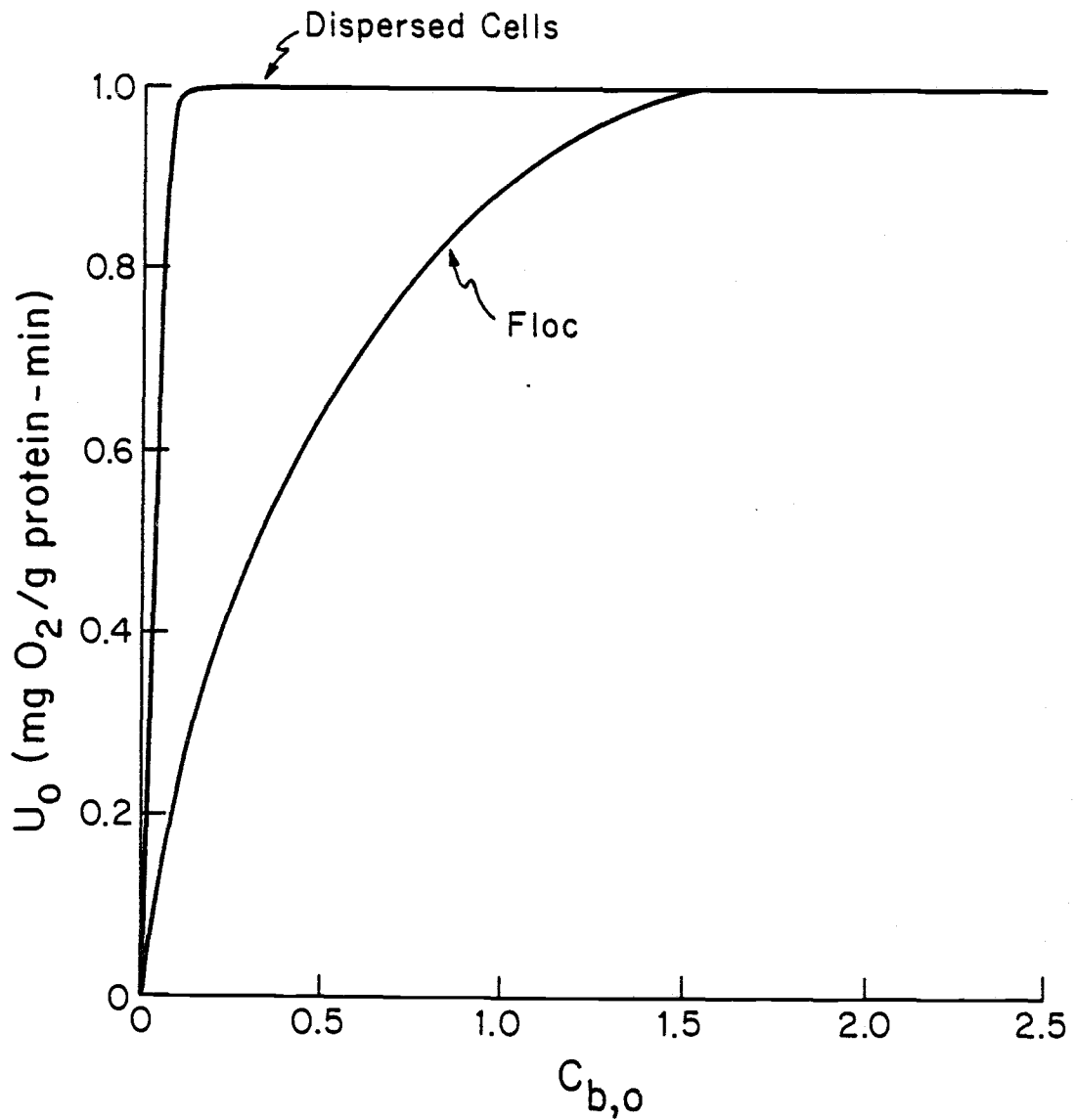
Figure 2-5.   Specific Oxygen Uptake Rate vs. Bulk DO for
              Floc and Dispersed Culture Samples of Zoo-
              gloea Ramigera (after Krul, 1977).

limitations for pellets of this size. The effective diffusivity of oxygen within the pellets was estimated by measuring dye penetration; however, the values obtained by this method were unreasonably large.

Huang and Bungay (1973) used a microprobe to measure the dissolved oxygen gradient in and near a mounted fungal pellet. Air-saturated glucose medium was pumped past the mounted pellet. For a 6 mm diameter pellet, the DO fell to one-half saturation at the surface of the pellet and was undetectable beyond a depth of 135 microns. From the slopes of the measured oxygen gradients, the diffusivity of oxygen within the pellet was estimated to be 2.9×10^{-6} cm^2/s.

Kobayashi, et al. (1973) studied the oxygen uptake rate in mycelial pellets of Asperigillus niger, both theoretically and experimentally. The respiration rate of filamentous mycelia, which were assumed not to be subject to diffusional limitations, was found to follow Michaelis-Menten kinetics with a half-velocity coefficient for oxygen of 0.096 mg/ℓ. The mathematical model developed for pellets incorporated Michaelis-Menten kinetics and diffusional considerations. The specific rate of respiration was found to decrease significantly with increasing pellet size. The effectiveness factor (ratio of respiration rate of a pellet to the respiration rate of a non-diffusion limited culture) decreased to 13% at a pellet diameter of 10.2 mm. Good agreement was found between the theoretical analysis and the experimental data.

THE PURE OXYGEN CONTROVERSY

The first large-scale pilot plant study of the oxygen activated sludge process was performed at the Batavia Municipal Pollution Control Plant, Batavia, New York in 1969 (Albertsson, et al., 1970). Since

that time, oxygen systems have risen in popularity and are rapidly becoming one of the standard alternatives considered for new wastewater treatment plant construction.

Although several companies market oxygen activated sludge systems, the major commercial vendor is the Union Carbide Corp., which sells their units under the trade name of UNOX and LINDOX. Basically, the process differs from typical air systems by supplying dissolved oxygen for wastewater treatment from either cryogenically or molecular-sieve generated pure oxygen. The DO in the aeration basin for oxygen plants is typically 5 to 10 mg/ℓ as compared to 1 to 2 mg/ℓ for air plants.

Advocates of oxygen systems claim several advantages, including (Chapman, et al., 1976):

1. increased metabolism rates,

2. decreased sludge yields, and

3. higher degrees of settleability.

These claimed advantages have been observed by numerous investigators at full-scale plants and pilot plants treating both municipal and industrial wastewaters (see McWhirtier, 1978 and Chapman, et al., 1976 for reviews). These differences were usually attributed to the higher DO levels maintained with oxygen as compared to air systems. However, in several other studies these effects were not observed (see Kalinske, 1976 for a review). Key references concerning this controversy are reviewed below.

Ball and Humenick (1973), in a carefully controlled laboratory experiment, compared air and oxygen activated sludge systems in completely-mixed chemostat reactors using a soluble glucose-yeast extract substrate. They evaluated differences in growth kinetics, initial

settling velocity, zone settling characteristics, and sludge volume index (SVI). Unfortunately, mixing intensity in the air and oxygen systems were not equal; this is a common problem in laboratory studies (Drnevich and Stuck, 1977). No significant differences were found in the sludge yield, substrate removal kinetics, or settling characteristics under air and oxygen conditions while holding other variables constant.

Kalinske (1976) reviewed the literature on oxygen versus air systems, concentrating on controlled laboratory studies. It was concluded that, if DO levels above 2 mg/ℓ were maintained, air systems were comparable to pure oxygen systems in sludge settling and dewatering characteristics, treatment compatibility, effluent quality, and sludge production.

In a response for Union Carbide Corp., Chapman, et al. (1976) cited extensive pilot- and full-scale plant data indicating that oxygen systems could be operated at lower solids retention times (higher food to microorganism (F:M) ratios), and that oxygen sludges settle and compact more effectively than sludges from air systems. It was postulated that activated sludge floc in air systems are typically anoxic at the core due to the low DO maintained in the aeration basin.

Parker and Merrill (1976) continued the discussion, suggesting that the differences in settling characteristics observed by Chapman, et al., were the result of bulking sludge in the air systems. It was suggested that variations in mixing intensities may be a fundamental reason for the observed differences between air and oxygen systems.

Benefield, et al. (1977) compared the kinetics of substrate utilization and growth between laboratory-scale air and oxygen activated

sludge systems. Their data had high statistical variation and effluent COD was used as the variable to determine kinetic coefficients without considering the build-up of refractory materials. A biochemical analysis of the biomass was also conducted, including analysis for DNA, RNA, lipids, and carbohydrates. The DO did not significantly change these biochemical constituents. The results of this study are difficult to interpret since mixing intensity was not controlled to values typical for full-scale systems. Results showed a higher specific substrate utilization in the oxygen reactor with higher cell concentrations.

Sezgin, et al. (1978) developed a descriptive model of filamentous bulking, one of the major settling problems in activated sludge. It was postulated that at low DO levels, the growth rate of filamentous microorganisms exceeds that of flocculant bacteria within the interior of flocs. Due to diffusional limitations, it was further postulated that DO levels in typical air activated sludge plants may be substantially lower in the floc interior than in the bulk solution, thereby encouraging the growth of filaments.

To test these hypotheses, laboratory activated sludge systems were fed domestic sewage daily. One unit was maintained at a DO of approximately 2 mg/ℓ (typical of air systems) and another identical system at a DO of approximately 8 mg/ℓ (typical of pure oxygen systems). The two laboratory systems performed similarly in terms of substrate removal efficiency, effluent suspended solids, and sludge yield. However, the system operated at a DO of 2 mg/ℓ developed filamentous bacteria in greater numbers and with greater length, particularly in response to high organic loads in the influent. It was theorized that the DO in the floc interior was a major variable influencing the relative growth rates

of filamentous and floc-forming bacteria with low DO levels resulting
in faster growth of filamentous bacteria and consequent sludge bulking.

Palm, et al. (1980) continued with studies intended to verify the
theory of Sezgin, et al. (1978). Two laboatory, completely-mixed acti-
vated sludge systems were fed settled domestic wastewater and were
operated under identical conditions with the exception that the mixed
liquor DO in the units was different. The substrate removal rate with-
in the reactors was varied from 0.2 to 1.8 g COD/g MLVSS-day. A con-
sistent relationship was found between the substrate removal rate and
the DO necessary to prevent the development of large populations of
filamentous bulking. Higher substrate removal rates required higher
aeration basin DO levels to prevent filamentous bulking. It was stated
that the flocs within the laboratory reactors were of greater average
size than those present in full-scale systems. However, no floc size
data were presented.

ATP VIABILITY AND ADENYLATE ENERGY CHARGE

The Role of ATP in Microbial Metabolism

The role of ATP in microbial metabolism is described in detail in
many biochemistry textbooks (see Lehninger, 1970, for instance). A
brief description of this role is given below.

Adenosine triphosphate (ATP) is commonly termed the "energy cur-
rency" of the living cell. By providing temporary storage of chemical
energy, it provides a necessary link between the energy-producing reac-
tions of substrate degradation and the energy-requiring reactions re-
lated to cell growth. Upon hydrolysis, the terminal phosphate bond is

broken, releasing inorganic phosphate (P_i) and energy, and forming
adenosine diphosphate (ADP):

$$ATP + H_2O \rightleftarrows ADP + P_i \qquad (2\text{-}22)$$

Many biosynthetic reactions involve splitting of pyrophosphate (PP_i)
from ATP to form adenosine monophosphate (AMP):

$$ATP + H_2O \rightleftarrows AMP + PP_i \qquad (2\text{-}23)$$

The pyrophosphate generated in the above reaction is rapidly hydrolyzed
to inorganic phosphate:

$$PP_i + H_2O \rightleftarrows 2\ P_i \qquad (2\text{-}24)$$

In cellular metabolism, the above energy-releasing reactions are
coupled with energy-requiring biosynthetic reactions so that the energy
derived from hydrolysis of ATP is utilized to drive biosynthetic reac-
tions.

Regeneration of ATP from ADP occurs primarily by the process of
oxidative phosphorylation in aerobic metabolism. In this process,
reducing equivalents derived from organic substrates are carried to the
terminal electron acceptor, oxygen, and the energy generated is ulti-
mately used to rephosphorylate ADP. AMP can be converted into ADP by
the action of adenylate kinase before rephosphorylation to ATP:

$$AMP + ATP \underset{\text{kinase}}{\overset{\text{adenylate}}{\rightleftarrows}} 2\ ADP \qquad (2\text{-}25)$$

In general, catabolic reaction sequences contain regulatory en-
zymes that are activated by ADP or AMP, or inhibited by ATP; conversely,

many regulatory enzymes in ATP-consuming biosynthetic pathways are acti-
vated by ATP or inhibited by ADP or AMP. Because adenine nucleotides
play a central role in metabolic control, Atkinson (1969) introduced
the concept of "adenylate energy charge" as a fundamental metabolic
control parameter. The adenylate energy charge (AEC) is equal to one-
half the anhydride bound phosphate of high free energy divided by the
total adenylate pool:

$$AEC = \frac{[ATP] + 1/2 \ [ADP]}{[ATP] + [ADP] + [AMP]} \hspace{3cm} (2\text{-}26)$$

The AEC is a linear measure of the metabolic energy stored in the
adenine nucleotide species and has been compared analogously to the
charge of a battery.

The AEC may vary from zero, where the adenylate pool is composed
entirely of AMP, to one, where the pool is composed entirely of ATP.
At any intermediate value of energy charge, there is a range of possible
compositions. If the adenylate kinase reaction (Equation 2-25) is at
equilibrium, the relative concentrations of the three adenine nucleo-
tides is fixed at any specified value of AEC, as shown in Figure 2-6.
In this figure, the effective equilibrium constant for the adenylate
kinase reaction was assumed to be 1.2.

ATP as an Indicator of Viability

Because ATP functions as the primary intermediate energy carrier
within the cells of every known organism, it has been used as an indica-
tor of viable biomass in environmental samples, including soils (Lee,
et al., 1971), the oceans (Holm-Hansen and Booth, 1966), and microbio-
logical cultures (Chapman, et al., 1971), including activated sludge
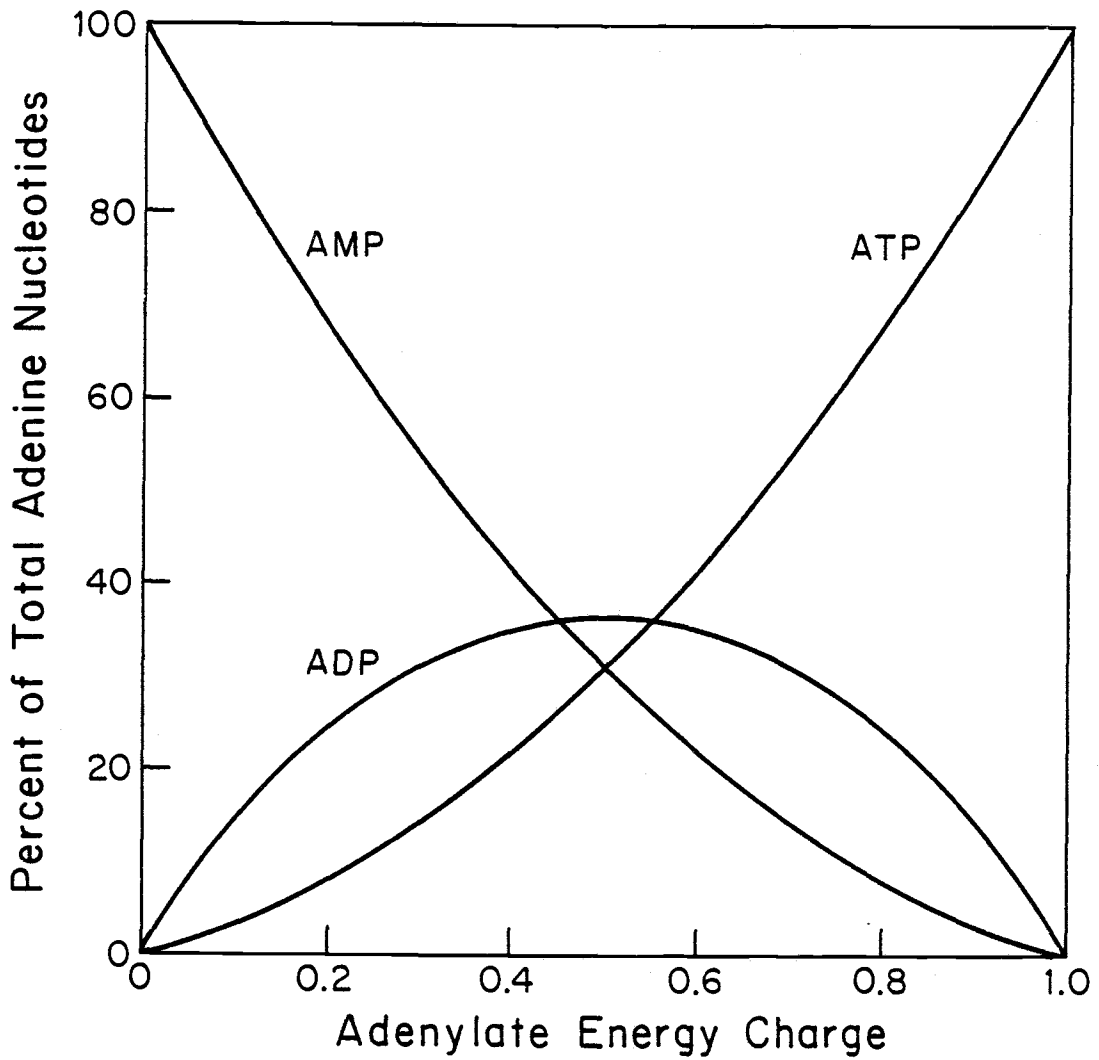
Figure 2-6.   Relative Concentrations of ATP, ADP, and AMP
             as a Function of Adenylate Energy Charge
             (after Atkinson, 1977).

(Patterson, et al., 1970). The accuracy of ATP measurements as indicators of viable biomass depend upon its association with living matter only and its presence as a relatively constant, predictable fraction in the biomass of diverse microorganisms. Most studies on the ATP content of microbial samples have supported the conclusion that ATP does not exist in measurable quantities in association with nonliving cells (Holm-Hansen and Booth, 1966) (Patterson, et al., 1970) (Hamilton and Holm-Hansen, 1967). Extracellular ATP has been shown to be rapidly degraded in microbial cultures (Patterson, et al., 1970). Furthermore, ATP content has been shown to correlate well with other measures of viability, such as plate counts (Chapman, et al., 1971) (Weddle and Jenkins, 1971) (Upadhyaya and Eckenfelder, 1975).

However, the ATP content of microorganisms has been shown to vary somewhat with species. Lee, et al. (1971) found that the ATP content per cell in soil microorganisms varied with the organism tested and with the growth stage of particular organisms, but the ATP content per unit of cell mass was confined within a relatively narrow range of 1 to 4 milligrams of ATP per gram of dry cell mass.

Knowles (1976) summarized data regarding ATP contents in microbial cultures from 30 separate studies. The ATP content for aerobic, heterotrophic growth appeared to vary with species, but was generally in the range of 2 to 10 micromoles per gram of cells (1 to 5 mg ATP/g cells). Chapelle, et al. (1978) have argued that, in situations where cell death is the result of nutrient limitations, intracellular ATP may decrease only gradually and ATP may, therefore, be associated with nonviable cells.

Effect of Environmental Variables on Energy Charge

Atkinson (1969) has found that regulatory enzymes from reaction sequences in which ATP is regenerated are highly active at low levels of energy charge and decrease sharply in activity as the charge increases above about 0.75. Conversely, regulatory enzymes from biosynthetic sequences that consume ATP exhibit very little activity at low levels of energy charge, and their activities increase sharply at charge values above 0.75. Response curves for regulatory enzymes involving ATP consumption and involving ATP regeneration tend to intersect at an energy charge value of about 0.85 (Figure 2-7). Therefore, Atkinson (1969) has postulated that, if the in-vitro enzyme response patterns observed reflect the behavior of enzymes in-vivo, the energy charge in living cells must be rather strongly stabilized in a range near 0.85. Any tendency for the energy charge to fall would be resisted by the consequent increase in the rate of ATP regeneration and decrease in the rate of ATP utilizing reactions, and a tendency to rise would be opposed by oppositely directed changes.

Since 1960, a significant amount of research has been directed at determining whether the ATP content or energy charge of a cell changes with environmental conditions, substrate or nutrient concentrations, growth rate, or other factors. Early studies concerning the effects of batch culture growth phase on ATP content achieved dissimilar results. Forrest (1965) claimed that the ATP pool of Streptococcus faecalis decreased throughout the logorithmic growth phase. In contrast, Franzen and Binkley (1961) reported that the ATP content of E. Coli (in mg ATP/g dry weight) was independent of growth rate, but that both cell mass and
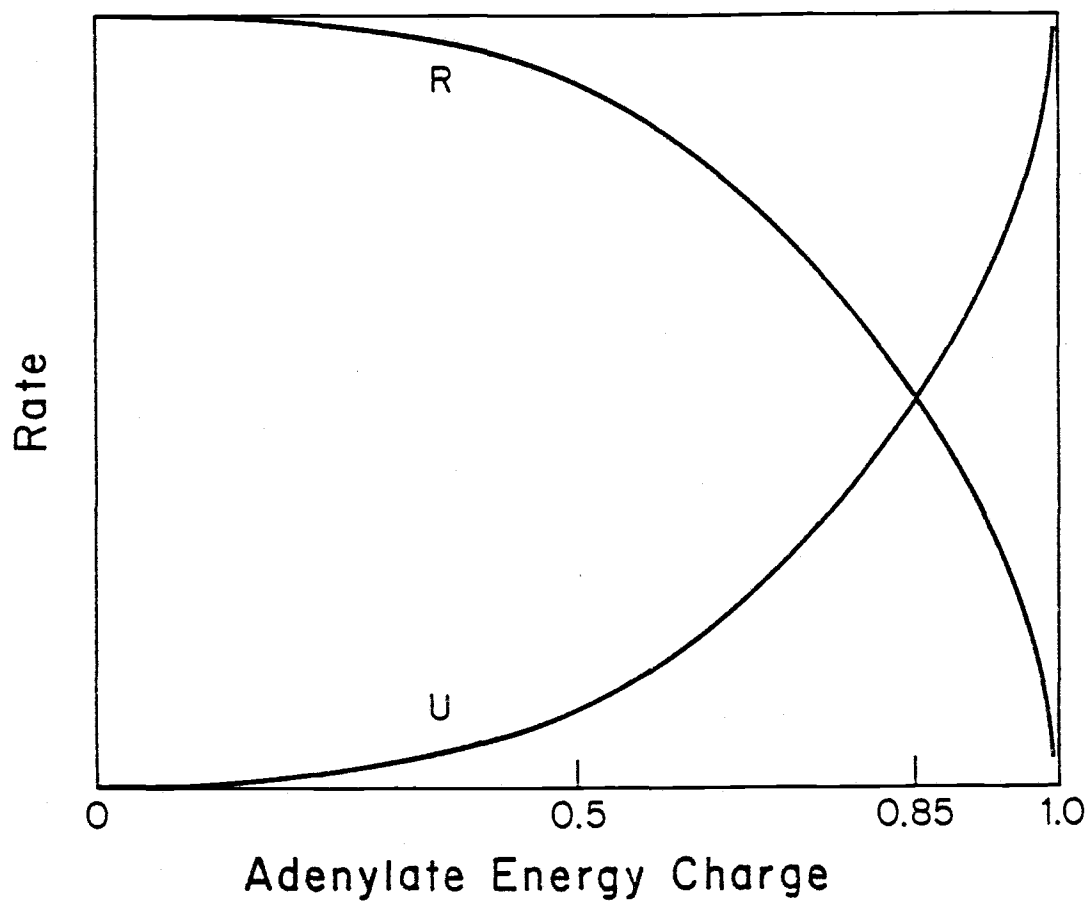
Figure 2-7. Theoretical Response Curves of Enzymes Involved in
             Regulation of ATP-Utilizing (U) and ATP-Regenerating
             (R) Metabolic Sequences (from Atkinson, 1969).

ATP content per cell increased with increases in growth rate. Neidhardt and Fraenkel (1961) found that the ATP content (as micromoles per gram of protein or gram dry weight) of Aerobacter (Klebsiella) aerogenes varied with growth rate.

More recent studies, using continuous culture, have tended to show a constant ATP pool over a wide range of growth rates (Harrison and Maitra, 1969) (Chapman, et al., 1971) (Dolezal and Kapralek, 1976). This may be largely due to improved analytical methods for extraction and analysis of ATP. Atkinson (1977) has shown that energy charge values reported for microbial cultures have increased dramatically since 1950, when values as low as 0.2 were reported, to the 1970's, when few studies reported values outside the range of 0.8 to 0.9.

Harrison and Maitra (1969) investigated the ATP content of Klebsiella aerogenes, a facultative bacteria, in glucose-limited chemostat culture at growth rates from 0.02 to 0.5 hr^{-1} and at varying DO levels. The ATP/ADP ratio and energy charge were approximately constant with changes in DO above the critical value ($\sim$ 0.2 mm Hg or 0.01 mg/ℓ), but fell when the culture was grown under oxygen limited conditions and was at a minimum in anaerobically grown cultures (Table 2-1). It was also reported that the steady-state ATP content was independent of growth rates over 0.1 hr^{-1} and was lower in cells growing under nitrogen-limited conditions with glucose in excess than in glucose-limited culture. Lower ATP contents at growth rates below 0.1 hr^{-1} were attributed to reduced viability. The sudden addition of extra glucose or succinate to a glucose-limited culture increased the respiration rate of cells, but the ATP content quickly returned to the steady-state value after initial perturbations. This tight control over ATP levels

Table 2-1. Steady-State Concentrations of Glucose-6-Phosphate and Adenine Nucleotides in Cells Grown at Different Dissolved Oxygen Tensions. (After Harrison and Maitra, 1969.)

DO TENSION[1] (mm Hg)	CELL CONC. (mg/cm^3)	CONCENTRATION (μ moles/g dry weight)						
		G6P[3]	ATP	ADP	AMP	TOTAL ADENINE NUCLEOTIDES	ATP/ADP RATIO	ENERGY CHARGE
420	3.8	0.9	8.3	-	-	-	-	-
220	4.2	0.5	6.6	-	-	-	-	-
57	4.3	-	5.9	-	-	-	-	-
5.3	4.3	2.8	6.1	2.1	0.4	8.6	2.9	0.83
3.0	4.3	2.6	6.5	2.2	0.4	9.1	3.0	.84
<0.2-2.5[2]	4.2	0.2	6.2	2.3	0.5	9.0	2.7	.82
<0.2	2.5	1.2	3.7	2.9	0.8	7.4	1.3	.70
0.0	0.7	12.4	3.7	3.9	1.1	8.7	0.9	.65

[1] mm Hg x 0.05 $\simeq$ mg/ℓ O_2

[2] Oscillating transition state

[3] G6P = Glucose-6-Phosphate

NOTE: Growth rate was glucose-limited at a growth rate of 0.18 hr^{-1}.

was attributed to the central regulatory role that adenine nucleotides play in cell metabolism.

An important question in biological wastewater treatment, where substrate and dissolved oxygen concentrations are often very low, is what impact substrate or oxygen starvation has upon the cellular adenylate content and balance. Chapman, et al. (1971) measured the adenylate energy charge in Escherichia coli during growth on glucose as relatively constant at 0.8. During the stationary phase after cessation of growth, or during starvation in carbon-limited cultures, the energy charge declined slowly, over a period of time taking from 1 to 60 hours, during which all the cells retained their viability (i.e., were capable of forming colonies on complete medium). Following this phase, the energy charge declined rapidly, accompanied by a proportional loss in viability. It was concluded that growth can occur only at energy charge values above about 0.8, that viability is maintained at values between 0.8 and 0.5, and that cells die at values below 0.5. The response of E. coli, a facultative bacteria, to temporary anaerobiosis was only a slight reduction in energy charge at the onset of anaerobiosis after which the normal energy charge (approximately 0.8) was recovered within one hour.

Several mechanisms by which bacteria maintain a high energy charge during a temporary substrate shortage have been uncovered. The mechanisms include excretion of AMP (increases energy charge while reducing the total adenylate pool), use of previously stored polymers or other endogenous substrates, and use of other high energy phosphate nucleotides such as GTP or UTP (Knowles, 1976). These measures, together with strict energy conservation except for essential maintenance reactions,

presumably allow the bacteria to retain its viability during extended
periods of starvation.

SUMMARY

Over the past 50 years, numerous microbiological studies on a wide
variety of aerobic, nonflocculant bacterial species grown on simple
carbonaceous substrates such as glucose have not identified any physio-
logical or metabolic change resulting from changes in the DO above the
critical level (generally less than 0.1 mg/ℓ) and below hyperbaric oxy-
gen concentrations. Below the critical DO, changes in metabolic path-
ways and in dominant cytochromes have been noted among facultative bac-
teria. Some substrates, specifically aromatic compounds, polyunsatu-
rated fatty acids, and aliphatic hydrocarbons, require the incorporation
of oxygen by an oxygenase to be metabolized. If the oxygenase reaction
is rate-limiting in the metabolic sequence of substrate degradation,
then the intrinsic half-velocity coefficient for oxygen for some of
these substrates may be substantially higher than for substrates that
do not require an oxygenase.

Research in environmental engineering and biotechnology has demon-
strated that microbial aggregates can be subject to diffusional limita-
tions if the aggregates are large and dense. Oxygen can be the dif-
fusion-limiting nutrient under certain conditions and the kinetic re-
sponse of microbial aggregates to oxygen diffusion limitations has been
effectively modeled.

ATP, the "energy currency" of the living cell, is an excellent
indicator of viable versus nonviable bacteria. Problems involved in
the use of ATP content of bacterial cultures as an indicator of viable

biomass include the variation in specific ATP content with bacterial species and the possible association of some ATP with dead bacteria.

Adenine nucleotides play a central role in metabolic control. Most recent research indicates that the adenylate energy charge of growing bacterial cultures is strongly controlled in the range of 0.8 to 0.9.

CHAPTER III

THEORY: MATHEMATICAL MODELS

Numerous mathematical models have been developed relating to the
activated sludge process. This section develops a series of mathemati-
cal models that describe the steady-state performance of an activated
sludge system under various conditions. These conditions include the
presence and absence of significant diffusional limitations. Particu-
lar emphasis is placed on the anoxic core model because this model pro-
vides the fundamental theoretical basis for this research study. The
development that follows is not entirely original but, rather, is a
compilation, digestion, and expansion of the models developed by numer-
ous researchers.

CASE I: MICROBIAL METABOLISM WITHOUT DIFFUSIONAL LIMITATIONS

General Model

The most widely accepted model of substrate utilization and micro-
bial growth in an activated sludge system has been presented by Lawrence
and McCarty (1970). The basic assumptions of this model are:

1) The mass specific substrate utilization rate (U_s) of
the microbial culture is related to the concentration
of the limiting nutrient (C) by a hyperbolic function
as proposed by Monod (1949):

$$U_s = \frac{k\ C}{K+C} \qquad\qquad (3\text{-}1)$$

where: k = maximum specific rate of substrate
utilization (mass substrate/mass
microorganisms-time)

C = concentration of the limiting nutrient (mass/volume)

K = concentration of the limiting nutrient at which the specific utilization rate is half-maximal (mass/volume)

2) The growth of microbial mass that results from substrate conversion into microbial cells can be described as:

$$(\frac{dX}{dt})_{growth} = YU_sX \qquad (3-2)$$

where: X = microorganism concentration (mass/volume)

t = time

Y = growth yield coefficient (mass microorganisms/mass substrate)

The product of U_s times X is simply the volumetric substrate utilization rate and Y is the mass of microorganisms synthesized from a unit mass of substrate.

3) The decay of microbial mass that results from natural death processes, endogenous respiration, and predation can be described by a first-order model:

$$(\frac{dX}{dt})_{decay} = bX \qquad (3-3)$$

where: b = decay coefficient (time^{-1})

Equation 3-1 assumes that all the microorganisms are exposed to the same nutrient concentrations present in the bulk solution. Therefore, diffusional limitations are not considered in this model.

Application of Equations 3-1 through 3-3 to the activated sludge process requires a definition of the system and the limiting nutrient. For treatment of organic wastewaters by aerobic microorganisms, the limiting nutrient is generally assumed to be the organic substrate.

Under these circumstances, Equation 3-1 becomes:

$$U_s = \frac{k\,C_s}{K_s + C_s} \tag{3-4}$$

where C_s is the concentration of the organic substrate and K_s is the concentration of the organic substrate at which the specific utilization rate is half-maximal.

A schematic flow diagram of the activated sludge process is shown in Figure 3-1. This figure shows an influent wastewater, with a flow rate Q and concentration of the organic substrate C_s^o, entering a reactor (aeration basin) that is assumed to be completely mixed and has a volume V. Utilization of the substrate by the microbial mass in the aeration basin (mass concentration = X) reduces the substrate concentration to C_s. From the aeration basin, the mixed liquor is sent to a clarifier, where the microbial mass is settled except for a small concentration X^e that escapes into the effluent. The settled microbial mass is returned to the aeration basin at a flow rate Q^r and a concentration X^r to once again degrade the organic compounds in the influent wastewater. However, the conversion of substrates in the influent wastewater into microbial cells results in an excess mass of microorganisms that must be removed from the system. This is generally accomplished by "wasting" mixed liquor from the aeration basin at a flow rate Q^w.

From mass balances on the substrate and microbial mass for the activated sludge system depicted in Figure 3-1, the equations that follow may be readily derived as described by Lawrence and McCarty (1970) or any textbook on biological wastewater treatment (see Metcalf and Eddy, 1970, for example). First, the average solids retention time
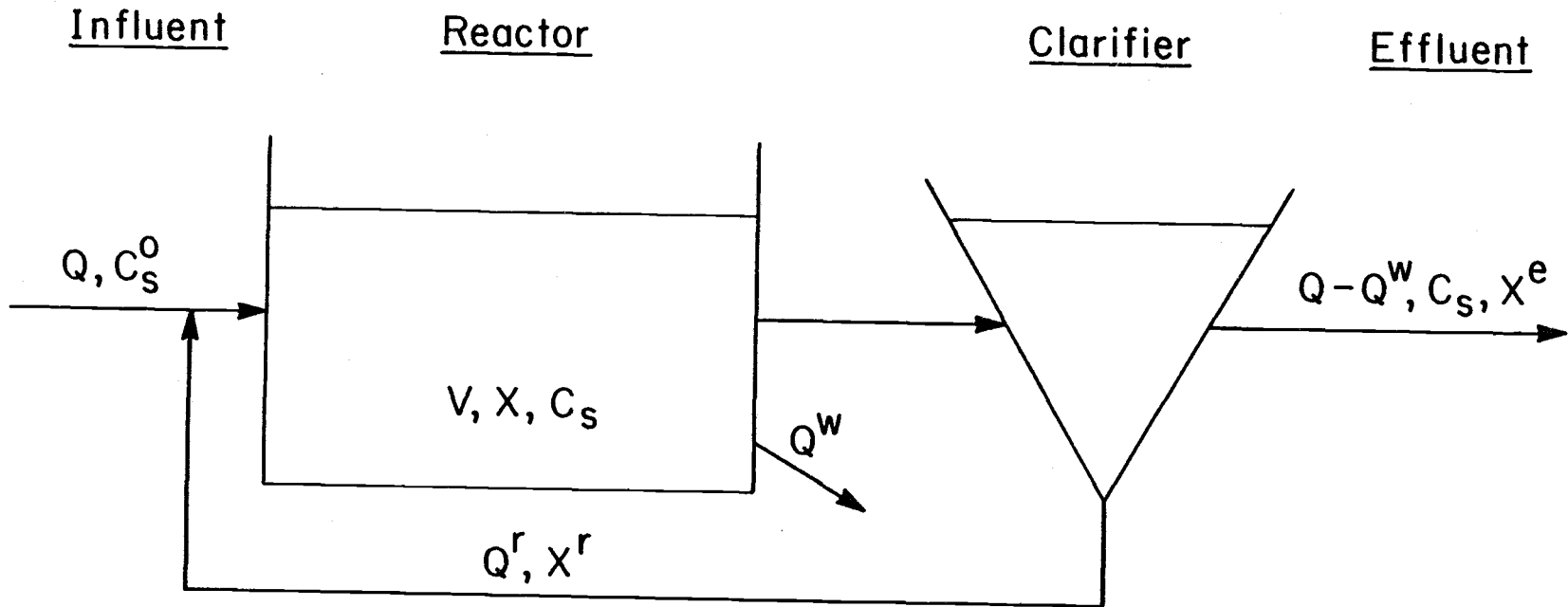
Figure 3-1.   Schematic Diagram of the Activated Sludge Process.

(SRT) in the system, θ_c, is given by:

$$\theta_c = \frac{XV}{Q^W X + (Q-Q^W) X^e} \qquad (3-5)$$

The general equations for this system are then:

$$\frac{1}{\theta_c} = YU_s - b \qquad (3-6)$$

with U_s as defined in Equation 3-4, and

$$U_s = \frac{C_s^o - C_s}{\theta X} \qquad (3-7)$$

where θ is the theoretical detention time in the aeration basin ($\theta = V/Q$).

The solution to the above equations in terms of the organic substrate concentration in the reactor and effluent, and the concentration of microbial mass that develops in the aeration basin is as follows:

$$C_s = \frac{K_s(1+b\theta_c)}{\theta_c(Yk-b)-1} \qquad (3-8)$$

$$X = \frac{\theta_c}{\theta} \frac{Y(C_s^o - C_s)}{1+b\theta_c} \qquad (3-9)$$

Viability Models

One serious limitation of the model proposed by Lawrence and McCarty (1970) has been that the mass concentration of microorganisms (X), generally approximated as total volatile suspended solids, does not represent active or viable cells (X_a) only, but includes nonviable cells (X_n) and nonbiodegradable material from endogenous decay (X_i). In response to this limitation, McCarty (1972) has proposed that the

generation of nonbiodegradable remains can be modeled as:

$$\frac{dX_i}{dt} = (1-f)b\ X_a \qquad (3\text{-}10)$$

with an estimate of f, the biodegradable fraction of cells, as 0.8.
Furthermore, the equations associated with the original model (Equa-
tions 3-3 through 3-9) are appropriate only if the microorganism mass
concentration X is actually the active mass concentration X_a. Inclu-
sion of Equation 3-10 in the mass balances results in the following:

$$X_i = 0.2b\ \theta_c\ X_a \qquad (3\text{-}11)$$

The fundamental mechanism by which a nonviable component (X_n) is
generated is not entirely clear and, therefore, quantitation and model-
ing of this component has proven difficult. Both Nelson and Lawrence
(1980) and Grady and Roper (1974) have experimentally determined that a
significant nonviable component exists separate from the nonbiodegrad-
able microbial remains. Grady and Roper (1974) have proposed a first
order decay coefficient for the rate of death (loss of viability) of
viable cells:

$$\frac{dX_n}{dt} = b_n X_a \qquad (3\text{-}12)$$

Williamson and Nelson (1976) have modeled the nonviable mass as:

$$X_n = nX_a \qquad (3\text{-}13)$$

where n is a variable parameter that reflects diffusional limitations
or other environmental conditions that cause viable cells to become
nonviable. Using Equation 3-13 to define the nonviable mass and sum-
ming the components of the total microbial mass:

$$X = X_a + X_i + X_n = (1 + (1-f)b\theta_c+n)X_a \qquad (3\text{-}14)$$

where X is the total microbial mass concentration in the aeration basin.

Williamson and Nelson (1976) and Grady and Roper (1974) proposed that nonviable cells decay at a much slower, and perhaps negligible, rate as compared to viable cells. The effect of such an assumption in the model is that the nonviable fraction may become a substantial fraction of the total microbial mass, particularly at long solids retention times (SRT's).

DIFFUSIONAL MODELS

The development of a diffusional model for suspended microbial flocs requires the use of several simpifying assumptions. First, the floc is assumed to be a mathematically definable shape, usually a sphere, with a uniform microbial density and a constant resistance to diffusion throughout. This assumption conflicts with known conditions in large part, but must be viewed as necessary to achieve a manageable mathematical model. Bacterial flocs have been observed to generally have a lower density at greater distances from the center and differences in floc density and diffusion coefficient between flocs of different size have been reported (Mueller, et al., 1966b; Lamotta and Shieh, 1979). Furthermore, bacterial flocs are irregularly shaped and may present a much higher surface area to the bulk liquid than would a spherical particle. Mueller, et al. (1966b) found that the floc volume to surface area ratio provided a better fit of data to a mathematical model than did the nominal diameter, as described previously. However,

it was also found that the use of various shapes as alternatives to the sphere did not significantly improve model agreement with the experimental data. Therefore, a spherical-shaped floc is assumed in this study for purposes of developing a mathematical model.

The second major assumption is that the diffusional resistance of any liquid boundary layer is small compared to that of the floc particle itself and can be ignored. This assumption has been verified analytically for floc size and mixing intensities typical of activated sludge (Mueller, et al., 1966b; Baillod and Boyle, 1970; and Matson, et al., 1972).

A third assumption is that the system is at steady-state with respect to time.

General Model

The general steady-state model for diffusion and reaction within a spherical microbial floc has been developed by Mueller, et al. (1966b):

$$D(\frac{d^2C}{dr^2} + \frac{2}{r} \frac{dC}{dr}) = \rho U \qquad (3-15)$$

where   D = diffusion coefficient for the limiting nutrient

C = concentration of the limiting nutrient at radius r within the floc

U = mass specific nutrient utilization rate at radius r

ρ = mass density of bacteria within the floc.

The derivation of Equation 3-15 was presented previously. The boundary problem for this differential equation is defined with the following conditions:

$$\text{BC 1:} \qquad \text{at } r=R, \; C=C_b \tag{3-16}$$

$$\text{BC 2:} \qquad \text{at } r=0, \; \frac{dC}{dr} = 0 \tag{3-17}$$

where R is the radius of the floc and C_b is the concentration of the limiting nutrient in the bulk solution.

The ultimate form of Equation 3-15 and its solutions depend upon the mathematical expression used for the specific utilization rate, U. Most generally, U has been modeled with a Monod-type expression:

$$U = \frac{k \; C}{K+C}$$

with symbols as described previously.

A complication exists, however, in that microbial metabolism usually involves two major reactants, the electron donor and the electron acceptor; most frequently, these are an organic substrate and oxygen, respectively. Because of this, U has been modeled as:

$$U = k \; \frac{C_s}{K_s+C_s} \cdot \frac{C_o}{K_o+C_o} \tag{3-18}$$

where the subscripts s and o refer to the organic substrate and oxygen, respectively, and the major symbols as previously defined. Alternately, U has been modeled by the following two equations:

$$U = k \; \frac{C_s}{K_s+C_s} \quad , \text{ or} \tag{3-19}$$

$$U = k \; \frac{C_o}{K_o+C_o} \quad , \tag{3-20}$$

whichever is less. The first expression (Equation 3-18) indicates that there is an interrelationship between the organic substrate and oxygen

in terms of their effect on the overall reaction rate and has been utilized to model the kinetics of activated sludge flocs (University of California, 1979). It results in a continuous mathematical function and is, therefore, suitable for numerical solutions. The second model (Equations 3-19 and 3-20) has been used by Williamson and McCarty (1976) to model biofilm kinetics. Due to the biochemical separation between rate-controlling reactions involving the electron donor (organic substrate) and those involving the electron acceptor (oxygen), this model intuitively appears to be more appropriate. However, it results in a discontinuous mathematical expression and is more complex to solve.

The distinction between these two alternate models, however, may not be of extreme importance. In particular, for aerobic metabolism of an organic substrate, the situations in biological reactors are limited where the concentration of the organic substrate and dissolved oxygen would be in the range necessary for the two models to differ substantially in their predictions of kinetic behavior.

Specific Models

Verification and application of the general diffusional model has generally required the use of simplifying assumptions to make the model more manageable. To make such simplifications, it is first necessary to define which species, organic substrate or oxygen, is diffusion-limiting. The diffusion-limiting nutrient is defined as the species which, theoretically, would first be utilized to essentially zero concentration at some point within the floc. Definition of the diffusion-limiting species depends upon the relative stoichiometric utilization of substrate versus oxygen and the respective diffusion coefficients.

To develop the mathematical relationships, consider a general metabolic reaction for organic substrate degradation by aerobic bacteria:

$$\nu_s \, S + \nu_o \, O_2 + \begin{array}{c} growth \\ requirements \end{array} \rightarrow CO_2 + H_2O + Cells \qquad (3\text{-}21)$$
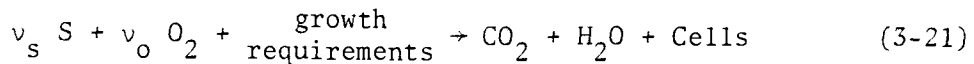
in which ν_s and ν_o are the stoichiometric reaction coefficients for the organic substrate (S) and dissolved oxygen, respectively. The mass stoichiometric ratio of oxygen to substrate utilization, γ, can then be defined as:

$$\gamma = \frac{\nu_o}{\nu_s} \cdot \frac{MW_o}{MW_s} \qquad (3\text{-}22)$$

where MW_o and MW_s are the molecular weights of oxygen and the organic substrate, respectively. The mathematical relationships that define the diffusion-limiting species are then as follows: If

$$C_{b,s} < C_{b,o} \cdot \frac{D_o}{D_s} \cdot \frac{1}{\gamma} \quad , \qquad (3\text{-}23)$$

then the organic substrate is diffusion-limiting; if

$$C_{b,o} < C_{b,s} \cdot \frac{D_s}{D_o} \cdot \gamma \quad , \qquad (3\text{-}24)$$

then oxygen is diffusion-limiting, where $C_{b,s}$ and $C_{b,o}$ are the bulk solution concentrations of substrate and oxygen, respectively. The above relationships were developed in their basic form by Wuhrmann (1963) for biological flocs, as described previously, and by Williamson and McCarty (1976) for bacterial films. With these relationships defined, two specific cases of the general diffusional model can be considered: the case where substrate is diffusion-limiting and the case where oxygen is diffusion-limiting.

CASE II: SUBSTRATE IS DIFFUSION-LIMITING

If the organic substrate is diffusion-limiting as defined by Equation 3-23, then it can be reasonably assumed that the concentration of the organic substrate controls the reaction rate throughout the bacterial floc. The half-velocity coefficient for oxygen is so small compared to that for most organic substrates that the case where oxygen is not diffusion-limiting (is not first utilized to near zero concentration within the floc), but is utilized to a concentration low enough to have some effect on kinetics is an impractical occurrence. Therefore, for this case, the general diffusional model can be simplified to yield:

$$D\left(\frac{d^2 C_s}{dr^2} + \frac{2}{r}\frac{dC_s}{dr}\right) = \rho k \frac{C_s}{K_s + C_s} \tag{3-25}$$

CASE III: OXYGEN IS DIFFUSION-LIMITING

If dissolved oxygen is the diffusion-limiting reactant as defined by Equation 3-24, then the simplifying assumption frequently considered is that the bacterial utilization rate is zero-order with respect to oxygen; that is, the utilization rate is independent of DO for any DO above zero. The reasonableness of this assumption depends upon the value of the intrinsic half-velocity coefficient for dissolved oxygen. Based upon the data of Longmuir (1954), it appears that the half-velocity coefficient for oxidation of a carbonaceous substrate by bacteria with a diameter of near one micron (typical of activated sludge) is between about 0.001 and 0.01 mg/ℓ. Thus, the assumption of intrinsic zero-order kinetics is judged to be reasonable for application to the

treatment of organic wastes by biological processes such as activated sludge.

Because the specific utilization rate is essentially zero-order with respect to DO, the DO may be utilized to essentially zero concentration within a floc, resulting in an anoxic condition for some portion of the floc volume around the center. Consequently, this model has been termed the anoxic core model (see Figure 3-2). Furthermore, the organic substrate concentration must limit the reaction rate throughout the aerobic portions of the floc. Thus, the appropriate mathematical expressions for the anoxic core model are:

$$D(\frac{d^2C}{dr^2} + \frac{2}{r}\frac{dC}{dr}) = \rho k \frac{C_s}{K_s + C_s} \tag{3-26}$$

for $r > r_a$, the radius of the anoxic core, and

$$D(\frac{d^2C}{dr^2} + \frac{2}{r}\frac{dC}{dr}) = 0 \tag{3-27}$$

for $r \leq r_a$. The above two equations state that the specific substrate utilization rate is zero within the anoxic core and is described by a Monod-type relationship with respect to the organic substrate concentration in the aerobic portions of the floc. The obvious solution to Equation 3-27 is that C_s is constant for $r \leq r_a$.

Equations 3-26 and 3-27 result in a discontinuous mathematical function at $r = r_a$ and no straightforward solution. A further simplification is possible, however, if the bulk substrate concentration, $C_{b,s}$, is sufficiently large such that the drop in substrate concentration, ΔC_s, between the floc surface and the center of the floc is small compared to $C_{b,s}$. In this case, the substrate concentration at all points within the floc is approximately equal to the concentration in the bulk
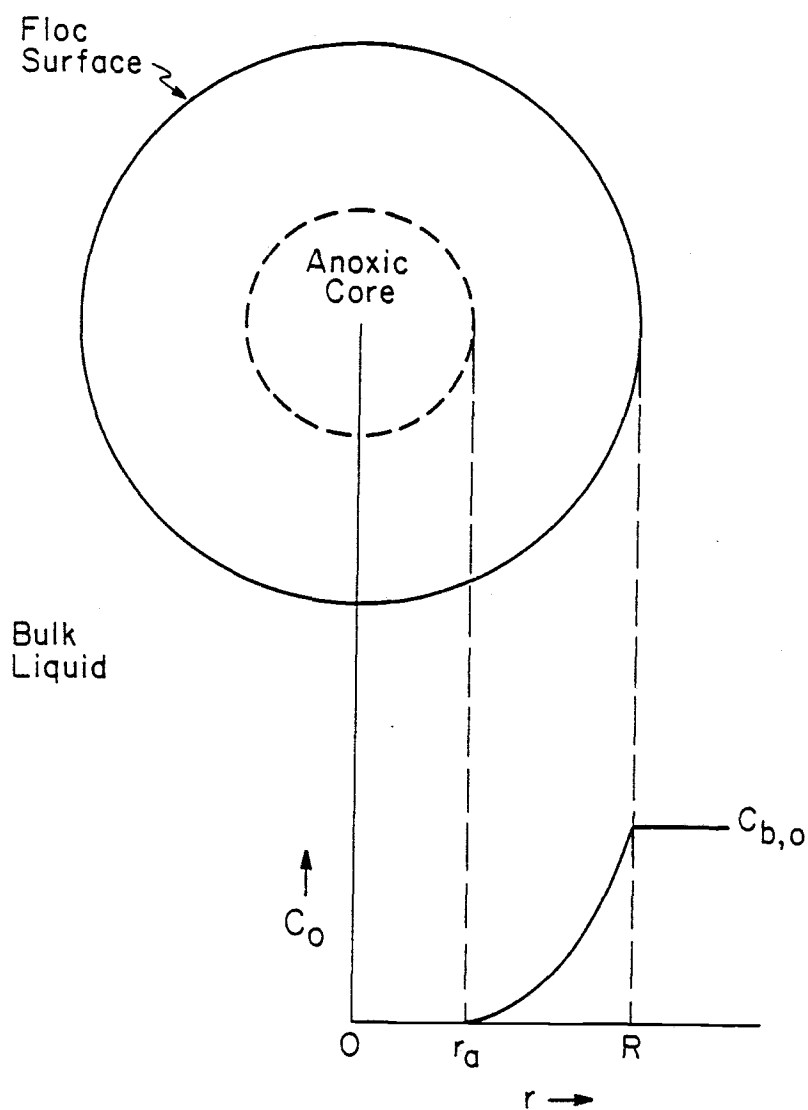
Figure 3-2. Anoxic Core Model for a Spherical
Activated Sludge Floc.

liquid and, therefore, a constant specific substrate utilization rate
for the aerobic volume may be defined based on the bulk substrate con-
centration

$$U' = k \frac{C_{b,s}}{K_s + C_{b,s}} = constant \qquad (3-28)$$

Equation 3-26 may then be modified to reflect the constant utilization
rate as follows:

$$D\left(\frac{d^2C}{dr^2} + \frac{2}{r}\frac{dC}{dr}\right) = \rho \ U' \qquad (3-29)$$

for $r > r_a$.

As described previously (Chapter II), Mueller, et al. (1966b) de-
veloped the relationship between the bulk DO and the radius of the
anoxic core:

$$\frac{6 \ D_o \ C_{b,o}}{\rho \ U'_o \ R^2} = 2\left(\frac{r_a}{R}\right)^3 - 3\left(\frac{r_a}{R}\right)^2 + 1 \qquad (3-30)$$

with symbols as described previously. Furthermore, they related the
overall utilization rate based on the total mass in the floc (U) to the
constant utilization rate in the aerobic portion (U') by the ratio of
the aerobic volume to the total volume of the floc:

$$U = U'\left[1 - \left(\frac{r_a}{R}\right)^3\right] \qquad (3-31)$$

A critical DO (C_o^c) may be defined for a flocculant bacterial cul-
ture in accordance with the anoxic core model. This corresponds to the
minimum DO at which the largest floc under consideration is maintained
in a fully aerobic state:

$$C_o^c = \frac{\rho \ U'_o \ R^2_{max}}{6 \ D_o} \qquad (3-32)$$

APPLICATION OF THE ANOXIC CORE MODEL

A parameter frequently found useful in the description of diffusional limitations is the effectiveness factor (E), defined as the ratio of the reaction rate in the presence of diffusional limitations divided by the theoretical reaction rate in the absence of any diffusional limitations. Mathematically, E can be defined as the specific substrate utilization rate in a flocculant or other culture subject to diffusional limitations (U) divided by the theoretical specific utilization rate if all cells were exposed to the bulk nutrient concentrations (U'):

$$E = \frac{U}{U'} \qquad (3\text{-}33)$$

For the anoxic core model, this factor is equal to the ratio of the aerobic floc volume to the total floc volume (see Equation 3-31).

An additional postulate of this research study is that, after some period of anoxia, the bacteria within the anoxic core will become nonviable and lose their ATP. Consequently, the extent of the anoxic core that develops can also be determined as a reduction in the ATP viability. Specifically, the ratio of the aerobic volume to the total volume of floc should be equal to the ratio of the measured ATP viability (mass ATP/mass cells) to the theoretical ATP viability in the absence of diffusional limitations. Thus, Equation 3-33 can be expanded to express all these relationships:

$$E = \frac{U}{U'} = 1 - (\frac{r_a}{R})^3 = \frac{T}{T'} \qquad (3\text{-}34)$$

where: T = the measured ATP viability based on total microbial mass

T' = the theoretical ATP viability in the absence of diffusional limitations

and other symbols are as previously described. Equation 3-34 states that the development of an anoxic core due to oxygen diffusional limitations can be measured as a reduction in the specific utilization rate and as a reduction in ATP viability in direct proportion to the volume of the anoxic core. Furthermore, Equation 3-30 defines the radius of the anoxic core that will develop based on a mathematical analysis of diffusion and reaction in a spherical particle. It requires, however, measurement of parameters that are not easily quantified, including the diffusion coefficient for oxygen within the bacterial floc, the density of bacterial cells within the floc, and the diffusional radius of the floc.

The anoxic core theory as developed is capable of predicting improvements in process performance with increasing DO as claimed for pure oxygen activated sludge systems. First, increased metabolism rates are indicated directly by Equation 3-34, wherein oxygen diffusion limitations are predicted to result in an anoxic core and in reductions in the specific utilization rate U in proportion to the volume of the anoxic core. Second, decreased sludge yields are predicted if the decay coefficient for the nonviable bacteria in the anoxic core is substantially smaller than that for viable bacteria in the aerobic portions as proposed by Williamson and Nelson (1976) and Grady and Roper (1974).

The final claim of pure oxygen advocates is that of improved settleability. This claim cannot be predicted directly, but a reasonable theory can be advanced based upon the anoxic core model. It is

this author's postulate that oxygen diffusion limitations and develop-
ment of an anoxic core in large flocs results in a competitive disad-
vantage for the highly flocculant bacteria that form large, dense flocs.
This results in an activated sludge culture that consists of moderate-
sized floc of perhaps less than maximum density or in a proliferation
of filamentous growth from flocs. Such floc would not settle as rapidly
as the large, dense floc that would develop in the absence of any oxygen
diffusion limitations. A theory of increased filamentous growth in
activated sludge floc as a result of oxygen diffusional limitations has
been developed and confirming data presented by Sezgin, et al. (1978)
and Palm, et al. (1980).

CHAPTER IV

EVALUATION OF THEORY AND PREVIOUS RESEARCH

Development of a series of mathematical models that describe diffusion and reaction within bacterial flocs was presented in the previous chapter. However, several of the parameters in the models, including the diffusion coefficient, the bacterial density, and the diffusional radius, have proven to be extremely difficult to measure experimentally. Several research studies have attempted to verify the predictions of the mathematical models by developing experimental procedures that approximate these parameters or by fitting the models to experimental data and backing out values for these parameters. These studies have provided qualitative verification of the mathematical models, but have frequently obtained extremely different values for the diffusion coefficient, bacterial density, and diffusional radius of bacterial flocs. Consequently, the first purpose of this chapter was to review these studies and the methods used to quantify the various parameters, to evaluate the values obtained for the parameters, and to determine whether an average value or range of values can be defined for a typical activated sludge floc. A second purpose was to apply typical values for the various parameters to the activated sludge process and to evaluate the conditions under which oxygen diffusional limitations may be important.

Several research studies have already been conducted at Oregon State University regarding the effect of DO on the ATP viability of activated sludge. The final purpose of this chapter was to review

those studies and evaluate the results obtained in terms of the theory developed in the previous chapter.

EVALUATION OF THE DIFFUSIONAL MODEL

Parameters

1. Floc Density

A summary of values reported for the dry biomass density (ρ) within several research studies is presented in Table 4-1.

Mueller, et al. (1966a) conducted the most extensive measurements of activated sludge floc densities to date (see Chapter II for a description of methods). The values reported for Mueller, et al. (1966a) were calculated from the formula:

$$\rho = \rho_W (1- P_W/100) \qquad (4-1)$$

where ρ_W is the wet density and P_W is the percentage water in the floc. The values for the dry biomass density that result from this calculation, however, are unreasonably high. The average value of P_W reported for eight activated sludge plants was 80 percent, while the water content of isolated bacterial cells grown under a variety of conditions has been determined to be about 80 percent (McKinney, 1962). Consequently, Mueller's data indicated a dry bacterial density equal to and, in some cases, greater than the dry weight density of a bacterial cell. Therefore, the method of Mueller, et al. (1966a) must be severely questioned with regard to measurement of the percentage water in floc and the resultant dry biomass density of bacterial floc.

Table 4-1. Summary of Biomass Densities as Measured by Several Researchers

Researcher	Method	Biomass Type	ρ^{1} (mg/cm^3)
Mueller, et al. (1966a)	Determination of wet and dry density	Samples from 8 activated sludge plants	214^{2} $(104\text{-}262)^{3}$
Mueller, et al. (1966b)	Method of Mueller (1966a)	Batch cultures of Zoogloea Ramigera	403
Baillod and Boyle (1970)	Method of Mueller (1966a)	One activated sludge sample Batch cultures of Z. Ramigera	224 392
Lamotta and Shieh (1979)	Not given	Laboratory activated sludge with nitrifying bacteria	57
Miura, et al. (1975)	Direct visual and gravimetric measurement	Fungal pellets	19-57
Williamson and McCarty (1976b)	Gravimetric determination of biofilm mass, microscopic measurement of thickness	Vacuum-filtered biofilm of nitrifying bacteria	$50\text{-}80^{2}$ $(40\text{-}110)^{3}$

[1] density as milligrams of dry biomass per cubic centimeter of floc or film volume

[2] average

[3] range

Unfortunately, very few other studies have attempted to measure floc density. Within the biotechnology field, measurements of the density of fungal pellets have been made. Due to the large size of fungal pellets (1 to 10 mm) and the spherical growth form, direct visual and gravimetric measurements are possible so that the range of densities reported by Miura, et al. (1975) for various fungal species are reliable estimates. Fungal pellets, however, are comprised of entangled mycelial strands and their density may be different from that of a bacterial floc.

The range of densities reported by Williamson and McCarty (1976b) are included in Table 4-1 as representative values for biofilms. Since biofilms are aggregates of bacterial cells similar to activated sludge floc, the density of bacteria within biofilms provides some indication of the probable density of bacteria in flocs. Furthermore, relatively direct and reliable methods are possible in the measurement of biofilm densities.

2. Floc Size

A summary of values reported for floc size in activated sludge systems is given in Table 4-2. The most extensive measurements of floc size in pilot-scale and full-scale activated sludge plants were conducted by Mueller, et al. (1966a), Finstein and Heukelekian (1967), and Parker, et al. (1972). The nominal diameter measurements of Mueller, et al. (1966a) were based on the measured wet and dry density of the floc and the number of floc particles per unit volume as previously described (Chapter II). These measured densities, however, resulted in a calculated percentage of water in the floc that is unreasonably small,

Table 4-2. Summary of Floc Size Measurements by Several Researchers
(expanded from Knudsen, et al. 1980)

Researcher	Type of System	Method of Measurement	Floc Size (microns)	
Mueller, et al. (1966a)	Samples from 8 activated sludge plants	Equivalent nominal diameter	Mean:	42
			Range:	21-115
Mueller, et al. (1966b)	Batch cultures of Z. Ramigera	Equivalent nominal diameter	Range:	40-124
Englande and Eckenfelder (1972)	Laboratory and pilot-scale activated sludge	Maximum and minimum microscopic dimension	Max:	39-160
			Min:	23-99
Aiba, et al. (1965)	One activated sludge plant	Equivalent diameter	Range:	62-109
Finstein and Heukelekian (1967)	Samples from 4 activated sludge plants	Horizontal chord from microscopic projection	Mean:	60-172[1]
			Mode:	30-90[1]
			Range:	20-200
Parker, et al. (1972)	Activated sludge pilot plant	Filtration, staining, and microscopic measurement of maximum particle dimension	Range:	0.5-1000
			Primary Mode:	1[1]
			Secondary Mode:	300[1]
Lamotta and Shieh (1979)	Laboratory activated sludge with nitrifying bacteria	Microscopic counting cell (details not given)	120	
Knudsen, et al. (1980)	Laboratory activated sludge	Maximum dimension	Mode:	6-35[1]
				600-1200[2]

[1] weighted by number of particles

[2] weighted by equivalent volume or mass

as previously described. Therefore, the accuracy of the nominal diameter measurements must be questioned also.

With the exception of Mueller's data, and that of Aiba, et al. (1965), who used a method similar to Mueller's, all the research studies in Table 4-2 utilized visual microscopic measurement of the floc size, although the exact method of measurement varied (maximum dimension, minimum dimension, horizontal chord, etc.). It is obvious, however, that the major differences in floc size reported in Table 4-2 are not explainable by differences in the exact method of measuring the floc under the microscope. For example, Parker, et al. (1972) report a maximum floc size of approximately 1000 microns in an activated sludge pilot plant, while Finstein and Heukelekian report a maximum of 200 microns in four full-scale plants. Parker, et al. (1972 and 1976) discussed the role of mixing intensity in shearing large flocs and suggested that mixing intensity is the primary variable controlling floc size in activated sludge systems. Undoubtedly, differences in aeration-basin mixing intensity and in exposure to shear forces in pumps accounts for a substantial part of the differences evident in Table 4-2. However, the influence of various environmental variables on the microbial ecology of activated sludge systems probably also had a substantial effect.

Inspection of the mathematical models of diffusion and reaction within a spherical particle presented in the previous chapter shows that these equations are only applicable to a single floc particle of given diameter. For a broad distribution of floc particle sizes such as is present in an activated sludge system, the appropriate solution must be obtained for each increment of floc size and then summarized

over the entire floc size distribution. Therefore, average values of floc size are not appropriate for application of the mathematical model. Furthermore, the appropriate weighting of the floc size distribution for application of the mathematical model is mass (or volume) and not number of particles. Knudsen, et al. (1980) found that volume-weighting caused the floc size distribution to be heavily shifted towards the maximum floc size because the larger floc particles, although few in number, contained the majority of the bacterial mass. Means and modes based on the number of particles per unit volume, such as those presented by Finstein and Heukelekian (1967) and Parker, et al. (1972) are misleading in terms of the floc size increment that incorporates most of the bacterial mass.

3. Diffusion Coefficient

An additional parameter in the mathematical models is the diffusion coefficient (diffusivity) for the limiting nutrient within the bacterial floc. A summary of values reported for the diffusion coefficient of several nutrients in various types of biomass, including bacterial flocs, is given in Table 4-3.

The diffusion coefficients reported by Mueller, et al. (1966b), Lamotta and Shieh (1979), and Huang and Bungay (1973) are based on curve-fitting to experimental data and are a product of the methods utilized to estimate the density and diffusional radius. This dependency is clearly shown by the data of Mueller, et al. (1966b), wherein the use of two different methods for estimating the diffusional radius resulted in an order of magnitude difference in the curve-fitted diffusion coefficient. The use of a large fungal pellet by Huang and

Table 4-3. Summary of Diffusion Coefficient Measurements by Several Researchers (expanded from Matson, 1976)

Researcher	Type of Biomass	Procedure	Reactant	Diffusivity ($\times 10^5$ cm^2/s)	D_b/D_w[1] ($\times$ 100%)
Mueller, et al. (1966b)	Batch cultures of Z. Ramigera	Curve fit to oxygen uptake data with diffusional diameter estimated as:	Oxygen		
		1) specific surface		0.18±.073	7
		2) nominal diameter		2.05±.84	80
Lamotta and Shieh (1979)	Suspended nitrifying culture	Curve fit to ammonia uptake data	Ammonia	.0089-.046	0.6-3
Pipes (1974)[2]	Suspended mixed culture	Two-chamber apparatus	Glucose	0.06-0.6	10-100
Matson and Characklis (1976)	Suspended mixed culture (laboratory)	Two-chamber apparatus	Oxygen	0.4-2.0	20-90
			Glucose	0.06-0.21	10-30
	Samples from 3 activated sludge plants		Oxygen	0.85-1.4	40-60
			Glucose	0.18-0.35	30-50
Huang and Bungay (1973)	Mounted fungal pellet	Derived from slope of DOC gradient	Oxygen	2.5×10^{-6}	10
Tomlinson (1966)	Bacterial slime	Reaction product analyses	Oxygen	1.5	60
Williamson and McCarty (1976)	Vacuum-filtered biofilm of nitrifying bacteria	Two-chamber apparatus	Oxygen	2.2	90
			Ammonia	1.25	80
			Nitrite	1.2	90
			Nitrate	1.35	90

[1] D_b = diffusivity in the biomass, D_w = diffusivity in water

[2] as reported by Matson (1976)

Bungay (1973), however, allowed direct measurement of the pellet dia-
meter and dry weight so that the derived diffusion coefficient may be
representative of the true value.

Direct measurements of diffusion coefficients generally utilize a
two-chamber apparatus wherein a concentration gradient is established
between the two chambers and diffusion of the reactant between the two
chambers requires passing through a sample of the biomass. Williamson
and McCarty (1976b) developed such a two-chamber apparatus by modifying
a standard Millipore filter apparatus. Because the biofilms utilized
in the kinetic experiments were dispersed bacterial floc vacuum-filtered
upon a membrane filter, direct insertion of the biofilm into the appar-
atus was possible without any disturbance of the biofilm. The diffu-
sion coefficients obtained for the biofilm by this method were only
slightly smaller than the corresponding diffusion coefficients in water.

Direct measurement of diffusion coefficients for bacterial floc is
not possible without concentrating the floc and fitting them into some
type of template suitable for a two-chamber experimental apparatus.
Matson and Characklis (1976) utilized a modified Millipore filtration
apparatus similar to that of Williamson and McCarty to measure diffu-
sion coefficients for activated sludge floc. A perforated brass tem-
plate was packed with concentrated bacterial floc and inserted between
the two chambers. The oxygen diffusion coefficient obtained for labor-
atory activated sludge cultures by this method decreased with increasing
solids retention time and with lower carbon to nitrogen ratios in the
feed. Activated sludge from three full-scale plants was also tested,
resulting in measured diffusion coefficients of approximately 50 percent
of the corresponding value in water.

4.   Typical Values

The above review of values reported for the three parameters in
the diffusional model demonstrates the difficulties involved in experi-
mentally measuring these parameters, the dependence of values derived
for one parameter by curve-fitting techniques on the experimental
methods utilized to estimate the other parameters, and the wide range
in values that are possible. However, the values listed below are
typical values for bacterial flocs in activated sludge systems as best
can be determined from the data in Tables 4-1 through 4-3:

Parameter	Typical Value	Range
Dry weight density, ρ (mg/cm^3)	75	10-150
Diffusion coefficient (D_f/D_w)	0.50	0.10-0.90
Floc radius (microns)	-	1-1000

The typical value for density is based largely on biofilm measurements
because relatively direct and reliable measurements are possible and
because no method has been developed to date that accurately measures
the density of a bacterial floc. The typical value given for the dif-
fusion coefficient (reported as the ratio of the diffusion coefficient
in the floc to that in water) was based largely on the work of Matson
and Characklis (1976) since their study involved the most direct mea-
surement that is feasible and because the values reported appeared to
be reasonable in comparison to relatively reliable values reported for
biofilms and fungal pellets. The data reported in Table 4-2 for floc
size indicates that this parameter varies widely within activated sludge
systems. Therefore, establishment of a typical value is not realistic

and application of the mathematical models must consider the complete floc size distribution.

Application to Activated Sludge

Application of typical values for the various parameters in the diffusional models to operating conditions typical of full-scale activated sludge plants provides an indication of the conditions under which oxygen diffusion limitations occur.

Activated sludge plants are designed and operated over a wide range of loadings, but are generally categorized as high-rate, conventional, or extended aeration plants. Typical loadings for these categories are generally described in terms of the mass of organic substrate applied or removed per unit time per unit mass of microorganisms present, frequently termed the food to microorganism (F:M) ratio. If expressed in terms of the mass of substrate removed, then the F:M ratio is identical to the specific substrate utilization rate, U_s, and can be defined mathematically as:

$$F:M = U_s = \frac{C_s^o - C_s}{\theta X} \qquad (4-2)$$

where C_s^o and C_s are the influent and effluent substrate concentrations, respectively, θ is the aeration basin detention time, and X is the concentration (dry weight basis) of microorganisms. Generally, the substrate concentration and utilization rates are expressed in terms of the biochemical oxygen demand (BOD). Typical values of the F:M ratio, or U_s, for high-rate, conventional, and extended aeration plants are 1.0, 0.3, and 0.1 g BOD/g biomass-day, respectively.

With typical values for the floc density and diffusion coefficient as described in the previous section and U_s defined as described above, a simple analysis of the importance of oxygen diffusional limitations can be accomplished. The following assumptions are utilized in the analysis that follows:

1. The steady-state model of Lawrence and McCarty (1970) for an activated sludge system with a completely mixed aeration basin is applicable (see Chapter III for development of this model).

2. The wastewater treated is domestic sewage, with a composite chemical formula of $C_{10}H_{19}O_3N$ (McCarty, 1972). The following coefficients apply to this wastewater:

    Y = 0.65 g biomass/g BOD_L b = 0.05 days^{-1}

    k = 5 g BOD_L/g biomass-day K_s = 50 mg BOD_L/l

    where BOD_L represents the ultimate BOD of the wastewater and is approximated as the theoretical oxygen demand (2.0 g O_2/g substrate for domestic sewage as described above).

3. The typical values of U_s previously described are in terms of g BOD_L/g biomass (dry weight)-day.

4. The composite chemical formula for cells is $C_5H_7NO_2$.

The model of Lawrence and McCarty can then be applied to describe the system and its performance in terms of the solids retention time (θ_c), the concentration of substrate (as BOD_L) in the effluent (C_s), and the net yield (Y_{net}) of biomass according to the following previously derived equations:

$$\theta_c = \frac{1}{YU_s - b}$$

(4-4)

$$Y_{net} = Y - \frac{b}{U_s}$$

(4-5)

$$C_s = \frac{K_s(1+b\theta_c)}{\theta_c(Yk-b)-1} \tag{4-6}$$

Furthermore, using the calculated net yield, an overall stoichiometric equation for substrate conversion into microbial mass can be developed, yielding the stoichiometric oxygen requirement (γ) and the specific oxygen utilization rate (U_s) according to the equation:

$$U_o = \gamma\, U_s \tag{4-7}$$

These calculations are described below for the high-rate process example:

1. $\theta_c = \dfrac{1}{YU-b} = \dfrac{1}{0.65(1.0)-0.05} = 1.7$ days

2. $Y_{net} = Y - \dfrac{b}{U} = 0.65 - \dfrac{0.05}{1.0} = 0.60$ g biomass/g BOD_L

3. $C_s = \dfrac{50(1+0.05\cdot1.7)}{1.7(0.65\cdot5.0-0.05)-1} = 17.1$ mg BOD_L/l

4. The balanced stoichiometric equation is then

    $$C_{10}H_{19}O_3N + 8.26\ O_2 \rightarrow 0.85\ C_5H_7NO_2 + 5.61\ CO_2$$

    $$+ 0.15\ NH_4^+ + 0.15\ HCO_3^- + 6.15\ H_2O$$
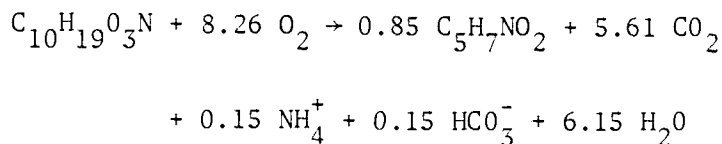
    and the stoichiometric requirement for oxygen is

    $$\gamma = \frac{8.26\ \text{moles}\ O_2}{1.0\ \text{moles substrate}} \cdot \frac{32\ \text{g/mole}}{200\ \text{g/mole}} \cdot \frac{1}{2.0\ \text{g } BOD_L/\text{g substrate}}$$

    $$= 0.66\ \text{g } O_2/\text{g } BOD_L$$

Similar calculations for the other two loading rates yields the results shown in Table 4-4.

Table 4-4. Typical Operating Characteristics for the Activated Sludge Process

Process Loading	U_s $(\dfrac{g\ BOD_L}{g\ biomass-day})$	θ_c (days)	Y_{net} $(\dfrac{g\ biomass}{g\ BOD_L})$	C_s (mg BOD_L/1)	γ (g O_2/g BOD_L)	U_o $(\dfrac{g\ O_2}{g\ biomass-day})$	$C_s \dfrac{D_s}{D_o} \gamma$ (mg/1)
High Rate	1.0	1.7	0.60	17.1	0.66	0.66	2.45
Conventional	0.3	6.9	0.48	4.23	0.73	0.22	0.67
Extended Aeration	0.1	67	0.15	1.33	0.92	0.092	0.27

For the purposes of determining whether oxygen diffusional limitations are important in activated sludge plants, the anoxic core model was applied and the critical DO calculated in accordance with Equation 3-32:

$$C_o^c = \frac{\rho}{6} \frac{U_o}{D_o} R^2$$

The major assumption of this model is that the specific oxygen utilization rate (U_o) is constant throughout the floc. This assumption may not be completely valid for all the examples being considered. However, even if U_o is decreasing with increasing penetration into flocs, the calculated value of U_o represents an average utilization rate and unless changes in U_o with floc radius are severe, the predicted value of C_o^c should be a reasonable estimate.

Application of Equation 3-32 to the oxygen utilization rates calculated in Table 4-4 yields the curves in Figure 4-1. These curves were based on typical values for the density and diffusion coefficient of 75 mg/cm^3 and 1.3 x 10^{-5} cm^2/sec ($\approx$50% x D_w) as developed in the previous section. Figure 4-1 shows, for example, that a bulk DO of approximately 2.2 mg/ℓ is necessary at the conventional loading rate to maintain flocs with a radius of 300 microns in a fully aerobic state. Higher loading rates result in a larger C_o^c for the same floc radius due to the increased specific utilization rate. Therefore, the development of an anoxic core is much more likely in the high-rate process.

Figure 4-1 also emphasizes the importance of floc size in the possible development of an anoxic core within activated sludge flocs. For example, if the floc size measurements of Finstein and Heukelekian (1967) are typical (maximum radius $\simeq$ 100 microns), then a bulk DO of
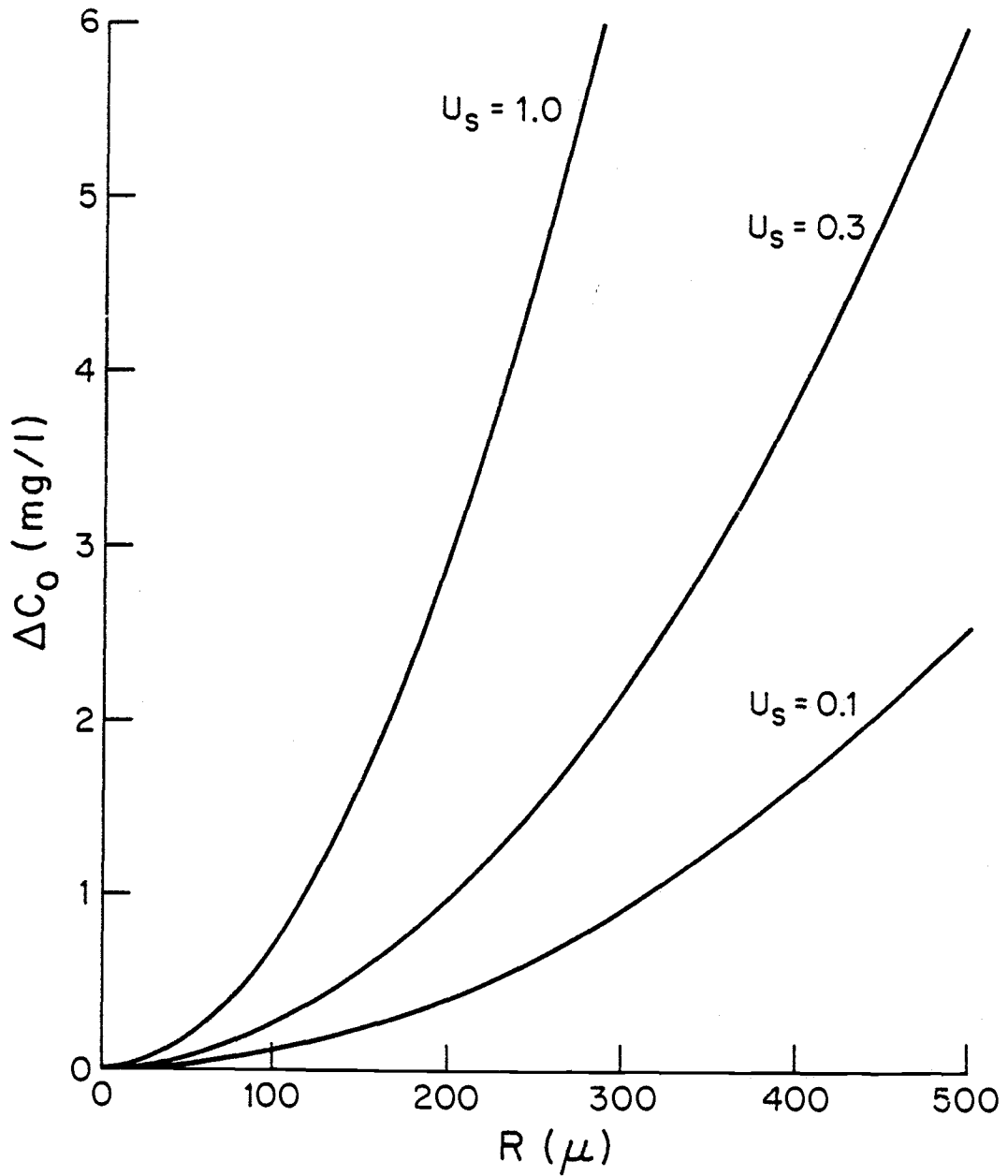
Figure 4-1.   Reduction in DO within Activated Sludge Flocs as
              a Function of Floc Radius and Substrate Utiliza-
              tion Rate (U_s is in units of g BOD_L/g biomass-
              day).

1.0 mg/ℓ would maintain fully aerobic conditions in all flocs even at the high loading rate. However, if the floc size measurements of Parker, et al. (1971) and Knudsen, et al. (1980) are typical (maximum radius ≃ 500 microns), then development of anoxic conditions within a major portion of the floc volume could occur at a bulk DO of 1.0 to 2.0 mg/ℓ (typical of air activated sludge), particularly at the conventional and high loading rates.

A major question in evaluating whether oxygen diffusion limitations are important in the treatment of organic wastewaters by the activated sludge process is whether oxygen, rather than the organic substrate, is the diffusion-limiting reactant. The mathematical relationships that define the diffusion-limiting species were developed in the previous section (Equations 3-23 and 3-24). These state that if

$$C_{b,o} < C_{b,s} \frac{D_s}{D_o} \gamma \tag{3-23}$$

then oxygen is the diffusion-limiting species.

The following assumptions and values are utilized in the analysis of Equation 3-23 for the examples being considered:

1. The ratio D_s/D_o in bacterial flocs is the same as that in water. This assumption is supported by the experimental studies of Williamson and McCarty (1976b) and appears to be reasonable with respect to the measurements of Matson and Characklis (1976).

2. The diffusion coefficient for oxygen in water at 20°C is 2.5 x 10^{-5} cm^2/s and that for large, organic molecules, such as would be present in domestic wastewater, is 0.5 x 10^{-5} cm^2/s (see Perry, 1973).

With the above assumptions and the calculated values of C_s (=$C_{b,s}$) and γ given in Table 4-4, the value of $C_{b,s} \frac{D_s}{D_o} \gamma$ was calculated for the

three loading rates considered and is listed in Table 4-4. The values indicate that the bulk DO would have to be less than 2.45, 0.67, and 0.27 mg/ℓ in order for oxygen to be the diffusion-limiting species for the high-rate, conventional, and extended aeration process examples.

The above calculations lead to the conclusion that air activated sludge systems operated at DO levels of 1-2 mg/ℓ are not oxygen-limited except at very high loading rates. However, the many assumptions that entered into these calculations must be considered and evaluated in terms of their applicability to full-scale activated sludge plants.

First, evaluation of whether oxygen or the organic substrate is the diffusion-limiting species depends primarily on the calculated value of the substrate concentration in the aeration basin according to the Lawrence and McCarty model. The key element in this model, as previously described, is the assumption of a Monod-type expression (Equation 4-3) for the specific utilization rate. While this type of expression has been experimentally verified numerous times for pure bacterial cultures utilizing a single, soluble organic substrate, its accuracy in predicting substrate concentrations in activated sludge systems must be regarded as very approximate. Activated sludge systems treat wastewaters containing a wide variety of organic compounds, both soluble and particulate, with a highly mixed culture of bacteria and higher organisms. The difficulties encountered in application of the Lawrence and McCarty model are reflected, in large part, by the wide range of kinetic coefficients that are reported for complex organic substrates such as domestic sewage (see Nelson and Lawrence, 1980).

Second, the assumption of steady-state conditions for the examples does not correlate well with actual plants, where the influent flow

rate and substrate concentration vary from day to day and hour to hour. Although oxygen diffusional limitations may not be important at the steady-state condition, they may become important during shock loads, when the substrate concentration in the aeration basin rises and utilization rates increase.

Third, the assumption that the aeration basin is completely mixed with a uniform substrate concentration throughout is not appropriate for many full-scale plants. Most large activated sludge plants utilize long, rectangular basins that more nearly approximate plug-flow rather than completely-mixed conditions. Substrate concentrations in plug-flow reactors decrease from the influent to the effluent end so that oxygen may be the diffusion-limiting species in a major portion of the reactor.

EVALUATION OF PREVIOUS RESEARCH

Several research studies have been conducted at Oregon State University regarding the effect of DO on the ATP viability of activated sludge. The results of these studies are presented in this section along with an analysis of these results.

Study No. 1

Edlund (1976) and Eckley (1976) operated two identical laboratory-scale activated sludge reactors using a filtered raw sewage and nutrient broth mixture as substrate (COD $\simeq$ 200 mg/ℓ). The mixing intensity factor, G, for both reactors was in the range of 94-133/sec, typical of full-scale activated sludge plants (Parker, et al., 1971). One reactor was operated at a DO of 2 mg/ℓ (simulating air systems) and the other

at 8 mg/ℓ (simulating oxygen systems). Results of their study are given in Table 4-5.

The bacterial cultures that developed were extremely filamentous over the entire range of solids retention times (SRT's) as indicated by the high SVI values. The substrate concentration in the effluent (C_s), measured as soluble COD, was similar for the two reactors over the range of SRT's tested. Sludge production was larger in the oxygen reactor at SRT's of 0.5 and 1.0 days, and then was lower in the oxygen reactor at θ_c's of 2.0, 5.0, and 10.0 days. However, the most consistent result of this study was in terms of the specific ATP viability (T), which was approximately twice as large in the oxygen reactor as in the air reactor over the entire range of SRT's (see Figure 4-2).

Williamson and Nelson (1976) evaluated these data in terms of the anoxic core model. The reduced specific viability and the increased sludge production in the air system was reasonably predicted by assuming that a large fraction of the microbial mass in the air system was inactive due to anoxic conditions and that this inactive mass had a decay coefficient (b) of zero.

To determine whether the data of Edlund and Eckley were the result of oxygen diffusional limitations in the air system, the theory developed in the preceding section is applied. First, it is necessary to determine whether oxygen was the diffusion-limiting species as defined by Equation 4-9. As shown in previous examples, the stoichiometric ratio of oxygen to substrate utilization (γ) varies only from about 0.6 to 0.9 over a wide range of loading rates so that the major variable in this equation is the bulk substrate concentration. For the values of γ stated above, it can be seen that the ambient substrate concentration

Table 4-5. Results of Edlund and Eckley (1976)

θ_c (days)	SVI (mg/1)		C_s (mg COD/1		Sludge Production (g/day)		T (mg ATP/g VSS)	
	A[1]	B[2]	A	B	A	B	A	B
0.5	320	630	82	63	1.3	1.7	1.25	2.44
1.0	470	250	52	40	1.3	1.7	1.15	2.57
2.0	620	240	41	37	2.4	1.9	1.38	2.25
5.0	260	410	33	37	1.9	1.2	0.99	2.04
10.0	370	260	30	45	1.0	0.6	1.03	2.03

[1] A = reactor with DO = 2 mg/1
[2] B = reactor with DO = 8 mg/1
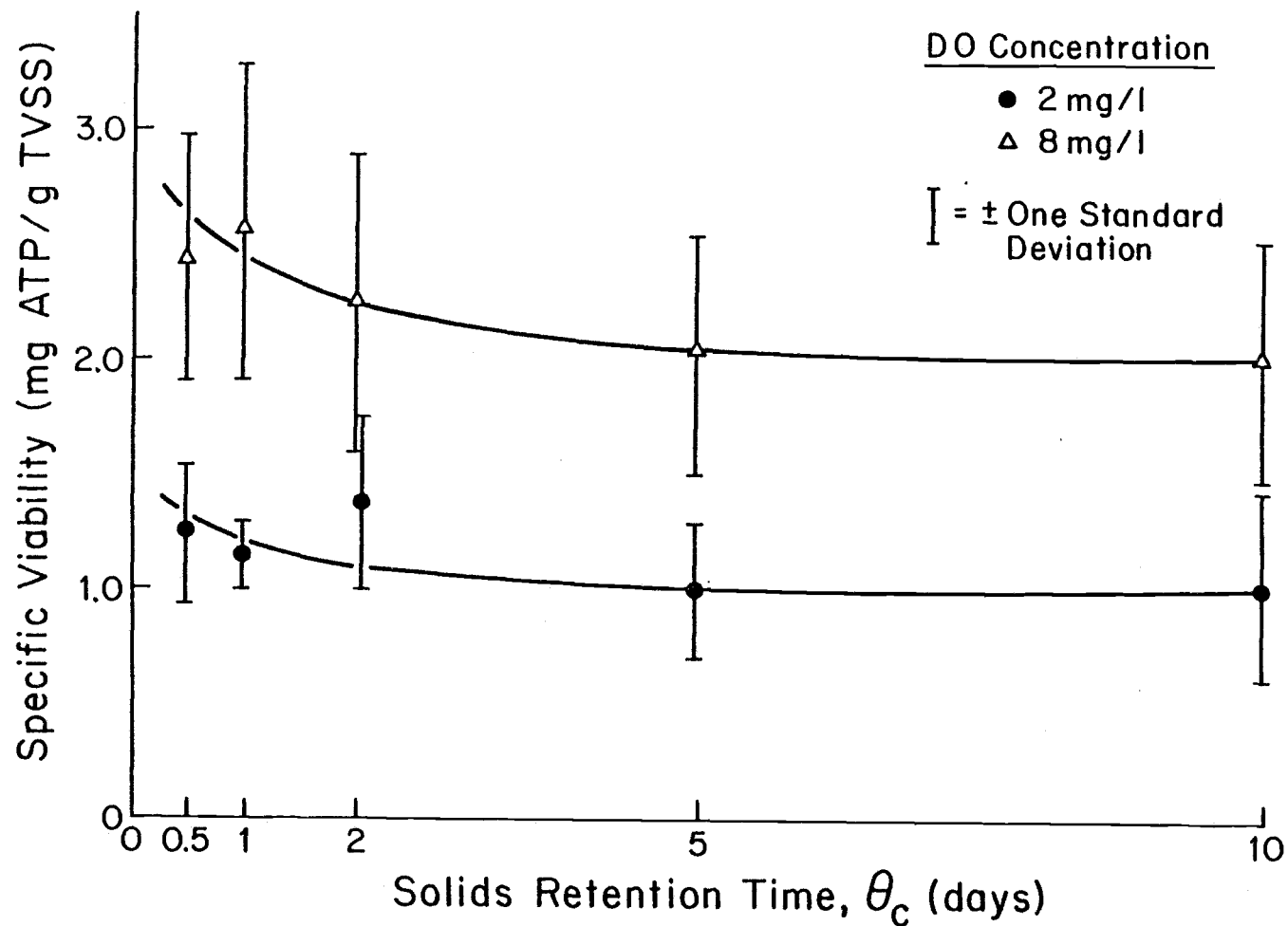
Figure 4-2.   Specific Viabiity versus θ_c (from Edlund and Eckley, 1976).

would have to be approximately three to four times the bulk DO for oxygen to be the diffusion-limiting species. Unfortunately, measurement of the biodegradable substrate concentration in the reactors was masked by the relatively high level of nonbiodegradable compounds typical of domestic sewage. From the data in Table 4-5, it appears that the non-biodegradable fraction of the total soluble COD was 30 to 40 mg/ℓ. Therefore, only at SRT's of 0.5 and 1.0 days was the biodegradable substrate concentration in the reactor high enough to reliably determine that oxygen was the diffusion-limiting species. For the operation at SRT's of two, five, and ten days, it appears that oxygen was probably not the diffusion-limiting nutrient, although definite conclusions are not possible.

A second requirement for the development of oxygen diffusion limitations is that the bacterial flocs must be large enough for dissolved oxygen to be completely utilized before penetrating to the core. Unfortunately, no floc size measurements were included in this study and, therefore, it is not possible to assess whether oxygen diffusion limitations provide a reasonable explanation of the data.

Study No. 2

Lasswell (1977) utilized the same soluble sewage-nutrient broth substrate as the previous study. In the first phase of this study, two laboratory-scale reactors were operated as chemostats with a detention time of one day. The reactors were allowed to come to a steady-state (assumed to occur after three SRT's) at each of four different DO levels from 2 to 20 mg/ℓ. The flocs that developed in the chemostat reactor were characterized as small and without any filamentous growth.

Results of this phase are shown in Figure 4-3. The specific ATP viability increased significantly with increasing DO and reached a maximum of 1.5 mg ATP/g VSS. This maximum viability was somewhat smaller than the maximum value of 2.6 mg ATP/g VSS found in the first study at a SRT of one day. Also, the ratio of ATP viability at a DO of 8 mg/ℓ to that at 2 mg/ℓ was 1.4 in this study and 2.2 in the first study. These disparities may have been the result of different cultures or the smaller floc size in this study as compared to the first study.

The COD removal increased slightly, but not significantly, with increased DO and viability. This suggests that the biodegradable fraction of the effluent COD was small. The effluent COD ranged from 32 to 45 mg/ℓ.

The second phase of this study was designed to determine whether differences in the microbial culture between air and oxygen systems could explain the observed increases in ATP viability with increasing DO. Two solids-recycle reactors were operated at DO levels of 2 and 8 mg/ℓ and with θ and θ_c equal to six hours and five days, respectively, until a steady-state was acheived. At that time, the specific viabilities in both reactors were measured. The aeration rate was then altered to give a DO of 8 mg/ℓ in the reactor that previously was at 2 mg/ℓ, and 2 mg/ℓ in the reactor that was previously at 8 mg/ℓ. The specific viabilities in both reactors were then monitored over the following 48 hours.

Mixing intensities (G values) were held constant throughout the experiment. The flocs that developed were small, but settled well. No filamentous growth was noted. The soluble effluent COD was between 30 and 34 mg/ℓ in both reactors.
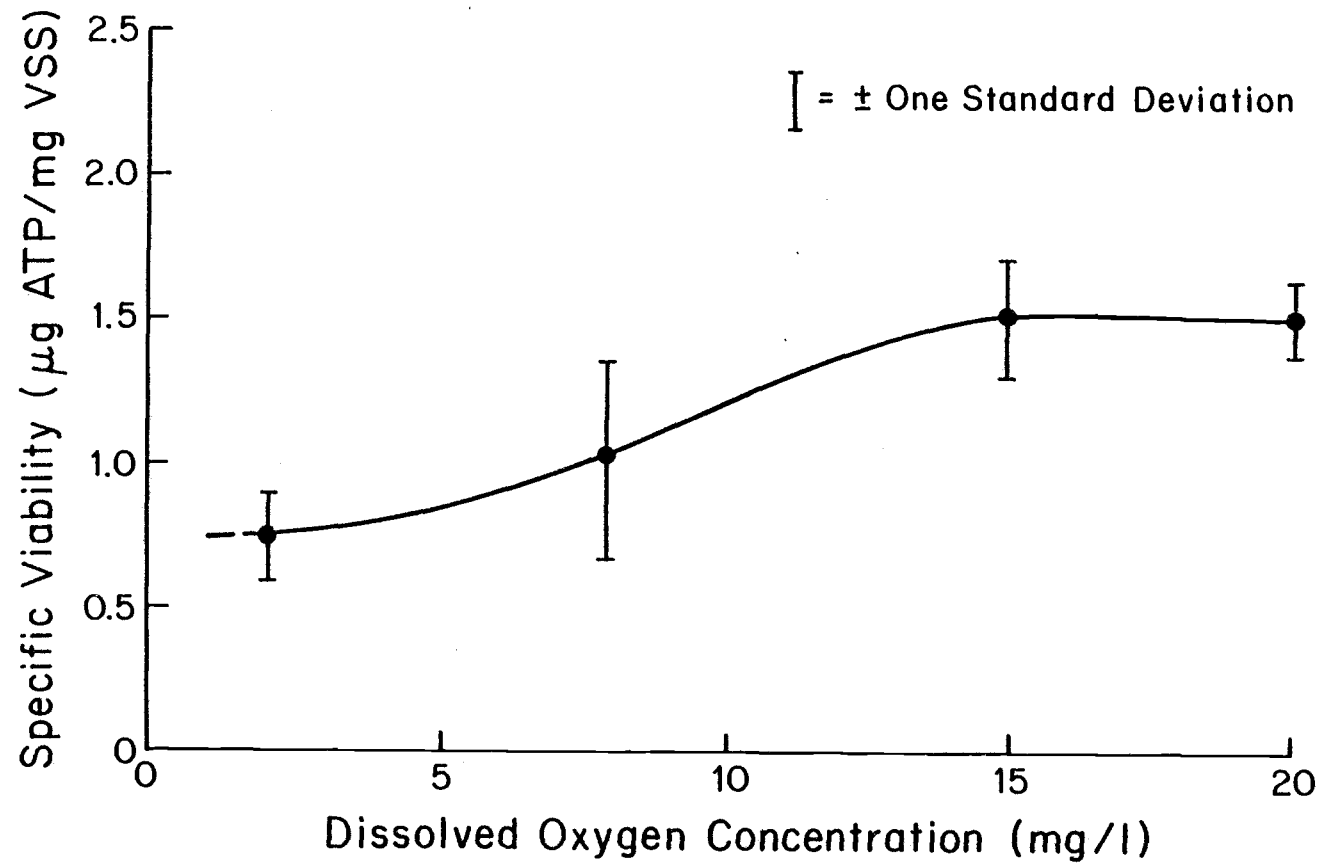
Figure 4-3. Effect of DOC on Specific Viability (after Lasswell, 1977).

Results of this phase are shown in Figure 4-4. The specific via-
bilities in the two reactors at steady-state were measured as 1.3 and
2.2 mg ATP/g VSS at DO values of 2 and 8 mg/ℓ, respectively. These are
the values in Figure 4-4 at time (t) equals zero. After the aeration
was altered, the specific viability in each reactor responded directly
to the change in DO and approached the initial (t = 0) steady-state
viability for the alternate reactor within 24 hours.

These results indicate that the differences in specific viability
observed in the two reactors at different DO values were not due to
differences in the microbial populations. At a mean SRT of five days,
the populations in the reactors could not have shifted completely with-
in a one-day period.

Evaluation of these data within the anoxic core theory, however,
is again difficult. The existence of a sizeable nonbiodegradable frac-
tion of the organic material in the feed precludes accurate measurement
of the concentration of biodegradable organic substrates in the ef-
fluent. Therefore, it is not possible to determine whether oxygen was
the diffusion-limiting species. Furthermore, floc sizes were reported
to be small in both phases of this study, which casts doubt upon the
appropriateness of the anoxic core model in explaining the observed
differences in ATP viability.

Study No. 3

Knudsen, et al. (1980) operated two identical laboratory activated
sludge reactors with phenol as the organic substrate. The solids re-
tention time was maintained at three days, the hydraulic detention at
12 hours, and the mixing intensity factor (G) at 155 per second in both
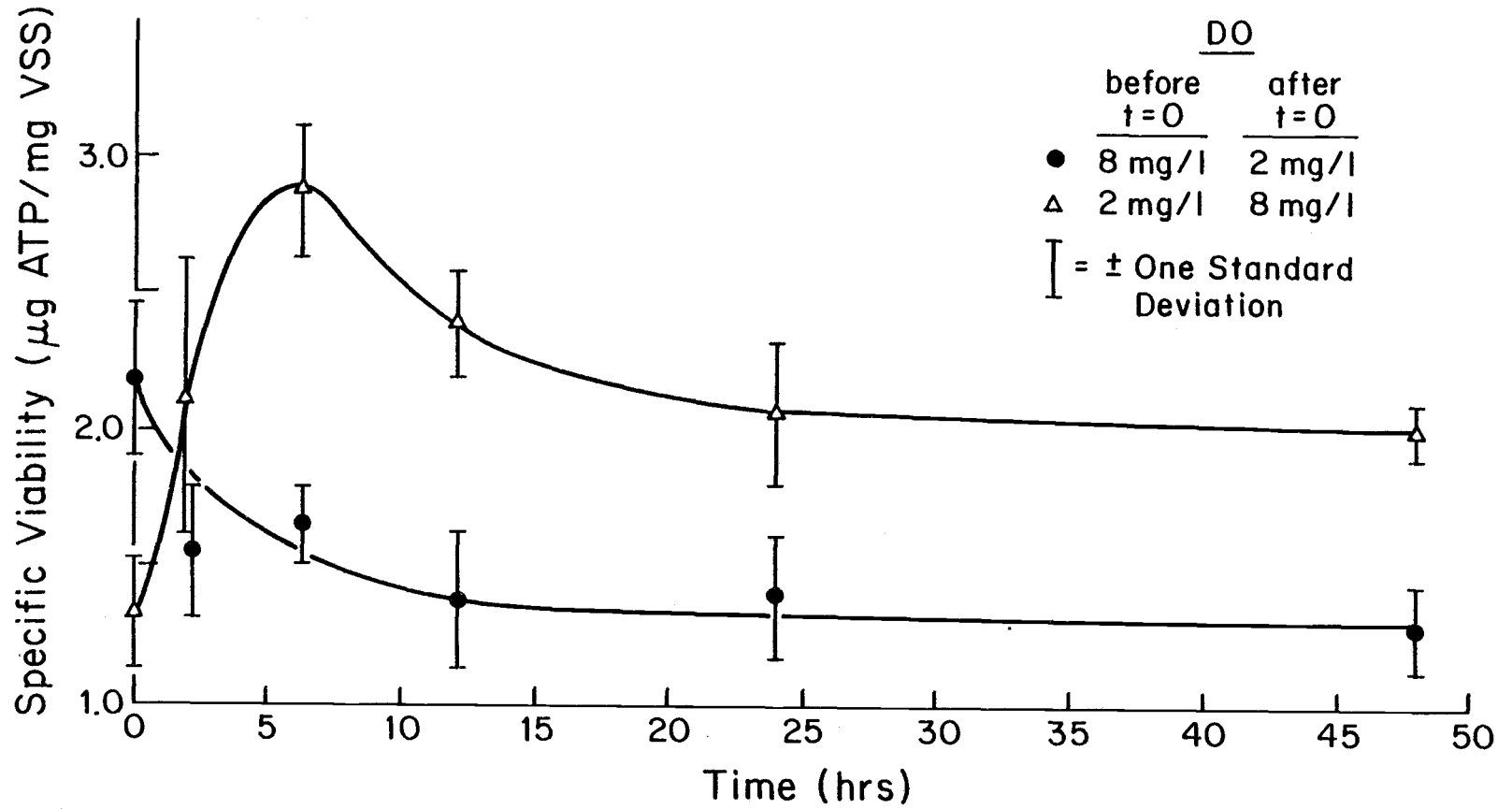
Figure 4-4. Dynamic Response to Change in DO (after Lasswell, 1977).

reactors. Each reactor was operated to steady-state at DO levels of 1.5, 5, and 15 mg/ℓ. Parameters measured included influent and effluent phenol concentration, the maximum substrate utilization rate (k) and the half-velocity coefficient (K_s) by the method of Williamson and McCarty (1974), the mixed liquor specific viability, and floc size.

Results of this study are shown in Figures 4-5 and 4-6. Figure 4-5 shows that the mixed liquor suspended solids (indicative of net yield) declined slightly with increasing DO and that the effluent phenol concentration declined significantly with increasing DO in both reactors.

Both the specific viability and the maximum substrate utilization rate (based on VSS) increased with increasing DO from 1.5 to 15 mg/ℓ (Figure 4-6). Calculation of the maximum substrate utilization rate on an ATP basis, however, showed a relatively constant k over the complete range of DO levels tested.

Floc size determinations indicated that the vast majority of the microbial mass was in flocs with a maximum dimension larger than 800 microns in both reactors. As described in previous examples, flocs of this size are likely to be subject to significant oxygen diffusion limitations, particularly at high loading rates (see Figure 4-1). Furthermore, evaluation of Equation 4-9, which defines whether oxygen is the diffusion-limiting species, indicates that the bulk substrate (phenol) concentration would have to be greater than approximately 1.6 times the bulk DO for oxygen to be the diffusion-limiting species. This condition is met for the phenol concentrations present in both reactors at DO levels of 1.5 and 5 mg/ℓ, but not at a DO of 15 mg/ℓ. However, at the 15 mg/ℓ DO, the reactors were considered to not be subject to any
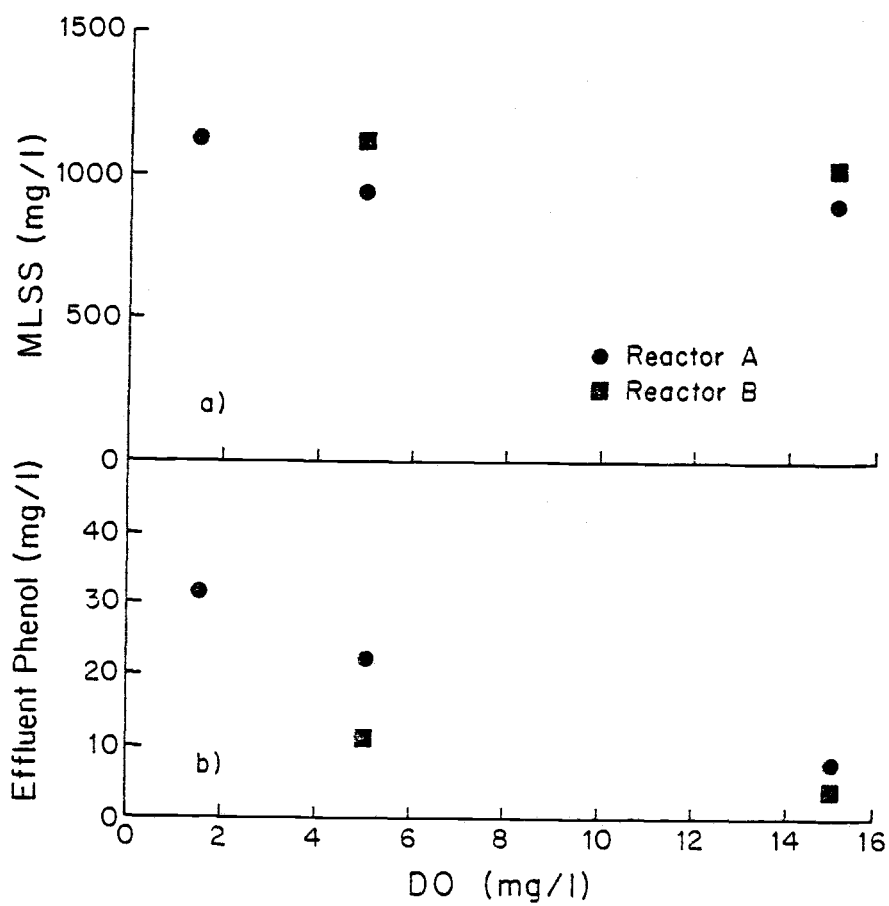
Figure 4-5.   Mixed Liquor Suspended Solids and
              Effluent Phenol Concentration (after
              Knudsen, et al., 1980).
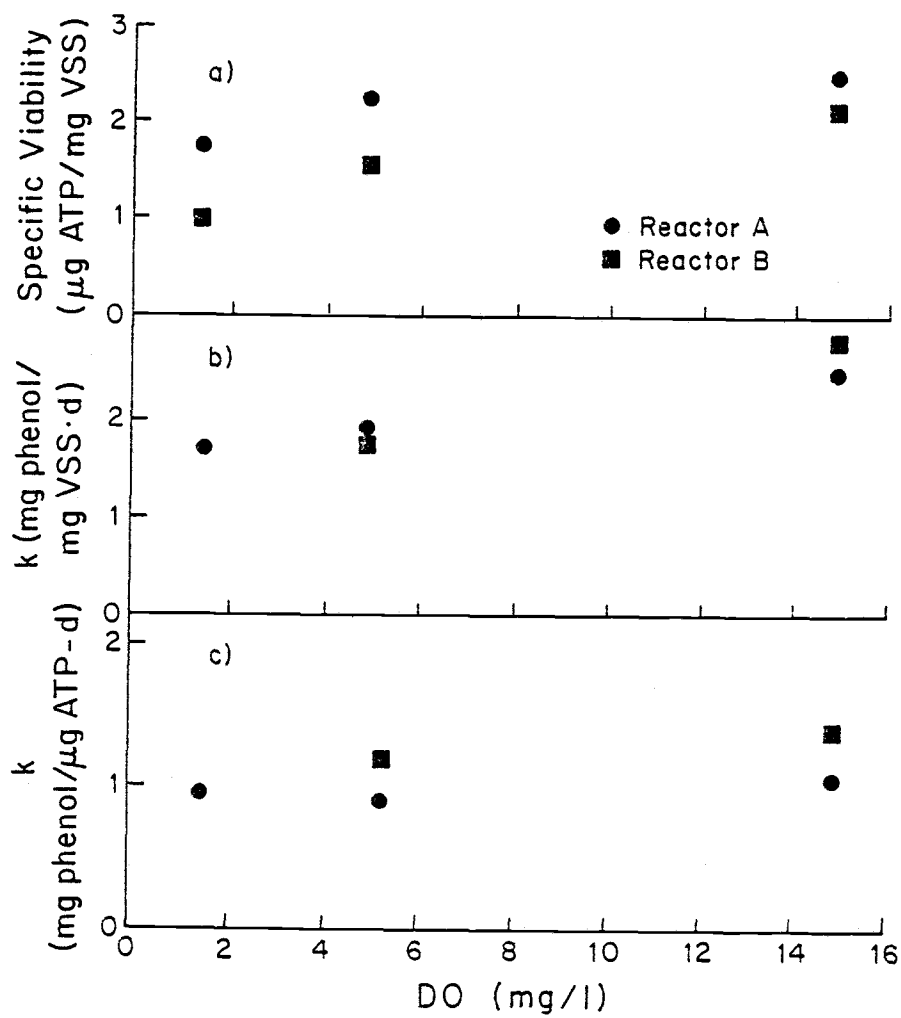
Figure 4-6. Specific Viability and Maximum
Substrate Utilization Rates (after
Knudsen, et al., 1980).

diffusional limitations and to not contain an X_n component of the micro-
bial mass. Therefore, interpretation of these results in terms of the
anoxic core theory is reasonable.

Knudsen, et al. (1980) evaluated these data in terms of the anoxic
core theory and development of a nonviable portion (X_n) of the total
microbial mass. Using assumed values for the bacterial density and
diffusion coefficient in the floc, the nonviable fraction (n) was pre-
dicted from the mathematical model and compared to that measured as the
ratio of ATP viability in the oxygen diffusion-limited cases (DO = 1.5
and 5 mg/ℓ) to that at a DO of 15 mg/ℓ as shown in Table 4-6. The lack
of close agreement between the measured and predicted values of n can
be attributed to the lack of accurate determinations of floc size and
the assumed values for the density and diffusion coefficient.

Study No. 4

Titus (1980) operated two laboratory-scale activated sludge reac-
tors using primary effluent from full-scale activated sludge plants as
feed. The reactors were filled with activated sludge from the full-
scale plant and the reactors operated at the same hydraulic detention
time as the full-scale plants. The DO in one reactor was controlled at
13.0 ± 1.0 mg/ℓ and that in the other at 2.0 ± 0.5 mg/ℓ by varying the
aeration gas composition, similar to the three previous studies. Con-
sequently, the laboratory-scale activated sludge operation simulated
the full-scale plant operation in all respects except for the DO main-
tained in the aeration basin and the maintenance of a constant tempera-
ture of 20°C.

Table 4-6.   Measured and Predicted Ratio of
Inactive to Active Mass

Steady-State DO (mg/ℓ)	$n = X_n/X$			
	Reactor A		Reactor B	
	Measured	Predicted	Measured	Predicted
1.5	0.5	1.0	1.4	0.7
5	0.1	0	0.5	0.2
15	0	0	0	0

The reactors were operated at three different full-scale plants, including two runs at a pure oxygen plant. The reactors were operated for a 20-hour period with ATP, TSS, and VSS analyses performed at four-hour intervals and floc size measurements taken at the beginning and end of each run. It was assumed that effects of oxygen diffusional limitations on ATP viability would be manifested within the 20-hour period of operation as indicated by Study No. 2 (see Figure 4-4).

Results indicated no difference in the specific ATP viability between the two reactors at any of the four plants where the reactors were operated. Floc sizes were generally less than 200 microns but flocs up to 800 microns were noted at one plant.

Because the results indicated that floc sizes at the three plants tested were too small to result in oxygen diffusional limitations at a DO of 2 mg/ℓ, floc size determinations alone were conducted on aeration basin samples from four other full-scale plants, including one pure oxygen plant. Results indicated a maximum floc size of 200 microns at three of the four plants and 300 microns at the remaining plant. A very interesting result was that the two pure-oxygen plants exhibited the smallest floc sizes observed and the absence of significant filamentous growth, which was present in all samples from air activated sludge plants.

CHAPTER V

GENERAL RESEARCH PLAN AND EXPERIMENTAL METHODS

GENERAL RESEARCH PLAN

Background

Formulation of an appropriate research plan for this study required a review of the literature, of the theory and resultant models, and of previous, directly related research, as presented in Chapters II through IV. The following conclusions were derived from this review and were utilized in formulating the research plan presented later in this chapter:

1. In general, the DO at which a dispersed bacterial culture is maintained has no effect on the kinetics of substrate removal or any other aspect of metabolism above a very low, critical level (less than 0.1 mg/ℓ). Substrates that require oxygenases in their degradative pathway may result in a higher critical DO if the oxygenase reaction is rate-limiting in the overall metabolic pathway and if the oxygenase enzyme exhibits a much lower affinity for oxygen than the terminal cytochrome oxidase. However, this does not appear to be a common occurrence.

2. Flocculant bacteria, such as those that develop in an activated sludge system, may be subject to diffusional limitations if the bacterial aggregates formed are

large and dense. Oxygen may be defined as the dif-
fusion-limiting species where the bulk substrate con-
centration is approximately 1.5 to 8 times the DO,
depending upon the substrate and the growth condi-
tions, as defined in the mathematical model (Equa-
tions 3-23 and 3-24).

3. ATP is a good indicator of bacterial viability.
Furthermore, the adenylate energy charge of growing
bacterial cultures is strongly controlled in the
range of 0.8 to 0.9.

4. The effects of oxygen diffusional limitations can be
described mathematically by the anoxic core model.
This model predicts that substrate utilization rates
and ATP viability in a bacterial aggregate will de-
crease in direct proportion to the fraction of aggre-
gate volume that becomes anoxic, and that the anoxic
volume can be predicted from a mathematical model of
diffusion and reaction. Previous research studies have
provided substantiation of this model; however, addi-
tional verification is necessary.

The following problem areas were also defined within the review
process:

1. The parameters within the mathematical models of dif-
fusion and reaction within microbial aggregates, in-
cluding the biomass density, the diffusion coefficient,
and the equivalent diffusional radius have proven to

be very difficult to measure experimentally. The various methods utilized by different researchers to quantify those parameters have often not produced comparable results. In particular, no method has been developed to date that produces reasonable and nonsubjective measures of the bacterial density within bacterial aggregates characteristic of the activated sludge process.

2. The conditions that are conducive to the development of oxygen diffusional limitations include large aggregate size and a high bacterial density within the aggregate, high oxygen utilization rates, and high bulk substrate concentrations. Laboratory activated sludge systems necessarily include a completely-mixed aeration basin which, at SRT's representative of the activated sludge process (1 to 10 days) typically leads to quite low concentrations of biodegradable substrates in the bulk solution. Furthermore, for reasons that are not completely understood, laboratory activated sludge systems frequently develop bacterial aggregates that are both filamentous and substantially larger than typical for full-scale plants. Therefore, laboratory activated sludge systems may not effectively model conditions at full-scale plants.

3. Problems involved in the use of ATP as an indicator of viable biomass include the variation in specific

ATP content with bacterial species and the possible association of some ATP with nonviable bacteria.

4. The research conducted previously at Oregon State University indicated that the ATP viability in steady-state activated sludge systems increased with increasing DO even when it appeared that substrate was the diffusion-limiting species (Study No. 1) and when the flocs were small in size (Study No. 2). This research indicates the possibility that DO may have an effect on ATP viability apart from that caused by diffusional limitations.

5. The possibility exists, depending on the nature of the substrates and bacteria, that bacteria subject to anoxia may be able to shift to anaerobic pathways and to utilize substrates at a rate sufficient to maintain their viability and normal ATP content. If this shift occurs in typical activated sludge systems, then the predictions of the model regarding the cor-relation of ATP concentration with the anoxic volume are not valid.

Research Plan

Based on the review described above, the research program for this study was divided into three phases.

The first phase was designed as a preliminary research phase to test several basic assumptions within the anoxic core model, to develop methods, and to address some of the problem areas described above. The

specific purposes of this phase were:

1. To confirm, for a dispersed bacterial culture, that
    the maintenance of DO levels above that typically
    maintained at air activated sludge plants (1 - 2
    mg/ℓ) has no effect upon the culture in terms of the
    kinetics of substrate utilization or ATP viability.

2. To test various methods for the extraction of ATP,
    to compare these methods, and to select one method
    for use in subsequent research.

3. To measure the adenylate energy charge of a mixed cul-
    ture maintained at long SRT's and to evaluate, based
    on these measurements, the possibility that signifi-
    cant quantities of ATP are associated with nonviable
    bacteria.

The purpose of the second phase of research was to confirm the
applicability of the anoxic core model to laboratory-grown flocculant
bacterial cultures. This confirmation was anticipated to be threefold
in showing the interrelationship between the anoxic volume that develops
within a bacterial aggregate, as predicted by the mathematical model,
and the reduction in ATP viability and in substrate utilization rates
that results from development of an anoxic core.

The third phase of this research study was designed to determine
whether oxygen diffusional limitations are important in full-scale acti-
vated sludge plants. The general approach for this phase was to obtain
samples of mixed liquor from several activated sludge plants and to sub-
ject the samples to simple, short-term batch experiments that would

allow a correlation between respiration rate and DO in accordance with the anoxic core model.

A detailed research plan and description of the methods utilized in each of these three phases is presented at the beginning of Chapter VI (phase 1), Chapter VII (phase 2), and Chapter VIII (phase 3).

EXPERIMENTAL APPARATUS AND PROCEDURES

The principal equipment utilized in this study included two 7-liter laboratory-scale activated sludge reactors, two 1-liter batch reactors, and two BOD bottle respirometers. This equipment and the methods utilized in their operation are described below.

Activated Sludge Reactors

The activated sludge reactors utilized throughout the laboratory phases of this study were Horizon Ecology, Co. Bio-Oxidation Systems (Figure 5-1). These reactors consisted of a glass outer containment vessel and a glass inner cone to insure complete mixing in the reactor and quiescent conditions for settling in the central, integral settling tube. The plexiglass top of the reactors was fitted with a rubber gasket to provide a seal on reactor contents. A three-pronged glass fitted diffuser in the bottom provided aeration and mixing. For operation in the chemostat mode, the clarifier tube and inner cone were removed and the waste sludge pump disconnected.

The total liquid volume in these reactors was 6.0 to 7.0 liters, depending on operating conditions within given experiments. Clarifier tubes of two different sizes were utilized: the first was 25.4 cm long and 4.5 cm in diameter, the second was 26.0 cm long and 7.3 cm in
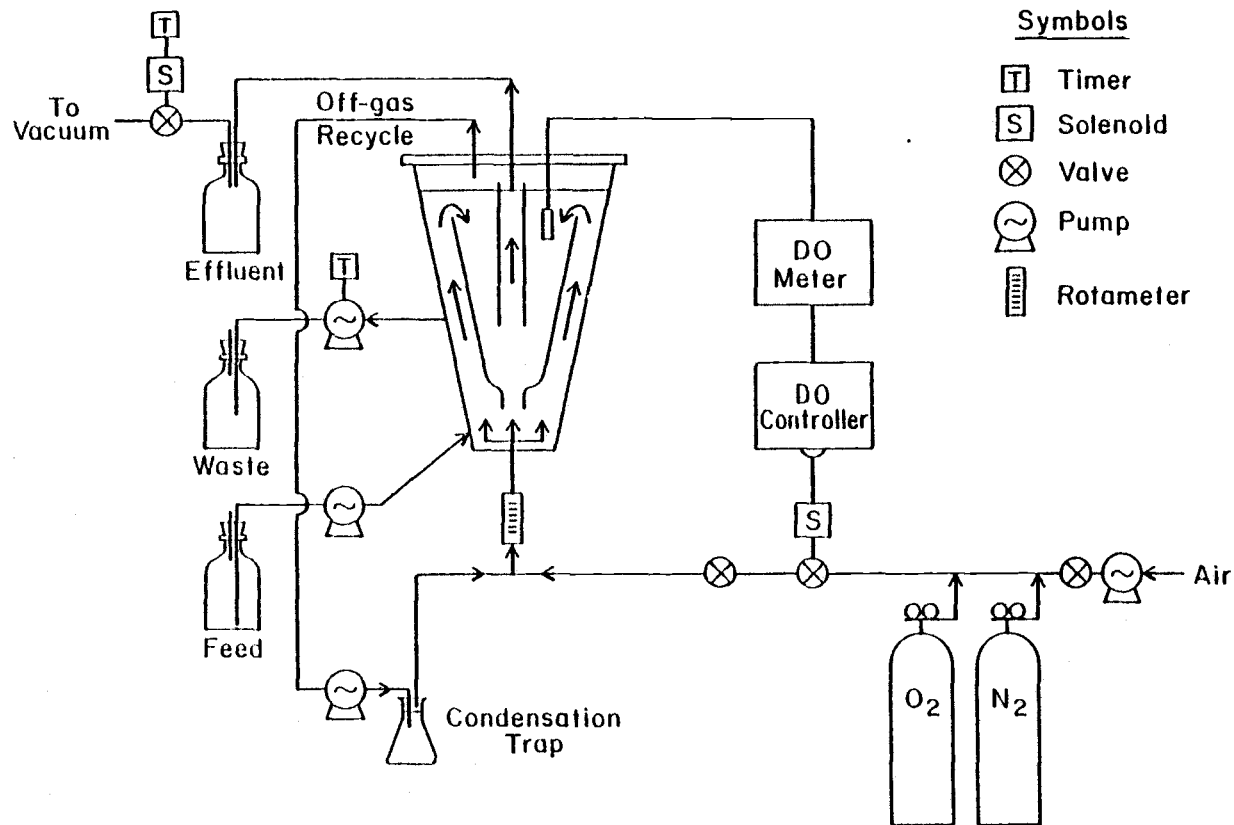
Figure 5-1. Schematic Diagram of Activated Sludge Reactors.

diameter. The clarifier tube was chosen to provide the desired hydraulic detention time in the clarifier under the influent flow conditions of particular experiments.

Feed was pumped to and waste sludge removed from the reactors by peristaltic pumps. The waste sludge pump was connected to a timer and operated continuously for five minutes on, followed by 55 minutes off. This was done to minimize clogging of the small tubing associated with small, continuous flow rates.

Off-gases from the reactors were recycled using two identical diaphragm pumps (Neptune Dyna-Pump, model 2). This was done to provide a constant aeration gas flow rate while maintaining a preset DO. The total aeration gas flow rate was maintained at 2.5 ℓ/min during all phases of the laboratory experiments, resulting in an average mixing intensity, or G factor, of approximately 140 s^{-1} at a reactor volume of 6.5 ℓ, according to the formula presented by Fair, Geyer, and Okun (1968):

$$G = (\frac{ghQ}{\nu V})^{1/2}$$ (5-1)

where: G = mixing intensity (s^{-1})

g = acceleration of gravity (980 cm/s^2)

Q = aeration gas flow rate (2.5 ℓ/min)

ν = kinematic viscosity (1.004 x 10^{-2} cm^2/s @ 20°C)

V = fluid volume (6.5 ℓ)

h = diffuser depth (32 cm)

The slightly different reactor volumes utilized during various phases of this study did not have a significant effect upon the calculated G factor.

The DO in each reactor was continuously monitored by a YSI polaro-graphic dissolved oxygen probe immersed in the mixed liquor and a YSI Model 54 dissolved oxygen meter. Electronic controllers manufactured specifically for this research were utilized to control the DO in the reactors. The controller was designed to be plugged into the recorder output terminals of the YSI meter and responded to meter output below a preset level with activation of a solenoid valve, allowing supplemen-tal aeration gas to flow to the reactor. A valved manifold allowed selection of oxygen, nitrogen, air, or any mixture of these to be used as the supplemental aeration gas. By appropriate selection of this composition, the DO in the reactor could be very tightly controlled to within the deadband limits of the electronic controller. For most ap-plications, it was possible to control the DO within five percent of the control setting.

The reactors were set up in a constant temperature room at 20°C. This maintained the reactor contents at 20 ± 1°C in all phases of this study.

Reactor Feed Composition

Phenol was utilized as the carbonaceous substrate throughout the laboratory phases of this study. Phenol was selected as the substrate for the following reasons:

1. Rapid degradation of phenol involves oxygenation of the aromatic ring through the action of specific oxygenases and subsequent cleavage of the ring. Con-sequently, under anoxic conditions such as develop in

the core of larger, rapidly respiring bacterial aggregates, the utilization of phenol to provide cellular energy is not possible. Therefore, the possibility that bacteria could retain their viability (as reflected by ATP content) by switching to anaerobic degradation pathways when subjected to anoxia was eliminated.

2. The bacterial degradation of phenol is of general interest to the environmental engineering community because of its toxicity, its ability to inhibit certain aerobic bioreactions, and its presence, sometimes in high concentrations, in effluents from refineries, coal gasification plants, coke ovens, and phenolic resin manufacturing (Neufeld, et al., 1980).

3. Sensitive analytical techniques are available for the rapid determination of phenol in aqueous solution. Specifically, the 4-aminoantipyrine colorimetric test is capable of measuring phenol concentrations in the microgram per liter range and is relatively simple and rapid (Standard Methods, American Public Health Association, 1976, hereinafter referred to as Standard Methods).

4. Previous studies at Oregon State University have utilized phenol as substrate. The mixed cultures grown exhibited a large floc size and the absence of settling problems due to growth or filamentous bacteria

that are very common to the operation of laboratory activated sludge systems.

It should be noted, in regard to the first point above, that at the outset of this study the literature indicated that anaerobic pathways for the degradation of phenol were not known to exist (Hayaishi, et al., 1975). During this study, literature has appeared that demonstrates anaerobic degradation of phenol occurs (Neufeld, et al., 1980). However, the bacteria involved are strict anaerobes and the possibility that bacteria acclimated to respiring aerobically with phenol as substrate could switch to anaerobic pathways when subject to anoxia does not appear likely. Nevertheless, this possibility was evaluated within Phase 2 of this study as described in Chapter VII.

In addition to phenol, mineral salts were added to the feed solution to provide the micronutrients necessary for microbial growth. The required concentrations of major micronutrients were calculated by compiling an approximate chemical composition of bacteria as listed in Table 5-1 and by conservatively assuming a yield factor for conversion of phenol into bacterial cells (1.0 g cells/g phenol).

The chemical feed composition used for Phase 1 studies is:

Constituent	Concentration (mg/ℓ)
Phenol	1000
NH_4Cl	500
K_2HPO_4	250
KH_2PO_4	125
$NaHCO_3$	500
$MgSO_4$	75
$CaCl_2$	25
$FeCl_3$	7.5
1N H_2SO_4 or 1N NaOH	To maintain pH ≃ 7

Table 5-1. Nutrient Requirements of Bacteria in Relation to
           Feed Composition

Composition of Bacteria		Nutrient Concentrations in Feed Solution	
Element	% of Total[1]	Required[2]	Provided
Carbon	50	limiting	765
Nitrogen	10	100	131
Phosphorus	0.25	2.5	45
Calcium	0.7	7.0	9.0
Potassium	0.5	5.0	112
Magnesium	0.5	5.0	7.4
Sulfur	0.7	7.0	9.8
Iron	0.1	1.0	1.5

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[1] Major source: McKinney, 1962.

[2] Based on an assumed yield of 1.0 g bacterial cells/g phenol.

The nutrients listed above were dissolved in dechlorinated tap water. Tap water contains sufficient concentrations of trace elements necessary for bacterial growth under the conditions of this study. The major nutrient concentrations provided by the above feed composition are listed in Table 5-1 in relation to the conservatively estimated concentrations required for balanced bacterial growth. As shown, all major nutrients were present in excess so that the carbonaceous substrate was the limiting species. Additionally, the net yields observed during the laboratory phases of this study were well below the estimate used to calculate the required nutrient concentrations in Table 5-1. This provided an additional safety factor in establishing phenol as the limiting nutrient.

During phase two of this research the feed strength was reduced to 500 mg/ℓ phenol and all other constituent concentrations reduced by one-half.

Reactor Operation

The laboratory activated sludge systems were operated as mixed culture reactors throughout this study. Mixed cultures were utilized, rather than pure cultures, to provide the closest correlation to conditions that exist in full-scale activated sludge systems treating municipal and industrial wastewaters. The reactors were seeded with mixed liquor from the Corvallis, Oregon Municipal Wastewater Treatment Plant and with Hydrobac[1], a commercial product of dry-preserved bacteria adapted to the degradation of phenolic compounds. Previous studies have

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[1] Polybac Corporation, New York, NY.

shown the difficulty of establishing mixed cultures capable of phenol degradation (Knudsen, et al., 1980) (Beltrame, et al., 1980) and it was judged that addition of an adapted seed bacteria would aid in the rapid establishment of an adapted mixed culture.

A major problem in the operation of laboratory-scale biological reactors is the growth of biofilms. The surface area to volume ratio is large in laboratory systems and the contribution of biofilm growth to the observed substrate utilization kinetics may be very significant. To limit biofilm contributions, the reactor walls were scraped twice daily with a rubber blade on the end of a long glass rod. When operated in the chemostat mode, this procedure was relatively simple and appeared to provide a relatively clean reactor wall during reactor operation. Operation of the laboratory systems as solids-recycle reactors, however, required the insertion of the glass inner cone and the settling tube. Not only did these components increase the surface area several times, but they limited accessibility to surfaces so that the biofilm scraping procedure was not completely successful. The contribution of biofilm growth to the observed in situ kinetics of these systems was not determined, but may have been significant.

The difficulties involved in biofilm growth for reactor operation in the solids-recycle mode were overcome in two ways. First, kinetic tests were generally performed within the batch reactors on samples withdrawn from the main reactor. Because the batch reactors were thoroughly cleaned prior to all kinetic tests and because the batch tests were of short duration (generally less than four hours), biofilm growth was not a problem. Second, the later experiments within Phase 2 utilized a fill-and-draw procedure for operation in the solids-recycle

mode. This procedure required the removal of the inner cone and settling tube and allowed the reactor surfaces to be successfully scraped of biofilm growth.

The feed tubing of laboratory-scale biological reactors also has a significant surface area and biofilm growth within feed tubing can be a problem. To prevent bacterial growth within the feed bottle and feed tubing, a 1:100 dilution of a commercial five-percent sodium hypochlorite solution was used to wash the feed bottles prior to filling with feed solution and was pumped through the feed tubing once daily, followed by thorough rinsing with tap water. Additionally, the feed bottle was replaced daily with a clean bottle containing freshly prepared feed solution.

The influent flow rate to the main reactors was determined by measuring the quantity of feed solution withdrawn from the feed bottle over a one-day period. Additionally, during periods of critical measurements, the influent flow rate was checked once or twice daily by disconnecting the feed tube and placing it in a 100-ml graduated cylinder and then recording the time required to fill a specified volume. During measurement of the influent flow rate by this second method and during the disinfection procedure for the feed tubing, feed solution was manually fed to the reactors at a rate consistent with the set pumping rate.

Batch Reactors

Two batch reactors were utilized in the determination of bacterial kinetics within this study. The reactors were 1-liter glass vessels with an outer water jacket for constant temperature operation (Kontes Glass Co.). The batch reactors were placed on magnetic stirrers and a

magnetic stirring bar was inserted in the bottom of each reactor vessel for continuous mixing. Although calculation of an average mixing intensity factor, G, was not possible for the magnetic mixing apparatus, the rotation speed of the magnetic mixer was selected so that the observed mixing velocity was similar to that in the main reactor. Water from a constant temperature bath was circulated through the outer water jacket for constant temperature operation. For the laboratory phases of this study, all reactor temperatures were 20°C.

The plexiglass cover for each batch reactor was drilled with holes for insertion of a DO probe, a pH probe, an air diffuser, a feed tube (if needed), and for sampling with a pipette. A schematic diagram of the batch reactors and associated apparatus is shown in Figure 5-2. Both the pH and DO probes were continuously immersed in the reactor contents to provide continuous monitoring of the pH and DO. The DO meter was connected to an electronic controller identical to that utilized for controlling the DO in the main reactors. An aquarium pump was utilized to supply aeration gas (either air or oxygen) and was plugged into the DO controller. The maximum aeration gas flow rate was 0.9 ℓ/min. The DO in the batch reactors was then controlled by the on and off cycling of the aquarium pump.

The batch reactors were utilized in two different modes. First, they were utilized to determine the kinetic coefficients k and K_s in accordance with the "infinite dilution" procedure of Williamson and McCarty (1974). For operation in this mode, the completely-mixed batch reactors were filled with one liter (or an aliquot diluted to one liter) of culture and the DO control system was set to maintain the same DO as present in the corresponding main reactor. The batch reactors were
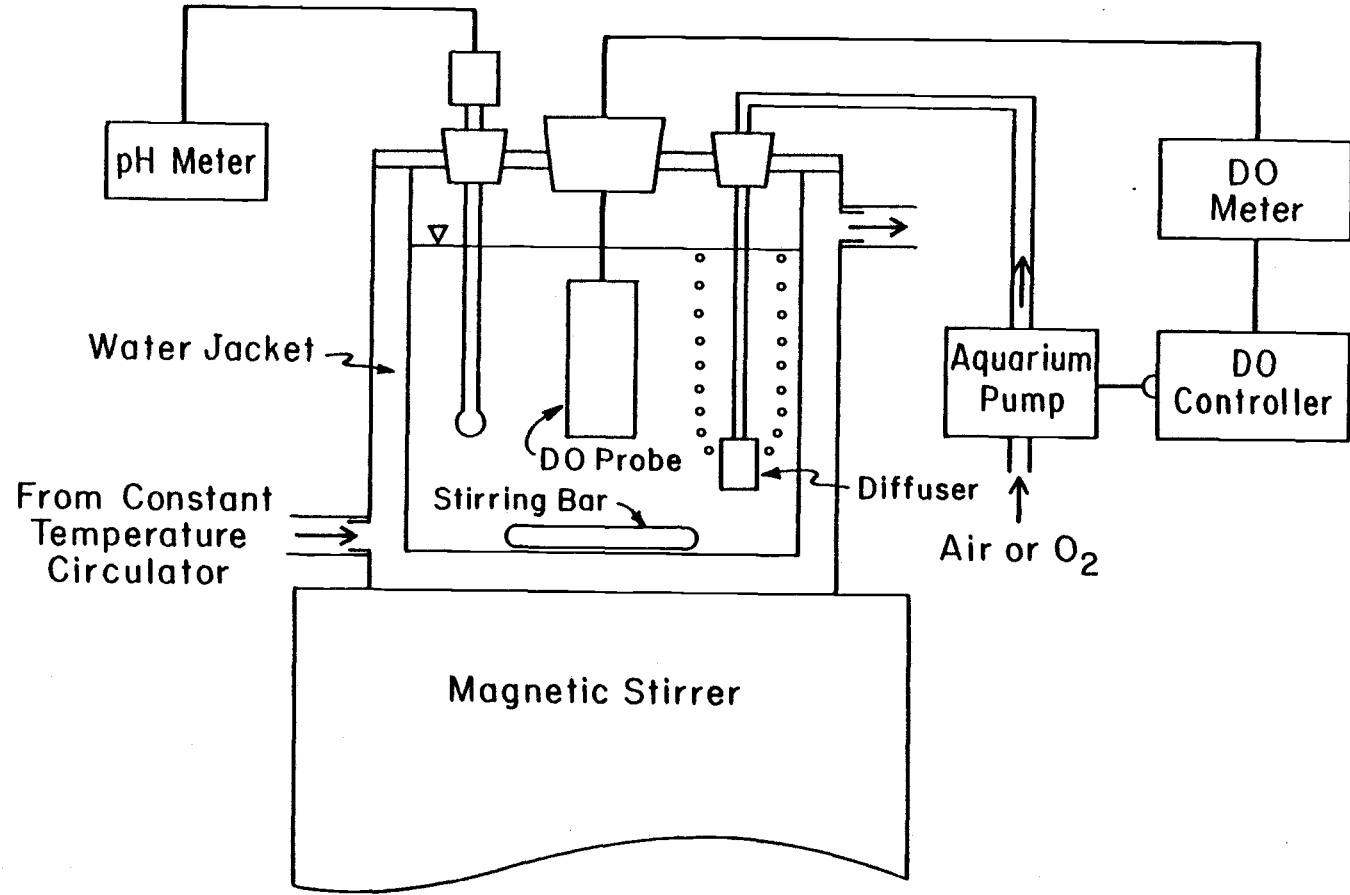
Figure 5-2. Schematic Diagram of Batch Reactors.

then continuously fed a concentrated feed solution at a low flow rate. No effluent was drawn off except for small amounts withdrawn for analytical purposes and, under these conditions, the change in reactor volume over the few hours that the experiments were conducted was negligible. It was assumed in this test that bacterial growth over the short time involved was small and that a steady-state condition was reached.

The basic kinetic equation that applies to this infinite dilution batch technique was presented by Williamson and McCarty (1974) as

$$\frac{kC_s^*}{K_s + C_s^*} = \frac{QC_s^o}{X\,V} \tag{5-2}$$

where C_s^* is the steady-state substrate concentration, Q is the flow rate and C_s^o the substrate concentration in the concentrated feed solution, and other symbols are as previously defined. By subjecting samples of culture to a range of mass loading rates, $M(=Q \cdot C_s^o)$, a range of steady-state substrate concentrations is obtained and a Lineweaver-Burke plot of $1/C_s^*$ versus $\frac{X\,V}{QC_s^o}$ can be utilized to define the kinetic coefficients k and K_s by linear regression.

The second mode in which the batch reactors were utilized included miscellaneous short-term kinetic studies in phase 2 and phase 3. These uses are described in Chapter VII and VIII.

The feed solution was initially pumped to the batch reactor using a Gilmont two-milliliter micrometer syringe attached to a timing motor as described by Williamson and McCarty (1974). However, this method required the removal, refilling and replacement of the syringe during reactor operation, a procedure that was difficult and was not ideal for the intended steady-state conditions of the reactor. Therefore, Phase

2 experiments utilized a Sage Instruments Model 355 syringe pump. This
pump would accept up to a 50 cc syringe so that replacement of the
syringe was not necessary during reactor operation. Furthermore, the
syringe pump was infinitely adjustable and was found to produce the
desired flow rate accurately and reproducibly.

BOD-Bottle Respirometer

A BOD-bottle respirometer was utilized to rapidly define the res-
piration rate versus DO curves under given initial conditions. The
respirometer consisted of a standard BOD bottle and a YSI Model 5720A
DO probe and Model 54 DO meter (see Figure 5-3).

Operation of the respirometer was as follows. First, the BOD
bottle was filled with the test culture or a dilution of the test cul-
ture. Substrate was added to the culture sample prior to filling the
BOD bottle and the sample was aerated, if necessary. Next, a teflon
stirring bar was placed in the bottom of the BOD bottle, the bottle was
set on a magnetic stirrer, the DO probe was inserted into the bottle,
the stirrer started, and the DO was read every 15 seconds to three
minutes as necessary to adequately define the DO versus time curve. A
piece of corrugated cardboard was placed between the BOD bottle and the
magnetic stirrer to prevent heat transfer from the stirrer to the
liquid sample.

The YSI Model 5720A DO probe has a self-contained stirring boot.
However, the stirring action from this boot was judged to be too in-
tense, perhaps contributing to floc shear, and the magnetic stirring
apparatus was utilized instead. The initial respirometer apparatus in-
cluded a recorder that was plugged into the DO meter and allowed direct
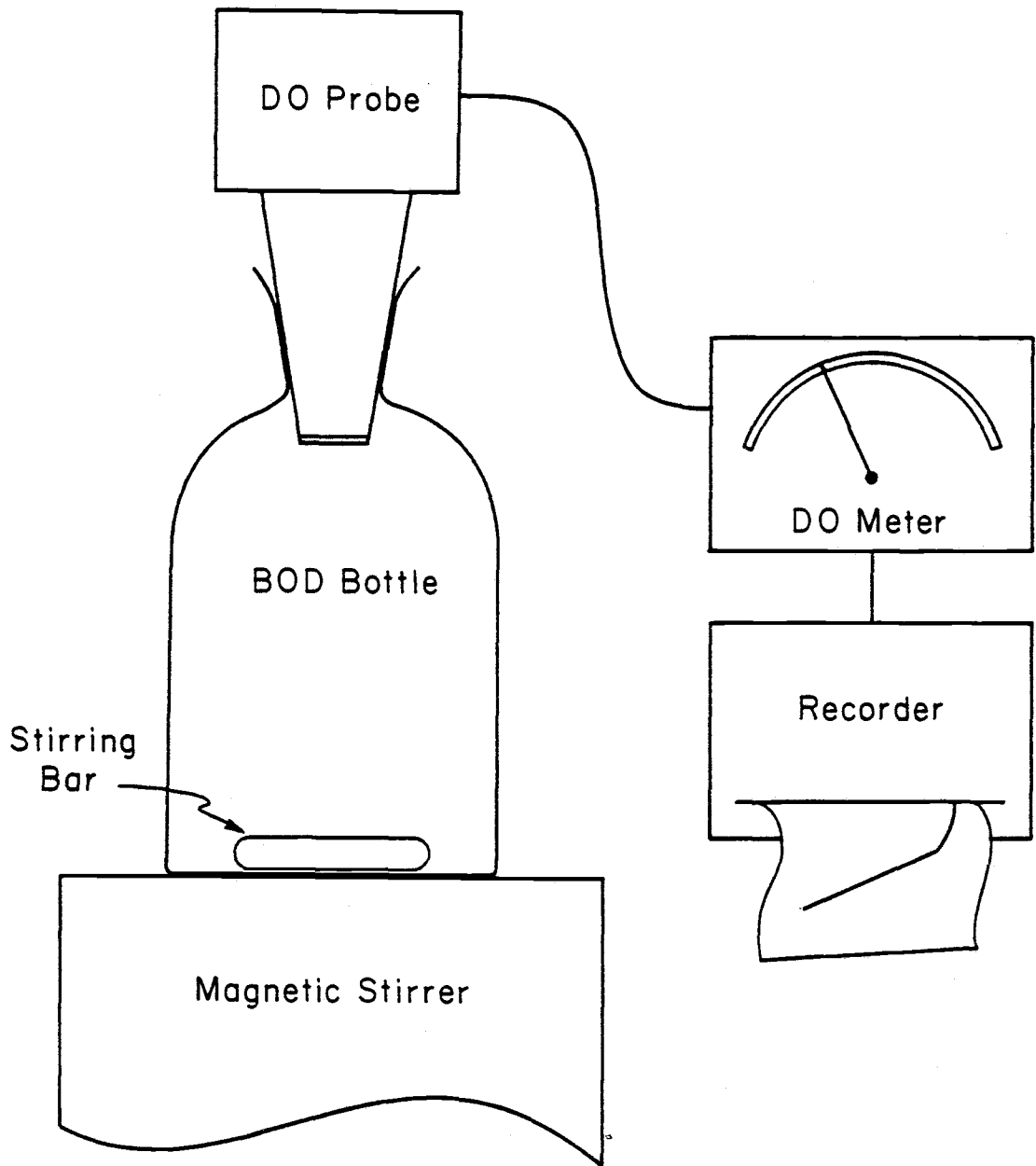
Figure 5-3. BOD Bottle Respirometer.

recording of the DO versus time curve. However, it was found that a more accurate curve could be produced by reading the DO meter directly at intervals checked on a stopwatch. Additionally, with the manual procedure, a high sensitivity (.005" thick teflon) membrane was utilized, allowing reading the DO meter to 0.025 mg/ℓ below 5 mg/ℓ. The meter scale was switched to allow readings up to 10 mg/ℓ.

A typical DO versus time curve produced by this method is shown in Figure 5-4a. The curve is characteristically linear at higher DO levels if the environmental conditions in the respirometer, including the substrate concentration, do not change significantly over the short time period involved in the test (5 - 30 minutes). Under these conditions, the reduction in the rate of oxygen depletion that occurs at lower DO levels is solely the result of oxygen limitations. Mueller, et al. (1967) showed mathematically that a quasi-steady-state condition can be assumed for diffusion of oxygen through bacterial floc particles in respirometric tests similar to those described above as long as the test took more than a few minutes.

Because a quasi-steady-state condition can be assumed at each DO along the DO versus time curve, Figure 5-4a may be converted into a plot of respiration rate versus DO by taking the slope at each DO (see Figure 5-4b). The respiration rate may be expressed as the mass-specific respiration rate, U_o, in mg O_2/g TSS-hr:

$$U_o = \frac{1}{X} \cdot \frac{d\,C_{b,o}}{dt} \tag{5-3}$$

In actual practice, the curve in Figure 5-4b was generated by calculating U_o for each interval between DO readings, correlating this with
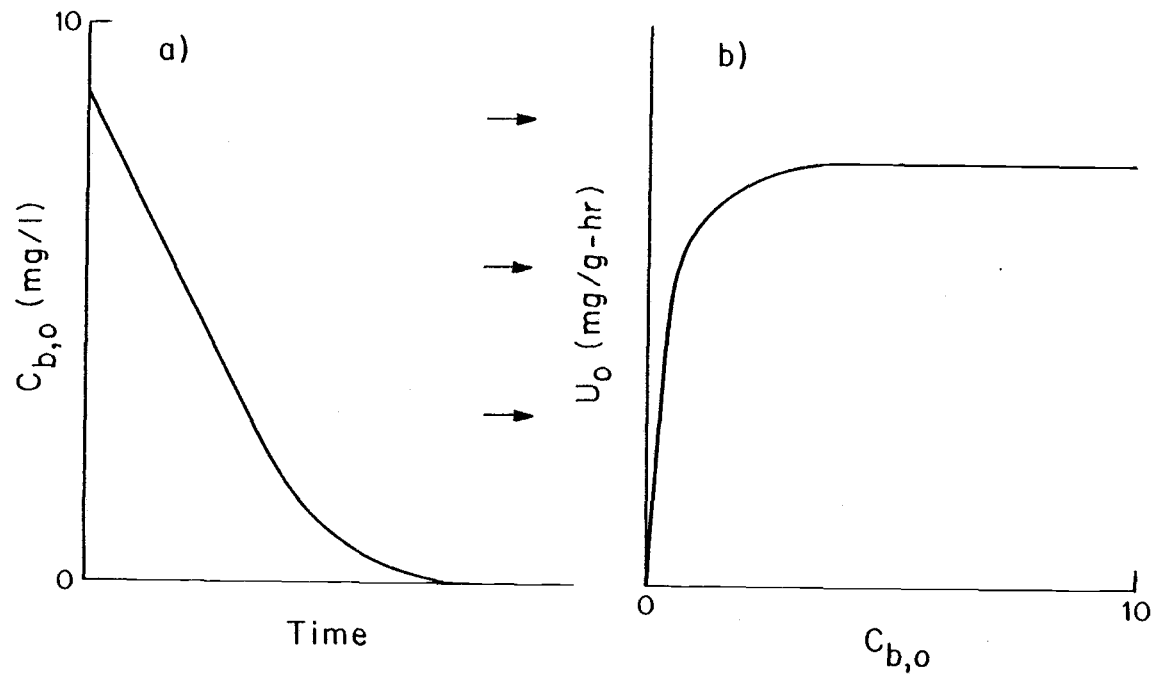
Figure 5-4. Development of the Respiration Rate versus Bulk DO
Curve from Respirometric Data.

the average DO over the interval, and then plotting those results and drawing the curve of best fit through application of the anoxic core model, where possible.

The rotary speed of the stirring bar in the respirometer bottle was maintained at approximately 200 RPM for all respirometer experiments. This was found to produce a mixing intensity that kept all particles in suspension, was similar to that in the main reactors, and did not contribute to shear of the bacterial aggregates. The effect of varying the time involved to complete the respirometric test was tested in Phase 2 and is described in Chapter VII.

Ultrasonicator and Blender

During Phase 2 studies, it was desired to disperse the floc in samples from flocculant cultures and to compare the kinetics of the dispersed samples with those of an undisturbed flocculant sample. Two methods were tested to achieve dispersion: ultrasonication and blending.

Banks and Walker (1977), among others, used ultrasonication to release bacteria undamaged from activated sludge flocs and to enumerate them by plating. It was shown that the maximum recovery of bacteria was a balance between their rate of release from the flocs and the rate at which they are killed by the ultrasonic treatment. An ultrasonication time of one to two minutes was found to generally be optimum.

The ultrasonicator utilized in this study was a Fisher Sonic Dismembrator, Model 300, with the intermediate tip. Power settings up to 60% (maximum for the intermediate tip) were tested to determine the optimum setting. The ultrasonication time was two minutes, in accordance

with the findings of Banks and Walker (1977). Samples (usually 300 ml) to be ultrasonically treated were poured into a 500 ml beaker and the ultrasonicator tip placed halfway into the solution.

Blending has been utilized by Mueller, et al. (1966b) and Lamotta and Skieh (1979) to reduce floc size and thereby evaluate intrinsic bacterial kinetics. The blender utilized in this study was a standard single-speed Waring blender (cat. no. 700A). The blending times were set at two minutes to be consistent with the ultrasonication procedure.

ANALYTICAL METHODS

Phenol

Phenol was utilized as the carbonaceous substrate throughout the laboratory phases of this research study. Aqueous phenol was analyzed by the 4-aminoantipyrine colorimetric method in accordance with Standard Methods (American Public Health Association, 14th edition, 1976). Samples containing less than 1.0 mg/ℓ were analyzed by Method B, which includes a distillation step, addition of reagents and development of the color, extraction and concentration of the color in chloroform, and spectrophotometric measurement of the color. Samples containing more than 1.0 mg/ℓ were analyzed by Method C, which involves direct measurement of the color in aqueous solution and excludes the chloroform extraction step of Method B. Filtration through a 0.45 micron membrane filter was utilized as the purification step rather than distillation because there were no known soluble interfering compounds in the culture solution.

Dissolved Oxygen

Dissolved oxygen concentrations were measured using YSI polaro-
graphic dissolved oxygen (DO) probes and YSI Model 54 electronic meters.
The meters were calibrated using water saturated with air in accordance
with the manufacturer's recommendations. Additionally, calibration at
the zero concentration point was accomplished using water depleted of
all dissolved oxygen by addition of sodium sulfite and a cobalt cata-
lyst. For accurate setting of the zero point in the respirometric
tests, a culture sample that had been allowed to deplete all dissolved
oxygen was utilized.

For continuous operation of the activated sludge units, the DO
probes were calibrated daily against standard solutions as described
above. Daily calibration resulted in only minor calibration adjust-
ments so that more frequent calibration was not deemed necessary. The
teflon membranes on the probes were replaced weekly or more frequently
if the daily calibration procedure found any drifting or erratic re-
sponse.

Suspended Solids

Both total suspended solids (TSS) and volatile suspended solids
(VSS) were analyzed according to the procedure described in Standard
Methods. Reeve Angel 934AH microfine glass fiber filters were utilized
throughout this study. Preparation of the filter included soaking in
distilled water overnight to leach out any soluble materials, drying
at 105°C for one hour, combustion of volatile matter at 550°C (15 min-
utes), and storage in a desiccator. Due to the limited accuracy of the

analytical procedures, all suspended solids analyses were performed on three separate samples and the average reported. When there was more than a ten percent variation between the low and high values, the analysis was repeated, if possible.

Floc Size Distribution

An important parameter in the mathematical models of diffusion and reaction within bacterial floc is the floc size. Various methods have been utilized to estimate the floc size in bacterial cultures, particularly in activated sludge systems. However, most of these methods involve visual sizing of the floc under a microscope with a calibrated grid or scalar (Knudsen, et al., 1980) (Finstein and Henkelkian, 1967) (Sezgin, et al., 1978). These procedures necessarily involve the subjective judgment of the analyst in defining a representative floc dimension. Furthermore, the nonuniformity of bacterial flocs prevents the establishment of a rigid procedure whereby the subjectivity of the analysis may be eliminated.

A search was undertaken during the initial phases of this study to identify a method that would provide a nonsubjective analysis of particle size for flocculant bacterial cultures. Based on the literature, it appeared that an electrozone particle (Coulter) counter was the most promising system. (Stockham and Fochtman, 1977) (Lamotta and Shieh, 1979). Basically, an electrozone particle counter defines particle volume as the particle is drawn through an orifice by measuring the reduction in current that occurs between charged electrodes on opposite sides of the orifice. It was felt that the particle size distribution measured by this method would not only be nonsubjective but, because

the measured parameter is particle volume, it would provide a very
reasonable estimate of the diffusional dimension for an equivalent
spherical particle. Furthermore, with an accurate accounting of the
particle volume distribution for a given volume of sample and a sus-
pended solids analysis, the average bacterial density could be cal-
culated, thereby providing a nonsubjective measure of the two most
important parameters in the mathematical model.

A Particle Data, Inc., Model 112 electrozone particle counter was
obtained and large orifice tubes with orifice diameters of 240, 480,
and 1100 microns. The instrument was tested on flocculant bacterial
cultures grown in the early stages of this study and on activated
sludge from the Corvallis, Oregon Wastewater Treatment Plant. It was
found that, although the average particle size was less than 100
microns in the cultures tested, it was necessary to use the largest
(1100 μ) orifice to prevent continual clogging of the orifice by an
occasional large particle. Even with this orifice occasional plugging
was a problem. Furthermore, it was found that bacterial floc tended to
stick to the sides of the orifice, interfering with the analysis.
Finally, it was found that the large orifice was subject to a very high
background noise so that it was not possible to detect particles of
less than about 250 microns in diameter and the accuracy of larger par-
ticle measurements was questionable. Technical staff at Particle Data,
Inc. said that this was a frequent problem with large orifices in simi-
lar applications. It was concluded that the electrozone particle coun-
ter was not well suited to measuring bacterial floc distributions in
the size range of interest in this study.

The only other method that was considered to be a viable alternative was that of Mueller, et al. (1968). As described in Chapter II, however, this method produced unreasonably high values for the bacterial density and, because the nominal diameter is derived from the density, this method was not judged to be suitable. Consequently, it was decided that visual sizing under a microscope was the only realistic method available for measuring floc size and that the subjectivity and lack of accuracy involved in such procedures would have to be recognized and tolerated.

Several techniques have been utilized in the visual sizing of floc under the microscope. However, the procedure of Sezgin, et al. (1978) was judged to be simple, direct, and capable of defining floc size as accurately as any other microsopic procedure. Basically, this procedure involves dilution of the sample from the aeration basin or reactor, mixing at approximately the same velocity gradient as present in the reactor, and transfer of one milliliter of the diluted culture to a counting chamber.

A wide-tip pipette (2 mm tip diameter) was utilized in the transfer of all culture samples and diluted samples. A binocular microscope at 100X with an ocular scalar was used to size flocs in the whole chamber or a known portion of it by their equivalent spherical diameter. The equivalent spherical diameter was taken as the average of the maximum and minimum dimensions for oblong-shaped flocs and was a matter of some judgment for very irregular-shaped flocs.

After sizing, the flocs were assigned to the size intervals 0-10, 10-30, 30-60, 60-100, 100-150, 150-200, 200-250, 250-300, 300-350, 350-400, 400-450, 450-500, 500-600, 600-700, 700-800, 800-900, 900-1000,

1000-1200, and 1200-1400 microns. Because the relative contribution of each floc to the total substrate utilization rate and ATP content of the culture is dependent on its mass, which is assumed to be directly proportional to its volume, and not on its equivalent diameter, the floc size distribution by count obtained by the above procedure must be converted into a floc size distribution by volume to allow application of the model. Since the volume of a floc is proportional to its diameter cubed, the mass of individual large floc particles in a distribution may be orders of magnitude greater than that of a small floc particle. For example, a 1000 μ-diameter floc contains 10^6 times the mass of a 10 μ-diameter floc. Consequently, it is appropriate to place emphasis on the accurate measurement of the floc size distribution for flocs in the larger size ranges. To do this, the microscopic counting chamber was divided into several fields. The first field was counted for all sizes of floc, the second field was counted for floc over a given size, perhaps 30 μ, the third field counted for floc over a given larger size, perhaps 100 μ, and so on. Additional 1-ml samples were analyzed as necessary to produce a reasonably smooth floc size distribution. The number of fields counted and the lower cutoff point for counting in a given field were determined as necessary to provide what was judged to be a representative floc size distribution.

COD

Chemical oxygen demand (COD) analyses were performed by the dichromate digestion ampule method, with procedures as described by Oceanography International Corp. (1978). Glass test tubes of 50-ml capacity with teflon-lined screw caps were utilized instead of glass-sealed

ampules. Sample and reagent quantities were reduced proportionately from those recommended by Standard Methods in order to provide a 15-ml total volume in the test tubes.

SVI and ISV

Sludge volume index (SVI) and initial settling velocity (ISV) were determined in accordance with Standard Methods. A one-liter graduated cylinder was utilized for the settling tests where sufficient culture volume was available. When it was not, a 100-ml graduated cylinder was utilized and its use noted.

pH

The measurement of pH was performed in accordance with Standard Methods using a sealed combination electrode.

Adenine Nucleotides (ATP, ADP, AMP)

Adenine nucleotides, particularly ATP, have primarily been analyzed using photometric measurement of light emission resulting from the bio-luminescent reaction of ATP with luciferin and luciferase, the substrate and enzyme involved in light emission by the firefly. The reactions involved are:

$$E + LH_2 + ATP \rightarrow E \cdot LH_2 - AMP + PP_i \qquad (5-5)$$

$$E \cdot LH_2 - AMP + O_2 \rightarrow E + oxyluciferin + CO_2 + AMP + light \quad (5-6)$$
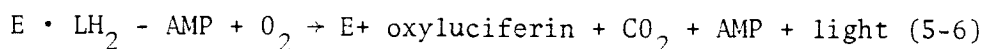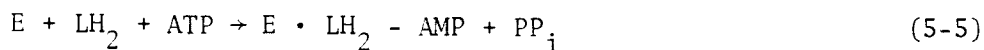
where PP_i = pyrophosphate, E = enzyme (luciferase), and LH_2 = luciferin.

The reaction is inherently very sensitive and detection of picomole quantities of ATP is possible with sophisticated instrumentation (Kimmich, et al., 1975). Furthermore, other adenosine phosphates may be speciated using the luciferin-luciferase bioluminescent reaction by enzymmatic conversion to ATP. ADP may be converted to ATP by incubation with the enzyme pyruvate kinase and the substrate phosphoenolpyruvate (PEP):

$$\text{ADP} + \text{PEP} \xrightleftharpoons[\text{kinase}]{\text{pyruvate}} \text{ATP} + \text{pyruvate} \tag{5-7}$$
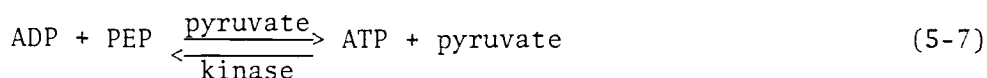
Adenylate kinase (myokinase) may be added to convert AMP to ADP:

$$\text{AMP} + \text{ATP} \xrightleftharpoons[\text{kinase}]{\text{adenylate}} \text{2ADP} \tag{5-8}$$

By coupling reaction 5-8 above with reaction 5-7, AMP can be quantitatively converted to ATP (Bostick and Ausmus, 1978). Kimmich, et al. (1975) found that 95 percent conversion of ADP and 90 percent conversion of AMP to ATP was accomplished using the above conversion reactions when dealing with picomole quantities of adenine nucleotides.

Extraction Methods

The accurate analysis of adenine nucleotides in bacterial cultures or other biological samples depends highly on their quantitative extraction from biological cells and preservation of the extract so that enzymmatic interconversion is prevented. Numerous agents have been tested and compared for extraction efficiency, including organic solvents, inorganic acids, and boiling buffers.

Lundin and Thore (1975) conducted the most extensive comparison to date regarding alternative extraction agents. Ten different extraction

methods were tested on five bacterial strains. The methods were compared with respect to the yield of adenine nucleotides, interference with the enzymmatic assay, reproducibility of the method, and stability of the extract. Analytical interference by bacterial enzymes not inactivated during the extraction step was found to be a major problem. However, these enzymes were largely inactivated in all of the extraction procedures by inclusion of ethylene diamine tetraacetate (EDTA) in the extraction agent. Results indicated that extraction with trichloroacetic acid was the method most closely reflecting actual levels of ATP, ADP, and AMP in intact bacterial cells. However, several other extraction agents including sulfuric and perchloric acids, and ethanol performed comparatively well and the practical advantages of these agents and their associated procedures was recognized.

Since the time of Lundin and Thore's notable study, however, little agreement has been reached regarding the most appropriate extraction procedure. Furthermore, techniques have been improved and most studies have demonstrated the adequacy of a variety of extraction techniques, including extraction with perchloric acid, nitric acid, ethanol, and boiling buffers, when appropriate experimental techniques are practiced (Chapman, et al., 1971) (Chappele, et al., 1978) (Holm-Hansen and Karl, 1978).

Within the microbiological community, the use of perchloric acid as the extractant for adenine nucleotides has become the most popular (Chapman, et al., 1971) (Atkinson, 1977). Although perchloric acid strongly interferes with the enzymmatic bioluminescent reactions, it can be removed by neutralization of the extract with potassium hydroxide and centrifugation of the potassium perchlorate precipitate.

Within the environmental studies community, the use of boiling buffers for extracting adenine nucleotides has become quite popular (Holm-Hansen and Karl, 1978) (Tobin, et al., 1978) (Patterson, et al., 1970). Boiling buffer techniques are relatively simple and direct and they avoid difficult pH neutralization and solvent extraction steps required by other methods. Furthermore, samples containing low concentrations of microorganisms may be filtered to concentrate the microorganisms and the filter pad inserted in the boiling buffer.

The major.problem with boiling buffer techniques appears to be the lack of achieving a 100°C temperature immediately after sample injection into the buffer. Holm-Hansen and Karl (1978) stated that the sample volume must be two percent or less of the buffer volume to achieve complete extraction. The use of large sample to buffer volume ratios may have been the primary cause of obtaining lower energy charge values than acid extraction methods in earlier studies such as those of Lundin and Thore (1975).

Due to the lack of agreement by various researchers on the most appropriate procedure for the extraction of adenine nucleotides and the finding that the performance of various extraction techniques is dependent on the nature of the sample, several extraction techniques were selected for comparative analysis within this study. Three different extractants were selected for this comparison: boiling tris buffer (pH 7.75), boiling glycine buffer (pH 10.0), and perchloric acid. These extractants have been recommended by Holm-Hansen and Karl (1978), Tobin, et al. (1978), and Atkinson (1977), respectively. EDTA was incorporated into all extraction agents to provide a final extract concentration of 2 mM as recommended by Lundin and Thore (1975). Additionally, both

boiling buffer extraction agents were tested using direct injection of sample into the boiling buffer and using filtration of the sample followed by insertion of the filter pad into boiling buffer, the procedure utilized in previous studies on the activated sludge process (Patterson, et al., 1970) (Nelson and Lawrence, 1980) (Williamson and Nelson, 1976).

A description of the procedures utilized within each of the five methods is described below:

METHOD I (20 mM Tris + 2 mM EDTA, pH 7.75)

A. Filtration Method

1. Filter rapidly five or ten ml of culture through a microfine glass fiber filter.

2. Transfer the filter pad to a 15 ml polycarbonate centrifuge tube containing nine ml of Tris-EDTA buffer in a boiling water bath.

3. Heat for five minutes.

4. Transfer the centrifuge tube to an ice water bath.

5. After 15 minutes, remove the centrifuge tube from the ice water bath, make up the volume to ten ml with buffer, and centrifuge (20,000 x g, 2°C, 15 min.).

6. Transfer five ml of supernatant to a screw-cap test tube and freeze at -20°C until analysis.

B. Direction Injection Method

1. Rapidly pipet 1 ml of sample into a 100 ml beaker containing 50 ml of boiling Tris-EDTA buffer.

2. Continue boiling the extract on the hot plate for two minutes.

3. Transfer the beaker containing the extract to an ice water bath.

4. After 15 minutes, remove the beaker from the ice water bath, make up the volume to 50 ml with distilled water, and transfer the extract to a 50-ml polycarbonate centrifuge tube.

5.   Centrifuge (20,000 x g, 2°C, 15 min.).

6.   Transfer five ml of supernatant to a screw-cap
     test tube and freeze at -20°C until analysis.

METHOD II (10 mM Glycine + 2 mM EDTA, ph 10.0)

A.   Filtration Method

1. - 6. similar to Method IA.

B.   Direct Injection Method

1. - 6. similar to Method IB

METHOD III (Perchloric Acid)

A.   Direct Method

1.   Rapidly pipet five ml of culture into a 15 ml
     polyethylene centrifuge tube containing 1 ml
     of 35% perchloric acid plus 20 mM EDTA.

2.   Mix on a vortex mixer for ten seconds.

3.   Transfer centrifuge tube to an ice water bath
     for 15 minutes.

4.   Neutralize the extract to pH 7.4 with IN KOH.

5.   Centrifuge (20,000 x g, 2°C, 15 min.) to remove
     the precipitate.

6.   Transfer five ml of supernatant to a screw-cap
     test tube and freeze at -20°C until analysis.

Analysis

Extracts of microbial cultures were stored frozen at -20°C and
were thawed for analysis, generally within 30 days after extraction.
Lundin and Thore (1975) found that the initial rate of decay for adenine
nucleotide extracts at -20°C was less than 0.2 percent per day when 2
mM EDTA was incorporated into the extract. Therefore, decay of the
extracts during the short storage periods involved in this study should
have been minimal.

All reagents used in the analysis, including ATP, ADP, and AMP standards, phosphoenol pyruvate, pyruvate kinase, and adenylate kinase were obtained from Sigma Chemical Company. Stock solutions of the sodium salts of ATP, ADP, and AMP (2 mM) were prepared in Tris-EDTA buffer, pH 7.75 and five-ml quantities were stored in individual test tubes at -20°C until needed. After thawing, dilutions were made in Tris-EDTA, the stock solution discarded, and the working standard stored for up to one day at 4°C. This procedure was similar to that of Lundin and Thore (1975), who found negligible decay of adenine nucleotide standards when the above procedures were utilized.

Adenylate kinase (Sigma M3003) was obtained from Sigma as a suspension in 3.2 M ammonium sulfate. To reduce the inhibition of bioluminescence caused by the presence of ammonium sulfate, the adenylate kinase suspension was centrifuged and the pellet dissolved in 20 mM Tris buffer, pH 7.75, to its original volume as recommended by Lundin and Thore (1975). Pyruvate kinase (Sigma P9136) was obtained as a dry powder.

The luciferin-luciferase solution was prepared from crude firefly lantern extract (Sigma FLE-50) by addition of five ml of 100 mM Tris buffer, pH 7.4, to one vial containing the soluble extract from 50 mg of dried firefly lanterns. The resulting firefly lantern extract solution contained approximately 0.05 M potassium arsenate and 0.02 M $MgSO_4$, according to Sigma. The enzyme mixture was stored overnight at 4°C to reduce the level of background light emission. After aging, the insoluble residue was removed by centrifugation and the supernatant stored in an ice water bath for up to eight hours during analysis. Sufficient firefly enzyme solution was prepared for the number of samples to be

analyzed during a day. If more than five ml was required, the contents of two or more vials were combined due to the considerable variation that occurs between the individual vials of firefly lantern extract.

The photometer utilized to quantify the light emission from the bioluminescent reaction of ATP with luciferin and luciferase was an Aminco Chem-Glow photometer, Model J4-7441 equipped with an Aminco integrator-timer (Model J4-7462A). This equipment was specifically designed for measurement of light emission from bioluminescent reactions. The integrator-timer recorded the integrated area under the relative intensity versus time curve for a preset time interval, generally ten seconds.

Both the peak height and initial ten-second integrated area were observed and recorded for ATP analyses. Lundin and Thore (1975b) recommend the initial rate of rise or peak height of the relative intensity versus time curve as most indicative of ATP content. This study found that the results obtained from utilization of peak height or ten-second integrated area were generally comparable. However, standard curves using peak height frequently resulted in a more linear relationship; therefore, peak height was utilized in all analyses of ATP presented in this study.

The procedures utilized in the analysis of ATP, ADP, and AMP in sample extracts were adapted from those of Holm-Hansen and Karl (1978) and are described below:

1. Thaw sample extracts and one set of two mM standards of ATP, ADP, and AMP in a room temperature water bath.

2. Prepare diluted standards and working standards of ATP, ADP, and AMP in Tris-EDTA buffer. Subject the working

standards to the identical procedure used for extrac-
tion of adenine nucleotides from culture samples.

3.   Pipet 200 μℓ of sample extract into each of three 6 x 50
     mm cuvetts labeled A, B, and C, and make the following
     additions to each:

Tube A: Add 100 μℓ of a solution containing 100 mM
          Tris and 12 mM $MgSO_4$, pH 7.4.

Tube B: Add 100 μℓ of a solution containing 100 mM
          Tris, 12 mM $MgSO_4$, 0.50 mM phosphoenol pyru-
          vate, and 150 IU/ml pyruvate kinase, pH 7.4.

Tube C: Add 100 μℓ of a solution containing 100 mM
          Tris buffer, 12 mM $MgSO_4$, 0.50 mM phosphoenol
          pyruvate, 150 IU/ml pyruvate kinase, and 125
          IU/ml adenylate kinase, pH 7.4.

Blanks, ATP standards, and standard mixtures of ATP,
ADP, and AMP are prepared as above.

4.   Incubate all tubes for one hour at 20°C, place in a
     boiling water bath for five minutes to inactivate all
     enzymes, and allow to adjust to room temperature
     (approximately 30 minutes).

5.   Place each cuvette in turn in the photometer sample
     chamber, inject 50 μℓ of firefly lantern extract
     solution, and record the obtained peak height and
     ten-second integrated area.

6.   Calculate a standard curve for the ATP standards.
     Calculate the efficiency of conversion of ADP and AMP
     to ATP from the mixed standards.

7.   The molar concentration of ATP obtained from the above
     analysis then represents the concentration of ATP
     (Tube A), ATP + ADP (Tube B) and ATP + ADP + AMP
     (Tube C). From these results, the individual concen-
     trations of ATP, ADP, and AMP are obtained and the
     energy charge calculated in accordance with Equation
     2-26.

ATP standard curves were generated at the beginning and end of
analytical periods involving adenine nucleotide analysis to detect any
changes in the enzyme potency of the firefly lantern extract solution.

In general, these curves were not significantly different and corrections for loss of activity in the firefly lantern extract solution were not necessary.

CHAPTER VI

PHASE 1 EXPERIMENTS

PURPOSE AND RESEARCH PLAN

Purpose

As discussed in Chapter II, the results of numerous previous stud-
ies have not produced a consensus of scientific and engineering opinion
regarding the possible effects of DO on biomass yield, substrate utili-
zation kinetics, settleability, or other biomass characteristics in
activated sludge systems. The major theory that has been advanced to
explain differences in biomass characteristics that apparently result
from higher DO levels is the anoxic core theory (Chapman, et al., 1976)
(Jewell and MacKenzie, 1972) (Knudsen, et al., 1980). A major purpose
of the literature review associated with this study was to identify
other theories that might possibly explain how such differences could
result from higher DO levels. A number of hypotheses were generated;
however, none were judged to be realistic when compared to the total
weight of evidence provided by numerous scientific investigations.

In particular, microbiological studies using pure cultures of non-
flocculant bacteria have provided very strong evidence that DO has no
effect on any measureable property of dispersed bacterial cultures as
long as it is maintained above a very low, critical level (less than
about 0.1 mg/ℓ) (see Chapter II for a review). Therefore, the anoxic
core theory was developed in this study as the only reasonable theory
for explaining differences in bacterial kinetics, yield, settleability
and viability in activated sludge systems operated at different DO
levels.

Some studies concerning the effect of DO on the activated sludge process, however, have shown differences between systems that were identical except for the DO maintained, differences not readily explained by the anoxic core theory. In particular, research conducted previously at Oregon State University indicated that the ATP viability in activated sludge systems maintained at high DO levels (8 - 20 mg/ℓ) was substantially higher than that in systems maintained at a lower DO (2 mg/ℓ), even when application of the general diffusional model indicated that substrate was probably the diffusion-limiting species (Study No. 1) and when the bacterial aggregates were small in size (Study No. 2) (see Chapter IV for a review of these studies). These studies suggested the possibility that maintenance of higher DO levels (above 2 mg/ℓ) may have an effect on ATP viability apart from that caused by diffusional limitations, even though no realistic theory was identified within the literature review that could explain such an effect.

The primary purpose of the phase 1 experiments, therefore, was to confirm that, for a dispersed bacterial culture (no significant diffusional limitations), operation at a high DO typical of oxygen activated sludge systems (8 - 20 mg/ℓ) has no effect on substrate utilization kinetics, biomass yield, ATP viability, or adenylate energy charge when compared to operation at lower DO levels typical of air activated sludge systems (1 - 2 mg/ℓ). Harrison and Maitra (1969) found that, for a pure culture of nonflocculant bacteria grown in a chemostat at a rapid growth rate (D = 0.18/hr), the DO maintained had no effect on the concentration of adenine nucleotides or the bacterial yield as long as it was maintained above the critical concentration of approximately 0.1

mg/ℓ. Therefore, the emphasis of this research was placed on longer cell retention times typical of the activated sludge process.

The second major purpose of this phase was to develop methods. Primarily, this involved a comparison of the five different ATP extraction procedures described in Chapter V.

The third purpose of the phase 1 experiments was to evaluate the possibility that some ATP may be associated with nonviable bacteria, as hypothesized by Chappelle, et al. (1978). The model developed by Williamson and Nelson (1978) assumes that ATP is associated only with viable cells and that reductions in specific ATP content are, therefore, a direct indicator of a reduction in viable biomass. However, the existence of a sizeable nonviable fraction of the biomass has been documented for activated sludge systems operated at long cell retention times (see Chapter III). The association of a substantial quantity of ATP with this nonviable fraction would be contrary to the assumption of Williamson and Nelson (1978) and would require modification of the model.

The determination of whether the nonviable fraction of the biomass in biological reactors operated at long cell retention times might contain substantial quantities of ATP was planned to result primarily from energy charge measurements. Previous studies have shown that the energy charge is strongly stabilized in the range of 0.8 to 0.9 in growing bacterial cultures and that a substantial reduction in energy charge occurs when cells become nonviable, although some ATP may remain (see Chapter II for a review). Therefore, the association of a substantial portion of the total ATP pool with nonviable cells would be indicated

by culture energy charge levels substantially below the 0.8 - 0.9 range found for growing bacterial cultures.

A possible effect of DO on bacterial metabolism that may have some importance for certain industrial wastewaters involves the utilization of oxygen in oxygenation reactions. In particular, the degradative pathway for aromatic compounds, such as phenol, involves an oxygenation reaction prior to cleavage of the aromatic ring. Furthermore, the affinity of the oxygenase for oxygen may be substantially lower than that of the terminal cytochrome oxidase. If the oxygenase reaction becomes rate-limiting at low DO levels, then the critical DO for substrates that involve an oxygenation reaction may be substantially higher than for substrates that do not involve an oxygenation reaction (see Chapter II for a more detailed discussion). Since phenol was the substrate planned for use in all laboratory phases of this research, it was decided that a brief investigation of the critical DO associated with phenol degradation was warranted and might help clarify the importance of DO to oxygenation reactions. While phenol is only one of many substrates that require oxygenations, the oxygenase enzymes involved in the degradation of phenol have a broad substrate specificity; furthermore, the K_m (O_2) determined for the isolated enzyme phenol hydroxylase is among the highest reported (0.64 mg/ℓ) (Hayaishi, et al., 1975).

Research Plan

The research plan for Phase 1 was to operate the two main reactors as identical mixed culture chemostats except for the maintenance of a high DO (15 mg/ℓ) in one and a lower DO (2 mg/ℓ) in the other. All other operational parameters, including the hydraulic retention time

(θ), pH, mixing intensity, and operational procedures were to be equal. At least two hydraulic retention times were to be evaluated.

It was reasoned that the lack of settling competition in the chemostat and the low substrate concentration that would result at long hydraulic retention times would result in the establishment of a very dispersed culture and, consequently, the absence of significant diffusional limitations for oxygen. Steady-state measurements were to include ATP and other adenine nucleotides, total suspended solids (representative of the net yield), effluent substrate concentration, and kinetic coefficients (k and K_s) by the infinite dilution batch procedure. A statistical evaluation of these data were then planned to reveal any correlation with DO.

EXPERIMENTAL PROCEDURES

The two main reactors were set up as identical chemostats. The DO control of Reactor A was set at 2.0 mg/ℓ and that of Reactor B at 15.0 mg/ℓ. The feed contained 1000 mg/ℓ phenol as described in Chapter V and was fed by a dual head peristaltic pump. Daily measurements of the bacterial cultures included pH, influent flow rate (and resultant hydraulic retention time), effluent substrate (phenol) concentration, reactor suspended solids, and a microscopic examination to determine the approximate floc size and types of higher organisms.

Although the dual head feed pump produced approximately the same flowrate to each reactor, the maintenance of an equivalent hydraulic retention time in each required small adjustments in the reactor volume. This was done by adjusting the height of the effluent withdrawal tube. The pH in each reactor was maintained near 7.0 by adjusting the

acid or base addition to the feed solution. The aeration gas flow rate to each reactor was the same, as described in Chapter IV, resulting in equal mixing intensities.

At first, reactor startup was accomplished by filling the reactor with diluted feed solution, adding the microbial seed, aerating for one to two days to allow development of a substantial culture, and then turning on the feed pump at the rate necessary to provide the desired hydraulic retention time. However, this procedure inevitably resulted in reactor failure (washout) due to the inhibitory effects of phenol at high concentrations (see Yang and Humphrey, 1975, for a discussion of phenol degradation and inhibition kinetics). Consequently, the reactor startup procedure was changed so that the feed pump was initially turned on at a very slow flow rate and, when the culture had established itself sufficiently and was approaching a steady-state condition, the influent flow rate was increased. It was found that if the phenol concentration climbed to more than 10 - 20 mg/ℓ, reactor failure inevitably resulted. Consequently, the increase in influent flow rate was kept small (less than 50% increase) and the feed rate was set back if a high phenol concentration began accumulating in the reactor.

Initially, the effluent was withdrawn continuously by a vacuum effluent tube at the top of the reactor. However, a significant amount of foaming occurred at the top of the reactor and, due to the very low flow rates being maintained, a major portion of the liquid withdrawn from the reactor was as foam. Furthermore, analysis of reactor and effluent suspended solids indicated a substantial difference, a difference attributed to foam fractionation. Consequently, the vacuum supply to the effluent tube was switched to a timer. The timer was set to

remain open for about one minute and the timing cycle was adjusted to the influent flow rate so that no more than a five percent change in volume occurred during the time the vacuum was off. In addition to solving the foam fractionation problem described above, this procedure proved desirable also in that a relatively large volume (∿ 250 ml) of culture was available for analytical purposes at the end of each cycle without the necessity of disturbing the normal operation of the reactor.

The reactors were brought to a steady-state condition at hydraulic retention times of approximately one and one-half days and five days. The occurrence of steady-state conditions was determined when all daily measurement parameters, including reactor suspended solids, pH, and effluent substrate concentration ceased to exhibit any distinctive increasing or decreasing trend. This generally required a time period of four to eight times the hydraulic retention time at the final influent flow rate. Steady-state measurements of adenine nucleotides, kinetic coefficients, suspended solids, and effluent phenol concentration were then taken in the following three to four days.

Comparative measurements with the five selected ATP extraction procedures were conducted during the period prior to steady-state measurements. This was done because these experiments did not require a true steady-state condition, only the existence of relatively constant conditions for the period of the tests (approximately four hours for completion of all extractions). Also, this allowed a decision to be made on the extraction procedure to be used in this study prior to measurement of the steady-state adenine nucleotide concentrations for Run 1-1. Prior to the comparative tests, each of the extraction procedures was practiced until consistent results were obtained.

Because samples from the main reactors and from the batch reactors during the test for kinetic coefficients contained phenol concentrations in the microgram-per-liter range, the rapid inactivation of bacterial respiration was essential to accurate measurements of the phenol concentration. For small sample volumes (less than ten ml), this was done by immediate filtration through a 0.45 μ membrane filter similar to the procedure described by Williamson and McCarty (1974). Larger volumes (up to 250 ml) were rapidly aspirated into a flask containing five ml of 1 + 9 H_3PO_4 and five ml of 100 g/ℓ $CuSO_4 \cdot 5 H_2O$.

RESULTS

Comparison of ATP Extraction Procedures

A comparison of the five selected ATP extraction methods was performed as the reactors approached the steady-state condition during Run 1-1. A complete description of the procedures associated with each of these extraction methods is given in Chapter V. The results of these comparative tests were evaluated by four main factors:

1. ATP Concentration. The result of a comparatively low ATP concentration is indicative of either a lack of complete extraction or lack of immediate inactivation of ATP-hydrolyzing enzymes.

2. Total Adenine Nucleotides (ATP + ADP + AMP). A comparatively low concentration is indicative of a lack of complete extraction.

3. Energy Charge. A comparatively low energy charge is indicative of a lack of immediate inactivation of enzymes.

4. Standard Deviations. These are indicative of the
reproducibility and precision of the method. Pre-
cision was particularly desirable for the determined
ATP concentration and energy charge.

The first comparative test included only the four boiling buffer
methods (IA, IB, IIA, IIB). Results of this test are shown in Table
6-1. Method IB (direction injection into boiling Tris buffer, pH 7.75)
produced the highest ATP concentration for both Reactor A and Reactor B
of the four boiling buffer methods. Total adenine nucleotides were
similar for Method IA and IB and were substantially lower for Methods
IIA and IIB. The energy charge was near 0.8 in both Reactor A and B for
Methods IB, IIA, and IIB but was substantially lower with Method IA.

The standard deviation of the three or four measurements of ATP
and energy charge was substantially lower for the direct injection
methods (IB and IIB) than for the filtration methods (IA and IIA).

To summarize the data in Table 6-1, Method IB (direct injection
into boiling Tris buffer) was clearly superior to the other three boil-
ing buffer techniques. Method IA was comparable in extraction effi-
ciency for total adenine nucleotides, but the lower ATP concentration
and energy charge obtained and the relatively large standard deviations
were indicative of a lack of immediate inactivation of ATP-hydrolyzing
enzymes. Methods IIA and IIB were inferior in extraction efficiency.

A comparison of all five of the selected extraction techniques,
including perchloric acid extraction (Method IIIA) and the four boiling
buffer techniques, is shown in Table 6-2. Perchloric acid extraction
has become widely accepted within the microbiological community as

Table 6-1.   Comparison of ATP Extraction Methods, Test No. 1.

PARAMETER	EXTRACTION METHOD*							
	IA		IB		IIA		IIB	
	Value	Std. Dev.	Value	Std. Dev.	Value	Std. Dev.	Value	Std. Dev.
Number of Measurements	4	-	3	-	4	-	3	
REACTOR A								
ATP (μM)	0.301	.032	.403	.008	.236	.048	.344	.004
ADP (μM)	.144	.032	.100	.060	.056	.015	.102	.030
AMP (μM)	.120	.030	.074	.008	.038	.037	.038	.026
Energy Charge	.66	.05	.79	.01	.80	.11	.82	.02
ATP+ADP+AMP (μM)	.565	.054	.577	.061	.330	.062	.484	.040
REACTOR B								
ATP (μM)	.288	.027	.341	.017	.256	.015	.279	.008
ADP (μM)	.127	.032	.072	.030	.054	.015	.071	.031
AMP (μM)	.077	.040	.074	.009	.044	.020	.050	.008
Energy Charge	.72	.08	.78	.01	.80	.05	.79	.01
ATP+ADP+AMP (μM)	.492	.058	.487	.036	.354	.029	.400	.033

*   IA = Filtration and insertion into boiling Tris buffer, pH 7.75.
    IB = Direct injection into boiling Tris buffer, pH 7.75.
   IIA = Filtration and insertion into boiling glycine buffer, pH 10.0.
   IIB = Direct injection into boiling glycine buffer, pH 10.0.

Table 6-2.   Comparison of ATP Extraction Methods, Test No. 2.

PARAMETER	EXTRACTION METHOD*									
	IA		IB		IIA		IIB		IIIA	
	Value	Std. Dev.	Value	Std. Dev.	Value	Std. Dev.	Value	Std. Dev.	Value	Std. Dev.
Number of Measurements	4	-	4	-	4	-	3	-	4	-
REACTOR A										
ATP (µM)	1.93	0.14	2.52	0.22	1.35	0.09	1.95	0.00	2.71	0.50
ADP (µM)	0.74	0.67	0.46	0.23	1.22	0.09	0.56	0.04	0.30	0.04
AMP (µM)	1.17	0.82	0.13	0.17	0.38	0.24	0.29	0.17	0.32	0.12
Energy Charge	0.60	0.12	0.88	0.06	0.67	0.07	0.80	0.04	0.86	0.04
ATP+ADP+AMP (µM)	3.84	1.07	3.11	0.36	2.95	0.27	2.80	0.17	3.33	0.52
REACTOR B										
ATP (µM)	1.69	0.21	2.40	0.03	1.44	0.09	1.83	0.12	2.43	0.47
ADP (µM)	1.49	0.47	0.49	0.24	1.42	0.13	0.90	0.09	0.50	0.28
AMP (µM)	0.65	0.58	0.13	0.11	0.53	0.05	0.12	0.10	0.13	0.17
Energy Charge	0.64	0.10	0.88	0.02	0.63	0.03	0.80	0.03	0.88	0.02
ATP+ADP+AMP (µM)	3.83	0.78	3.02	0.27	3.39	0.17	2.85	0.18	3.06	0.57

*   IA = Filtration and insertion into boiling Tris buffer, pH 7.75.
    IB = Direct injection into boiling Tris buffer, pH 7.75.
   IIA = Filtration and insertion into boiling glycine buffer, pH 10.0.
   IIB = Direct injection into boiling glycine buffer, pH 10.0.
  IIIA = Direct injection into perchloric acid.

achieving essentially 100-percent extraction of adenine nucleotides and immediate inactivation of ATP-hydrolyzing enzymes (Atkinson, 1977). Therefore, the basic purpose of this comparative test was to determine whether any of the simpler boiling buffer techniques could compare in extraction efficiency and inactivation of enzymes.

Comparison of the five methods in Table 6-2 shows that Method IB (direct injection into boiling Tris buffer) was the only boiling buffer technique comparable to perchloric acid extraction (Method IIIA) in terms of the ATP concentration and energy charge obtained. The filtration methods (IA and IIA) suffered from a low energy charge, indicating a lack of complete and/or rapid inactivation of ATP-hydrolyzing enzymes.

The increased concentration of total nucleotides with Method IA is not realistically explained as improved extraction efficiency due to the comparatively low ATP concentration and energy charge obtained and due to the lack of corresponding results within Test No. 1 with this method (see Table 6-1). Rather, this appears to be the result of experimental errors in the analytical procedure. Specifically, the enzymes that are added to convert ADP and AMP to ATP may not have been completely inactivated prior to measurement of bioluminescent light emission, which would account for the comparatively high values obtained for ADP and AMP concentration. Because extracts from each extraction procedure were processed as individual batches in the adenine nucleotide assay, the occurrence of a procedural error within the assay would tend to result in erroneous values for all samples extracted by that procedure. The evaluation of relatively large standard deviations for ADP and AMP concentrations determined by Method IA samples tends to confirm the

explanation of experimental error as the cause of the comparatively high yield of total adenine nucleotides.

Method IIB (direct injection into boiling glycine buffer, pH 10.0) performed consistently in comparison to perchloric acid extraction but was significantly lower in both extracted ATP concentration and energy charge. Method IB was, therefore, evaluated as the only boiling buffer method that was comparable to perchloric acid extraction. Also, Method IB produced generally smaller standard deviations in ATP concentration, as compared to perchloric acid extraction, probably the result of its exclusion of the difficult pH adjustment procedure associated with Method IIIA. Therefore, Method IB (direct injection into boiling Tris buffer, pH 7.75) was selected as the extraction technique for all further analyses of ATP and adenine nucleotides within this study.

Run 1-1

The first run was designed to test the effect of the two different DO values at a hydraulic detention time, θ, of approximately five days. Upon initial startup of the reactors, the cultures were quite flocculant and bacterial aggregates up to 500 microns in diameter were present in both reactors. Additionally, there was a wide variety of higher organisms, including protozoa and rotifers. After the influent flow rate was set at its final level, however, and the reactors began to approach a steady-state condition, the size of bacterial aggregates became smaller and smaller and the variety of higher organisms reduced. At the steady-state condition, the cultures in both reactors consisted primarily of spherical-shaped bacteria that occurred in chains of from ten to 50 microns in length and free-swimming ciliates. There were

some bacteria in aggregates, but these were rare and the aggregates were small (less than 20 microns in diameter). Consequently, the establishment of mixed cultures that were not subject to significant diffusional limitations was judged to be successful.

A listing of reactor suspended solids, pH, effluent phenol concentration, and hydraulic retention time (θ) is given in Appendix A (Table A-1) for the period beginning at the final setting of the influent flow rate and ending following the conclusion of all steady-state measurements.

Although periodic adjustment of the aeration gas composition was necessary during the initial stages of reactor operation to maintain tight control of the DO, the system stabilized as the culture approached the steady-state condition and the control system maintained the set DO within about a five-percent variation without difficulty. Maintenance of the same hydraulic retention times in the reactors required only occasional and minor adjustments to the reactor volume in accordance with the measured influent flow rates. Maintenance of approximately the same pH in each reactor required periodic adjustment of the acid or base added the feed solution, particularly after the aeration gas composition was changed. At the steady-state condition, however, the pH in each reactor was relatively constant and adjustment to the acid or base addition were not necessary.

The culture parameters measured at the steady-state condition, including effluent phenol concentration, suspended solids, ATP concentration and adenylate energy charge, were very similar between the two reactors (see Table 6-3). However, sampling procedures for effluent phenol utilized only 100 ml of sample. The sensitivity of the

Table 6-3.   Summary of Steady-State Conditions, Run 1-1.

PARAMETER	NO. OF MEASUREMENTS	REACTOR A Value	A Std. Dev.	B Value	B Std. Dev.
DO (mg/ℓ)	cont.	2.0	±0.1[1]	15.0	±0.5[1]
θ (hrs)	3	112	1.7	112	1.5
pH	3	6.90	0.10	6.92	0.08
Effluent Phenol (mg/ℓ)	3	<0.05	-	<0.05	-
TSS (mg/ℓ)	3	350	15	336	11
ATP (μM)	4	0.374	0.035	0.310	0.037
Energy Charge	4	0.80	0.021	0.78	0.010
Specific Viability (mg ATP/g TSS)	4	0.59	0.06	0.51	0.06
k (g phenol/g TSS-day)	1	3.90	0.32	4.16	1.95
K_s (mg/ℓ)	1	0.010	0.001	0.017	0.011

[1] Approximate deadband range of DO controller.

4-aminoantipyrine analytical method was only 0.01 mg/ℓ under these con-
ditions and it was recognized, after the steady-state measurements had
been completed, that a larger sample size and greater sensitivity would
have been desirable. Furthermore, the concentrations obtained were
quite variable within the range below 0.05 mg/ℓ. This was attributed,
at least partially, to the fact that the 100-ml sample for analysis was
withdrawn from the side of the reactor, a point relatively close to the
feed inlet, rather than the normal effluent withdrawal point at the top
of the reactor. Consequently, the statement that both reactors exhib-
ited an effluent phenol concentration of less than 0.05 mg/ℓ was felt
to be more representative than listing of an average concentration and
a standard deviation. The deficiencies in the analytical techniques
described above were corrected within the second run by changing the
sample withdrawal point to the top of the reactor and by using a full
250-ml sample for the phenol analysis.

A statistical evaluation of the data in Table 6-3 was performed by
the well-known test on the difference of two means, assuming a normal
distribution (Bethea, et al., 1975). This evaluation revealed that:

1. The hypotheses that the operational parameters, con-
   sisting of pH and hydraulic retention time, were equal
   could not be rejected at the 90-percent confidence
   level and, in fact, could not be rejected even at the
   50-percent level.

2. The hypotheses that the suspended solids (TSS), energy
   charge, and specific viability in Reactors A and B
   were equal were rejected at the 90-percent confidence

level but could not be rejected at the 99-percent

confidence level.

3.   The hypothesis that the ATP concentration in Reactors

A and B were equal was rejected at the 99-percent

level.

Consequently, it was concluded that the reactor operational para-
meters were not significantly different, that most of the culture para-
meters, including suspended solids, adenylate energy charge, and speci-
fic ATP viability, were not significantly different at a high statisti-
cal confidence level, and that the ATP concentrations were significantly
different between Reactors A and B at the steady-state. However, the
difference in ATP concentration between Reactor A and B was only about
20 percent. Furthermore, the ATP concentration was higher in Reactor A
(DO = 2 mg/ℓ), contrary to previous results that ATP concentration in-
creased with increasing DO (Williamson and Nelson, 1978).

Batch Test for k and K_s

The kinetic coefficients k and K_s were determined by the "infinite
dilution" batch procedure described previously. A summary of the data
obtained is given as Table 6-4, a Lineweaver-Burke plot of the data is
shown in Figure 6-1, and the resulting kinetic coefficients are listed
in Table 6-3. A significant amount of growth occurred during the two
to three hours the batch reactors were operated as shown in Table 6-4.
However, this growth rarely exceeded ten percent of the total mass and
it was judged that the contribution of the additional microbial mass to
the observed kinetics was not substantial and no correction was made
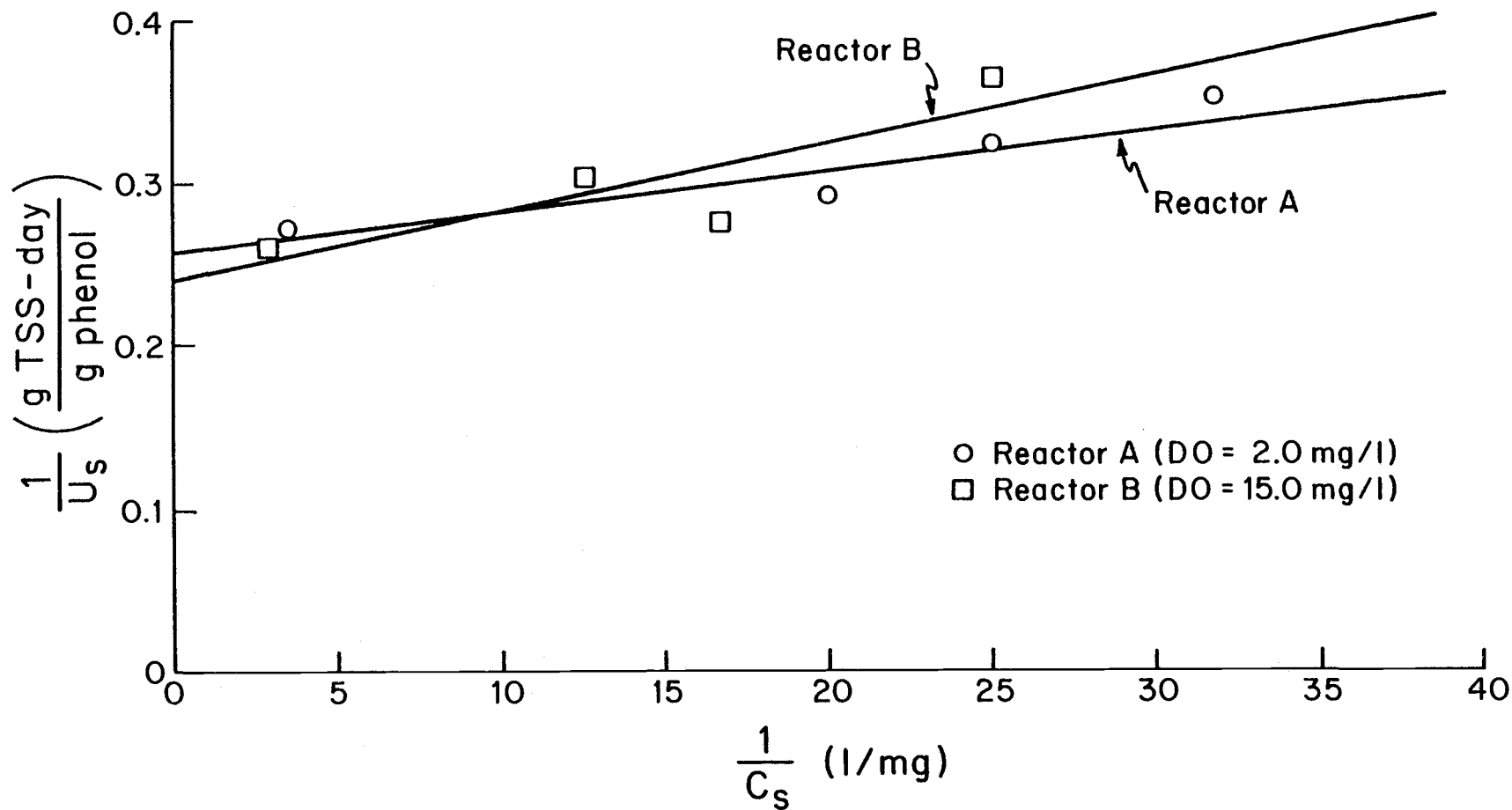
Figure 6-1.   Lineweaver-Burke Plot of Batch Reactor Data, Run 1-1.

Table 6-4. Batch Reactor Data Summary, Run 1-1.

REACTOR	Q (ml/min)	C_s^o (g/ℓ)	V (ml) initial	V (ml) final	X (mg/ℓ) initial	X (mg/ℓ) final	C_s (mg/ℓ)	$M=QC_s^o$ (g/day)	$U_s = \dfrac{M}{X_i\,V_f}$ (g/g-day)	$\dfrac{1}{U_s}$	$\dfrac{1}{C_s}$
A	.0127	50	500		338		no SS[1]	0.914			
		40					no SS	0.732			
		32.5		480		362	0.29	0.594	3.66	0.273	3.45
		30		475		375	0.05	0.549	3.42	0.292	20.0
		27.5		480		350	0.04	0.503	3.10	0.323	25.0
		25		480		362	0.03	0.457	2.83	0.353	33.3
B	.0361	20	500		346		no SS	1.04			
	.0127	50					no SS	0.914			
		35		470		388	0.35	0.640	3.85	0.260	2.86
		32.5		470		400	0.06	0.594	3.65	0.274	16.7
		30		480		388	0.08	0.549	3.30	0.303	12.5
		25		480		362	0.04	0.457	2.75	0.363	25.0

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[1] No steady-state condition achieved.

for this growth in the subsequent analysis. The parameters that were used in the analysis of the specific substrate utilization rate, U_s, were the initial microbial mass (X_i) and the final volume after small withdrawals for analytical purposes (V_f).

An additional problem was that the small sample volume (10 ml) withdrawn at 30-minute intervals was only sufficient for a maximum analytical sensitivity of 1.0 mg/ℓ because the rapid aqueous colorimetric test was employed. Since the half-velocity coefficient, K_s, was very low for these cultures, the steady-state phenol concentrations were generally well below 1.0 mg/ℓ and accurate measurement of the substrate concentration as a function of time was not possible for reactors approaching steady-state.

Therefore, the steady-state condition was assumed to be reached after two hours of operation with a measured phenol concentration of less than 1.0 mg/ℓ. The final sample was then taken with sufficient volume for analysis to 0.01 mg/ℓ using the more sensitive chloroform extraction analytical technique.

The DO control system performed very well during the batch tests and maintained the set DO within deadband limits of ± 5 percent. Because the aeration gas composition was different in the batch reactors, however, continuous monitoring and adjustment of the pH to equal that in the main reactor was necessary.

Linear regression of the batch reactor data, as shown in Figure 6-1, resulted in a good coefficient of correlation for Reactor A (r = 0.94) and a somewhat poorer correlation for Reactor B (r = 0.84). The standard deviations listed in Table 6-3 for the kinetic coefficient k and K_s were derived from the regression analysis and were relatively

small for Reactor A (coefficient of variance less than ten percent) but were relatively large for Reactor B (coefficient of variance near 50 percent) due to the poorer correlation for the Reactor B data.

The difference between the maximum substrate utilization rate (k) between Reactors A and B was less than ten percent and the half-velocity coefficients were similar, considering the difficulty involved in measuring K_s values in the microgram-per-liter range. A statistical evaluation of the kinetic coefficients obtained from the regression analysis yielded the following results:

1. The hypothesis that the two k values were equal could not be rejected at the 90-percent confidence level and, in fact, could not be rejected at even the 25-percent confidence level. However, this was largely the result of the large variance for k_B due to an outlier in the regression analysis.

2. The hypothesis that the two K_s values were equal could not be rejected at the 90-percent confidence level.

3. Elimination of the single outlier in the regression data for Reactor B (C_s = 0.06 mg/ℓ, U = 0.594 g/day) resulted in a dramatically improved correlation (r = .999) without substantially affecting the kinetic coefficients (k = 4.06 g/g-day, K_s = 0.019 mg/ℓ). Still, with the outlier point eliminated, the hypothesis that the two k values were equal could not be rejected at the 90-percent confidence level.

It was concluded that the kinetic coefficients k and K_s were not signi-

ficantly different between Reactors A and B at a high statistical confidence level.

Some of the individual batch reactor runs that did not produce a steady-state condition produced some interesting information about the kinetics of phenol degradation as shown in Figures 6-2 and 6-3. Figure 6-2 shows the response of batch Reactor A (DO = 2 mg/ℓ) to a mass substrate loading of 0.914 g/day. This response was typical of what would be expected from a bacterial culture exhibiting Monod-type kinetics. After an initial acclimation period, the culture responded to the high substrate concentration present with a constant utilization rate as indicated by the linearity of the substrate versus time plot for times greater than one hour. A maximum substrate utilization rate for this particular test was calculated as the difference between the rate of substrate accumulation, as reflected by the slope of the linear portion of Figure 6-2, and the mass substrate loading rate (M), divided by the TSS concentration, resulting in a k value of 3.88 g phenol/g TSS-day. This was very similar to the k value of 3.90 determined by linear regression of all the batch reactor data and tends to confirm both the applicability of Monod kinetics to the culture in Reactor A and the accuracy of the k value determined by linear regression of the batch reactor data.

The response of the culture from Reactor B (DO = 15 mg/ℓ) to substrate loading rates greater than the maximum utilization rate is shown in Figure 6-3. This response was very similar to batch Reactor A in exhibiting an initial acclimation or lag period of about one-half hour followed by a linear response indicative of the maximum substrate utilization rate. The k values derived from the linear portions of the
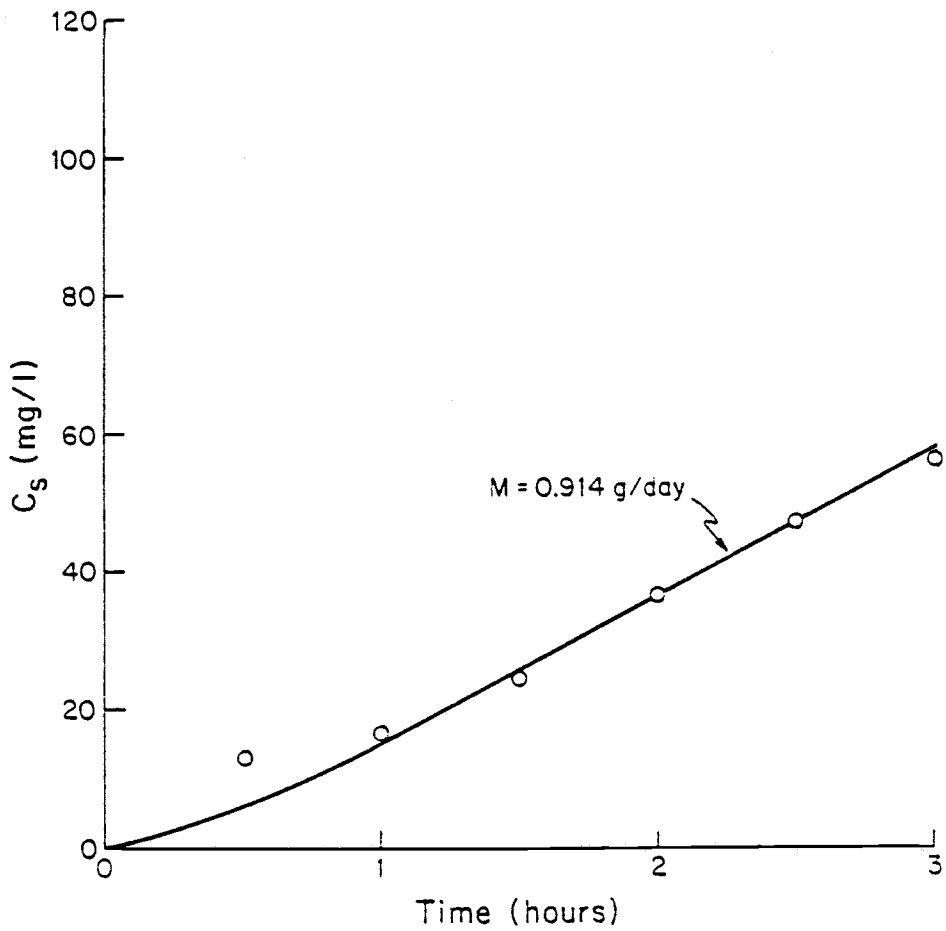
Figure 6-2. Substrate versus Time Plot for an "Infinite Dilution" Batch Reactor Test, Reactor A (DO = 2 mg/ℓ), Run 1-1.
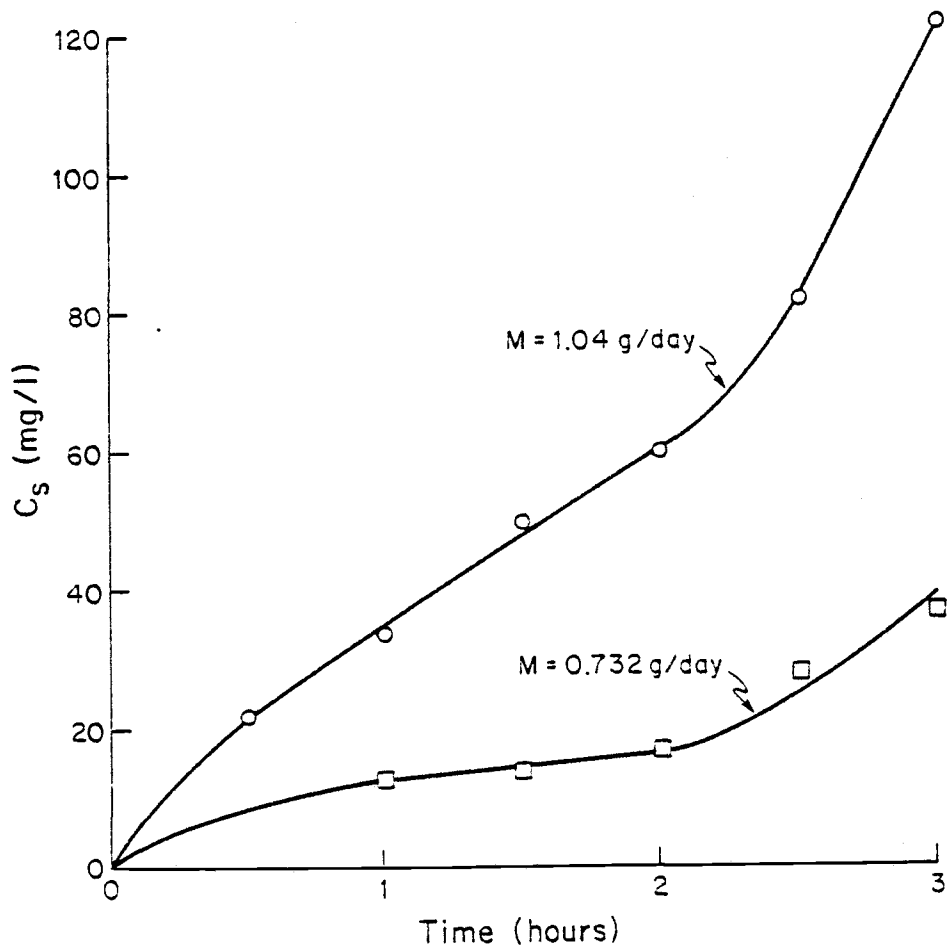
Figure 6-3. Substrate versus Time Plots for "Infinite Dilution" Batch Reactor Tests, Reactor B (DO - 15 mg/ℓ), Run 1-1.

curves in Figure 6-3 were 3.97 and 4.21 g phenol/g TSS-day for mass
loading rates of 0.732 and 1.04 g/day, respectively. These values were
very similar to the k value of 4.16 determined by linear regression of
all the batch reactor data and tend to confirm both the accuracy of the
k value derived by linear regression and the comparability of the k
values for Reactors A and B. A marked difference was illustrated by
Figure 6-3, however, in that an inhibited response was observed after
the culture had been exposed to high phenol concentrations for approxi-
mately two hours.

Run 1-2

The second run involved the same procedures and conditions as the
first except that a lower hydraulic retention time of approximately 1.5
days was maintained in the reactors. The bacterial culture in both
reactors remained quite dispersed throughout this run, similar to Run
1-1, and no significant change was observed in the nature of the domi-
nant bacterial species or higher organisms. A listing of reactor sus-
pended solids, pH, effluent phenol concentration, and hydraulic reten-
tion time is given in Appendix A (Table A-2) for the period beginning
at the final setting of the influent flow rate and ending following the
conclusion of all steady-state measurements.

A summary of operational and culture parameters at the steady-
state condition is given in Table 6-5. Operational parameters, includ-
ing the hydraulic retention time and pH were very similar between the
two reactors. Although there was a greater difference between the hy-
draulic retention times in Reactors A and B than in Run 1-1, the values
were still within a single standard deviation of each other (see the

Table 6-5.   Summary of Steady-State Conditions, Run 1-2.

PARAMETER	NO. OF MEASUREMENTS	REACTOR			
		A		B	
		Value	Std. Dev.	Value	Std. Dev.
DO (mg/ℓ)	cont.	2.0	±0.1	15.0	±0.5
θ (hrs)	3	39.4	1.1	38.8	0.8
pH	3	6.85	0.05	6.90	0.09
Effluent Phenol (mg/ℓ)	3	0.012	0.003	0.019	0.012
TSS (mg/ℓ)	3	430	12	443	15
ATP (µM)	4	.442	0.021	.421	.039
Energy Charge	4	0.82	0.02	.83	0.02
Specific Viability (mg ATP/g TSS)	4	0.57	0.04	0.52	0.07
k (g phenol/g TSS-day)	1	4.63	0.32	4.49	0.50
K_s (mg/ℓ)	1	0.011	0.003	0.028	0.006

statistical analysis below). The culture parameters for the two reactors, including the effluent phenol concentration, suspended solids, ATP concentration, adenylate energy charge, and specific ATP viability were also very similar between the two reactors. The improvements to the effluent sampling technique for phenol, as described in the previous section, resulted in an analytical sensitivity of 0.01 mg/ℓ and in a smaller variance in the measured concentrations than observed in Run 1-1. It should be noted that measurement of effluent phenol concentrations in the range found for Run 1-2 was extremely difficult. The volumetric substrate utilization rate in the reactors was approximately 0.007 mg/ℓ-s under the operating conditions of Run 1-2; consequently, even a one-second delay between the time of withdrawing the effluent sample and the time of complete inactivation of bacterial respiration would have a substantial effect on the measured phenol concentration. However, the sampling technique utilized within this run, which involved rapid aspiration of the effluent sample into an inactivating solution containing phosphoric acid and copper sulfate, seemed to produce good results, as indicated by the relatively low standard deviations calculated for the effluent phenol concentration.

Statistical evaluation of the data in Table 6-5 revealed the following:

1. The hypotheses that the true mean hydraulic retention time and pH in Reactors A and B were equal could not be rejected at the 90-percent confidence level.

2. The hypothesis that the true mean values for effluent phenol, suspended solids, ATP concentration, and speci-

fic ATP viability were equal between Reactors A and B
could not be rejected at the 90-percent confidence level.

Consequently, it is concluded that the reactor operational para-
meters were not significantly different and that the resultant culture
parameters, including suspended solids, effluent phenol concentration,
ATP concentration, adenylate energy charge, and specific viability,
were not signficantly different at a high statistical confidence level
between Reactors A and B.

Batch Test for k and K_s
___

Operation of the batch reactors in the infinite dilution mode for
determination of the kinetic parameters k and K_s was the same as that
described for Run 1-1. A summary of the data obtained is listed in
Table 6-6 and a Lineweaver-Burke plot of the data is shown in Figure
6-4. Linear regression of the batch reactor data resulted in a good
coefficient of correlation for both Reactor A (r = 0.96) and Reactor B
(r = 0.97) and very similar values for the maximum substrate utiliza-
tion rate, k. The K_s values derived were in the same range, but dis-
similar. However, the accuracy of the K_s evaluations is questionable
because all the steady-state substrate concentrations were substan-
tially larger than the K_s value. Therefore, the evaluation of K_s by
linear regression involved significant extrapolation of the data.

A statistical evaluation of the kinetic coefficients obtained from
the regression analysis revealed the following:

Table 6-6. Batch Reactor Data Summary, Run 1-2.

REACTOR	Q (ml/min)	$C_s^°$ (g/ℓ)	V (ml) initial	V (ml) final	X (mg/ℓ) initial	X (mg/ℓ) final	C_s (mg/ℓ)	$M=QC_s^°$ (g/day)	$U_s=\dfrac{M}{X\,V_f}$ (g/g-day)	$\dfrac{1}{U_s}$	$\dfrac{1}{C_s}$
A	.0127	30.0	500		210	215	no SS	0.549			
		25.0		470		260	0.37	0.457	4.63	0.216	2.70
		22.5		480		240	0.12	0.411	4.08	0.245	8.33
		20.0		480		240	0.04	0.366	3.63	0.275	25
B	.0127	25.0	500		215		no SS	0.457			
		22.5		470		240	0.19	0.411	3.98	0.251	5.26
		20.0		480		260	0.09	0.366	3.55	0.282	11.1
		17.5		480		250	0.08	0.320	3.10	0.322	12.5
		15.0		480		240	0.04	0.274	2.66	0.377	25
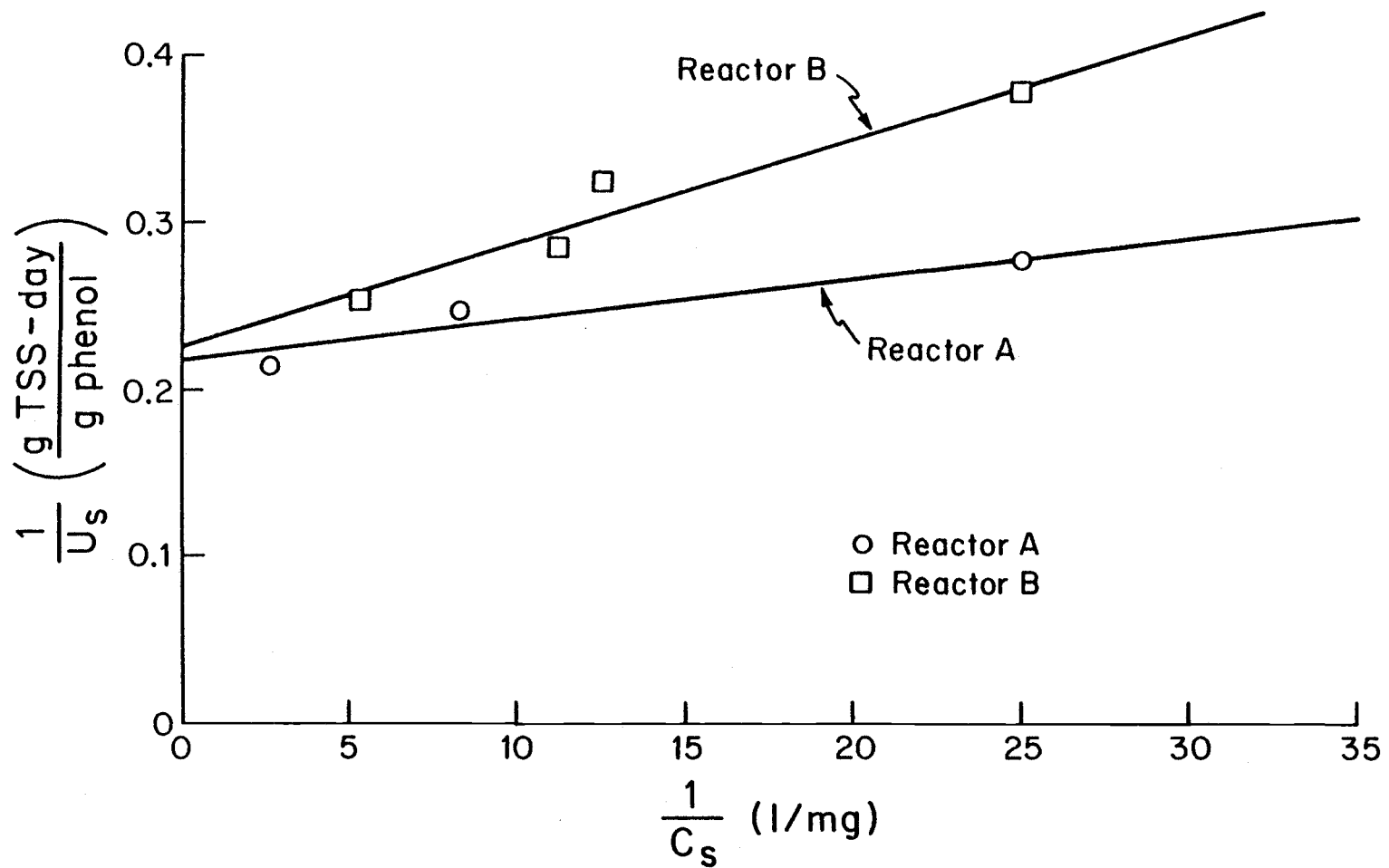
Figure 6-4.   Lineweaver-Burke Plot of Batch Reactor Data, Run 1-2.

1. The hypothesis that the true k values were equal in Reactors A and B could not be rejected at the 90-percent confidence level and, in fact, could not be rejected even at the 50-percent confidence level.

2. They hypothesis that the true K_s values were the same was rejected at the 99-percent confidence level.

Consequently, it is concluded that the measured k values were not significantly different, but the K_s were significantly different at a high confidence level. However, the difficulties involved in evaluating values of K_s in the range found in these tests must be recognized and, in view of these difficulties, the measured K_s values were similar.

A plot of substrate concentration versus time for a run that did not reach a steady-state is shown in Figure 6-5. Unlike the previous response curves (Figure 6-2 and 6-3), this curve indicates a directly increasing inhibition with increasing phenol concentration. The response shown by this figure indicates that the inhibiting effects of phenol at high concentrations (>10 mg/ℓ) may be quite complex, may involve a time response function, and furthermore, may vary with the exact species composition of a mixed culture.

Respirometric Response to DO

The final study area for this phase of research was the response of a mixed culture to low DO levels when phenol was the substrate. Specifically, it was desired to experimentally determine the respiration rate versus DO function and using these data to evaluate whether
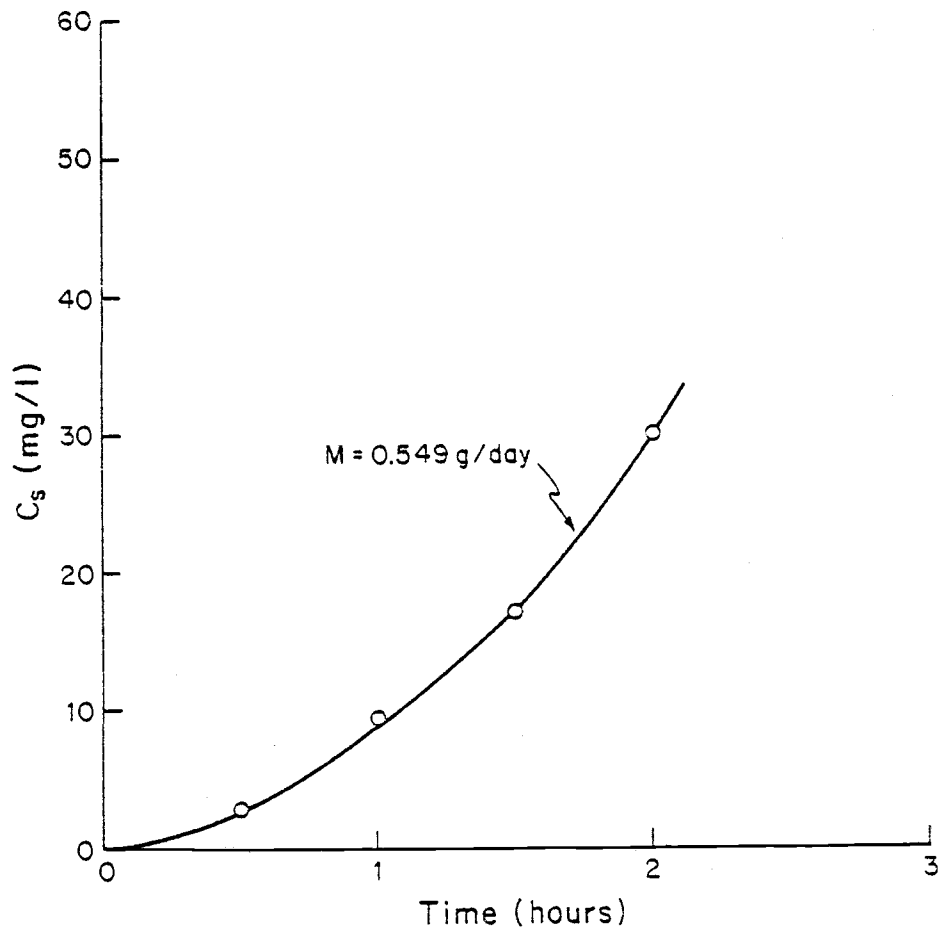
Figure 6-5. Substrate versus Time Plot for an
"Infinite Dilution" Batch Reactor Run,
Reactor A (DO= 2 mg/ℓ), Run 1-2.

the critical DO for phenol degradation is substantially higher than the very low values determined for bacteria degrading substrates that do not involve oxygenations. The simple batch respirometric test, as described previously, was utilized because it is rapid and sufficiently accurate to evaluate the alternative conclusions described above.

Culture samples from both Reactor A and Reactor B at the steady-state condition in Run 1-2 were taken for analysis. Sufficient feed was added to bring the initial phenol concentration to 50 mg/ℓ. Preliminary tests demonstrated the linearity of the DO versus time curve for DO levels above 1 mg/ℓ so that the final runs were begun at a DO of approximately 2 mg/ℓ. After adding substrate, both samples were aerated for five minutes to acclimate the culture, the DO probe inserted, and DO readings taken at 0.5 minute intervals initially and 0.25 minute intervals as the DO fell below approximately 1.0 mg/ℓ. The data obtained from these two runs are listed in Appendix A, Tables A-3 and A-4. The DO versus time curve for Reactors A and B are shown in Figures 6-6 and 6-7, respectively. These plots were linear for Reactors A and B down to a DO of approximately 0.2 to 0.3 mg/ℓ, indicating that the critical DO for phenol degradation by these cultures was quite low. The linearity of these curves above 0.3 mg/ℓ indicates that the bacterial respiration rate was unaffected by DO levels above 0.3 mg/ℓ.

The slope of the DO versus time functions, which are shown in Figures 6-6 and 6-7, represents the volumetric oxygen respiration rate, $U_{v,o}$, and this parameter was calculated for each interval between DO readings. The mass-specific respiration rate (U_o) was then obtained by dividing the volumetric rate by the suspended solids concentration in the batch reactor as listed in Tables A-3 and A-4. The mass-specific
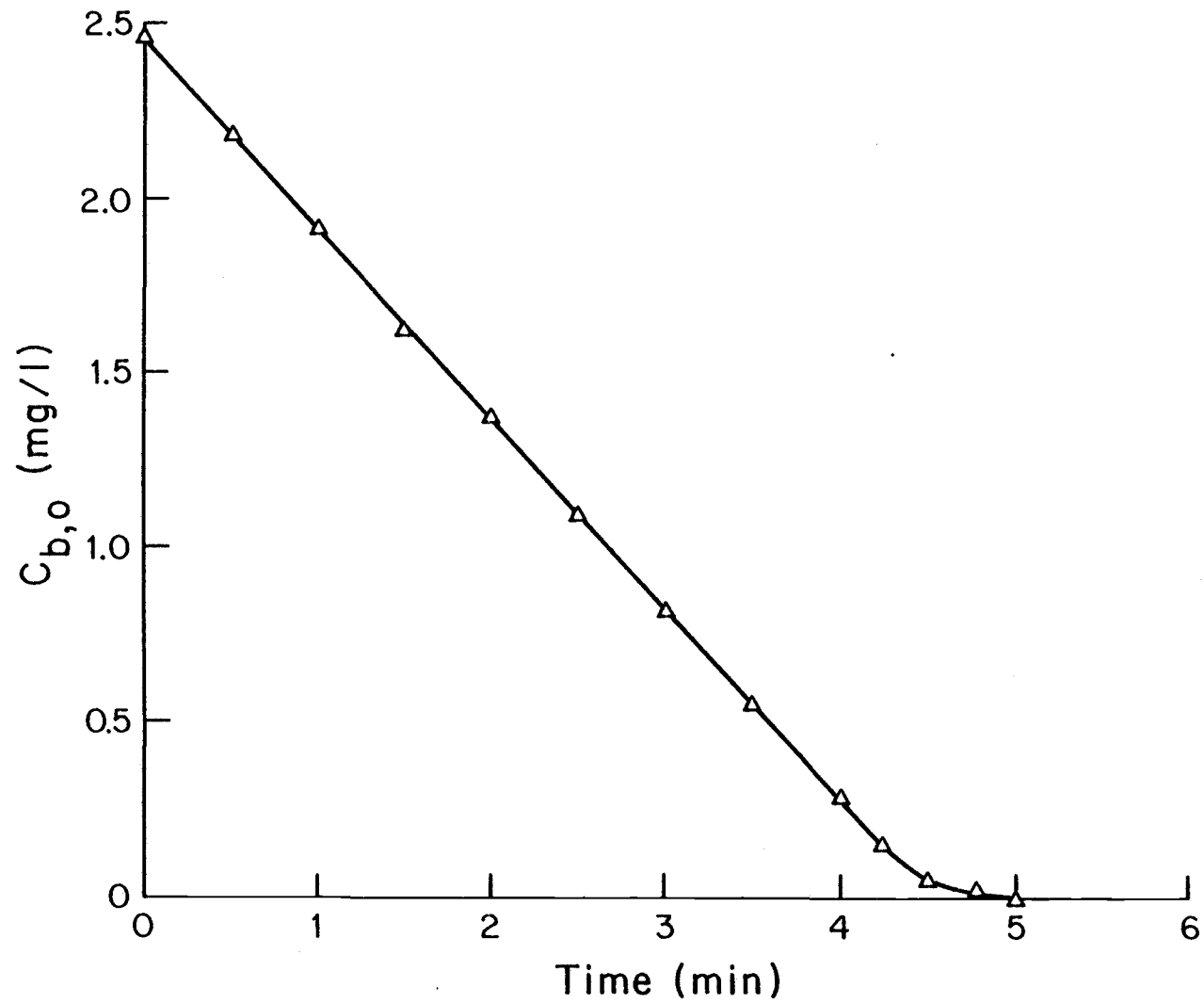
Figure 6-6. Bulk DO versus Time Curve for Respirometric Experiment, Reactor A, Run 1-2.
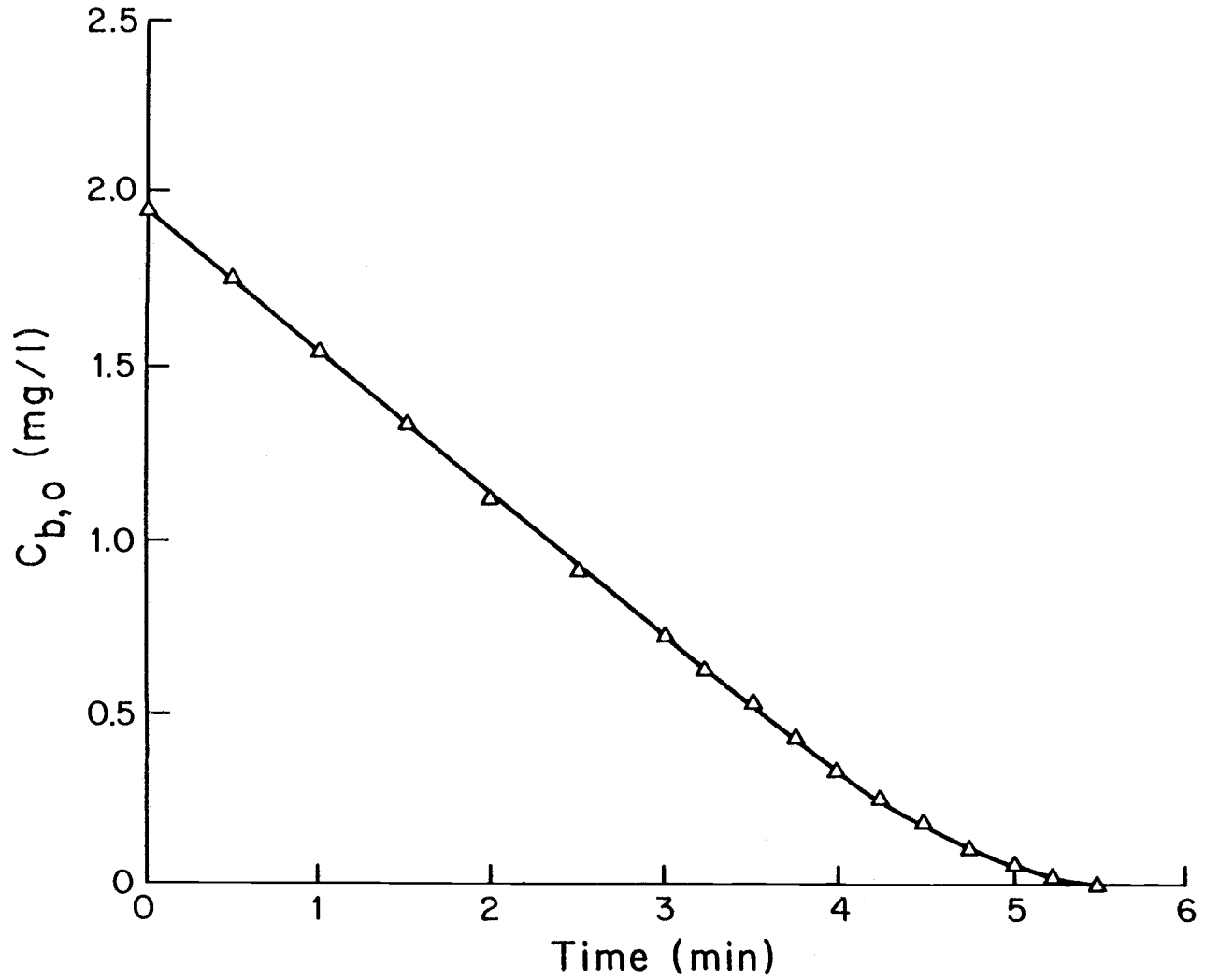
Figure 6-7.   Bulk DO versus Time Curve for Respirometric Experiment, Reactor B, Run 1-2.

respiration rate versus DO function was then plotted as shown in Figures 6-8 and 6-9.

The Monod function was fitted to these curves by linear regression of the data in Tables A-3 and A-4 on a Lineweaver-Burke plot and the resultant curves are shown as dashed lines in Figures 6-8 and 6-9. Because the linear portion of Figures 6-6 and 6-7 accurately define the maximum respiration rate, U_o^{max}, the Lineweaver-Burke regression line was forced through the y-intercept indicative of this U_o^{max} value. This analysis resulted in the determination of half-velocity coefficients for oxygen, K_o, of 0.044 mg/ℓ for Reactor A and 0.066 mg/ℓ for Reactor B. However, the Monod function did not provide a good fit to the data in either case. The actual data exhibited a sharp reduction in respiration rate as the DO fell below about 0.3 mg/ℓ that was not amenable to fitting with a Monod function.

In concluding whether the above-described data fit the Monod function, however, several points should be considered. First, the respirometric method utilized has limited accuracy in the range below 0.1 to 0.2 mg/ℓ due to a maximum readability of 0.025 mg/ℓ and the possible error associated with setting the zero point on the dissolved oxygen meter. Moreover, the data points associated with DO levels below 0.1 mg/ℓ have a disproportionately large impact on the K_o derived by linear regression a Lineweaver-Burke plot. However, the discrepancy between the data and the corresponding Monod functions shown in Figures 6-8 and 6-9 was sufficiently large that it could not be totally explained by limitations of the respirometric method utilized.

Although Longmuir (1954) found that the respiratory response of bacteria to DO followed Monod kinetics, earlier researchers found that
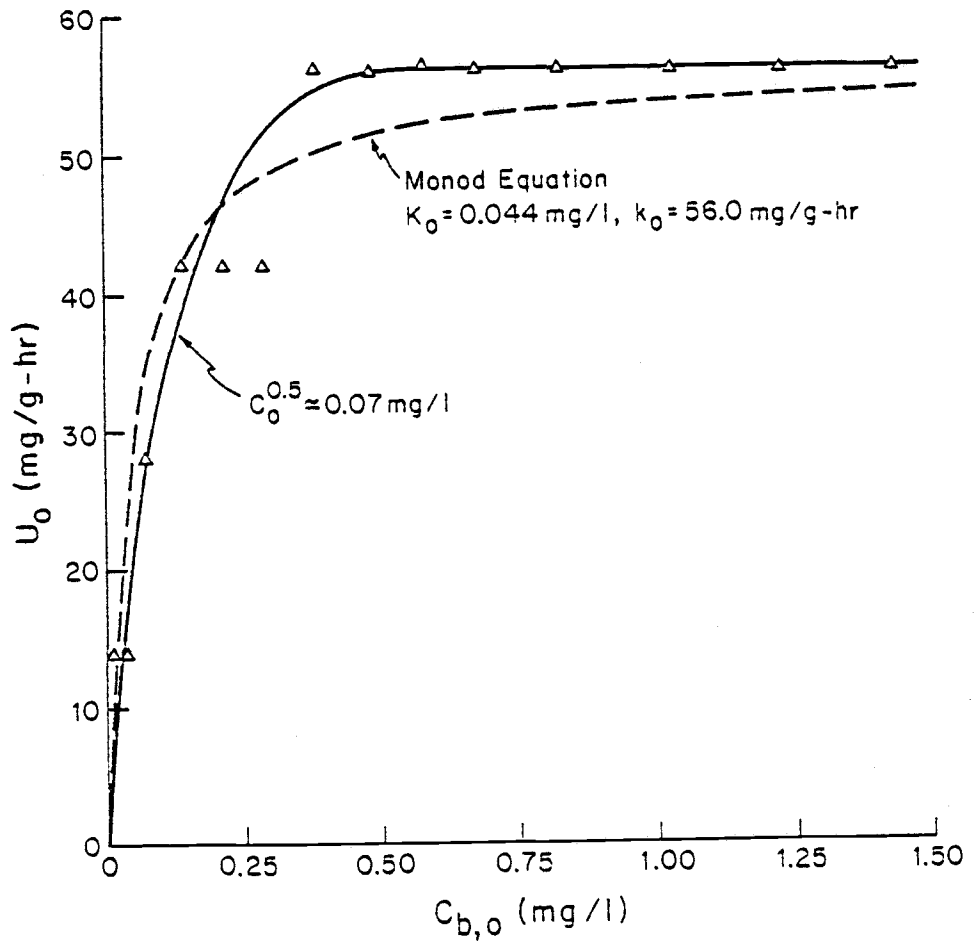
Figure 6-8. Specific Respiration Rate versus Bulk DO for Respirometer Test, Reactor A, Run 1-2.
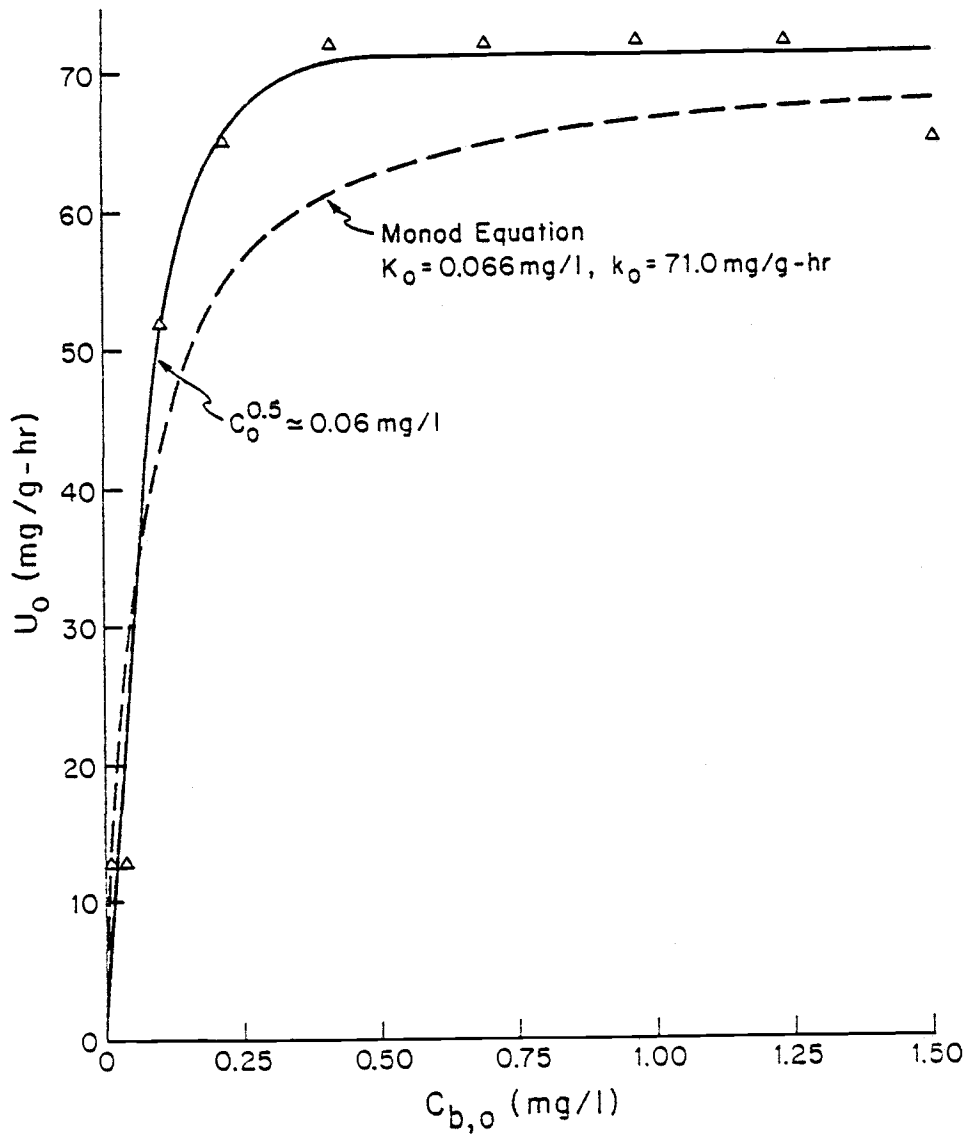
Figure 6-9.   Specific Respiration Rate versus Bulk
              DO for Respirometer Test, Reactor B,
              Run 1-2.

bacterial respiration rates were constant as a function of DO until a very low, critical level was reached, below which the specific respiration rate dropped rapidly (see Chapter II for a review). Furthermore, Chance (1957) found, based on an elaborate model of the bacterial cytochrome system, that the "critical DO" response observed by earlier researchers was theoretically explainable. More recently, Harrison (1972) has shown that bacterial response to DO levels below 0.1 mg/ℓ may be quite complex. However, all studies, including this one, have been limited by the ability to measure DO levels below 0.1 mg/ℓ accurately and reproducibly.

Because of the lack of fit for a Monod function, a curve of best fit was drawn for the respirometer data as shown in Figures 6-8 and 6-9. The bulk DO levels at which the respiration rate was half-maximal, represented as $C_o^{0.5}$, was approximately 0.07 mg/ℓ for Reactor A and 0.06 mg/ℓ for Reactor B.

DISCUSSION OF RESULTS

The comparison of the five selected ATP extraction procedures demonstrated the possible errors that may occur in ATP and other adenine nucleotide analyses when the extraction method is not tested for completeness of extraction and stability of the extract. In particular, the boiling buffer methods that included filtration of the microbial sample and immersion of the filter pad in boiling buffer were shown to result in substantially lower recoveries of ATP when compared to perchloric acid extraction. The possible reasons for this lower extraction efficiency include:

1. Filtration of a microbial culture alters the environmental conditions that the culture is exposed to and, in particular, may result in drying out the microbial cells. These environmental changes may alter the metabolic state of the microorganisms and result in a shift in the relative concentrations of adenine nucleotides.

2. The insertion of the filter pad into a test tube immersed in a boiling water bath tends to lower the temperature of the buffer below 100°C. Holm-Hansen and Karl (1978) have found that immediate exposure to 100°C temperatures is essential for complete extraction of adenine nucleotides and inactivation of ATP-hydrolyzing enzymes.

3. Concentration of the microbial sample on a filter pad may limit immediate access of the boiling buffer to microbial cells entrapped within the filter pad or at the bottom of the biofilm formed by filtration. As described above, lack of immediate exposure to 100°C temperatures may result in reduced ATP concentrations.

Previous studies on the activated sludge process have generally utilized filtration/boiling buffer methods. While the results of this study demonstrate the inferiority of these methods, it must be noted that the reproducibility of the two filtration/boiling buffer methods tested was good, indicating that the error involved in obtaining lower ATP concentrations was systematic and reasonably reproducible. Therefore, ATP data that have been derived using filtration/boiling buffer

methods probably reflect ATP concentrations lower than the actual value but large differences in ATP content between microbial cultures could not be reasonably attributed to deficiencies in the method. For future studies that require a concentration step such as filtration prior to extraction of adenine nucleotides, it is recommended that acid extraction be utilized similar to the procedures utilized by Chappelle, et al. (1978) or that methods be developed to insure the immediate exposure of the concentrated sample to 100°C if boiling buffer extraction is utilized.

The use of boiling glycine buffer, pH 10.0, as recommended by Tobin, et al. (1978), did not result in improved extraction efficiencies when compared to boiling Tris buffer, pH 7.75. In fact, Method IIB (direct injection into boiling glycine buffer) was consistently inferior to Method IA (direct injection into boiling Tris buffer) in terms of the ATP concentration extracted. The determination of why this inferiority existed would take a long-term, careful study comparing all steps within each procedure. For the purposes of this study, the utilization of glycine buffer, pH 10.0, was determined to not offer any experimental advantage to utilization of the more commonly used Tris buffer, pH 7.75. Furthermore, direct injection into boiling Tris buffer (Method IB) was demonstrated to be a straightforward and reproducible method and to be comparable in all respects to perchloric acid extraction. Therefore, this method was utilized within this study and is recommended for other studies.

Operation of the two chemostat reactors with all parameters the same except for the DO illustrated the lack of any substantial differences between the dispersed cultures that developed. For Run 1-1, the

pH and hydraulic retention time in the reactors was essentially iden-
tical and no significant difference could be demonstrated in any of
the culture parameters except ATP concentration at the statistical 99-
percent confidence level. For Run 1-2, no statistically significant
difference between the two reactors could be demonstrated for any of
the culture parameters at the 90-percent confidence level except for
the half-velocity substrate concentration, K_s. Considering the diffi-
culties involved in obtaining a steady-state system at the long deten-
tion times employed and in operating two reactors identically for the
long-time periods involved, it is reasonable to conclude that there was
no consistent difference between the dispersed microbial cultures that
was attributable to the differences in DO.

The evaluation of kinetic coefficients (k and K_s) for the phenol-
degrading cultures was subject to some difficulties. First, the in-
hibited response of the cultures to high phenol concentrations resulted
in difficulties in achieving a steady-state condition at higher mass
loading rates. Additionally, the low half-velocity coefficients that
were demonstrated to be characteristic of these cultures (0.01 - 0.03
mg/ℓ) resulted in extremely low and difficult to measure concentrations
of phenol at lower mass loading rates. Consequently, the "infinite
dilution" batch tests involved a narrow range of mass loading rates and
resultant steady-state substrate concentrations, and the regression
analysis occasionally resulted in high variances for the derived kine-
tic coefficients. In particular, the half-velocity coefficients de-
rived from the regression analysis must be regarded as approximate.
Despite these difficulties, the kinetic batch tests were successful in
demonstrating the lack of substantial differences in the kinetic

coefficients between the low-DO and high-DO cultures, particularly with respect to the maximum substrate utilization rate (k).

It is interesting to compare the kinetics of phenol degradation found within this study to those reported within previous studies. Table 6-7 lists the kinetic coefficients for the aerobic degradation of phenol as reported in several previous research studies. As shown, there is a substantial variation in the coefficients reported, particularly for the half-velocity coefficient, K_s. The half-velocity coefficients determined within this study (0.01 - 0.03 mg/ℓ) were substantially lower than those reported by other researchers, which were generally near 1.0 mg/ℓ except for the value of 245 mg/ℓ reported by Beltrame, et al. (1980).

The inhibitory response of bacterial respiration to high phenol concentrations is also a subject of distinct differences. Most microbiological studies have involved pure-culture bacteria in chemostats and have utilized the Haldane function (competitive inhibition) to describe the inhibitory actions of phenol. However, values for the reported inhibition constant (K_I) vary from about 100 to 500 mg/ℓ (see Table 6-7) and, as described by Yang and Humphrey (1975) and Chi and Howell (1976), no inhibition model has proved to be capable of describing steady-state performance over a wide range of growth rates and particularly the transient response of cultures to increased phenol concentrations. Within this study, inhibitory effects of phenol at high concentrations were observed but the response was varied, indicating that phenol inhibition may be quite species-dependent and may involve a time lag before the inhibitory properties of phenol take full effect. The other mixed-culture studies did not find any inhibitory

Table 6-7.   Kinetics of Aerobic Phenol Degradation.

RESEARCHER	TYPE OF SYSTEM	TYPE OF CULTURE	TEMPERATURE	θ_c or θ (hours)	k $(\frac{g\ phenol}{g\ TSS-day})$	K_s (mg/ℓ)	INHIBITION
Jones, et al.(1973)	Chemostat (2-stage)	Bacterium NCIB 8250	20°C	8.3	11.8	<1	K_1 = 110 mg/ℓ[3]
Hill & Robinson(1975)	Chemostat	Pseudomonas putida	30°C	3-12	24.6	<1	K_1 = 470 mg/ℓ[3]
Yang & Humphrey(1975)	Chemostat	Pseudomonas putida Trichosporan cutaneum (yeast)	30°C	3-20	19.8[1,2] 19.2	1.2 1.1	K_I = 224 mg/ℓ[3] K_I^I = 4%
Chi & Howell(1976)	Chemostat (transient experiments)	Pseudomonas sp.	28°C	3-10	17.7[4]	5.9[4]	K_1 = 227 mg/ℓ[3,4]
Beltrame, et al.(1980)	Solids-Recycle Reactor	Mixed	20°C	9-78	4.1	245	No inhibition
Knudsen, et al.(1980)	Solids-Recycle Reactor	Mixed	20°C	72	1.7-2.9	$\sim 2^5$	Inhibition not noted
This study	Chemostat	Mixed	20°C	112 39	3.9,4.2 4.6,4.5	0.010,0.017 0.011,0.028	Inhibition noted but not modeled

[1] Y = 0.5 g TSS/g phenol assumed.

[2] k = μ_{max}/Y.

[3] Haldane model, U = $kC_s/(K_s + C_s + C_s^2/K_1)$.

[4] Average of six runs

[5] Diffusional limitations may have contributed to the K_s value reported.

effect and, in particular, the study of Beltrame, et al. (1980) illus-
trated the lack of inhibition for phenol concentrations up to 360 mg/ℓ.
These data illustrate the fact that bacterial kinetics are a function
of the culture and the substrate. Particularly for mixed cultures, it
is not possible to describe any characteristic kinetics for a particular
substrate because the culture that develops is dependent on the feed,
the startup conditions, and the operational mode and characteristics of
the biological reactor.

As stated in the introduction to this chapter, an additional pur-
pose of the Phase 1 experiments was to determine whether substantial
quantities of ATP might be associated with the nonviable biomass frac-
tion in biological reactors operated at long cell retention times. The
energy charge measurements of Run 1-1 and 1-2 were of particular inter-
est in this regard. As listed in Table 6-3, the energy charge of the
steady-state cultures in Run 1-1 was near 0.8 (average of 0.79) in
Reactor A and B despite the long hydraulic retention time of nearly
five days. In Run 1-2, with a hydraulic retention time of about 39
hours, the energy charge was slightly higher (average of 0.825) as
shown in Table 6-5. Assuming an energy charge of 0.85 for viable cells,
a maximum energy charge of 0.5 for nonviable cells (Chapman, et al.,
1971), and an ATP-ADP-AMP equilibrium as shown in Figure 2-6, it was
calculated that less than ten percent of the total adenylate pool was
associated with nonviable cells in both Run 1-1 and Run 1-2. Moreover,
the ATP content of nonviable cells was calculated to be less than five
percent of the total ATP pool. This results primarily from the fact
that ATP becomes a smaller fraction of the total adenylate pool as the
energy charge falls. While the above assumptions provide only an

approximate analysis, it is apparent that the high energy charge levels measured is indicative of the presence of only a small fraction of the total ATP pool within nonviable cells. Therefore, the assumption of Williamson and Nelson (1978) that ATP is associated only with viable cells appears to be reasonable, even in biological reactors operated at long cell retention times.

Two additional points regarding the ATP measurements should be made. First, the specific ATP viability of the cultures was relatively low when compared to other related studies, particularly in Run 1-2 where, at a hydraulic retention time of about 39 hours, the specific ATP viabilities were in the range of 0.5 to 0.6 mg ATP/g TSS. The data of Patterson, et al. (1970), Williamson and Nelson (1978), and Nelson and Lawrence (1980) indicate a specific ATP viability of about 2.0 mg ATP/g TSS or more for an equivalent cell retention time. Possible explanations for this that were considered included poor extraction efficiency within the ATP extraction procedure, hydrolysis of ATP during storage, and errors in preparation of ATP standards. However, the ATP extraction efficiency of the method utilized was shown to be comparatively good as previously discussed, the high energy charge levels obtained eliminated the possibility of significant ATP hydrolysis during storage, and the ATP standards used were found to be comparable to freshly prepared standards. Therefore, the low specific ATP viability obtained was attributed to a lower specific ATP content for the cultures grown within this study as compared to the cultures of other studies. The literature summary of Nelson and Lawrence (1980) seems to confirm that the specific ATP content may vary substantially with bacterial species.

Second, the specific ATP content of the culture in Run 1-2 (θ = 39 hours) was very similar to that in Run 1-1 (θ = 112 hours). Results of other researchers, including Nelson and Lawrence (1980), Williamson and Nelson (1978) and Weddle and Jenkins (1971) indicate a decreasing specific ATP content with increasing cell retention time. The maximum substrate utilization rate (k) was substantially higher in Run 1-2 (average k = 4.56 g phenol/g TSS-day) as compared to Run 1-1 (average k = 3.98 g/g-day), indicating an increased viable fraction within the total cell mass, as expected. However, this increase was less than 15 percent and the expected corresponding increase in specific ATP viability may have been obscured by the limited precision of the analytical technique for ATP.

The final area of investigation for phase 1 of this study was the critical DO associated with phenol degradation for a dispersed bacterial culture. The respirometric studies were successful in showing a half-velocity DO ($C_o^{0.5}$) of less than 0.1 mg/ℓ and the lack of any effect for DO levels above 0.3 to 0.5 mg/ℓ (the critical DO). The difficulties involved in measurements of very low DO levels prevented the definition of more exact values for these parameters. However, the measured values of $C_o^{0.5}$ were far below the half-velocity coefficient reported for the enzyme phenol hydroxylase (0.64 mg/ℓ) and the $C_o^{0.5}$ of about 0.5 mg/ℓ derived from the data of Klein, et al. (1979) for a pure culture of the yeast Candida tropicalis. Furthermore, the $C_o^{0.5}$ was in the range reported by Longmuir (1954) for bacterial respiration by a variety of bacterial species with glucose as the substrate. Consequently, although phenol involves an oxygenation reaction in its degradative pathway, these results indicate that the response of bacteria degrading phenol

to low DO levels is not substantially different from that associated
with bacterial degradation of other substrates that do not require
oxygenations. The most probable reason for this is that the initial
oxygenation reaction is not rate-limiting in the overall sequence of
reactions involved in the degradation of phenol. A possible explanation
for the discrepancy between the findings of this study and those of
Klein, et al. (1978) is that the reaction pathways utilized by the
microorganisms within these respective studies may have been different.
Yang and Humphrey (1975) report that, after initial hydroxylation of
the benzene ring to form catechol, the yeast Trichosporan cutaneum
utilizes ortho oxidation while the bacterium Pseudomonas putida util-
izes meta oxidation and the degradative pathways are dissimilar after
that point (Yang and Humphrey, 1975). Consequently, the finding of a
higher half-velocity DO for a pure yeast culture by Klein, et al.
(1978) as compared to the mixed bacterial culture utilized within this
study may well have been the result of a general difference in the reac-
tion sequence involved in phenol degradation by bacteria versus yeasts.

Although this study showed that the response of a mixed bacterial
culture degrading phenol to low DO levels was not substantially differ-
ent from that reported by others for bacteria degrading substrates that
do not involve oxygenations, it is possible that utilization of other
oxygenase-requiring substrates may involve higher critical DO levels
or half-velocity DO levels. However, it is the author's judgment that
this possibility is not of great importance to biological treatment of
wastewaters for two reasons. First, although there is limited data,
the values of $K_m (O_2)$ reported for oxygenases isolated from bacteria
are generally quite low (less than 0.1 mg/ℓ) indicating that the

affinity of these enzymes for oxygen is generally high (Hayaishi, et al., 1975). Furthermore, the occurrence of aromatic and other compounds that require oxygenations in wastewaters is limited except for certain industrial wastewaters. A further investigation of this topic would be useful with particular application to those industrial wastewaters that contain substantial quantities of aromatic compounds and aliphatic hydrocarbons.

CONCLUSIONS

The following conclusions were derived from the results of the Phase 1 experiments:

1. Extraction of ATP and other adenine nucleotides by direct injection into boiling Tris buffer, pH 7.75, is a comparable method to the more accepted perchloric acid extraction technique in terms of extraction efficiency and inactivation of ATP-hydrolyzing enzymes. Additionally, this method is comparatively simple and more suitable for use by relatively inexperienced analysts. However, sample volumes must be small (less than two percent of the buffer volume) to achieve good results.

2. There is no consistent difference between mixed cultures of dispersed bacteria in terms of kinetics, net yield, ATP viability or energy charge when these cultures are grown at high DO levels typical of oxygen activated sludge systems (15 mg/ℓ) as compared to lower DO levels typical of air activated sludge operation (2 mg/ℓ).

Therefore, differences in activated sludge character-
istics that apparently result from the maintenance
of high DO levels can only be attributed to diffu-
sional limitations in large bacterial aggregates as
described within the anoxic core theory.

3.   The half-velocity DO ($C_o^{0.5}$) for phenol degradation by
a mixed bacterial culture was less than 0.1 mg/ℓ.
Consequently, although phenol involves an oxygenation
reaction in its degradative pathway, it does not
appear that the response of bacteria degrading phenol
to low DO levels is substantially different from that
associated with bacterial degradation of other sub-
strates that do not require oxygenations.

4.   The association of ATP with nonviable cells is not sub-
stantial even in biological reactors operated at long
cell retention times. Therefore, the assumption that
ATP is associated only with viable cells is reasonable
for purposes of model formulation. However, there may
be substantial variations in specific ATP content between
various bacterial species.

CHAPTER VII

PHASE 2 EXPERIMENTS

INTRODUCTION

The purpose of the Phase 2 experiments was to verify the anoxic core model for a laboratory activated sludge culture containing large, rapidly respiring flocs and to evaluate the potential for the occurrence of oxygen diffusional limitations within the activated sludge process. Originally, the research plan for this phase was to operate two identical activated sludge reactors at different DO levels (2 mg/ℓ and 8 mg/ℓ) to a steady-state condition where:

1. oxygen was the diffusion-limiting nutrient, and

2. the bacterial flocs were large enough and the respiration rate rapid enough to create substantial oxygen diffusional limitations within the reactor with the lower DO.

Steady-state measurements of the specific ATP viability and kinetic response to a range of DO levels were then to be correlated with the predictions of the mathematical model regarding the proportion of floc volume that was anoxic.

It was recognized, however, that several problems were likely to occur in fulfilling this original research plan.

1. The substrate concentration in a chemostat or activated sludge reactor maintained at long cell retention times typically becomes quite small. Therefore,

substrate is typically the diffusion-limiting species in completely-mixed activated sludge systems, particularly when a soluble feed of constant composition is utilized.

2. The development of a poorly settling or extremely filamentous sludge is common in laboratory activated sludge systems. The reasons for this are unclear although various researchers have proposed numerous theories, including the nature of the substrate, the type of mixing or mixing intensity, nutrient limitations, and others.

Anticipation of these difficulties led to a flexible research plan, wherein different operational modes could be tried in order to develop a stable activated sludge culture that was subject to oxygen diffusional limitations. These operational modes included conventional, pulse feed, and fill-and-draw operation. A description of the procedures associated with these operational modes is contained in the sections that follow.

RUN 2-1

Experimental Procedures

The first run in this phase utilized the two main laboratory reactors as conventional activated sludge units. Feed containing 500 mg/ℓ of phenol and other nutrients as described in Chapter V was continually pumped to each reactor. The DO in Reactor A was controlled at 2.0 mg/ℓ and that in Reactor B at 8.0 mg/ℓ. As in Phase 1, startup of the

reactors was accomplished by beginning at a very low flow rate with progressive increases until the final hydraulic retention time was reached, which was planned to be about eight hours.

The solids retention time (SRT) in the reactors was to be set at two days after the mixed culture was established. It was reasoned that this relatively short SRT would provide the best possibility for a relatively high phenol concentration in the effluent as necessary to insure that oxygen was the diffusion-limiting species.

The small clarifier tube was used to provide a high degree of settling competition and thereby encourage the development of large flocs. At a hydraulic retention time of eight hours, the detention time in the clarifier was approximately half an hour.

Daily reactor measurements included the hydraulic retention time (θ), pH, phenol concentration in the effluent (analytical sensitivity of 0.1 mg/ℓ), effluent suspended solids, reactor suspended solids, and sludge volume index. The automatically-timed sludge wasting pump was used to remove the majority of the desired wastage volume from the reactor and the remainder was wasted manually once daily.

Results

The daily data taken for Run 2-1 are contained in Appendix B, Table B-1. After setting the influent pump at its final setting, the suspended solids in the reactors were allowed to build up to about 1000 mg/ℓ before sludge wastage was initiated to maintain an SRT of 2.0 days. The effluent substrate concentration in both reactors had dropped below the detection limit of 0.1 mg/ℓ by that time and continued below 0.1 mg/ℓ during the entire run. After sludge wastage was initiated,

the reactors began approaching a steady-state condition. However, before the steady-state condition was reached, the reactor cultures were both overtaken by filamentous growth. The SVI in both reactors rose above 500 ml/g and the effluent suspended solids concentrations approached the concentration in the reactor, indicating that very little clarification was occurring in the clarifier. A preliminary run had achieved very similar results and it was found that a dense, flocculant sludge did not return to the reactors even after one month of continuous operation. Therefore, it was decided that the conventional operational mode would not achieve the dense, flocculant culture desired. This mode was abandoned and a modified operating mode initiated for Run 2-2.

Discussion

The occurrence of filamentous bacteria in dominant proportions has been a major problem in activated sludge plants treating both municipal and industrial wastewaters. Furthermore, this problem is particularly common in laboratory-scale activated sludge systems. A number of hypotheses have been advanced in attempts to explain the domination of filamentous bacteria over flocculant bacteria in these cases. A well-accepted general theory that has been proposed, however, is that somehow the filamentous growth form enables filamentous bacteria to compete successfully for low concentrations of soluble organic substrates, dissolved oxygen, or other nutrients that may be limiting (Pipes, 1978). More specifically, Sezgin, et al. (1978) have proposed that filamentous bacteria extend out into the medium from flocs, thereby exposing themselves to higher nutrient concentrations, whereas Pipes (1978) suggested

that the higher surface area/volume ratio may enable filamentous bacteria to utilize scarce nutrients more effectively.

It is the author's belief that the development of a highly filamentous culture within this run was related to the very low bulk substrate concentrations that were present in both reactors (less than 0.1 mg/ℓ phenol) in accordance with the theory of Sezgin, et al. (1978). With bulk substrate concentrations at this level, diffusional resistance within large, dense flocs results in even lower ambient substrate concentrations within the core of the flocs. Consequently, filamentous bacteria that extend from these flocs into the bulk medium and are exposed to the bulk substrate concentration can compete successfully with flocculant bacteria. Within this run, the bulk substrate concentration was so low that the culture became dominated by filamentous bacteria. The higher surface area/volume ratio of filamentous bacteria may explain why these bacteria were able to continue their domination of the culture even after most flocculant bacteria had been lost from the system.

Subsequent runs were therefore planned to utilize operational modes that would not result in continuously low concentrations of substrate.

RUN 2-2

Experimental Procedures

The operational mode of the activated sludge units was modified for the second run to provide a pulsed feed. The influent pump was plugged into a timer and the pump activated for approximately 1 minute out of a 12-minute cycle. The average influent flow rate was set to

maintain a hydraulic detention time of approximately 8 hours, as in Run 2-1. The small clarifier tube used in Run 2-1 was replaced with the larger clarifier tube, which resulted in a hydraulic detention time of approximately 1 1/4 hours in the clarifier. This was done to prevent hydraulic short-circuiting in the clarifier as a result of the pulse-feed operation. Other operational procedures were similar to Run 2-1.

The purpose of the pulsed-feed operational mode was to prevent the substrate concentration in the reactor from reaching a continuously low concentration where diffusional limitations would lead to the development of a filamentous culture, as occurred in Run 2-1. A disadvantage of the pulsed-feed mode, however, is that it does not result in a truly steady-state system. Consequently, it was recognized that verification of the anoxic core model would largely have to depend on batch kinetic tests within this run. In particular, steady-state correlations of specific ATP viability with DO were not anticipated to be practical experimentally.

Results

Description of Reactor Behavior

The daily data taken for Run 2-2 are listed in Table B-2 of Appendix B. Reactor A failed immediately after the final influent flow rate setting was reached. The mode of failure was similar to those that occurred during Phase 1 and the failure was attributed to the inhibitory or toxic effects of phenol. Reactor B, however, began with a high MLSS concentration and it was possible to set the SRT at two days immediately.

A typical plot of substrate concentration versus time over the 12-minute influent pumping cycle is shown in Figure 7-1. As shown, the reactor phenol concentration climbed to almost 7.0 mg/ℓ following the influent pump's on cycle, followed by a linear decline reflective of the maximum substrate utilization rate and a slowing decline as the substrate concentration approached zero. The half-velocity concentration for this curve was a little less than 1.0 mg/ℓ. The final substrate concentration (t = 12 minutes) was below analytical sensitivity (0.01 mg/ℓ).

Filamentous bacteria were again a problem in this run. Reactor B became quite filamentous on the fourth day of this run with an SVI of 580 ml/g and an effluent suspended solids concentration approaching that in the reactor. Reactor A was restarted by Day 5 but it became quite filamentous within three days in a manner similar to Reactor B. However, the filamentous bulking problem in this run was different from Run 2-1, wherein filamentous bacteria eventually dominated the reactors totally. In this run, the filamentous bacteria occurred primarily as projections from large flocs. The reactors bulked when these filaments so completely covered all the floc surfaces and when their projected length became so extreme that the floc particles settled only very slowly and compacted poorly. However, the dense core of these filamentous flocs remained throughout this run. Furthermore, the reactors recovered after each episode where in the filamentous bacteria became so prominent that bulking occurred so that the operational behavior of the reactors was cyclic.

Although the cultures in both reactors were highly filamentous on occasion and the operational behavior was somewhat unstable, the
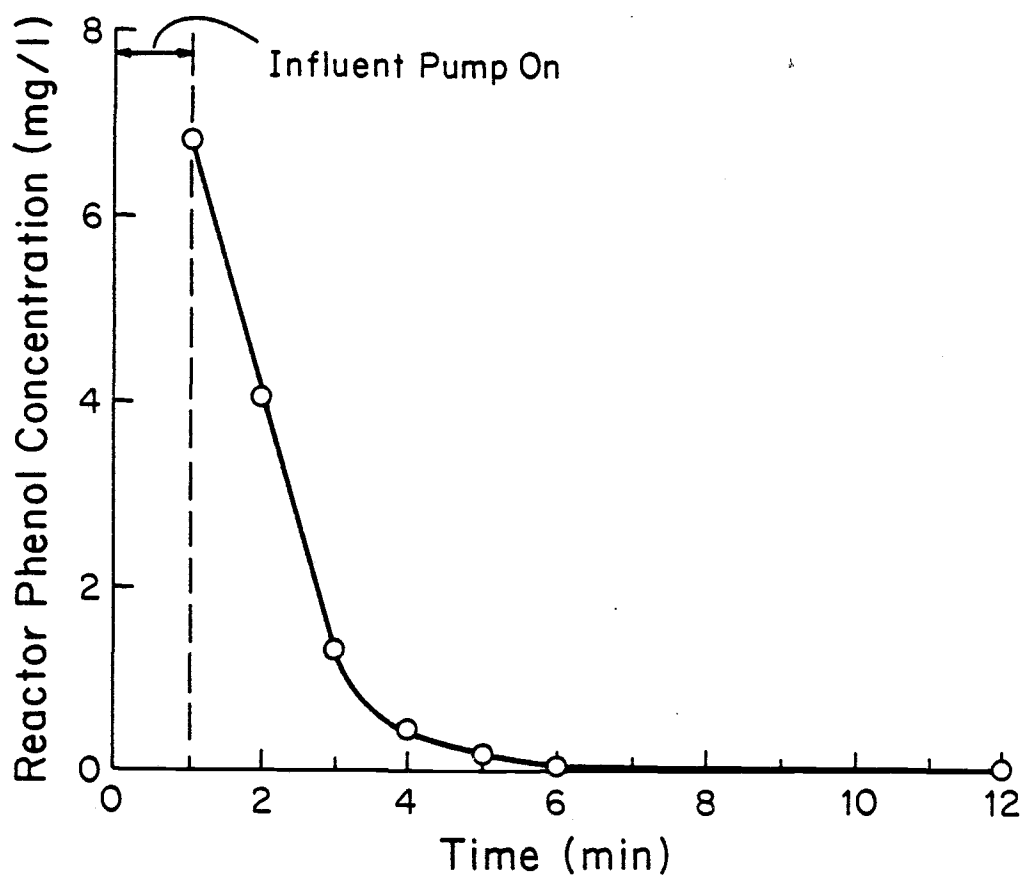
Figure 7-1.   Reactor Phenol Concentration versus Time for the
              Pulsed-Feed Operational Mode, Run 2-2, Day 3.

bacterial flocs were large as necessary for the creation of a substantial anoxic core. Therefore, batch respirometric studies were performed and floc size measurements were taken in order to confirm the applicability of the anoxic core theory and to evaluate the kinetic response of the flocculant culture to various DO levels.

Respirometer Studies and Floc Size Measurements

Floc size distributions were determined on several days within this run and the data from these are contained in Appendix B, Tables B-3 through B-7. The floc size measured included only the dense core of the floc and excluded any filamentous projections. Respirometric studies were also conducted, generally on the same day as the floc size measurements, and the data from these are contained in Tables B-8 through B-13 of Appendix B.

The first floc size distribution for Reactor B was taken on Day 4 and is illustrated in Figures 7-2 and 7-3. Figure 7-2 illustrates the relative number of floc within each size range. As shown, the largest number of floc occur in the smallest size range (0-10 microns) and there are greatly reduced numbers of floc in the large size ranges. In Figure 7-3, the floc size distribution has been adjusted to present the percent of total floc volume (assumed proportional to floc mass) that is incorporated within each size range. This is the appropriate distribution for application of the mathematical model and is the type of distribution presented for all subsequent floc size data. As shown in this figure, the total floc volume associated with the smallest size range (0-10 microns) was insignificant when compared to the larger size ranges and a major fraction of the total volume was contained in flocs
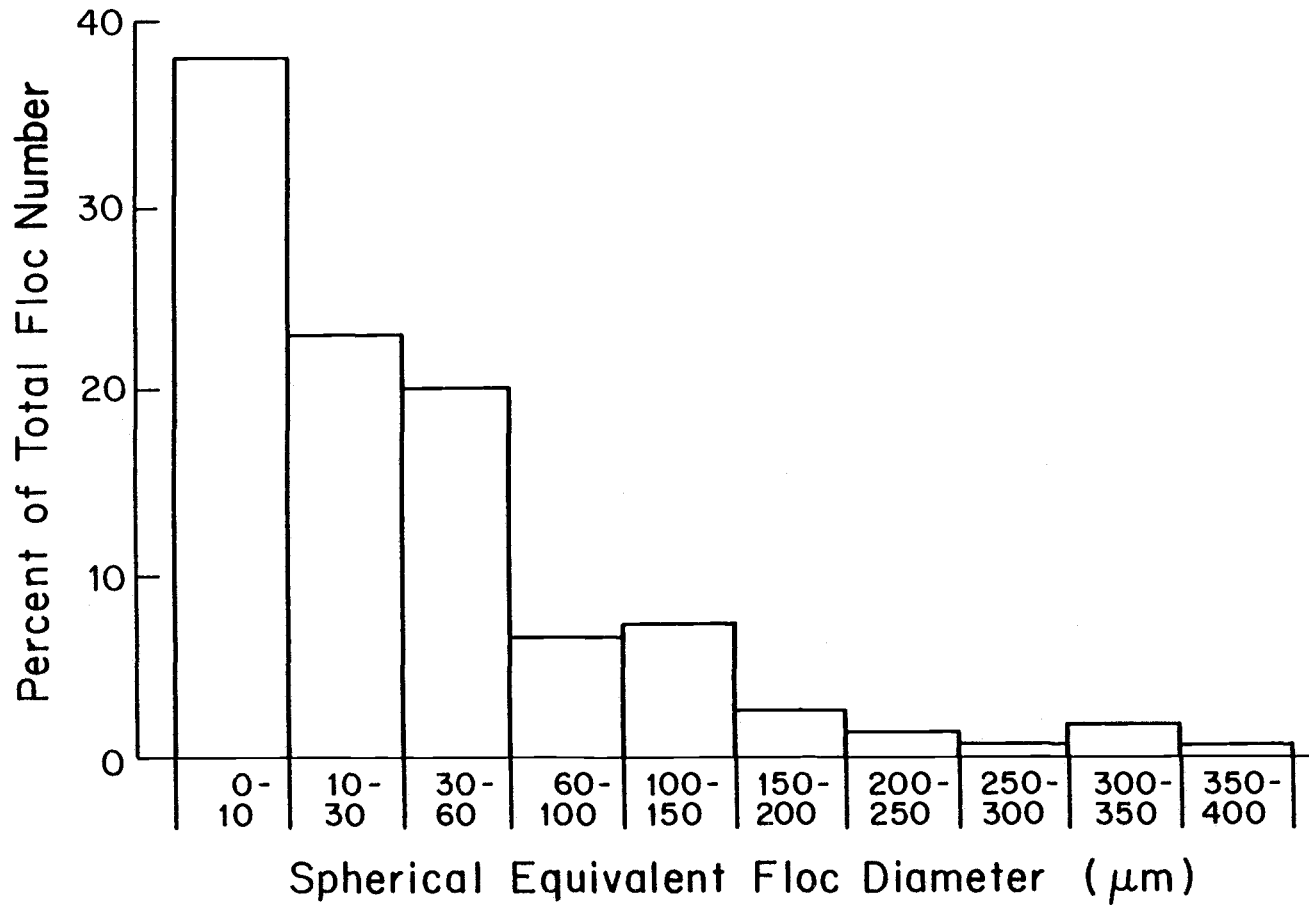
Figure 7-2. Floc Size Distribution by Number for Reactor B on Day 4 of Run 2-2.
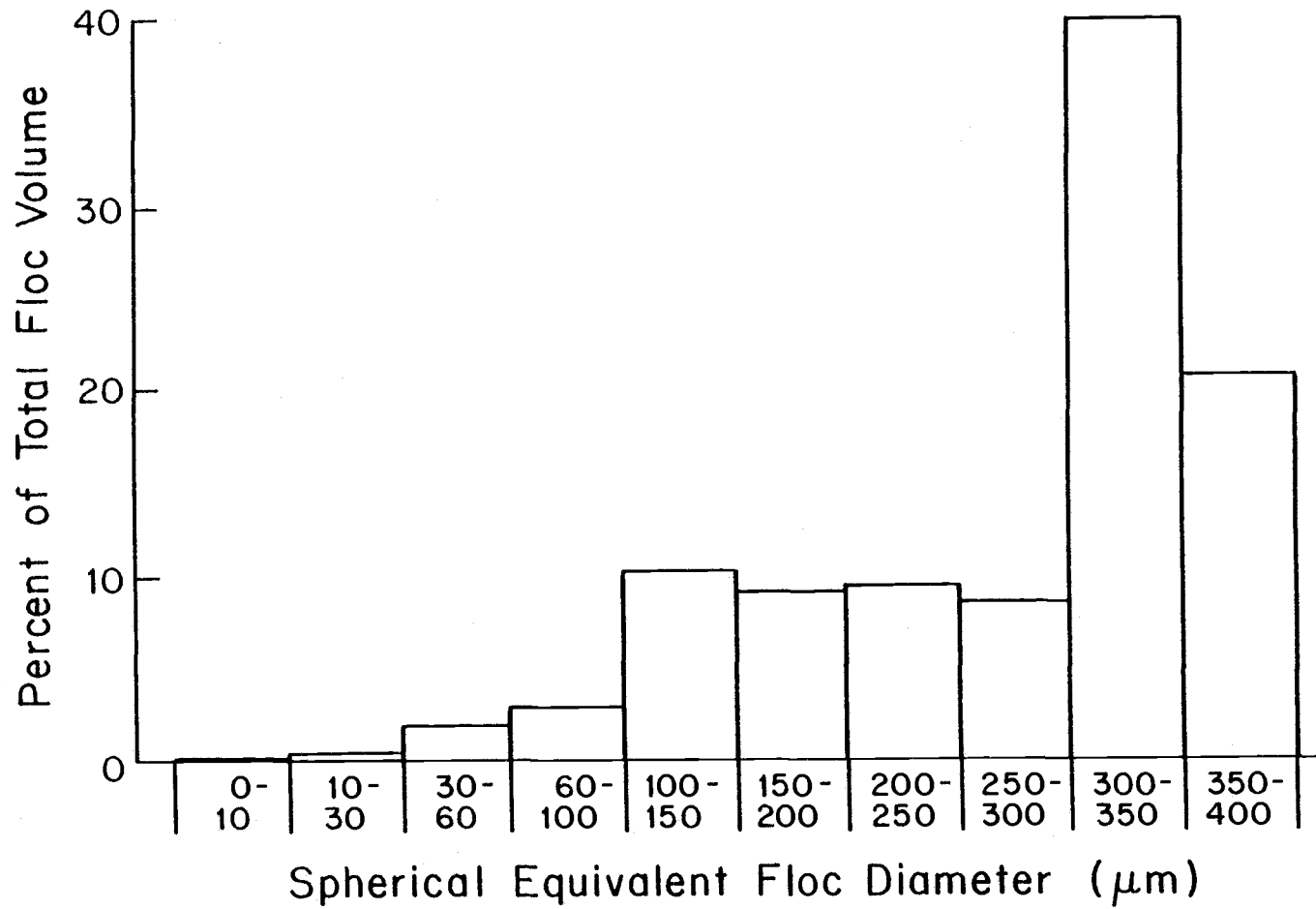
Figure 7-3.   Floc Size Distribution by Volume for Reactor B on Day 4 of Run 2-2.

larger than 300 microns. The maximum floc diameter on Day 4 was 400 microns.

Floc size distributions on Day 8 for Reactors A and B are shown in Figures 7-4 and 7-5, respectively. The median floc diameter in Reactor A was in the range of 350-400, while the median in Reactor B was the range of 300-350 microns. The maximum floc size in both reactors was 500 microns.

Floc size distributions on Day 12 for Reactors A and B are shown in Figures 7-6 and 7-7, respectively. These distributions illustrate a smaller floc size on this day as compared to Day 8. The median floc diameter in Reactor A was in the range of 100-150 microns and that in Reactor B was in the range of 150-200 microns. Reactor A and B exhibited maximum floc diameters of 300 and 250 microns, respectively.

The respirometric sudies within this run were performed by mixing a 1:9 proportion of 500 mg/ℓ feed solution to culture, which resulted in an initial phenol concentration of 50 mg/ℓ, and then aerating this mixture and transferring it into the respirometer bottle. This was done to ensure that oxygen would be the diffusion-limiting nutrient rather than phenol, that relatively constant environmental conditions would be present in the respirometer bottle over the short period required to completely deplete the dissolved oxygen, and that the bacteria would be respiring rapidly. The half-velocity substrate (phenol) concentration for these cultures was previously shown to be in the range of 1.0 mg/ℓ. Therefore, a phenol concentration of 50 mg/ℓ insured saturation kinetics even though a small depletion occurred during the respirometric test. The possible inhibitory effects of phenol was not
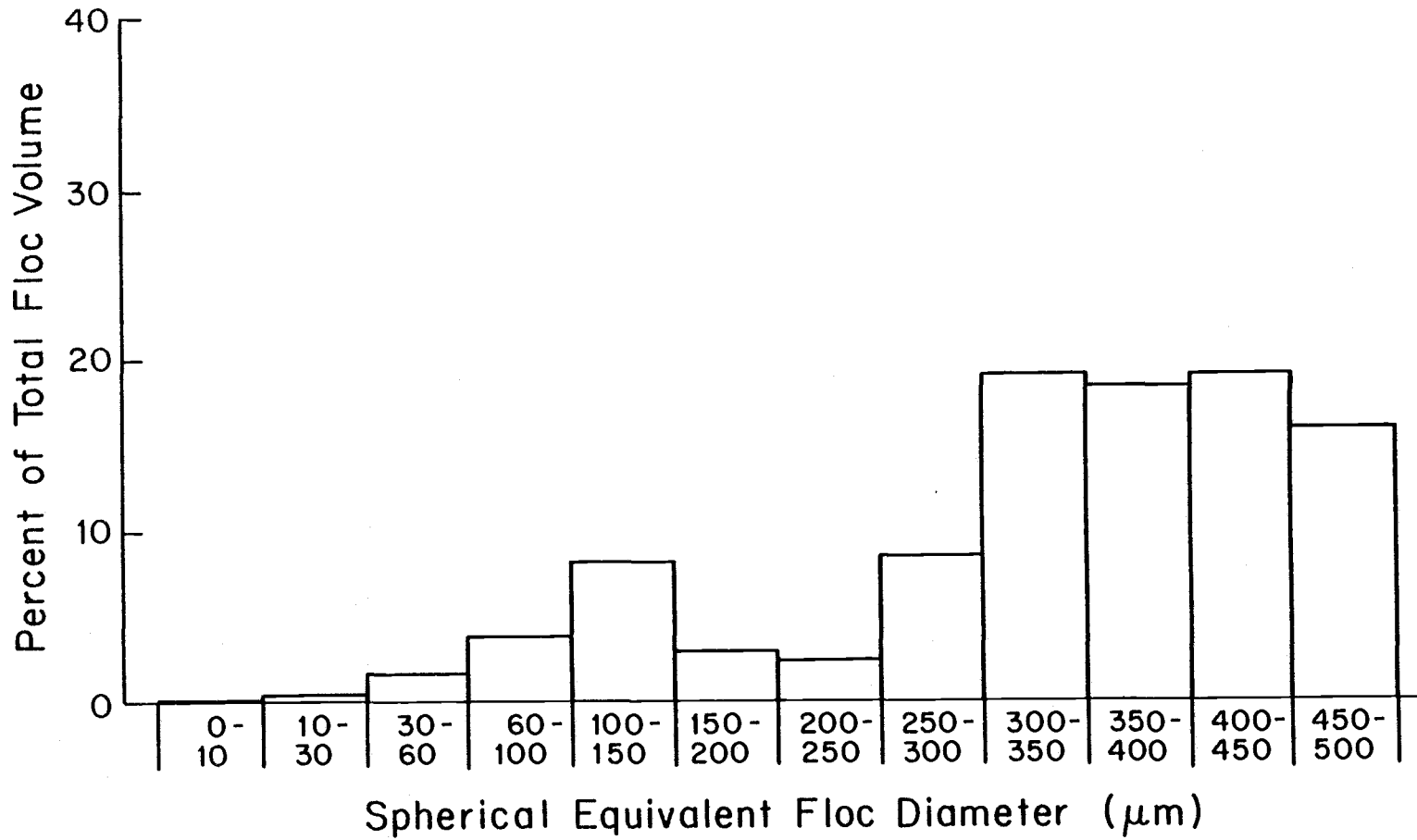
Figure 7-4. Floc Size Distribution for Reactor A on Day 8 of Run 2-2.
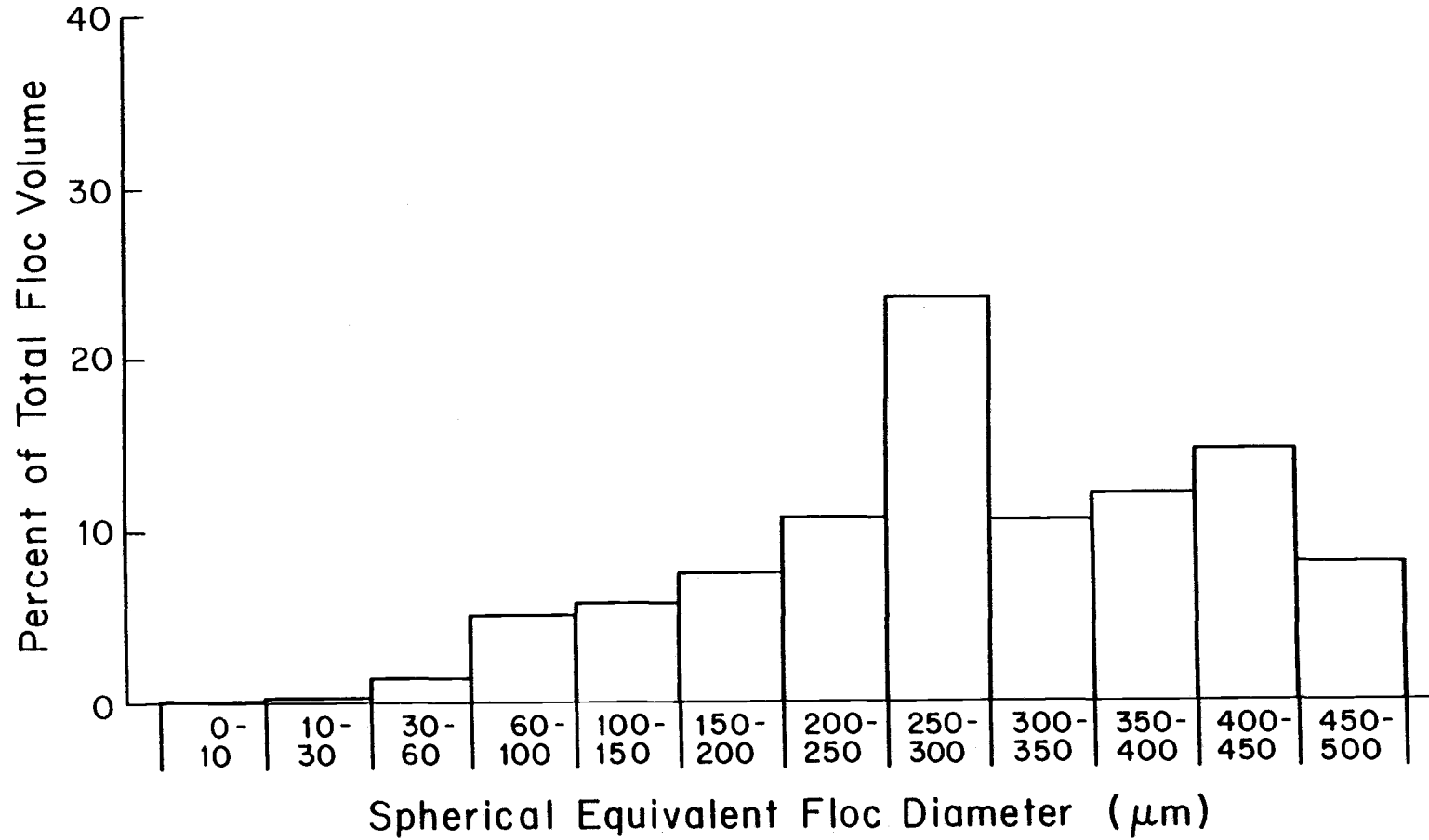
Figure 7-5. Floc Size Distribution for Reactor B on Day 8 of Run 2-2.
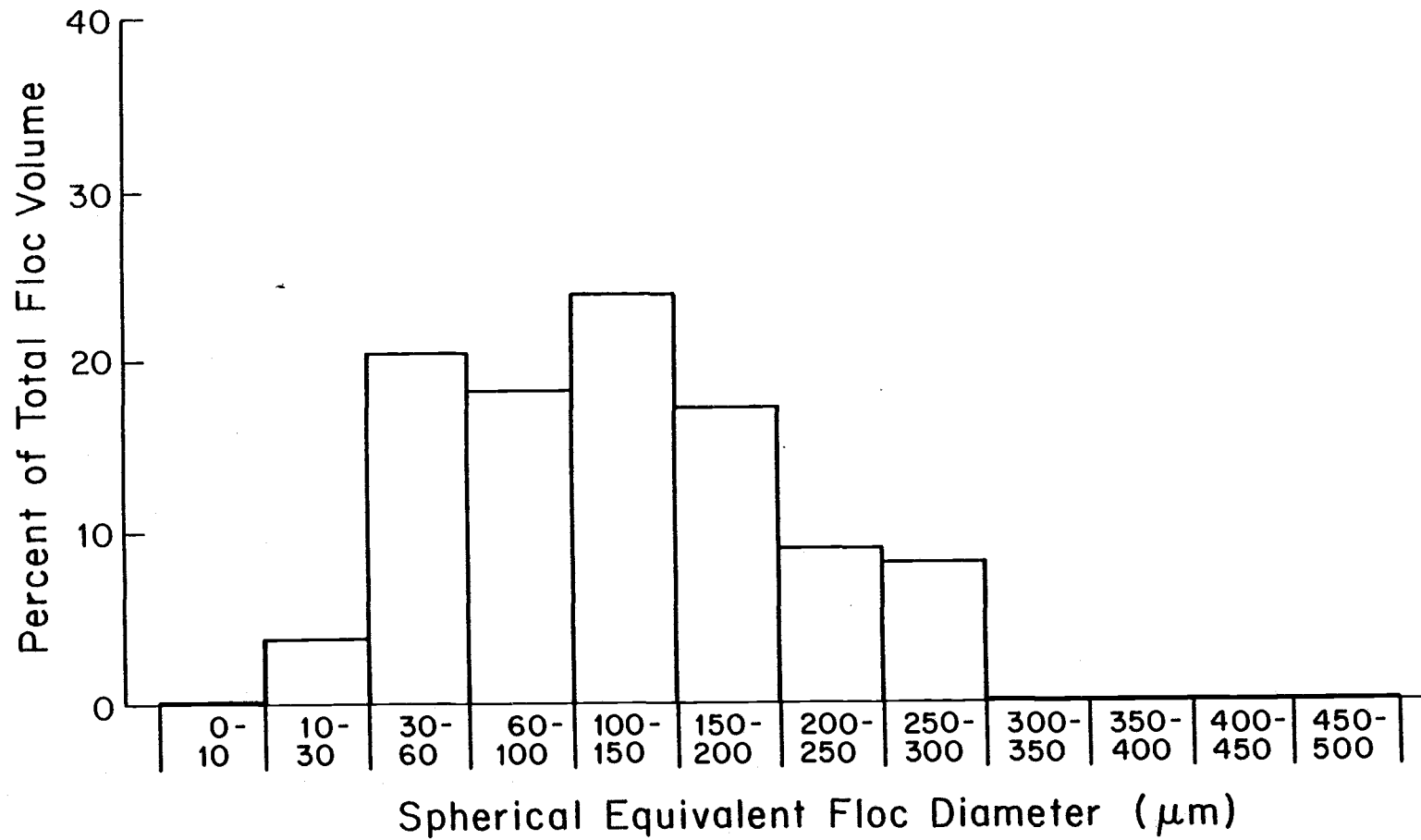
Figure 7-6. Floc Size Distribution for Reactor A on Day 12 of Run 2-2.
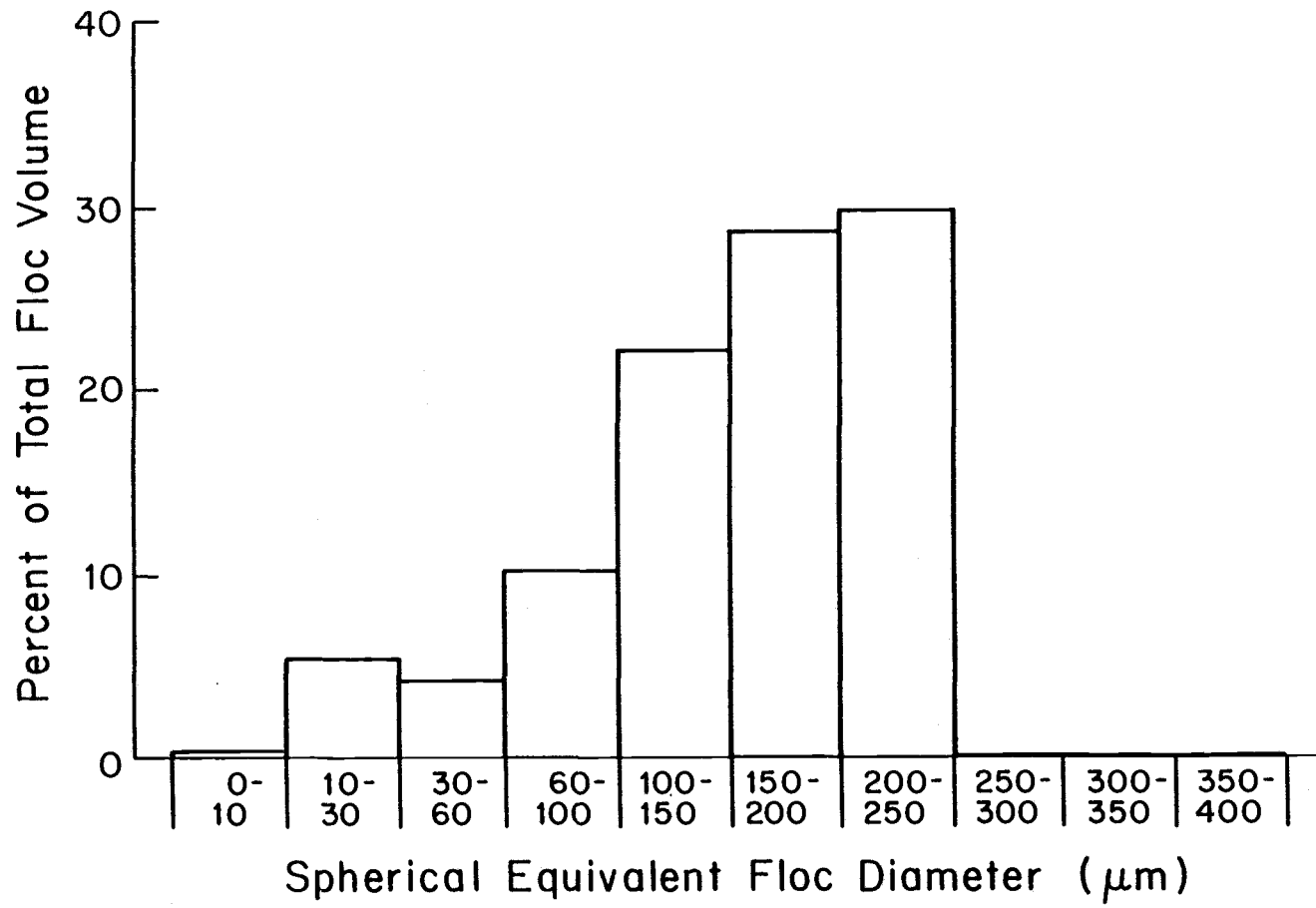
Figure 7-7. Floc Size Distribution for Reactor B on Day 12 of Run 2-2.

considered to be a problem due to the short time period involved in the respirometric tests.

The bulk DO versus time data obtained from the respirometric tests was converted into specific respiration rate versus bulk DO data by the methods described previously (see Chapter V and Appendix B, Tables B-8 through B-14). These data were analyzed, the anoxic core mathematical model was applied to the data, and the fitting parameter D_o/ρ was optimized (see Appendix D for details of model application). The resultant data points and the curve produced by application of the model were then plotted.

Results of this procedure are shown in Figures 7-8 through 7-12 for culture samples taken from Reactors A and B on Day 4, 8 and 12 of this run. A summary of these results is given in Table 7-1. As shown, the model fit to the data was very good ($r > 0.99$ for all data sets). A common discrepancy between the model and the data was evident, however, in that the model generally predicted higher than actual values of U_o when $C_{b,o}$ was low (less than 0.5 mg/ℓ) and predicted lower than actual values of U_o when $C_{b,o}$ was high (greater than about 0.5 mg/ℓ). Also, it was noted that the optimum value of D_o/ρ for fitting the model to the data ranged from 5.21×10^{-8} to 5.05×10^{-7} cm^5/s-mg and exhibited a distinct correlation with the average floc diameter (see Table 7-1). The fundamental reason for the above discrepancies is discussed later in this chapter. Also listed in Table 7-1 are model predictions of the bulk DO at which the respiration rate was 50 percent and 90 percent of the maximum ($C_o^{0.5}$ and $C_o^{0.9}$). Because the model fit was generally very good for bulk DO levels near the $C_o^{0.5}$, the predicted value of $C_o^{0.5}$ was in very good agreement with the data. The predicted value of
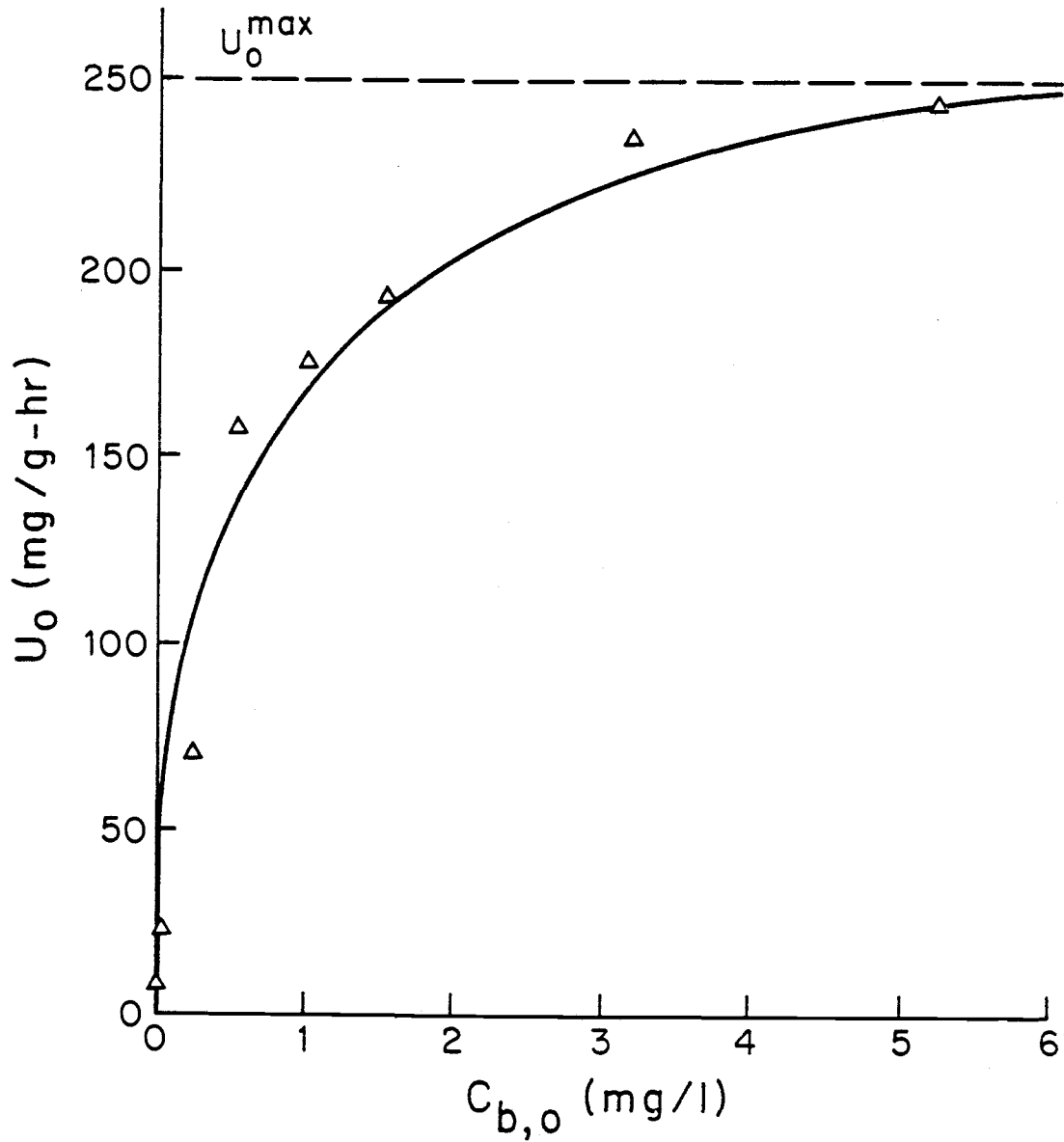
Figure 7-8.   Model Fit to Specific Respiration Rate versus
              Bulk DO Data for Reactor B on Day 4 of Run 2-2.
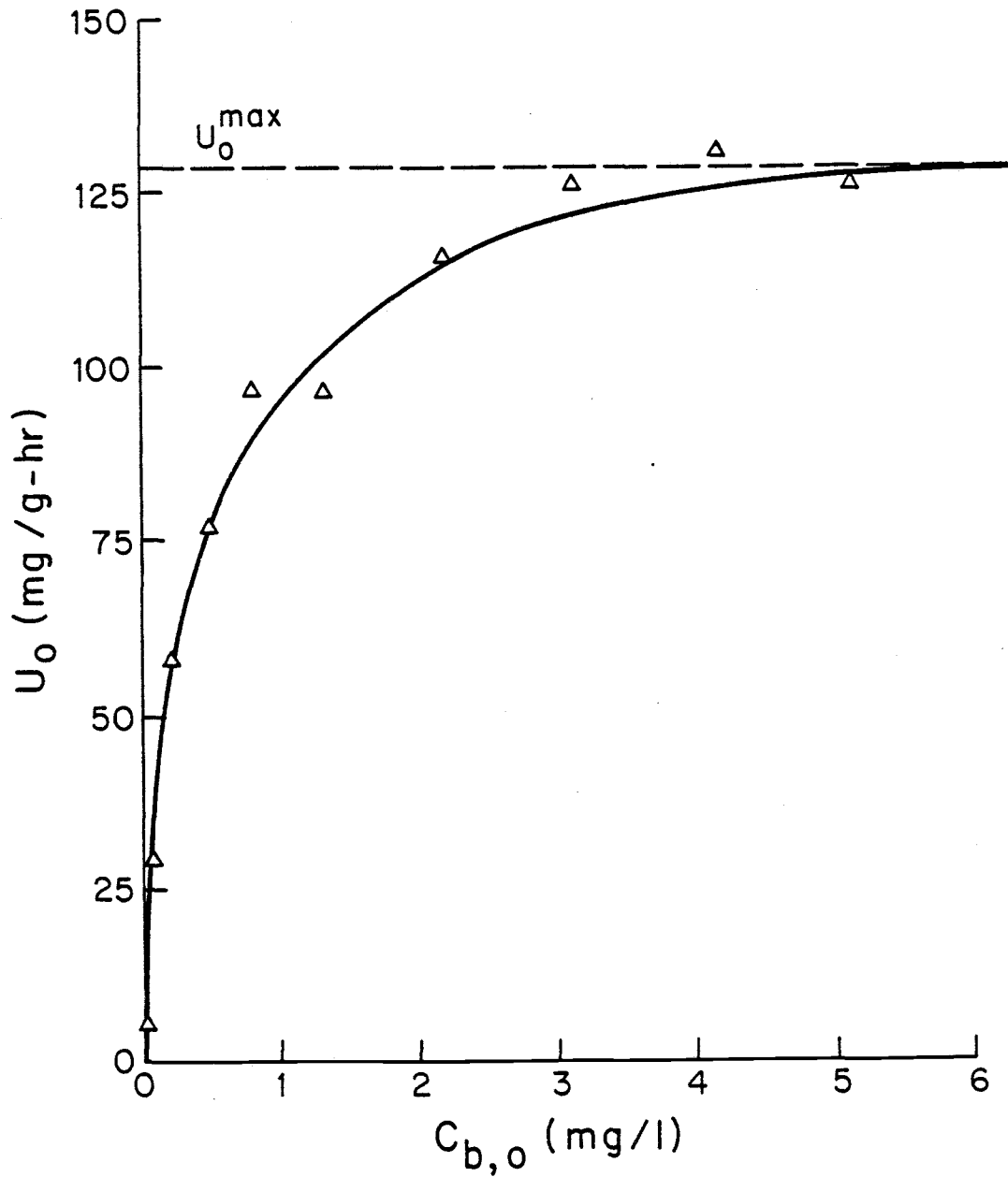
Figure 7-9. Model Fit to Specific Respiration Rate versus Bulk
DO Data for Reactor A on Day 8 of Run 2-2.
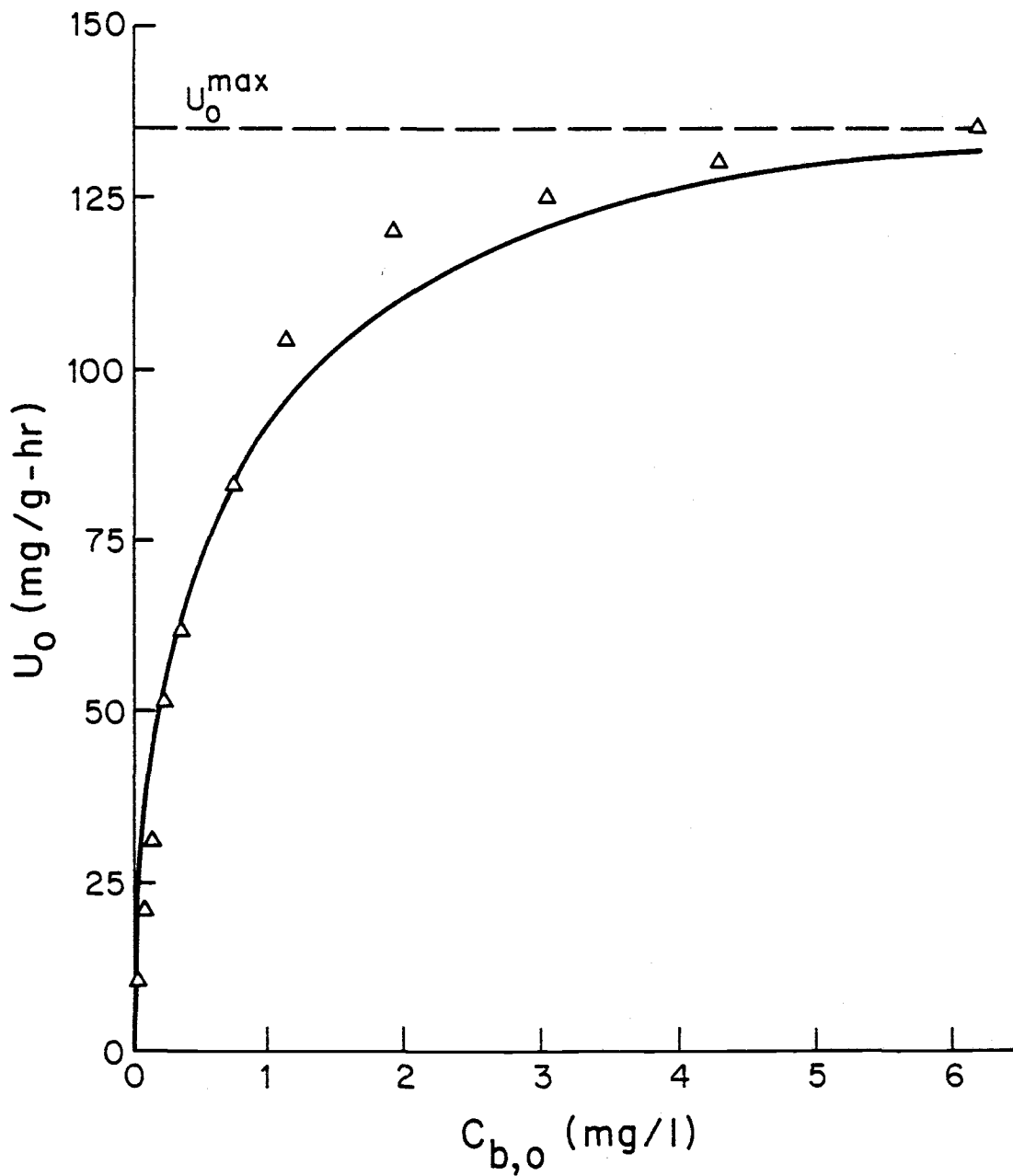
Figure 7-10. Model Fit to Specific Respiration Rate versus Bulk DO Data for Reactor B on Day 8 of Run 2-2.

Figure 7-11. Model Fit to Specific Respiration Rate versus Bulk
DO Data for Reactor A on Day 12 of Run 2-2.

Figure 7-12. Model Fit to Specific Respiration Rate versus Bulk
DO Data for Reactor B on Day 12 of Run 2-2.

DAY	REACTOR	d_{avg}^a (μ)	d_{max}^b (μ)	U_o^{max} (mg/g-hr)	$(D_o/\rho)_{opt}^c$ (cm^5/s-mg)	r^d	$C_o^{0.5}$ (mg/ℓ)	$C_o^{0.9}$ (mg/ℓ)
4	B	304	400	251	4.64×10^{-7}	0.9911	0.44	3.2 (2.3)[e]
8	A	371	500	128	5.05×10^{-7}	0.9947	0.31	2.2 (2.0)
	B	314	500	135	2.92×10^{-7}	0.9956	0.40	3.2 (2.3)
12	A	163	300	90	5.21×10^{-8}	0.9915	0.24	2.6 (1.6)
	B	178	250	76	7.09×10^{-8}	0.9940	0.28	2.2 (1.5)
ALL DATA					3.70×10^{-7}	0.9784		

[a] Average spherical equivalent floc diameter (volume-weighted basis).

[b] Maximum spherical equivalent floc diameter.

[c] Optimum value of D_o/ρ for fitting the anoxic core mathematical model.

[d] Coefficient of correlation.

[e] Number in parentheses was estimated directly from the experimental data.

$C_o^{0.9}$, however, was typically high when compared to the data because of the discrepancy between the model and the actual data in this region. Values of $C_o^{0.9}$ estimated directly from the data are included in parentheses in Table 7-1 to illustrate this discrepancy.

The critical DO (C_o^c) was also derived from the model for each of the respirometric tests listed in Table 7-1 according to Equation 3-32. However, the discrepancy between the data and the model determined for values of $C_o^{0.9}$ seemed to be magnified in estimating the critical DO. Furthermore, estimation of the critical DO directly from the data was exceedingly difficult due to the near asymptotic approach of the curves in Figures 7-8 through 7-12 to the maximum specific respiration rate (U_o^{max}). Therefore, analysis of the critical DO associated with these tests was not conducted until the discrepancies between the model and the data discussed previously were evaluated. This evaluation is described within the discussion section at the end of this chapter.

Miscellaneous Respirometric Tests

It was desired to confirm that the respirometric response of the flocculant cultures developed in this study to the bulk DO was the result of diffusional limitations for oxygen and not some inherent response of the bacteria themselves. To achieve this, attempts were made to disperse the bacteria within the flocculant cultures and compare the results of a respirometric test with the dispersed cells to that with the undisturbed flocculant culture. Two methods were tested to achieve dispersion: ultrasonication and blending. The procedures associated with these were described previously (see Chapter V).

Respirometer runs on Day 4 were conducted with an untreated cul-
ture sample and with an ultrasonically dispersed culture sample in an
attempt to compare the response of the natural flocculant culture to
that of the culture after the flocs had been dispersed.

Ultrasonic treatment of the flocculant culture was not successful
in completely dispersing the floc. Microscopic examination of the
ultrasonically-treated sample indicated that disruption of the dense
core of the flocs was not occurring. However, the ultrasonic treatment
was successful in shearing off the large quantities of filamentous bac-
teria that projected from the flocs. Results of this respirometric
test are shown in Figure 7-13. The untreated culture sample exhibited
a half-velocity bulk DO ($C_o^{0.5}$) of about 0.4 mg/ℓ, a 90 percent velocity
DO ($C_o^{0.9}$) of 2.7 mg/ℓ, and a critical DO (C_o^c) of about 6 mg/ℓ. The
ultrasonically-dispersed curve in Figure 7-13 illustrates a $C_o^{0.5}$ of
about 0.3 mg/ℓ, a $C_o^{0.9}$ of 1.6 mg/ℓ, and a critical DO of about 3 mg/ℓ,
which are somewhat lower than the corresponding values for the undis-
turbed culture, but not indicative of total dispersion, in agreement
with microscopic observations. Furthermore, Figure 7-13 illustrates a
substantially lower maximum utilization rate for the ultrasonically
dispersed culture, indicating that some bacteria were damaged by the
ultrasonic treatment even though the power applied was not sufficient
to disrupt the flocs. Attempts to achieve increased dispersion with
the ultrasonication by utilizing different power settings and different
treatment times were not successful and this method was abandoned for
the purposes of achieving dispersion of flocculant cultures.

Because the ultrasonic treatment was not effective in dispersing
floc, attempts were made on Day 5 to disperse the flocculant cultures
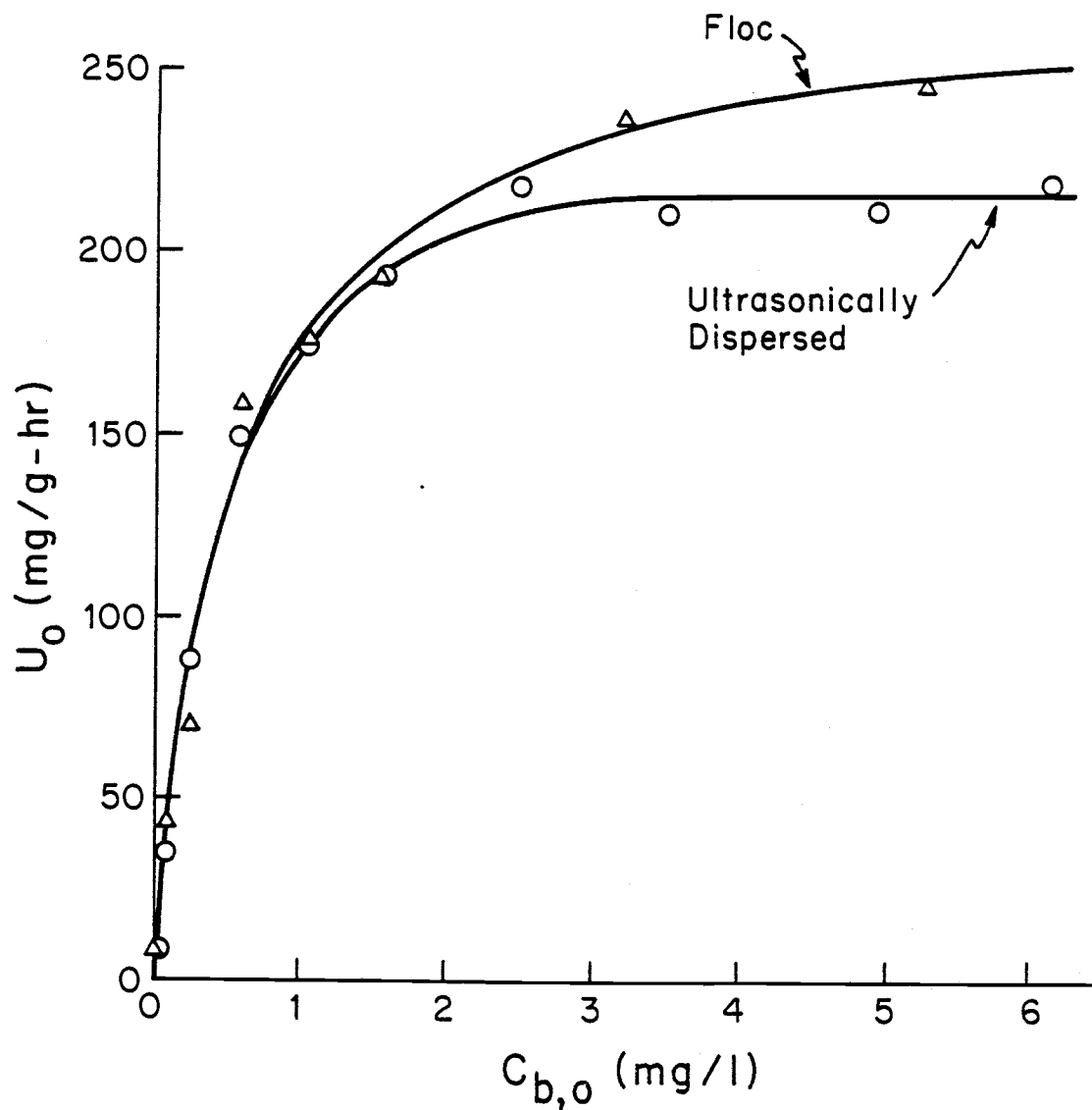
Figure 7-13. Comparison of Specific Respiration Rate versus Bulk
DO Curves for Floc and Ultrasonically Dispersed
Samples (Day 4, Reactor B, Run 2-2).

by blending. The specific respiration rate versus bulk DO curves are shown in Figures 7-14 and 7-15 for Reactors A and B, respectively. The untreated sample from Reactor A exhibited $C_o^{0.5}$, $C_o^{0.9}$, and C_o^c values of about 0.45, 1.9 and 5 mg/ℓ, respectively as compared to corresponding values of 0.25, 1.3, and 3 mg/ℓ for the blended sample. For Reactor B, the $C_o^{0.5}$, $C_o^{0.9}$, and C_o^c values were approximately 0.5, 2.1, and 5 mg/ℓ for the untreated sample and 0.3, 1.4, and 3 mg/ℓ, respectively, for the blended sample. Consequently, blending resulted in a substantial reduction in the diffusional limitations for oxygen but did not appear to be successful in producing total dispersion. Microscopic examination of the blended samples confirmed this postulate in that the floc sizes were substantially reduced, but flocs up to 100 microns in diameter were common. Blending did result in shearing off the filamentous growth projecting from flocs, similar to ultrasonication. However, the maximum respiration rates for the blended and untreated samples were similar, indicating that blending did not result in significant damage to bacterial cells. A summary of results from the ultrasonication and blending tests is given in Table 7-2.

Volumetric respiration rates within the respirometric tests conducted during this run varied from about 0.5 mg/ℓ-min to 2.5 mg/ℓ-min due to variations in the bacterial concentrations in the reactor samples and due to variations in the culture itself. To confirm the applicability of the respirometric test for describing the respiration rate versus bulk DO curve under varying volumetric respiration rates and resultant elapsed times for completion of the test, the results of two tests were compared as shown in Figure 7-16. One test utilized an undiluted (except for 10 percent feed solution) sample and the other a
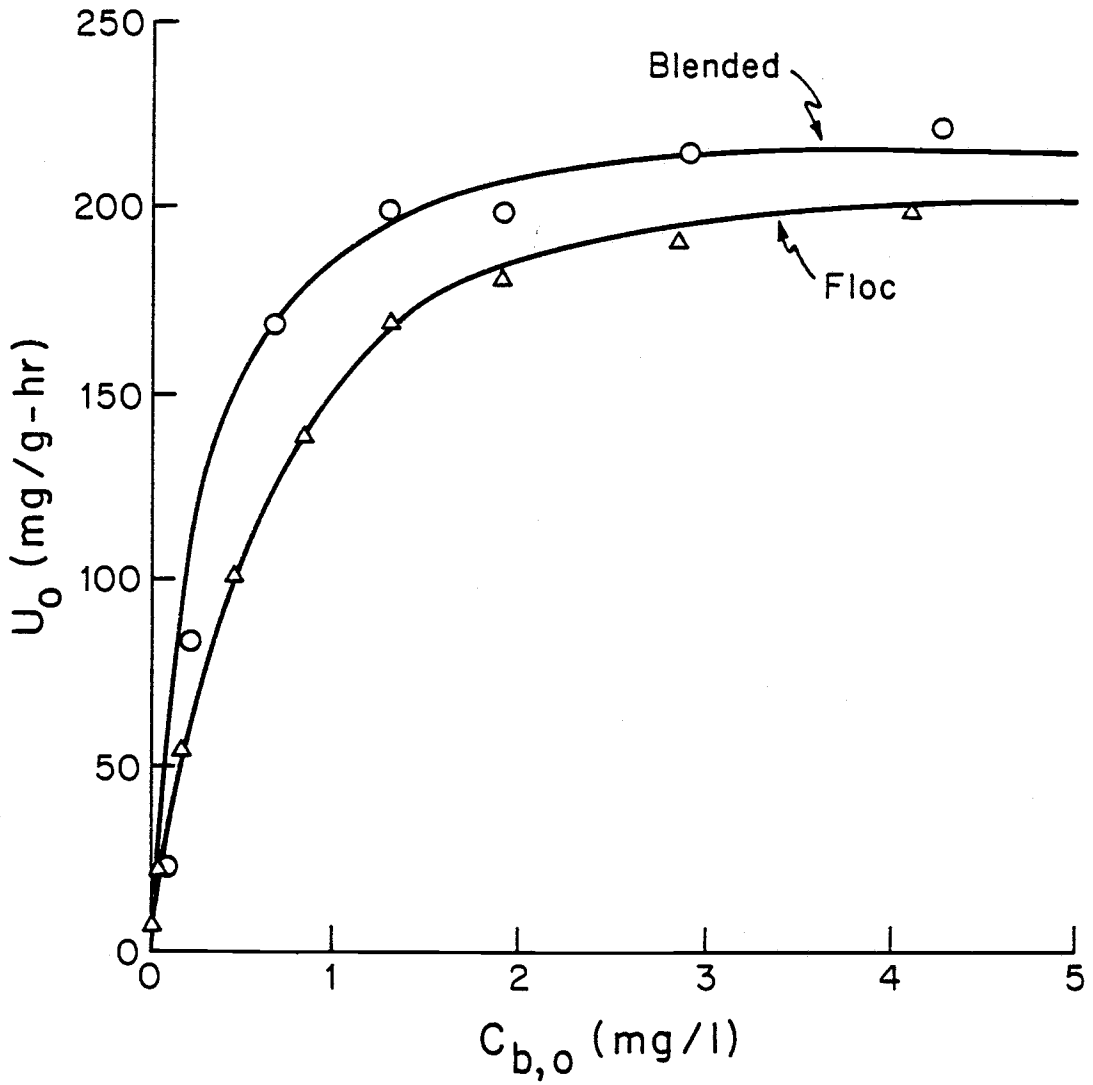
Figure 7-14. Comparison of Specific Respiration Rate versus Bulk
DO Curves for Floc and Blended Samples (Day 5,
Reactor A, Run 2-2).
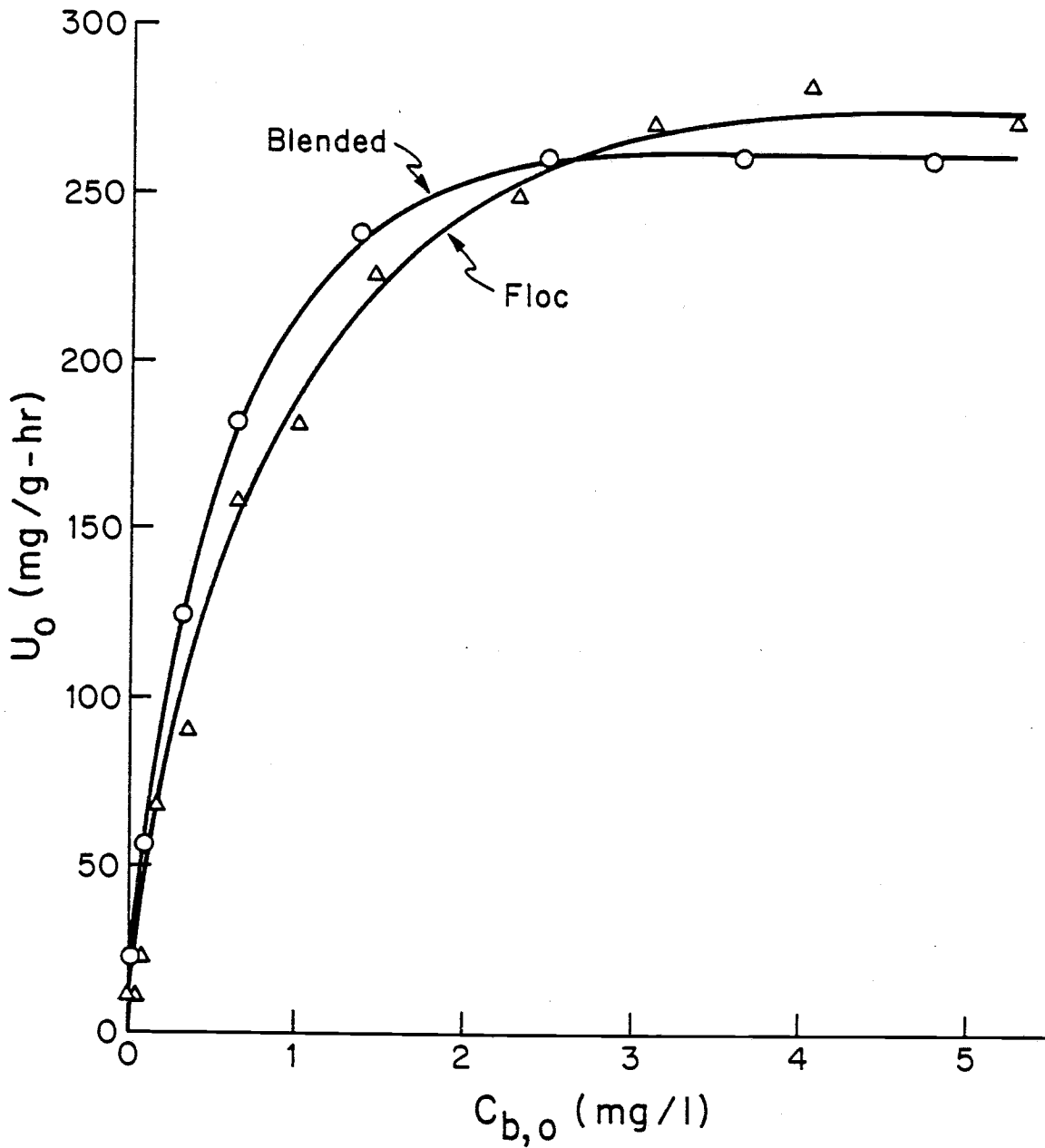
Figure 7-15. Comparison of Specific Respiration Rate versus Bulk
DO Curves for Floc and Blended Samples (Day 5,
Reactor B, Run 2-2).

Table 7-2. Summary of Miscellaneous Respirometric Tests, Run 2-2.

DAY	REACTOR	SAMPLE TREATMENT	d_{avg}	d_{max}	U_o^{max}	$C_o^{0.5}$ (mg/ℓ)	$C_o^{0.9}$ (mg/ℓ)	C_o^{c} [1] (mg/ℓ)
4	B	-	304	400	251	0.4	2.7	6
	B	ultrasonically dispersed	-	400	216	0.3	1.6	3
5	A	-	-	-	204	0.45	1.9	5
	A	blended	-	100	262	0.25	1.3	3
	B	-	304^2	400^2	273	0.5	2.1	5
	B	blended	-	100	262	0.3	1.4	3

[1] Values of C_o^{c} are approximate due to near asymptotic approach of curves to U_o^{max}.

[2] Assumed same as Day 4.
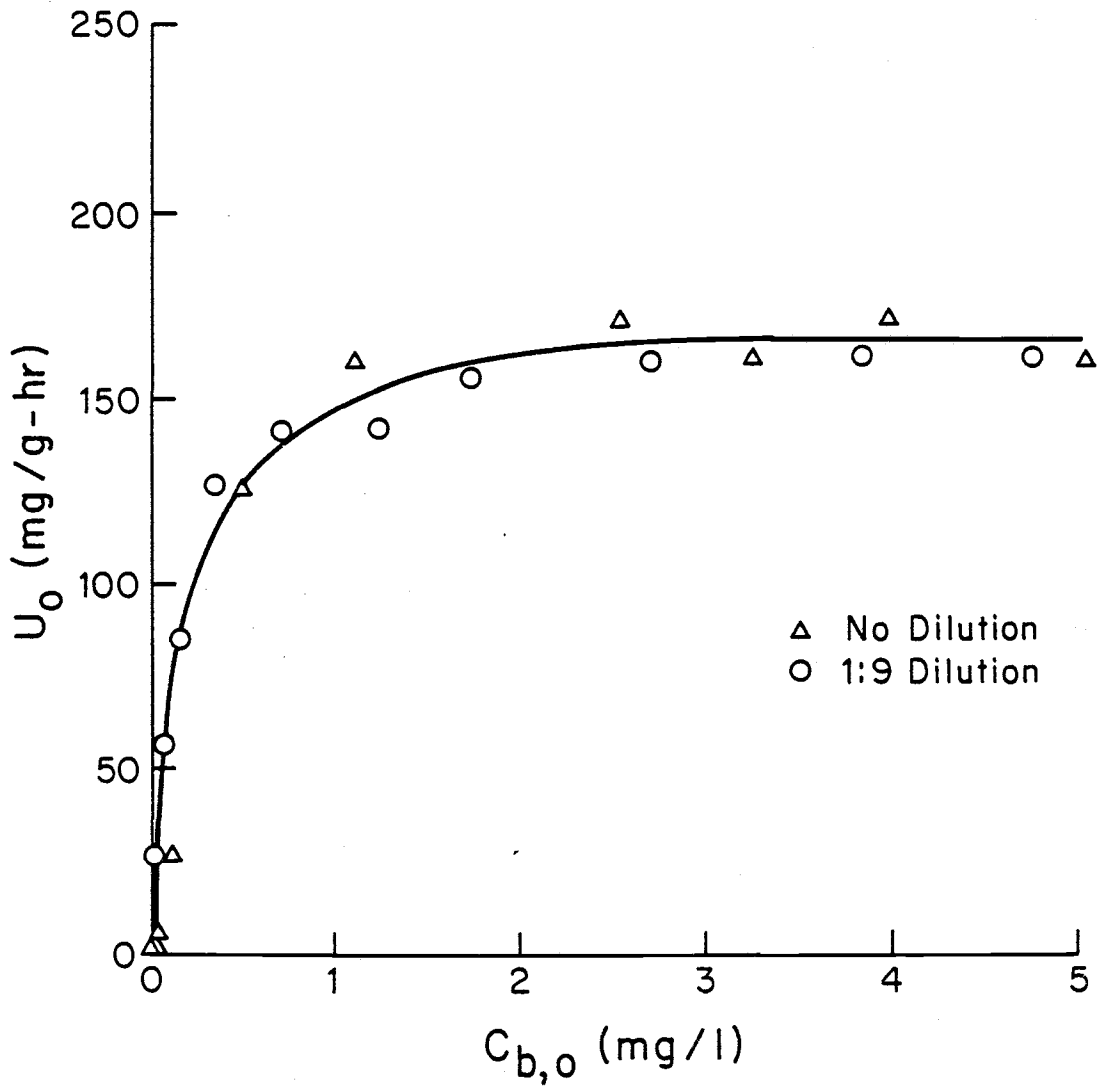
Figure 7-16.   Comparison of Specific Respiration Rate versus Bulk
                DO Curves for Diluted and Undiluted Culture Samples
                (Reactor B, Day 14, Run 2-2).

1:9 dilution of the culture. These tests were conducted on Day 14 when the suspended solids concentration in Reactor B was relatively high. The undiluted test required only five minutes to completely deplete 10 mg/ℓ of dissolved oxygen, incorporating a maximum volumetric utilization rate of 3.0 mg/ℓ-min, while the diluted sample proceeded at a maximum rate of approximately one-tenth that of the undiluted sample. As shown in Figure 7-16, a single curve described the respiration rate versus DO data despite the wide differences in the volumetric oxygen utilization rate. Therefore, the volumetric oxygen utilization rate and the resultant time required to complete the test does not appear to have any significant impact upon the respiration rate versus bulk DO curve produced.

A second question regarding the use of results from the respirometric tests to generate a specific respiration rate versus bulk DO curve was the effect of mixing speed in the respirometer bottle. As stated in Chapter V, a mixing speed of 200 RPM was selected for normal respirometer operation because this was judged to provide a mixing intensity in the respirometer bottle similar to that in the main reactors. To assess the effect of varying mixing speed in the respirometer bottle on the resultant specific respiration rate versus bulk DO curve, three respirometer tests were conducted on Day 15 with all conditions identical except that the mixing speed was varied. Data from these respirometric tests is listed in Appendix B (Table B-14) and the specific respiration rate versus bulk DO data are plotted in Figure 7-17. As shown, a single curve provided a reasonable description of the data obtained from all three tests. However, the data seemed to indicate a small, but noticeable, increase in the specific respiration rate at any
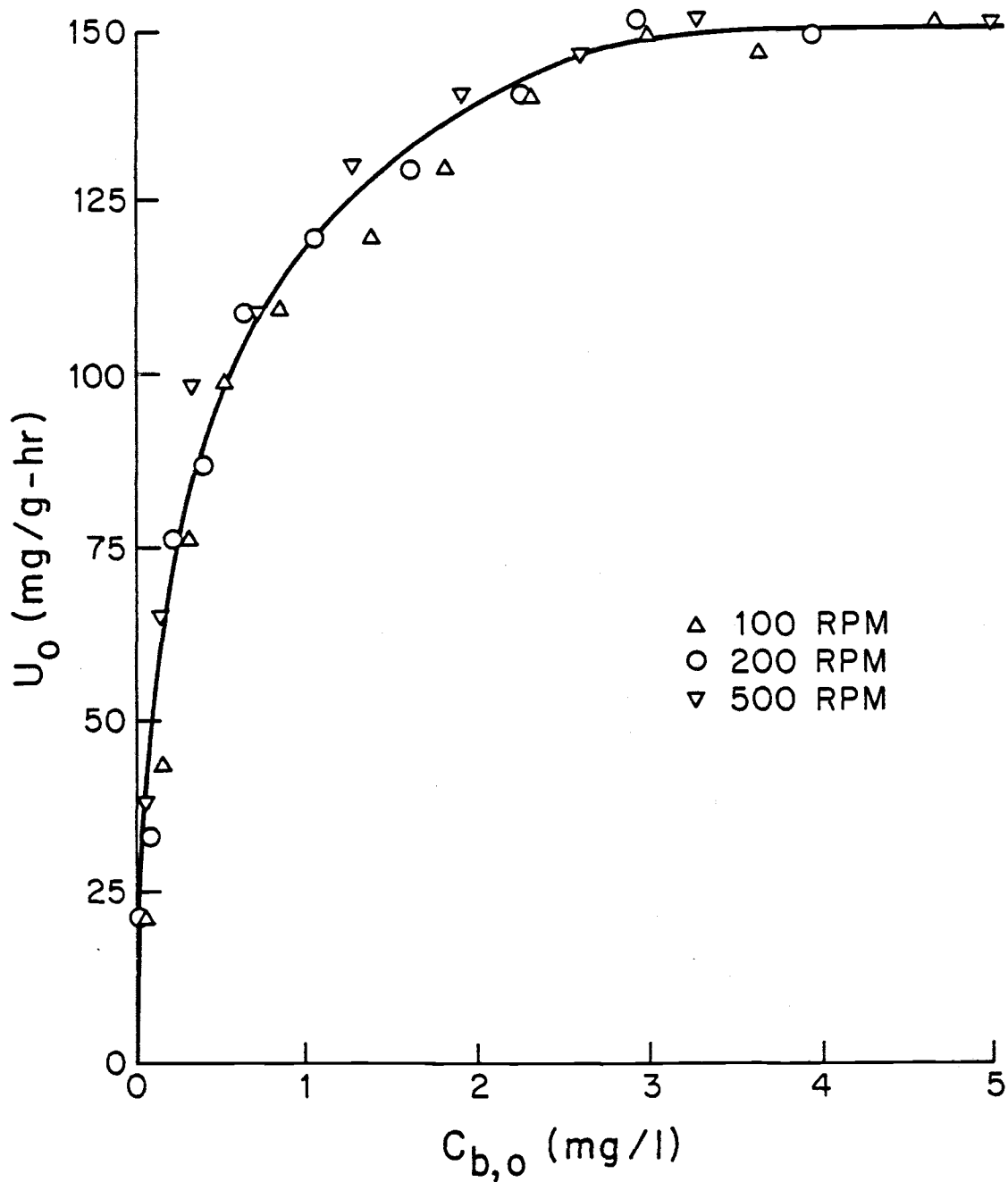
Figure 7-17. Effect of Mixing Speed on the Specific Respiration
Rate versus Bulk DO Curve (Day 15, Reactor B, Run
2-2).

given bulk DO for the test using the higher mixing speed. This is explainable as a reduction in the extent of the liquid boundary layer around flocs at higher mixing intensities. However, the effect of mixing speed was very small and the assumption within the model that the diffusional resistance of the liquid boundary layer is small compared to that of the floc itself is supported by these data.

Discussion

As listed in Table B-2, reactor operation during this phase was unstable and exhibited a cyclic pattern of filamentous bulking and recovery. The pulse-feed mode eliminated the extremely and continuously low concentration of substrate (phenol) that was believed to have caused the filamentous growth problem in Run 1-1. The reason why this mode did not result in a stable culture with good settling characteristics is not known with certainty. It did appear, however, that the occurrence of large numbers of filaments was related to floc size. The pattern that seemed to recur in the reactors was development of an increasing floc size followed by attachment of filaments to large flocs in increasing numbers. Eventually, the large floc became so filamentous that they did not settle in the clarifier and substantial amounts of microbial solids were lost from the reactors. It was also interesting to note during floc size measurements that small floc (up to 200 microns in size) were seldom covered with substantial numbers of filaments and that the extent of filamentous coverage of flocs increased with increasing floc size. The subject of filamentous bulking is discussed further at the end of this chapter.

Although reactor operation was somewhat unstable, the cultures that developed contained large flocs (up to 500 microns in diameter) and it was possible to utilize the respirometric test to illustrate the reduction in respiration rate that occurs when oxygen is diffusion-limiting and a substantial anoxic core develops within larger floc. A summary of results for the respirometric tests conducted during this run was given in Table 7-1. As shown, the bulk DO at which the respiration rate was half-maximal $(C_o^{0.5})$ varied from about 0.2 to 0.5 mg/ℓ and the DO at which the respiration rate was 90 percent of the maximum $(C_o^{0.9})$ was generally in the range of 2 to 3 mg/ℓ, depending on the floc size and the maximum specific respiration rate (U_o^{max}). Estimates of the critical DO (C_o^c) were not listed but generally appeared to be in the range of 4 to 10 mg/ℓ. When compared to the values determined for the dispersed culture in Phase 1 $(C_o^{0.5} \cong 0.05$ mg/ℓ, $C_o^c \approx 0.5$ mg/ℓ), the effect of diffusional limitations is apparent. The significance of these results in establishing whether oxygen diffusional limitations typically occur within full-scale activated sludge plants is discussed at the end of this chapter.

Blending resulted in a substantial reduction in floc size and a corresponding reduction in diffusional limitations for oxygen as indicated by lower values of $C_o^{0.5}$, $C_o^{0.9}$, and C_o^c. Ultrasonication was not effective in dispersing bacteria within flocs without damaging a substantial fraction of the bacterial cells.

The experiments conducted regarding the effect of mixing intensity and volumetric oxygen uptake rate on the results of the respirometric tests confirmed the usefulness of the respirometric test in defining the specific utilization rate versus bulk DO curve. The volumetric

oxygen uptake rate, which is inversely proportional to the time re-
quired to complete a respirometer test, did not have a significant im-
pact on the specific respiration rate versus bulk DO curve derived from
the respirometric data, as was expected.

The mixing speed in the respirometer bottle did have a very small
impact, with higher mixing speeds resulting in a small apparent reduc-
tion in diffusional limitations for oxygen (see Figure 7-17). The
anoxic core model developed within this study does not account for any
effects of mixing intensity because the diffusional resistance of the
liquid boundary layer is neglected as being small compared to the dif-
fusional resistance of the floc itself and because the floc are assumed
to be solid spheres with a constant diffusivity throughout. The small
effect of mixing speed shown in Figure 7-17 can probably be attributed
to a reduction in the diffusional resistance of the liquid boundary
layer. An additional possibility is that activated sludge floc are not
solid bacterial masses but contain channels and pores. Higher mixing
intensities would increase the transfer of the bulk solution through
these channels and pores and would result in an apparent reduction in
diffusional resistance of the floc itself.

The small effect of mixing speed illustrates that the above-
described assumptions in the anoxic core model may not be perfectly
valid. However, the small effect of mixing intensity (see Figure 7-17)
demonstrates the reasonableness of these assumptions in developing a
manageable model.

RUN 2-3

Experimental Methods

Operation of the reactors in the pulse-feed mode in Run 2-2 did not result in a stable steady-state condition. Furthermore, although the floc sizes that developed were large, the flocs were highly filamentous, which resulted in a high SVI and poor settling characteristics. Although Run 2-2 did produce good results in terms of illustrating the response of bacterial respiration rate to bulk DO for a culture composed of large flocs, it was desired to produce a culture that not only contained large flocs but whose settling and compaction behavior and extent of filamentous growth were more similar to typical full-scale activated sludge plants.

Operation in the fill-and-draw mode utilized a 7.0-liter reactor volume. Each morning, the air supply to the reactor was turned off and the contents allowed to settle for 15 minutes. After this settling period, supernatant was siphoned out of the reactor to a volume of 1.0 liter and the reactor was filled with 1.4 liters of feed solution (phenol concentration = 500 mg/ℓ) and 4.6 liters of tap water, resulting in an initial phenol concentration of 100 mg/ℓ. After filling, the air supply was turned on and aeration continued for approximately 12 hours. The same procedure was repeated each evening for a twice-per-day fill-and-draw operation.

Verification of the anoxic core model for this run was planned to result from respirometric and other batch tests. Therefore, only one reactor was operated and the DO in this reactor was controlled at 8 mg/ℓ

to allow development of the culture in the absence of significant oxygen diffusional limitations.

Daily suspended solids analyses on the reactor contents were taken immediately after the reactor was fed in the morning. The pH measurements were taken after all substrate had been depleted.

The solids retention time in the reactor was calculated from the following mass-balance equation:

$$\theta_c = \frac{V\,X}{(Q^e - Q^w)X^e + Q^w X} \tag{7-1}$$

where: θ_c = solids retention time (days)

V = reactor volume (7.0 liters)

X = reactor TSS (mg/ℓ)

Q^e = effluent volume per day (12.0 ℓ/day)

Q^w = wastage volume per day

X^e = effluent TSS (mg/ℓ)

Wasting was accomplished manually once per day prior to the evening feeding.

Respirometric tests were conducted during the 3 to 5 hour period immediately after feeding. Initially, the tests were conducted at t = 0.5 hours to ensure that saturation kinetics were observed. Later, respirometric tests were conducted at various times after feeding (t = 0) and the phenol concentration at that time recorded. A minimum phenol concentration of 25 mg/ℓ was assumed to ensure saturation kinetics with respect to the substrate (phenol).

Results

Daily data for the main reactor during Run 2-3 are contained in Appendix C, Table C-1. A flocculant, non-filamentous culture rapidly established itself during this run and it was possible to set the SRT at the desired level of 2.0 days on the second day of operation. The SRT was reset at 3.0 days on Day 17. During the 26-day period of operation, the settling behavior of the culture was very good, the SVI was fair to good (100-250 ml/g) and the effluent suspended solids were generally in the range of 20-50 mg/ℓ. Furthermore, reactor operational behavior was very stable in contrast to the two previous modes of operation.

A typical plot of reactor substrate (phenol) concentration and total suspended solids versus time is shown in Figure 7-18. The time required to completely deplete the initial phenol concentration of 100 mg/ℓ varied over the 26-day operational period of this run from about three to eight hours, depending on the culture and the biomass concentration. As shown in Figure 7-18, there was significant growth in the reactor as the substrate was depleted, with the reactor suspended solids rising 57 mg/ℓ during the time period required to deplete 100 mg/ℓ of phenol. The half-velocity coefficient for the substrate (phenol) was about one milligram per liter for the cultures developed during this run, similar to Run 2-2.

Floc Size and Respirometer Results

Floc size measurements were taken at about four-day intervals during this run and the floc size distributions generated are shown in
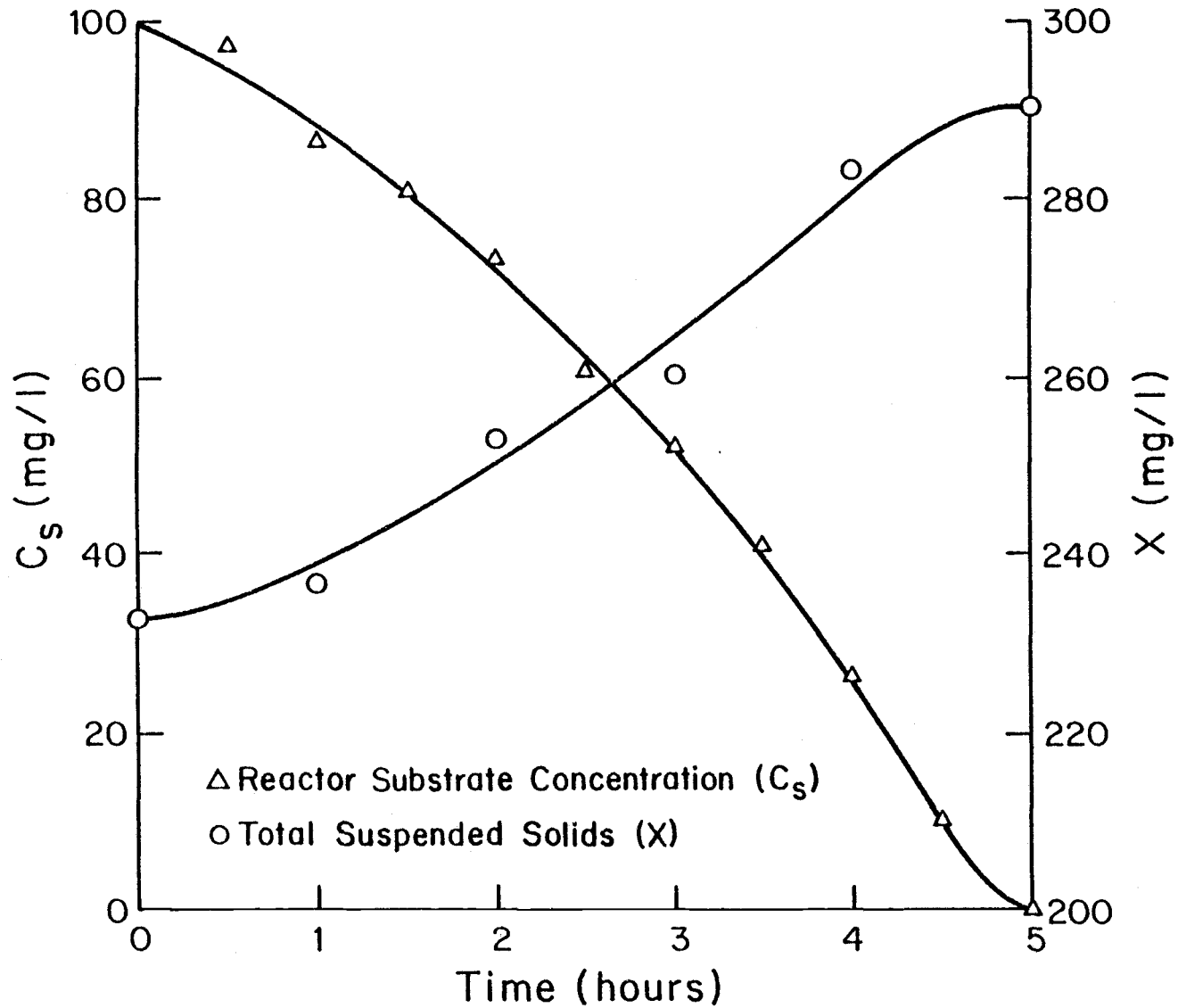
Figure 7-18.   Reactor Substrate Concentration and Total Suspended
Solids versus Time on Day 14 of Run 2-3.

Figures 7-19 through 7-23. The bacterial flocs within the reactor were initially moderate-sized, as shown in Figure 7-19, which illustrates the floc size distribution on Day 2 of this run. The median floc diameter on this day was in the 200-250 micron range and the maximum was 350 microns. By Day 6 the floc sizes in the reactor were increased with a median floc diameter in the 300-350 micron range and a maximum floc diameter of 450 microns (see Figure 7-20).

The floc size in the reactor continued to grow throughout this run and, on Day 9, the median floc diameter was in the 400-450 micron range and there were substantial numbers of floc in the 500-600 micron range (see Figure 7-21). On Days 14 and 19 the flocs were extremely large in size with a substantial fraction in the 1000-1200 micron range, as shown in Figures 7-22 and 7-23. The median floc diameter was in the 900-1000 micron range on Day 14 and in the 700-800 micron range on Day 19.

Respirometric tests were conducted on the same day as the floc size measurements so that the anoxic core mathematical model could be applied to results of the respirometric tests. The specific respiration rate versus bulk DO data produced from the respirometric tests were fit to the model by the methods described previously (see Appendix D for details of model application).

Results of the respirometric experiment at $t = 0.5$ hours on Days 2 and 6 are shown in Figures 7-24 and 7-25. The model fit to the data was very good with a coefficient of correlation (r) of greater than 0.995 in both cases. The half-velocity DO ($C_o^{0.5}$) predicted by the model was 0.14 mg/ℓ on Day 2 and 0.42 mg/ℓ on Day 6 while the 90 percent velocity DO ($C_o^{0.9}$) was 0.97 mg/ℓ on Day 2 and 3.1 mg/ℓ on Day 6. These
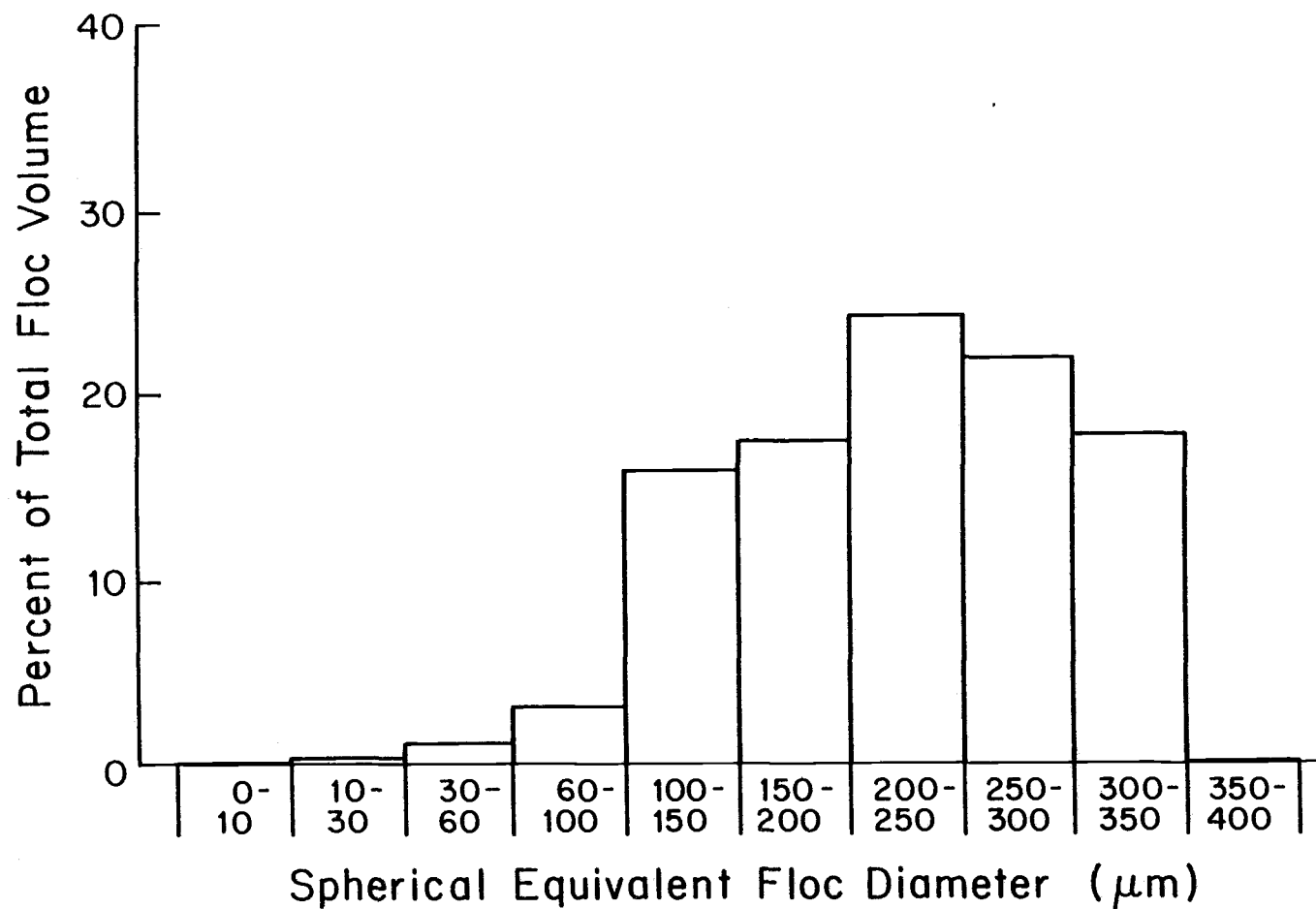
Figure 7-19.   Floc Size Distribution on Day 2 of Run 2-3.
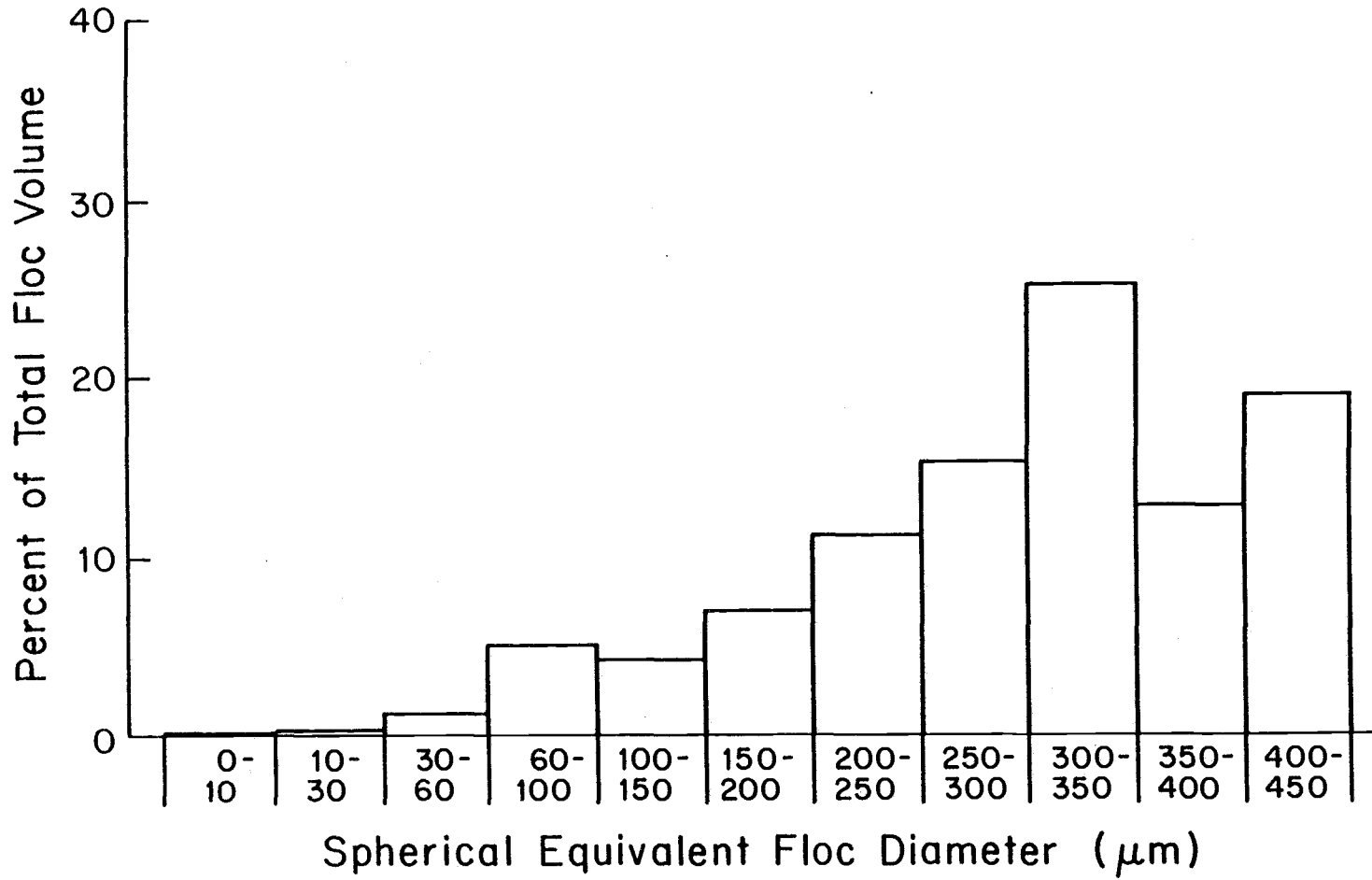
Figure 7-20. Floc Size Distribution on Day 6 of Run 2-3.
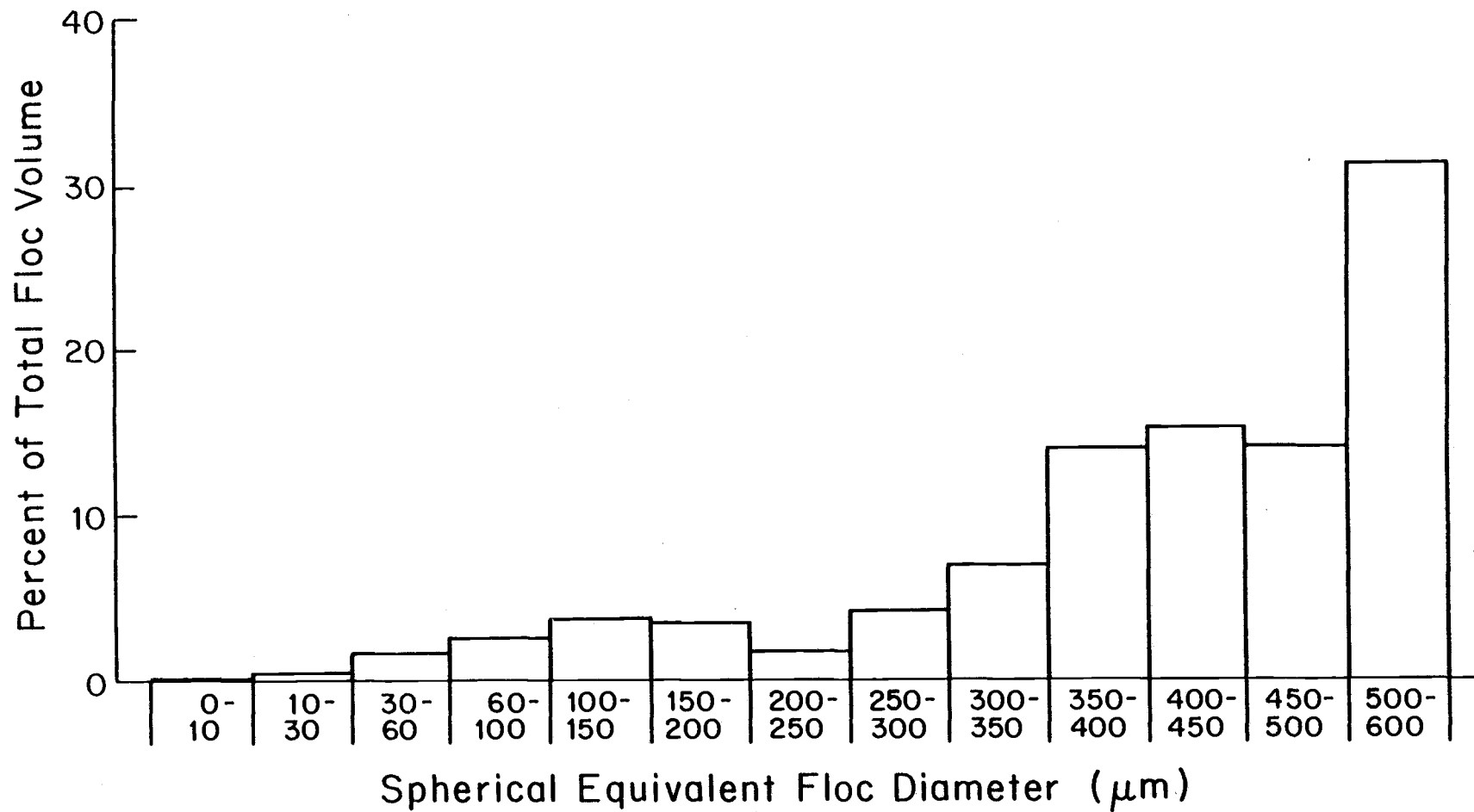
Figure 7-21. Floc Size Distribution on Day 9 of Run 2-3.
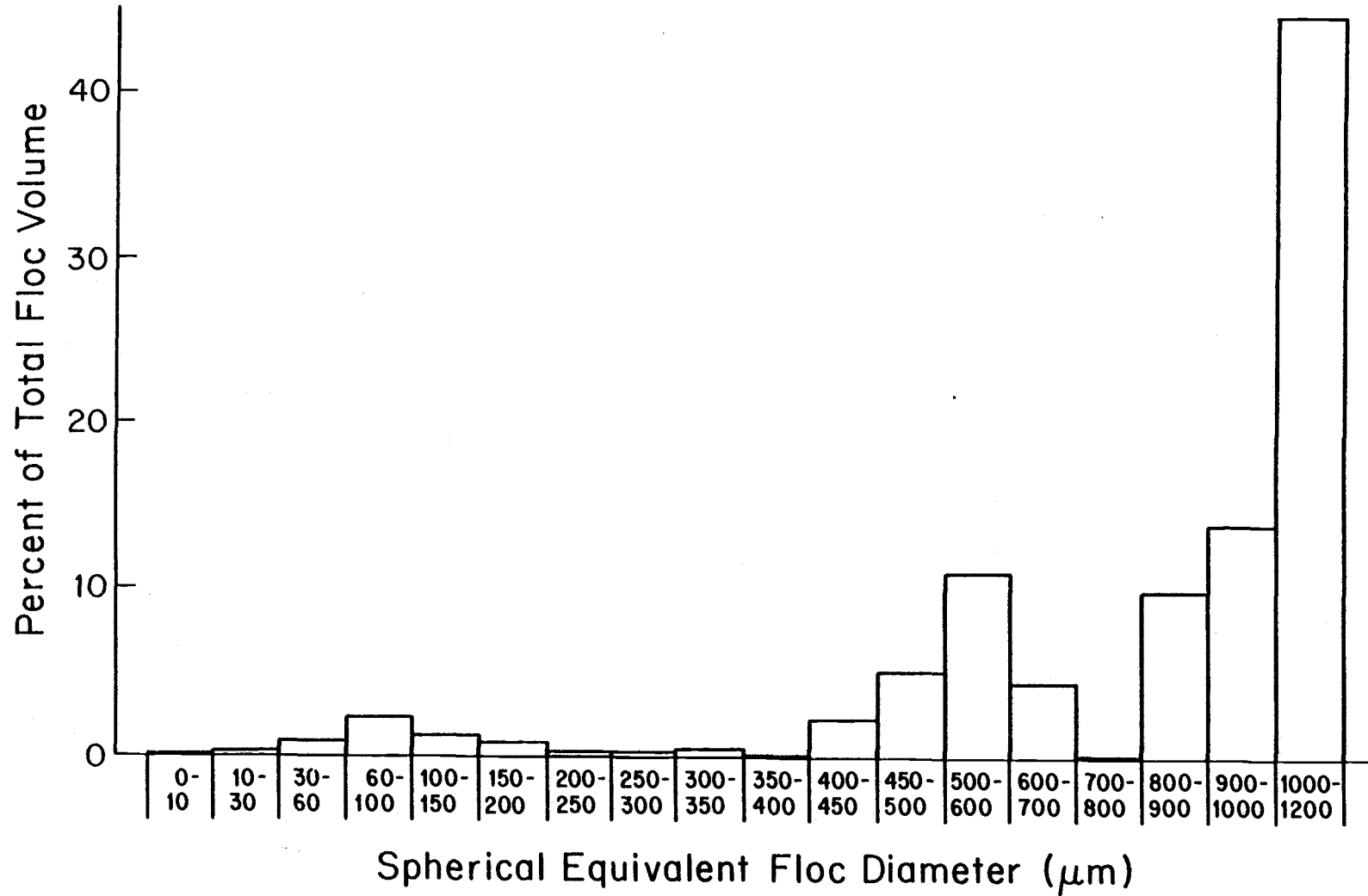
Figure 7-22.   Floc Size Distribution on Day 14 of Run 2-3.
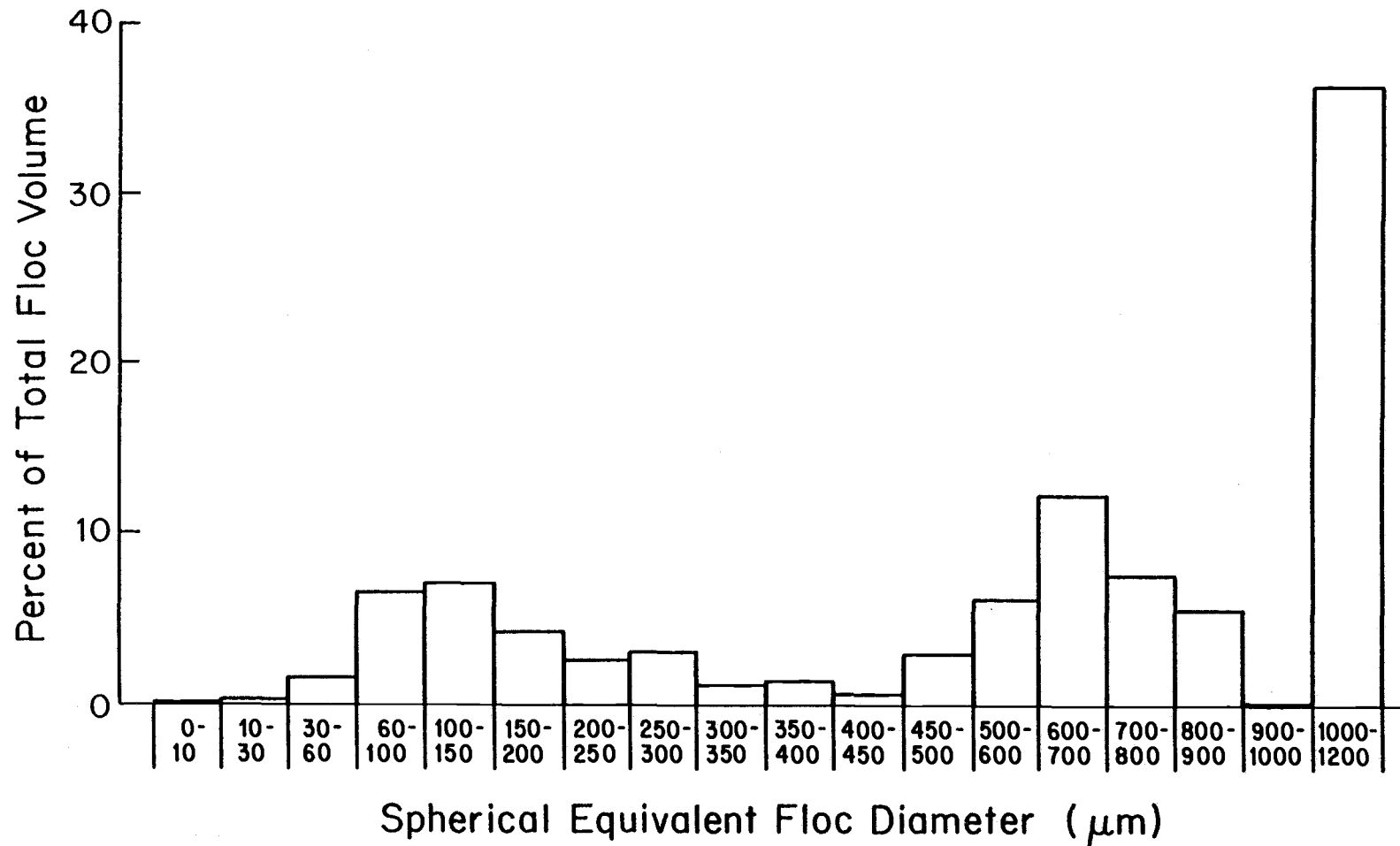
Figure 7-23.   Floc Size Distribution on Day 19 of Run 2-3.
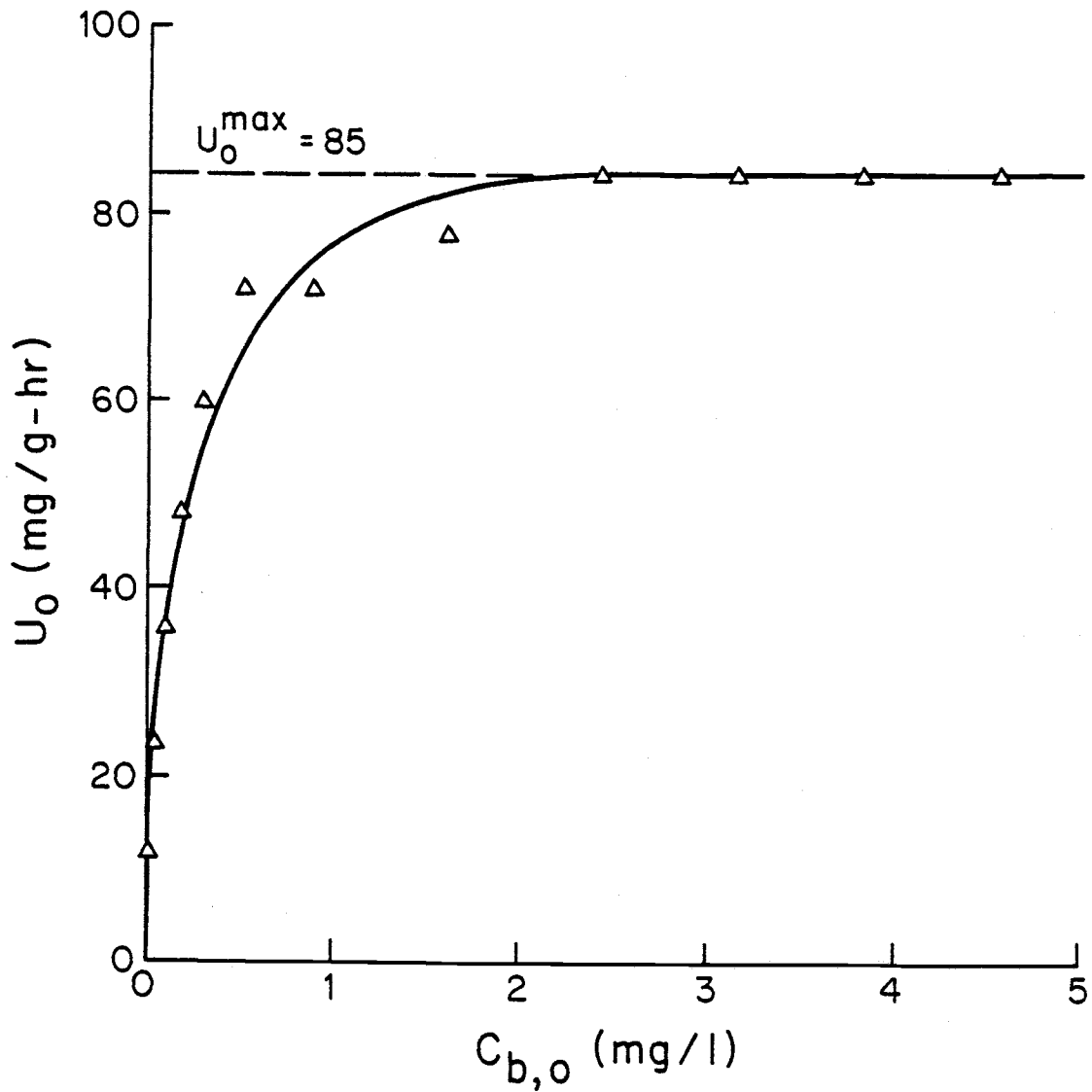
Figure 7-24. Model Fit to Specific Respiration Rate versus Bulk DO Data for Day 2 of Run 2-3 (t = 0.5 hours).
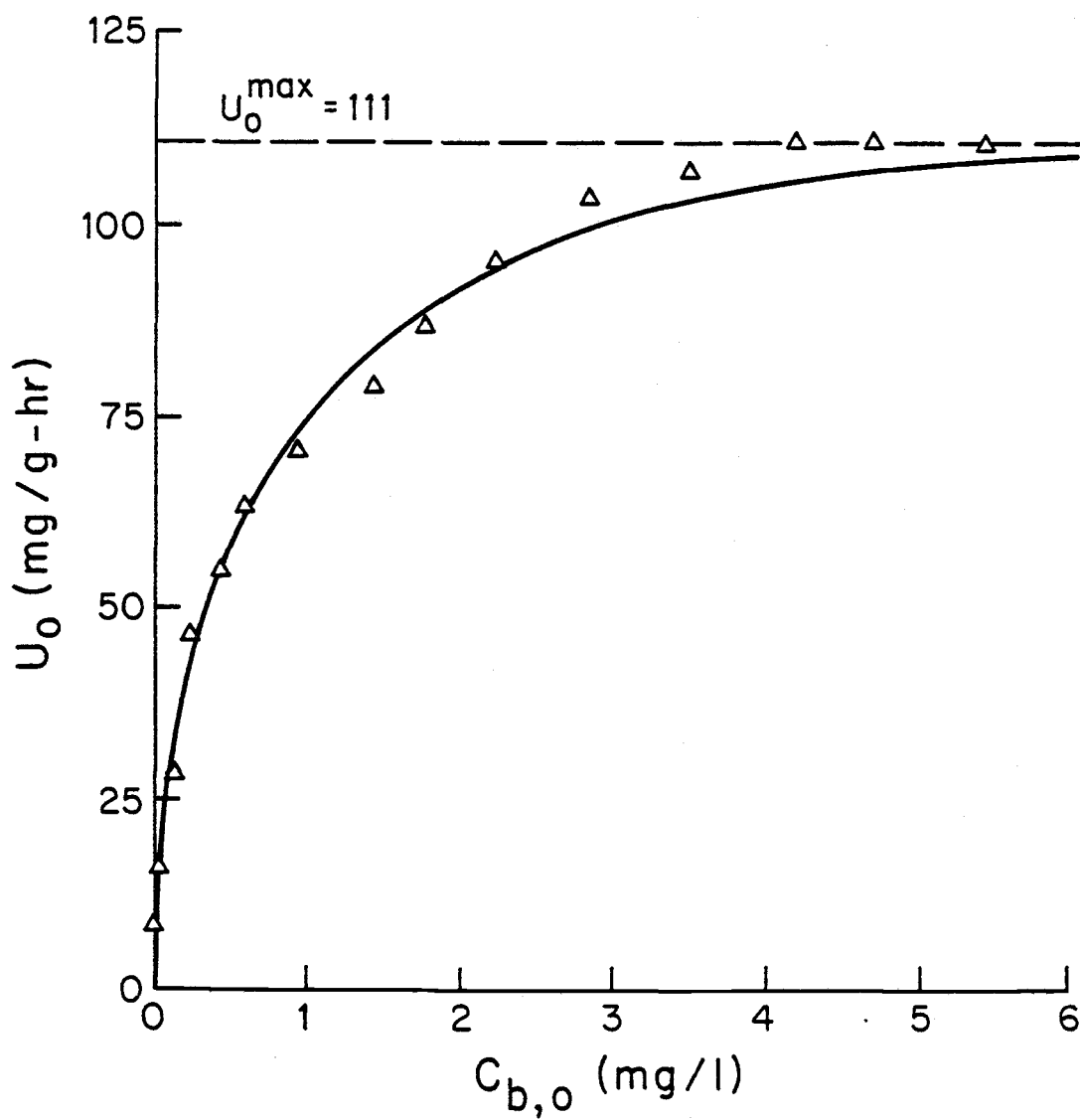
Figure 7-25. Model Fit to Specific Respiration Rate versus
Bulk DO Data for Day 6 of Run 2-3 (t = 0.5
hours).

parameters were substantially higher on Day 6 as compared to Day 2 due to a larger floc size on Day 6 (see Figures 7-19 and 7-20) and due to a higher maximum specific respiration rate (U_o^{max}) on Day 6 (111 versus 85 mg/g-hr).

The daily respirometric experiments were expanded on Day 9 to include three tests per day at intervals of one to two hours. Because the specific respiration rate increased during the three to six-hour period after the reactor was fed in the morning, these tests provided a comparison of the specific respiration rate versus bulk DO behavior at increasing values of $\dot{U}_o^{max}$ with approximately the same floc size distribution.

Results of the three successive respirometric tests on Days 9, 14, and 19 are shown in Figures 7-26, 7-27, and 7-28, respectively. The model fit was again good on these days with coefficients of correlation typically greater than 0.99. The $C_o^{0.5}$ for these tests was in the range of 0.4 to 0.7 mg/ℓ, which was substantially higher than previous tests due to the large floc size in the reactor on these days.

A summary of the model fit to the respirometric data obtained during Run 2-3 is given in Table 7-3. As shown, the average (volume-weighted basis) floc diameter ranged from 246 microns on Day 2 to 943 microns on Day 14 and the maximum floc diameter ranged from 350 to 1200 microns. The maximum respiration rate for these tests was generally below 100 mg/g-hr for $t \leq 1.0$ hours and increased to near 150 mg/g-hr as the substrate in the reactor was depleted.

The optimum value for D_o/ρ varied substantially from day to day with a minimum value of 2.47×10^{-7} cm^5/s-mg on Day 6 to a maximum of 1.62×10^{-6} cm^5/s-mg on Day 14. As in Run 2-2, the optimum value of
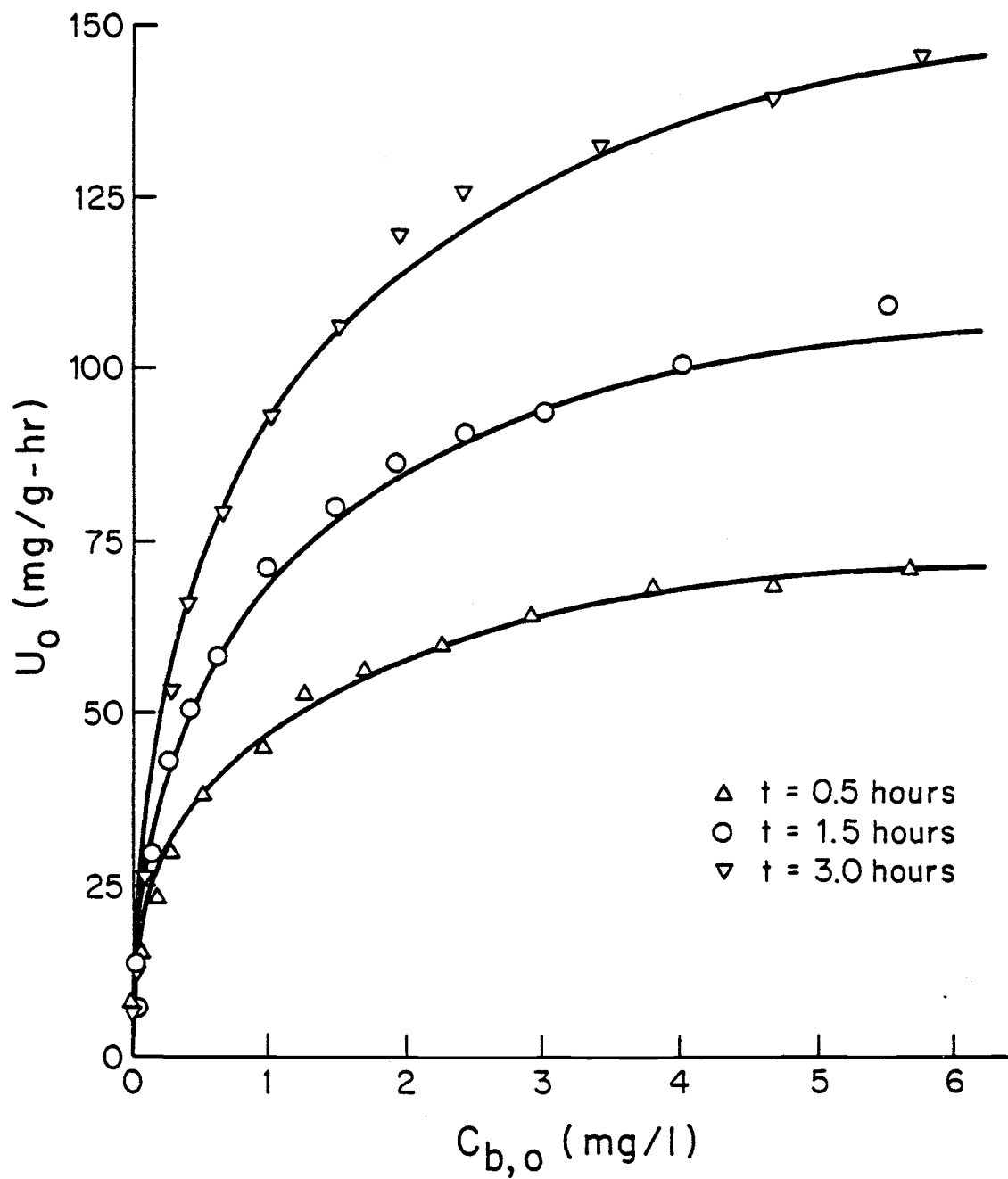
Figure 7-26. Model Fit to Specific Respiration Rate versus Bulk DO Data on Day 9 of Run 2-3.
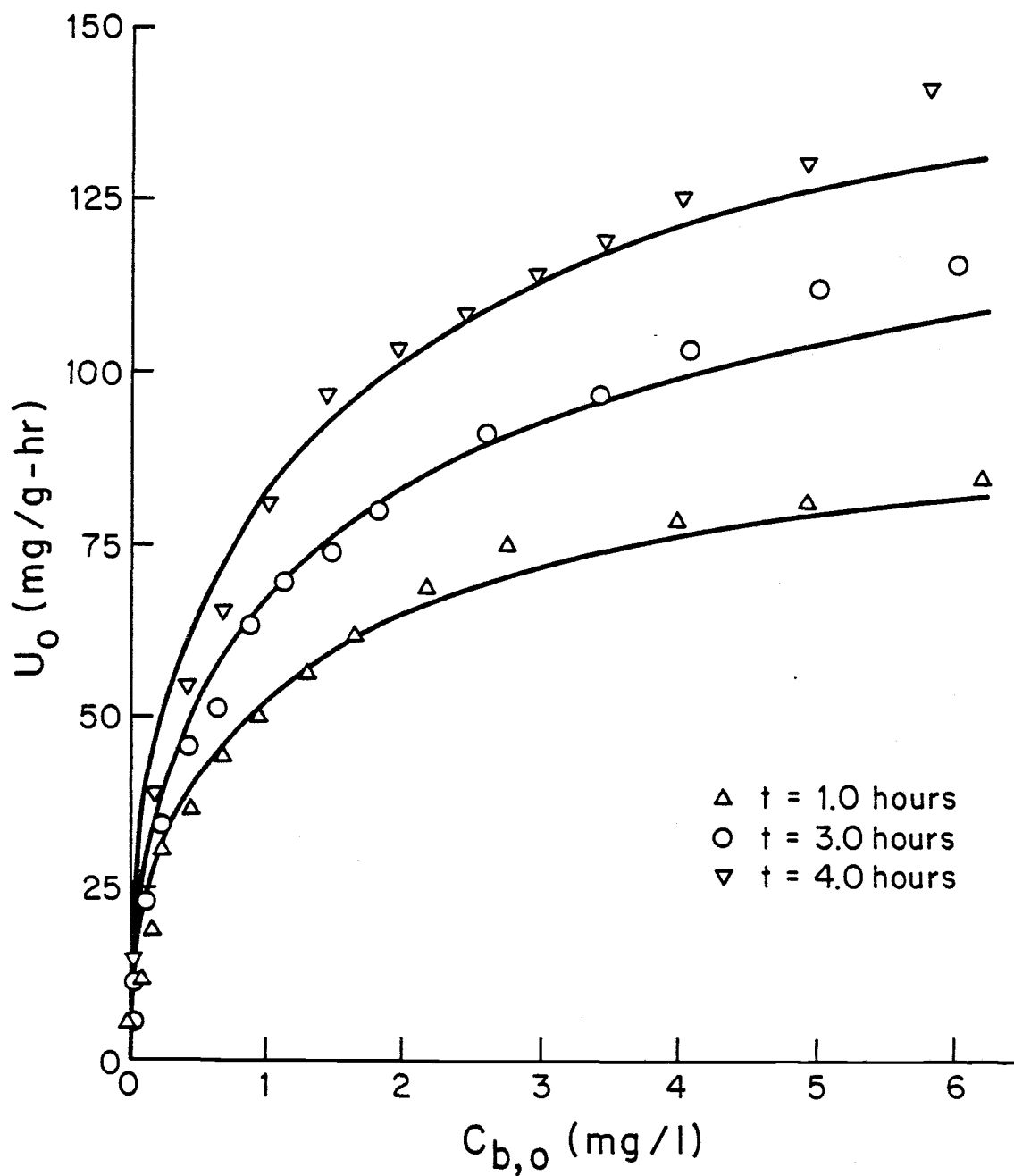
Figure 7-27. Model Fit to Specific Respiration Rate versus Bulk DO Data on Day 14 of Run 2-3.
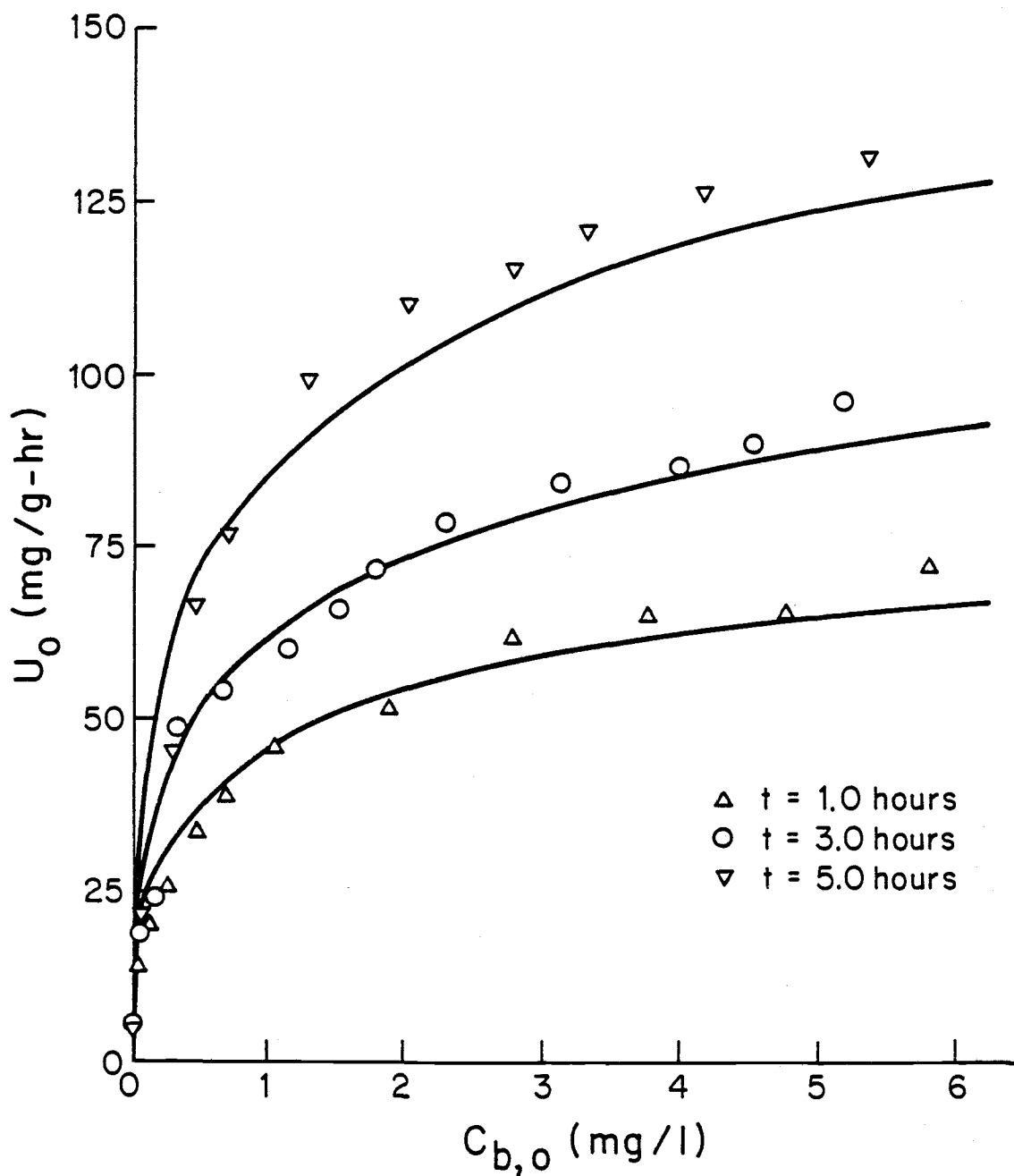
Figure 7-28. Model Fit to Specific Respiration Rate versus Bulk DO Data on Day 19 of Run 2-3.

Table 7-3. Summary of Model Application to Respirometric Data from Run 2-3.

DAY	t^a (hours)	d_{avg}^b (μ)	d_{max}^c (μ)	U_o^{max} (mg/g-hr)	$(D_o/\rho)_{opt}^d$ (cm^5/s-mg)	r^e	$C_o^{0.5}$ (mg/ℓ)	$C_o^{0.9}$ (mg/ℓ)
2	0.5	246	350	85	3.18×10^{-7}	0.9957	0.14	$0.97(1.1)^f$
6	0.5	304	450	111	2.47×10^{-7}	0.9977	0.42	3.1 (2.7)
9	0.5	456	600	73	2.79×10^{-7}	0.9971	0.49	3.5 (2.9)
	1.5			109	3.99×10^{-7}	0.9960	0.52	3.8 (3.4)
	3.0			151	4.86×10^{-7}	0.9952	0.58	4.3 (3.8)
14	1.0	943	1200	85	1.17×10^{-6}	0.9964	0.56	4.2 (3.1)
	3.0			115	1.31×10^{-6}	0.9963	0.67	5.1 (4.1)
	4.0			141	1.62×10^{-6}	0.9956	0.68	5.1 (4.3)
19	1.0	851	1200	72	6.30×10^{-7}	0.9930	0.41	5.2 (3.7)
	3.0			102	7.46×10^{-7}	0.9889	0.50	6.2 (4.9)
	5.0			147	9.89×10^{-7}	0.9870	0.54	8.0 (5.5)
ALL DATA					7.49×10^{-7}	0.9535		

a Time after feeding in the fill-and-draw operational mode.

b Average spherical equivalent floc diameter (volume-weighted basis).

c Maximum spherical equivalent floc diameter.

d Optimum value of D_o/ρ.

e Coefficient of correlation.

f Number in parentheses was estimated directly from the experimental data.

D_o/ρ appeared to be highly correlated with the average floc diameter. The model fit to the data was generally very good with a coefficient of correlation greater than 0.99 on all days except Day 19. As in Run 2-2, however, the model-predicted curve reflected a higher value of U_o than the actual data for low bulk DO levels (less than about 0.5 mg/ℓ) and reflected a lower value of U_o for higher bulk DO levels. A second interesting result was the comparative optimum value of D_o/ρ for the three successive respirometer tests conducted on Days 9, 14, and 19. As shown in Table 7-3, the optimum value of D_o/ρ increased with increasing time (and increasing U_o^{max}) on each day for the three tests. For example, on Day 9 the optimum value of D_o/ρ was 2.79×10^{-7} cm^5/s-mg at t = 0.5 hours (U_o^{max} = 73 mg/g-hr), 3.99×10^{-7} cm^5/s-mg at t = 1.5 hours (U_o^{max} = 109 mg/g-hr), and 4.86×10^{-7} cm^5/s-mg at t = 3.0 hours (U_o^{max} = 151 mg/g-hr). Because only a limited amount of growth occurred in the reactor over the time required to complete these three tests, it can be reasonably assumed that the floc size in these three tests was very similar. According to the model, the optimum value of D_o/ρ should therefore be very similar for these three successive tests. The fact that the optimum value of D_o/ρ increased from the first to the third respirometric test on all days this procedure was followed indicates a second discrepancy between the model and the data. These discrepancies are further discussed at the end of this chapter.

Also listed in Table 7-3 are model predictions of the bulk DO at which the respiration rate was 50 percent and 90 percent of the maximum. Because the model fit was very good for bulk DO levels near the $C_o^{0.5}$, the predicted value of $C_o^{0.5}$ was in very good agreement with the data. The predicted value of $C_o^{0.9}$, however, was typically high when compared

to the data because of the discrepancy between the model and the data in this region. Values of $C_o^{0.9}$ estimated directly from the data are included in parentheses in Table 7-3 to illustrate this discrepancy.

Miscellaneous Respirometric Tests

As in Run 2-2, attempts were made during Run 2-3 to disperse a sample of the flocculant culture by blending and to compare the results of a respirometric test with a blended culture to that with the undisturbed flocculant culture. An example of this type of comparison is shown in Figure 7-29 wherein specific respiration rate versus bulk DO curves are compared for a blended and an untreated culture sample on Day 6. The untreated culture sample exhibited a $C_o^{0.5}$ of 0.42 mg/ℓ, a $C_o^{0.9}$ of 2.7 mg/ℓ, and a C_o^c of about 5 mg/ℓ as compared to values of 0.09, 1.0, and 3 mg/ℓ, respectively, for the blended sample. Consequently, it can be seen that blending resulted in a substantial reduction in oxygen diffusional limitations by reducing the floc size. Microscopic observation of the blended sample indicated that the maximum floc size had been reduced to about 100 microns. The maximum floc size for the untreated culture sample was 450 microns and the average floc size was 304 microns (see Figure 7-20).

Discussion

Operation of the activated sludge reactor in the fill-and-draw mode produced a stable activated sludge culture. The settling behavior of the culture was good, the SVI was fair to good (100-250 ml/g), and the effluent suspended solids were generally in the range of 20-50 mg/ℓ. The fill-and-draw mode has previously been found to produce stable
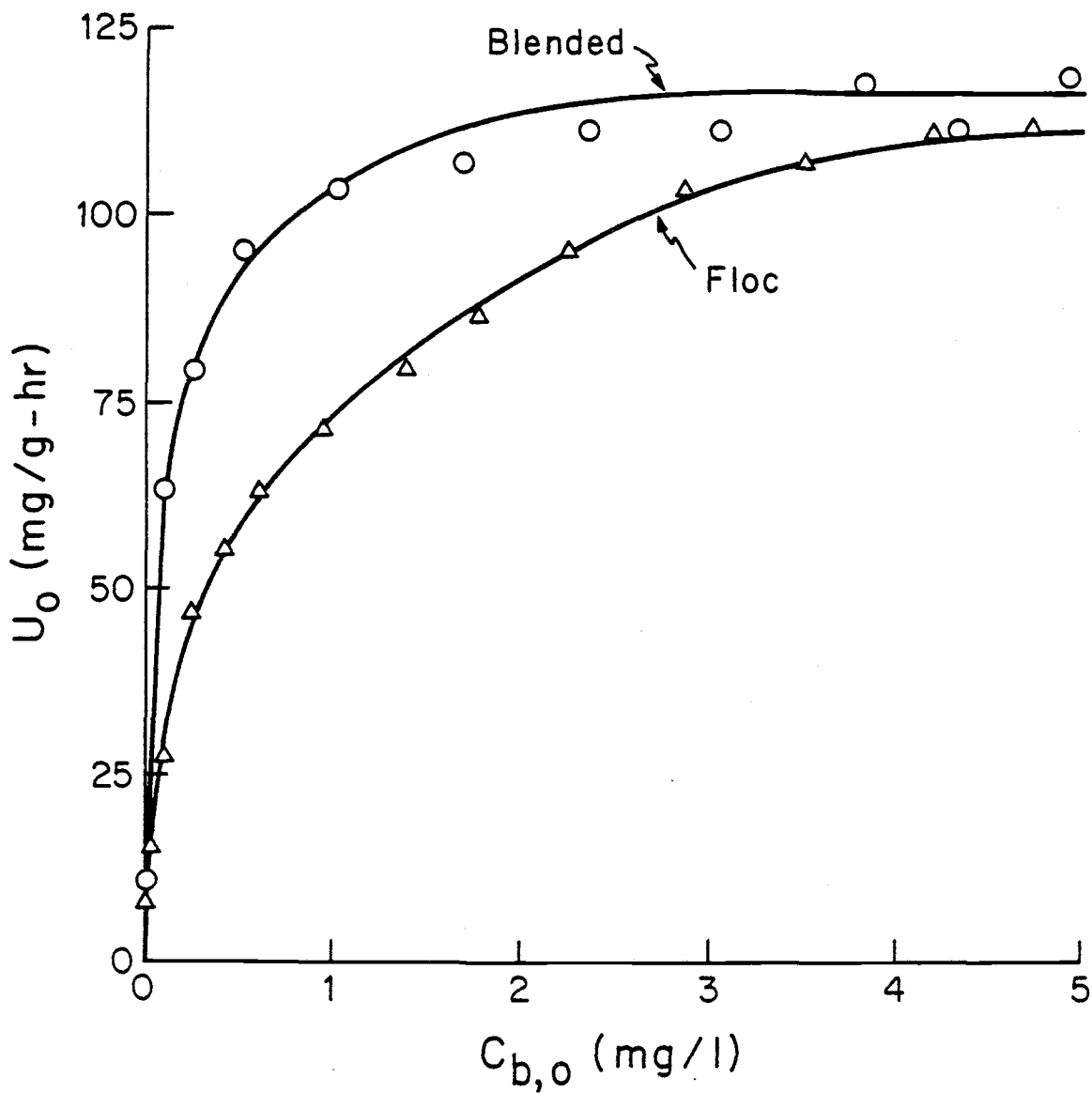
Figure 7-29. Specific Respiration Rate versus Bulk
DO on Day 6 of Run 2-3 (t = 0.5 hours).

operational behavior and to limit filamentous bulking where other modes have failed (Nelson and Lawrence, 1980).

The cultures developed within this run initially exhibited a moderate floc size (maximum diameter of 300 microns), but the floc size increased steadily until Day 14 when the maximum floc diameter was 1200 microns and the average floc diameter was nearly 1000 microns. It was interesting to note that the extent of filamentous attachment to the floc increased with the increasing floc size. It was found that floc particles less than about 300 microns in diameter rarely had a substantial coverage of filamentous bacteria while larger floc particles exhibited a high degree of surface coverage with filamentous bacteria. Diffusional limitations do not explain this phenomenon because both the substrate and dissolved oxygen concentrations in the reactor were typically higher than the concentrations necessary to produce diffusional limitations. Rather, the phenomenon of filamentous coverage of floc surfaces appears to be related to floc size by some other mechanism. A possible explanation is that only large flocs can accept substantial surface coverage of filamentous bacteria and still settle. Smaller floc that incur significant filamentous attachment may become unsettleable and be selectively eliminated from the system.

The respirometric experiments conducted during this run were successful in illustrating the effect of oxygen diffusional limitations in large, rapidly respiring activated sludge floc. A summary of results for these respirometric tests was given in Table 7-3. As shown, the $C_o^{0.5}$ varied from about 0.1 to 0.7 mg/ℓ and the $C_o^{0.9}$ varied from about 1.0 to 6.0 mg/ℓ, depending on the floc size and the maximum specific respiration rate. Estimates of C_o^c were not listed but appeared

to be in the range of 3 to 8 mg/ℓ. Blending of a culture sample was successful in reducing the maximum floc size to below 100 microns and in substantially reducing diffusional limitations for oxygen.

The fit of the anoxic core mathematical model to the data from the respirometric experiments was generally very good. As in Run 2-2, however, the fitted model tended to predict higher than actual values of U_o when $C_{b,o}$ was small (less than about 0.5 mg/ℓ) and lower than actual values of U_o when $C_{b,o}$ was high (see Figures 7-24 through 7-28). This discrepancy is discussed further in the subsequent section.

An additional discrepancy between the model and the data occurred on those days when three successive respirometric tests were performed (Days 9, 14, and 19). As listed in Table 7-3, there was a distinct pattern wherein the optimum value of D_o/ρ increased with time after feeding, which was directly correlated with an increasing maximum specific respiration rate. Direct application of the model dictates that the optimum value of D_o/ρ should not have changed in these successive experiments even though U_o^{max} changed if the floc size distribution remained the same. However, one contribution to this discrepancy was that the bacterial culture in the reactor was growing during this time and new growth in the reactor tended to be of a smaller floc size. This occurred because small, unsettleable flocs were removed from the reactor during the settling period but tended to regrow after feeding. It was not possible to evaluate a complete floc size distribution for each respirometric test because of the time involved (up to four hours to evaluate a single distribution). The development of a more dispersed culture as time progressed in the reactor does explain a trend

of increasing D_o/ρ with time after feeding because increased values of D_o/ρ reflect reduced diffusional limitations.

A second probable contribution to the discrepancy described above was that the optimum value of D_o/ρ was a "fitted" parameter and, as such, tended to be adjusted for all differences between the theoretical model and the actual system. Therefore, the behavior of the model with the fitted optimum value of D_o/ρ was not expected to be in perfect accord with the theory.

DISCUSSION AND CONCLUSIONS

Reactor Operational Behavior and Filamentous Bulking

A frequent problem in the operation of activated sludge systems is the occurrence of large numbers of filamentous bacteria that leads to settling and compaction problems (filamentous bulking). This problem is acute in the operation of laboratory-scale activated sludge systems and is frequent in the full-scale treatment of many high-strength industrial wastewaters (Pipes, 1978). Within this phase, three operational modes were tested for a laboratory-scale activated sludge system using soluble phenol as the sole substrate. Filamentous growth was a problem in each of these runs to some degree and two possible causes were identified.

Operation in the continuous flow mode (Run 2-1) resulted in very low concentrations of the soluble substrate in the completely-mixed aeration basin. Filamentous growth and bulking was a major problem within this run and the activated sludge culture eventually became dominated by filamentous bacteria. The fundamental cause for this was

postulated to be diffusional limitations for the substrate within acti-
vated sludge floc. Because the bacteria that form large, settleable
floc were diffusion-limited, the growth of bacterial forms that were
able to overcome these diffusional limitations by extending out into
the bulk medium (filaments) was preferred. The substrate (phenol) con-
centration in the reactor was so low that floc-forming bacteria were
eventually eliminated from the system and filamentous bacteria became
totally dominant.

The fundamental reason for the occurrence of very low substrate
concentrations in Run 2-1 was that the activated sludge culture was
highly adapted with an apparent half-velocity substrate concentration
(K_s) of less than 1.0 mg/ℓ. Consequently, the occurrence of diffu-
sional limitations for the substrate, and the postulated resulting
occurrence of filamentous bulking, can be correlated with the degree of
adaptation (as exhibited by a low value of K_s) of an activated sludge
culture. This may explain claims that certain types of substrates are
conducive to filamentous bulking in that highly adapted cultures typi-
cally develop. Within this study, the use of a highly adapted seed
explains the rapid development of a low effluent substrate concentra-
tion and of filamentous bulking problems.

Operation in the pulsed-feed mode (Run 2-2) was designed to pre-
vent the occurrence of continuously low bulk substrate concentrations
that occurred in Run 2-1 and thereby eliminate diffusional limitations
for the substrate and the resultant filamentous bulking problem. How-
ever, a second cause of filamentous growth was identified within this
run as large numbers of filaments were observed to project from large
flocs. The extent of filamentous coverage of flocs increased with time

of operation and with increasing size of the flocs until the settling behavior of the culture was so poor that bulking occurred and substantial bacterial solids were lost from the reactor. This pattern was repeated in a cyclic manner. The reason why filamentous attachment to large flocs occurred and eventually resulted in bulking is not known with certainty. However, a reasonable explanation is that only large flocs were capable of settling in the clarifier with a substantial filamentous attachment while the attachment of large numbers of filaments to smaller flocs rendered them unsettleable and resulted in their loss in the effluent.

Operation in the fill-and-draw mode improved the stability and settleability of the activated sludge cultures greatly. Substantial numbers of filaments were still present as projections from large flocs; however, the low concentration of bacterial mass in the reactor resulted in essentially discrete settling of floc particles and appeared to be more effective in eliminating highly filamentous floc that settled poorly from the system.

An interesting result of the ultrasonication and blending tests in Runs 2-2 and 2-3 was that both ultrasonication and blending were effective in shearing filaments from large flocs, even when their performance in reducing the size of the major floc mass was marginal. It is possible, therefore, that incorporation of higher shear forces within activated sludge aeration basins may be a practical method to reduce filamentous bulking problems.

To summarize, a first and primary cause of filamentous bulking is diffusional limitations in large floc. Chudoba, et al. (1973) and Houtmeyers, et al. (1980) have demonstrated that filamentous bulking

commonly results in laboratory activated sludge systems that utilize completely-mixed aeration basins and are continuously fed a synthetic substrate. Sezgin, et al. (1978) developed the correlation between diffusional limitations and filamentous bulking, although oxygen was emphasized as the diffusion-limiting species within their study. The frequent occurrence of filamentous bulking in laboratory activated sludge systems that utilize soluble feeds can frequently, the author believes, be attributed to diffusional limitations for the substrate. As the culture becomes highly adapted to the feed in these systems, the bulk substrate concentration in a completely-mixed reactor becomes extremely low and diffusional limitations become significant. Diffusional limitations for the carbonaceous substrate may also be the cause of filamentous bulking in the treatment of industrial wastewaters with a relatively constant composition of soluble substrates. Conversely, the variability of the substrates in the influent wastewater and the association of a large portion of the total organics with particulate matter may be a fundamental reason why full-scale activated sludge plants, particularly municipal plants, encounter filamentous bulking problems only occasionally. The inability of the cultures to adapt rapidly to changing substrates and the need to solubilize particulate matter before it can be transported across the cell membrane may prevent the utilization of substrates within most full-scale activated sludge plants to concentrations where diffusional limitations for the substrate become significant.

Diffusional limitations for oxygen may also lead to filamentous bulking, as demonstrated by Sezgin, et al. (1978) and Palm, et al. (1980). The filamentous bulking problems that occurred during this

phase, however, were not related to the bulk DO maintained and diffu-
sional limitations for oxygen is not a reasonable explanation.

The Anoxic Core Model

The anoxic core model provided a reasonable theoretical basis for
the response of activated sludge cultures to the bulk DO. Furthermore,
the model fit to the respirometric data was very good within this
phase. Two discrepancies between the model and the respirometric data
were observed in Runs 2-2 and 2-3, however, that require further dis-
cussion. First, there was a general tendency for the fitted model to
predict higher than actual values of U_o for bulk DO levels below the
$C_o^{0.5}$ and lower than actual values of U_o for high bulk DO levels.
Second, the optimum value of D_o/ρ for fitting the anoxic core model to
the respirometric data in Runs 2-2 and 2-3 varied over an order of mag-
nitude within both runs and appeared to exhibit a strong correlation
with floc size.

Figure 7-30 illustrates the correlation between the optimum value
of D_o/ρ and floc size, here presented as the average spherical equiva-
lent floc diameter for a given respirometric test. The dependence of
D_o/ρ on floc diameter is apparent within this figure. Several previous
researchers have found that the density of bacterial flocs tends to
decrease as the diameter increases (Lamotta and Shieh, 1979) and this
appears to be the fundamental reason for the correlation of D_o/ρ with
floc diameter within this study. Furthermore, the diffusivity of bac-
terial aggregates must be expected to exhibit an inverse proportionality
to the density. This explains the large variation in D_o/ρ as larger
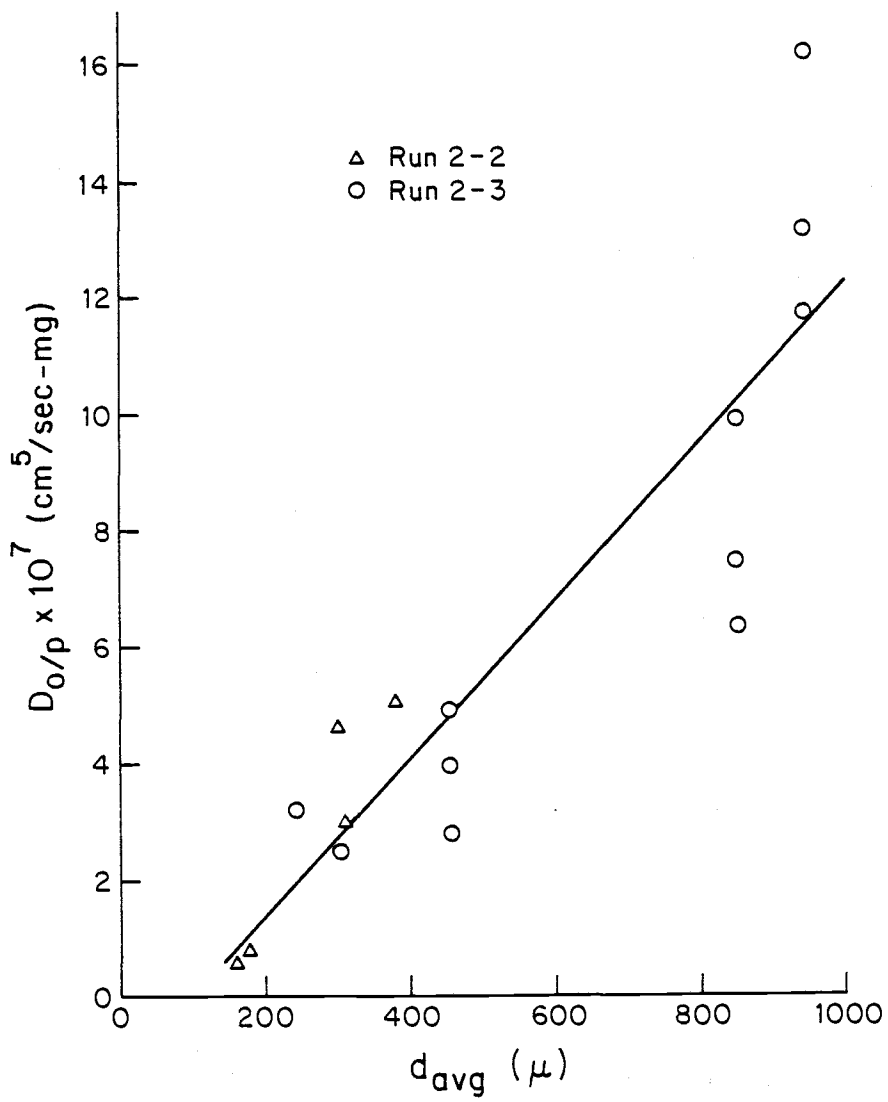
Figure 7-30. Comparison of Optimum Value of D_o/ρ with
Average Floc Size in Respirometric Tests.

bacterial floc are not only of lower density but also of higher diffusivity.

The correlation between D_o/ρ and floc diameter described above also explains the tendency of the model to predict higher than actual values of U_o when $C_{b,o}$ is high. Large flocs control the response of the culture at high bulk DO levels because these are the first to become diffusion-limited. The optimum value of D_o/ρ, however, reflects the density and diffusivity of the average-sized floc particle and when applied to the larger flocs, the extent of diffusional limitations is overestimated and the model predicts larger reductions in U_o than actually occur. Conversely, as the bulk DO becomes small, the smaller floc particles become diffusion-limited and tend to control the respirometric response of the culture. Application of the average value of D_o/ρ to these small flocs underestimates the extent that diffusional limitations are occurring; consequently, the model predicts higher than actual values of U_o at very low bulk DO levels.

Further applications of diffusional models to the activated sludge process must consider the dependence of floc density on the floc size. Additional data is needed to determine more exactly the relationship between floc density and floc size and the fundamental reason for this relationship.

Conclusions

1. The anoxic core model, as developed within this study, reasonably describes the response of bacterial aggregates such as occur in the activated sludge process to the bulk dissolved oxygen concentration.

2. The major problem involved in application of the anoxic core model is that the density of activated sludge floc decreases (and the diffusivity increases) as the diameter increases.

3. Diffusional limitations for oxygen are possible in activated sludge systems if the floc are large and respire rapidly. Evaluation of the potential for oxygen diffusional limitations, however, must consider the actual floc size and respiration rate of the culture in question.

4. Floc diameters in laboratory-scale activated sludge systems range up to about 1200 microns but the size distribution that develops depends on the type of reactor, operational mode, and on other operational parameters and startup methods.

CHAPTER VIII

PHASE 3 EXPERIMENTS

INTRODUCTION

The results of the Phase 2 experiments indicated that substantial oxygen diffusional limitations occur in large bacterial floc that are respiring rapidly. However, application of these results to full-scale activated sludge plants, where respiration rates are substantially smaller than those involved in the Phase 2 laboratory experiments, indicated that the bacterial floc must be quite large to produce diffusional limitations at the operational DO levels typical of air activated sludge plants. Furthermore, the small amount of data that is available indicates that floc sizes in full-scale activated sludge plants are typically much smaller than those observed within the laboratory reactors in Phase 2 of this study (see Chapter III). Therefore, evaluation of the results from Phase 2 indicated that the critical DO of typical full-scale activated sludge plants is probably below the 1-2 mg/ℓ level that is typical of air activated sludge operation and that oxygen diffusional limitations generally do not occur in these plants. However, it was considered possible that large flocs are present in full-scale activated sludge plants or that bacterial density within these flocs may be substantially higher than that found within the laboratory activated sludge systems operated in Phase 2 of this study. Consequently, it was desired to directly assess the possible occurrence of oxygen diffusional limitations within activated sludge cultures at full-scale plants.

The general research plan for Phase 3 was to obtain samples of activated sludge from several full-scale plants and to subject these samples to respirometric and other batch tests that would allow evaluation of the DO levels at which oxygen diffusional limitations occur. Evaluation of these results was expected to provide general conclusions regarding the minimum DO levels that are needed in typical activated sludge plants to maintain maximum bacterial respiration rates and regarding the claimed advantages of pure oxygen activated sludge systems.

EXPERIMENTAL PROCEDURES

Two basic tests were utilized within this phase. The first was the simple batch respirometric test, similar to those performed in Phase 1 and Phase 2. For these tests, grab samples of return sludge and primary effluent were obtained from the plant and combined in approximate proportion to the operating R/Q ratio (return sludge flow divided by primary effluent flow) at the plant. The initial conditions of this respirometric test, including substrate and biomass concentrations, were then identical to those at the initial point in a plug-flow aeration basin. Because the maximum respiration rate occurs at this initial point in a plug-flow aeration basin, the conditions existing in this respirometric test were the most severe, in terms of producing oxygen diffusional limitations, that could occur at the plant.

The basic output from the respirometric tests is a plot of bulk DO versus time for given initial conditions. Under certain conditions, this plot of bulk DO versus time may be converted into a plot of specific oxygen uptake rate versus the bulk DO (see Figure 5-4 and related discussion). The primary condition for this conversion is that no

significant reduction in specific respiration rate occurs during the respirometric test as a result of substrate depletion; that is, reductions in specific respiration rate must be solely attributable to oxygen limitations. In practical terms, this means that the substrate concentration in the mixed liquor sample inserted into the respirometer must be high enough to prevent significant reductions in the substrate concentration over the time period required for the respirometric test. The ability of primary effluents from the plants surveyed to maintain a constant respiration rate over the time period required for the respirometric test was doubtful, particularly for plants treating low-strength domestic wastewaters. Consequently, a synthetic feed solution consisting of 1000 mg/ℓ glucose was prepared as an alternate feed where low-strength primary effluents were encountered.

The second test that was conducted was a batch reactor simulation of a single pass through a plug-flow aeration basin. Grab samples of primary effluent and return sludge were obtained and combined in each of the two batch reactors in proportion to the plant's operating R/Q ratio (see Figure 5-2 for an illustration of the batch reactors). The DO in one batch reactor was set and controlled at a high level (8 or 15 mg/ℓ) and the DO in the other reactor at a lower level more typical of air activated sludge operation (1 or 2 mg/ℓ). Temperature in the batch reactors was controlled at 20°C. Specific respiration rates and specific ATP concentrations were then measured as a function of time.

To measure the specific respiration rate occurring within the batch reactor at any given time, an aliquot was withdrawn from the batch reactor and briefly aerated to increase the DO by 1-2 mg/ℓ. The aliquot was then inserted in a respirometer bottle and the DO depletion versus

time recorded. The specific respiration rate was then calculated as the slope of the DO versus time curve at the operational DO of the batch reactor.

Samples of primary effluent collected for purposes of conducting the previously-described respirometric and batch tests were analyzed for TSS and total COD. Return sludge samples were analyzed for TSS and for floc size distribution. Additionally, flow rates for the primary effluent and return sludge streams at the plant were noted for purposes of calculating the appropriate mixing ratio in the laboratory tests.

These tests were conducted at three separate plants in the immediate vicinity, including the Salem, Oregon Waste Water Treatment Plant (WWTP), the Albany, Oregon WWTP, and the Corvallis, Oregon WWTP. For the Corvallis and Albany plants, samples of primary effluent and return sludge were taken at the plants and transported to the university laboratory because the transportation time was less than 20 minutes. An experimental trailer containing all the necessary experimental equipment was set up and transported to the Salem, Oregon plant site for purposes of conducting the tests at that plant. Further details of the experimental procedures at each plant are described in the sections below.

Plant data and data associated with the respirometric and batch tests conducted at the three plants is contained in Appendix E for reference.

SALEM, OREGON WWTP

Description of Plant

The Salem, Oregon plant provides primary clarification and secondary biological treatment for wastewaters from the Salem metropolitan area, including a substantial industrial wastewater contribution. Secondary biological treatment is provided primarily by a pure oxygen activated sludge process. The pure oxygen activated sludge facility consists of three trains, each containing four completely-mixed cells in series. Mix and aeration in the cells is provided by prop-type mixers.

The primary industrial wastewater sources are several canneries and these provide a substantial organic loading during the late summer to early fall canning season. During this period of the year, the operators maintain extremely high DO levels in the aeration basin and contend that these are necessary to provide efficient and stable treatment of the high organic loadings received at the plant. Historically, performance of the pure oxygen activated sludge facility has been very good with an SVI typically less than 100 and effluent BOD_5 and TSS below 30 mg/ℓ on a monthly average basis.

Tests were conducted at the Salem, Oregon WWTP during the period of August 4 through August 8, 1980. During this period, large but intermittent quantities of high-strength beet canning wastewater were being received at the plant. The periods during which large quantities of beet-canning wastewater were being received was very noticeable as the influent and primary effluent became very red in color and the

tests described previously were generally performed during these periods.

During this five-day testing period, plant records showed that the primary effluent flow to the pure oxygen activated sludge plant was in the range of 13 to 18 MGD and the primary effluent BOD_5 was generally in the range of 100 to 150 mg/ℓ, except on August 8 when it was 278 mg/ℓ. These BOD_5 averages are somewhat misleading, however, in that much higher organic loadings were received during several periods as the result of cannery operation. Only two of the three aeration basin trains were in operation during this period and the aeration basin detention time averaged about three hours. Because the operators were anticipating bringing the third aeration basin train on line in the near future, they were wasting only small quantities of mixed liquor and the calculated SRT was between 30 and 60 days, which is longer than normal operation. The F:M ratio was in the range of 0.1 to 0.4 g BOD_5 removed/g MLVSS-day. The settling characteristics of the activated sludge were excellent, with an SVI of about 60 ml/g and a return sludge concentration of about 1.8 percent. The activated sludge culture contained essentially no filaments. The recycle (R/Q) ratio was 25-35 percent. A list of plant data as measured and compiled by the operators of the Salem plant during the period August 1 to August 10, 1980, is given in Table E-1 (Appendix E).

Description of Tests and Results

Testing began on Monday, August 4, 1980 and continued through Friday, August 8. The cannery wasteload during this week was initially light but became heavier and more consistent later in the week.

Results of the floc size measurements on August 4 are shown in Figure 8-1. The maximum floc diameter on this day was 150 microns and the median was in the 60-100 micron range.

As previously described, samples of return activated sludge and primary effluent from the plant were aerated (or oxygenated), combined in the respirometer bottle in proportion to the plant's operating R/Q ratio at the time the samples were taken, and the DO depletion recorded as a function of time. The resulting DO versus time curve is plotted in Figure 8-2. As shown, the DO versus time response was linear down to or below a DO of about 1.0 mg/ℓ, illustrating that sufficient substrate was present to maintain a relatively constant respiration rate over the time required to complete the respirometer test. Also, these results indicated that the DO at which oxygen diffusional limitations began was quite low. A second respirometer test was run with more frequent measurements of DO and the DO versus time curve was converted into a plot of specific respiration rate versus bulk DO according to previously described procedures (Chapter V). These results are shown in Figure 8-3 and illustrate a critical DO (C_o^c) of about 0.9 mg/ℓ, a 90-percent velocity DO ($C_o^{0.9}$) of about 0.5 mg/ℓ, and a half-velocity DO ($C_o^{0.5}$) of about 0.2 mg/ℓ. The maximum respiration rate (U_o^{max}) was 16.6 mg/g-hr.

On the second day of testing at Salem (August 5, 1980), both respirometric and batch tests were conducted. Some cannery wastes were being received during these tests as evidenced by a higher COD and a pink color in the primary effluent. Results of the respirometric test again indicated a constant respiration rate over the time period required to complete a respirometric test. Consequently, a specific
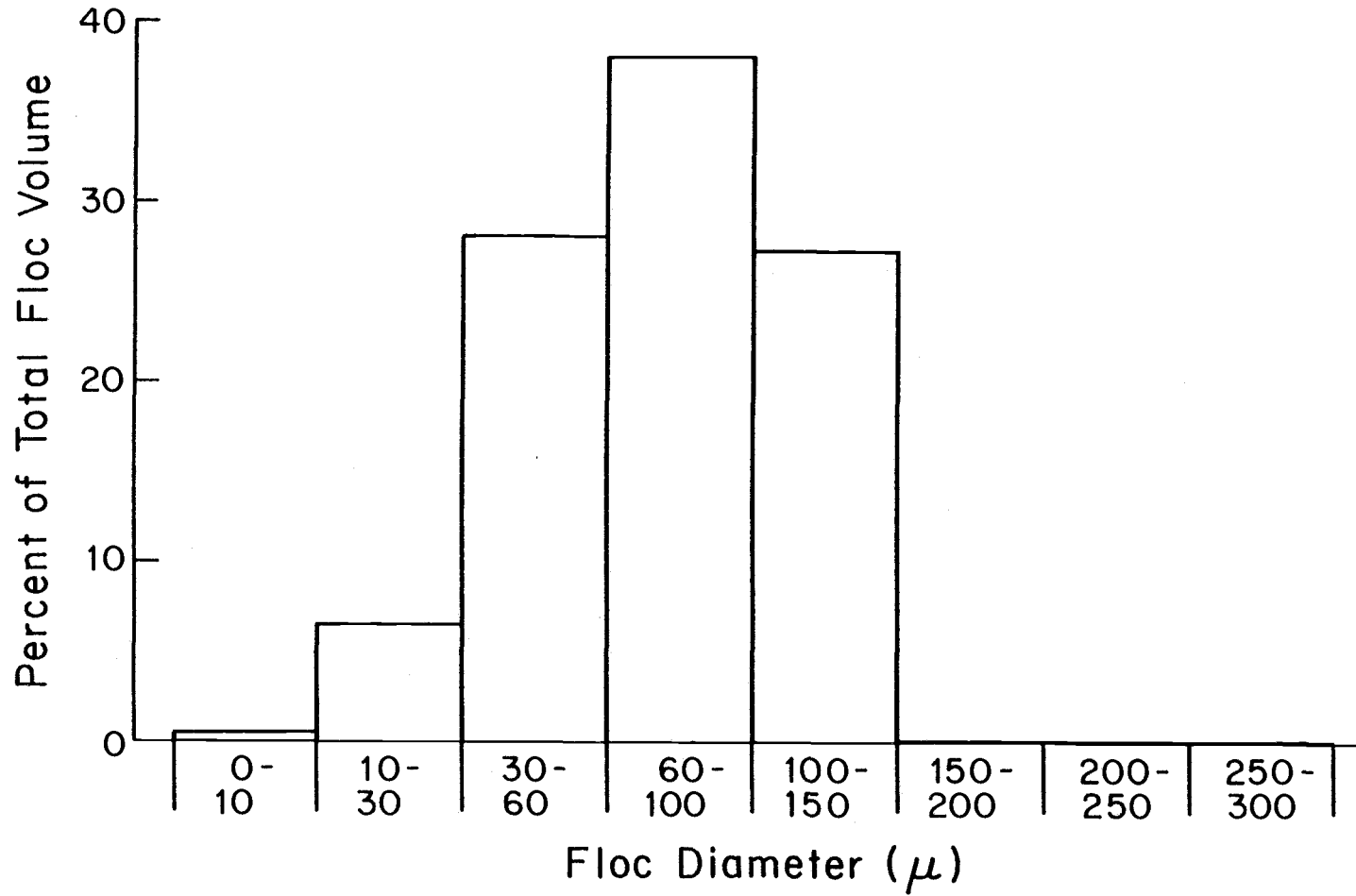
Figure 8-1.   Floc Size Distribution at the Salem WWTP on 8/4/80.
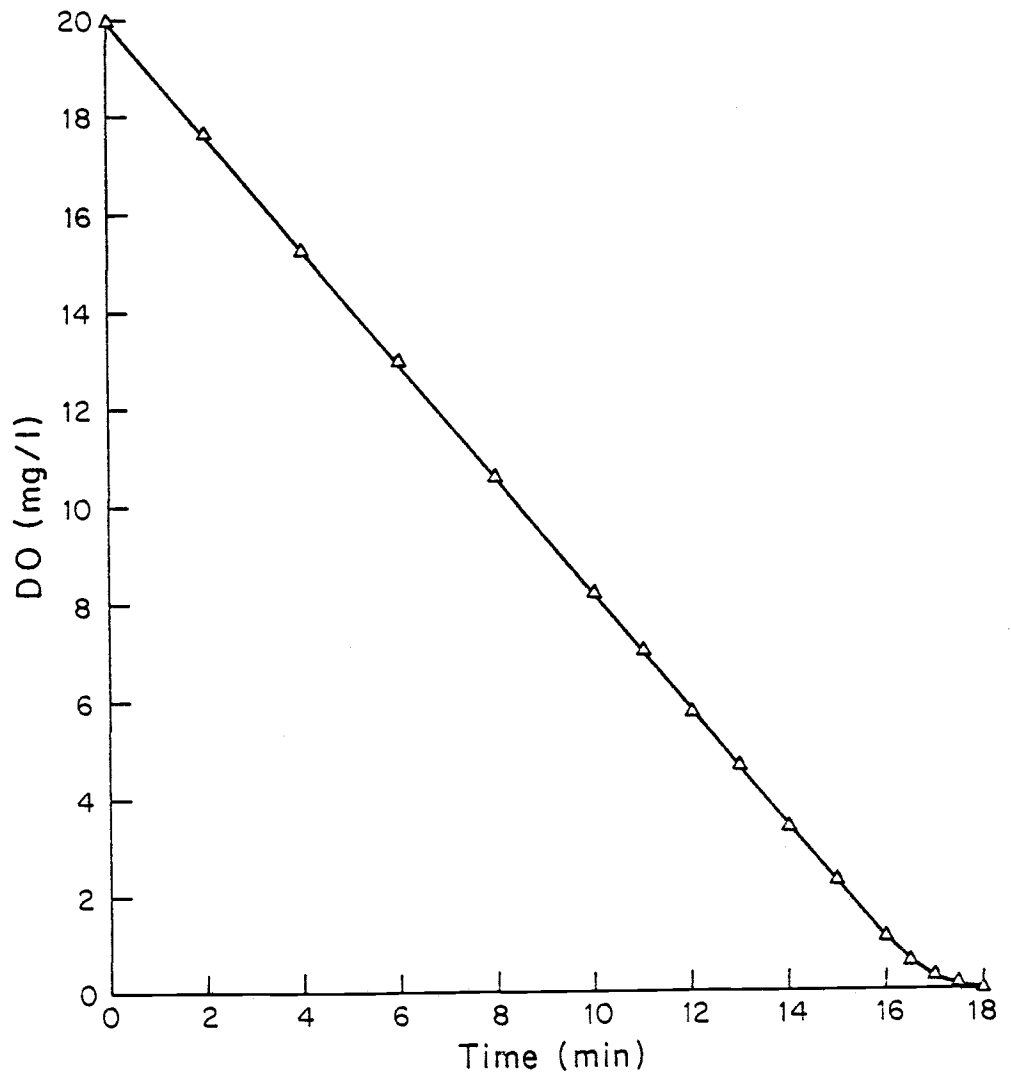
Figure 8-2. DO versus Time in Respirometric Test No. 1 at the Salem WWTP on 8/4/80.
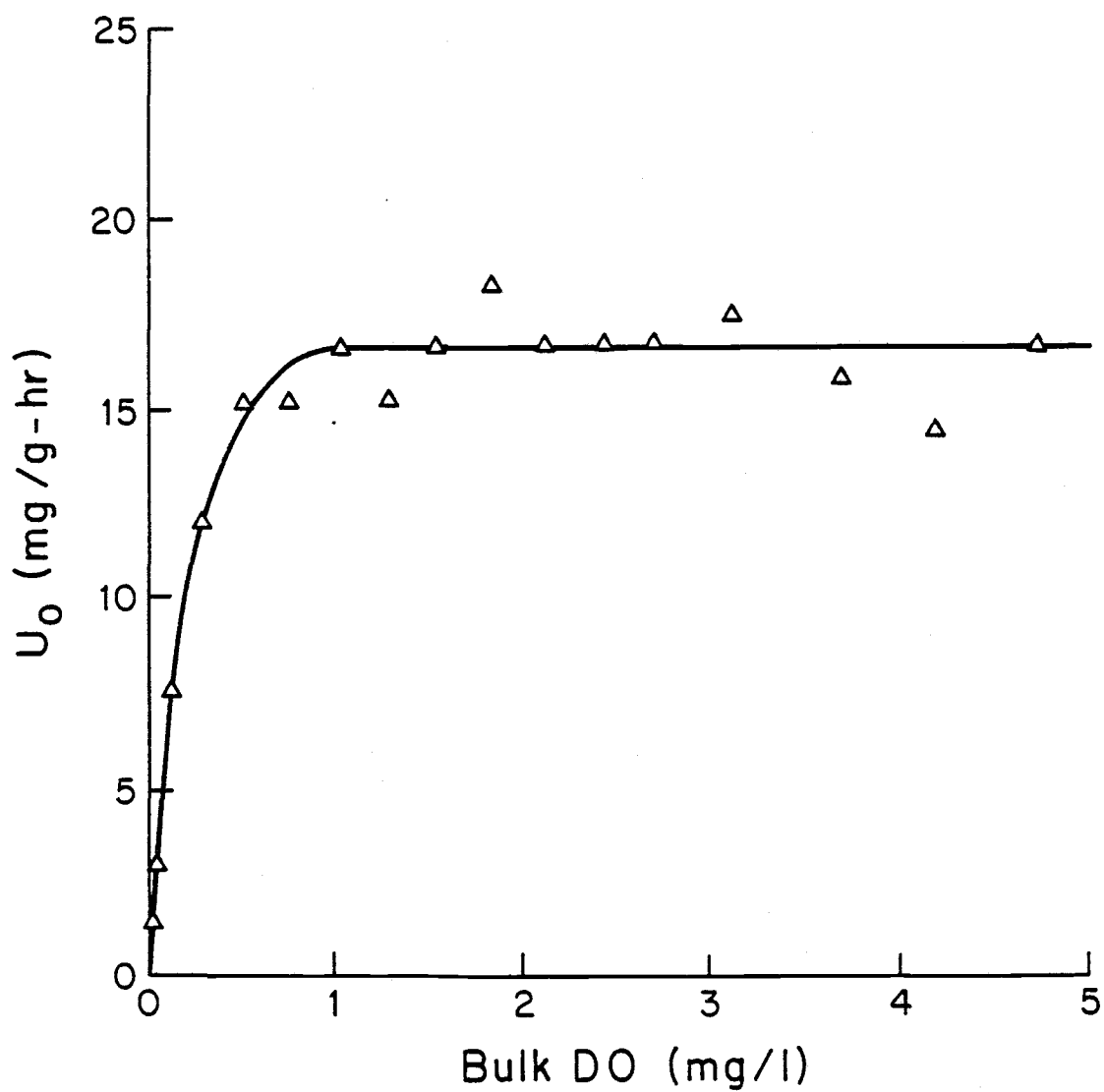
Figure 8-3. Specific Respiration Rate versus Bulk DO for Test No. 2 at the Salem WWTP on 8/4/80.

respiration rate versus time curve was developed as shown in Figure
8-4. These results indicated a maximum respiration rate of 23.4
mg/g-hr, slightly higher than the previous day, a C_o^c of about 1.0 mg/ℓ,
and $C_o^{0.9}$ of about 0.5 mg/ℓ, and a $C_o^{0.5}$ of about 0.2 mg/ℓ.

Batch tests (plug flow simulations) were also performed on August
5, 1980. These batch tests were conducted over a period of three hours,
which was the approximate detention time in the aeration basin at the
plant. Batch Reactor A was controlled at a DO of 15 mg/ℓ and Reactor
B at 2.0 mg/ℓ. The specific respiration rate and specific ATP content
measured during this batch test are presented in Figures 8-5 and 8-6.
As shown, the specific respiration rates in Reactors A and B were very
similar, as the results of the respirometer study would predict. Fur-
thermore, the specific ATP concentrations were very similar, indicating
a rapid climb during the first one-half hour as the microorganisms
respired rapidly and replenished their ATP supply, and exhibiting a
slight drop-off after about two hours as the substrate became depleted
and endogenous metabolism began.

The floc size distribution on August 6, 1980 was similar to that
previously measured with a maximum floc size of 150 µ and a median floc
size in the 60-100 µ size range (see Figure 8-7). A large cannery load
was being received on this day as evidenced by the very red color of
the primary effluent and the high COD of the primary effluent (486
mg/ℓ).

Data from the initial respirometer test on August 6, 1980 are
listed in Table E-7 of Appendix E. The maximum respiration rate (U_o^{max})
of nearly 70 mg/g-hr that was measured was substantially higher than
previous days as a result of the large cannery load. The volumetric
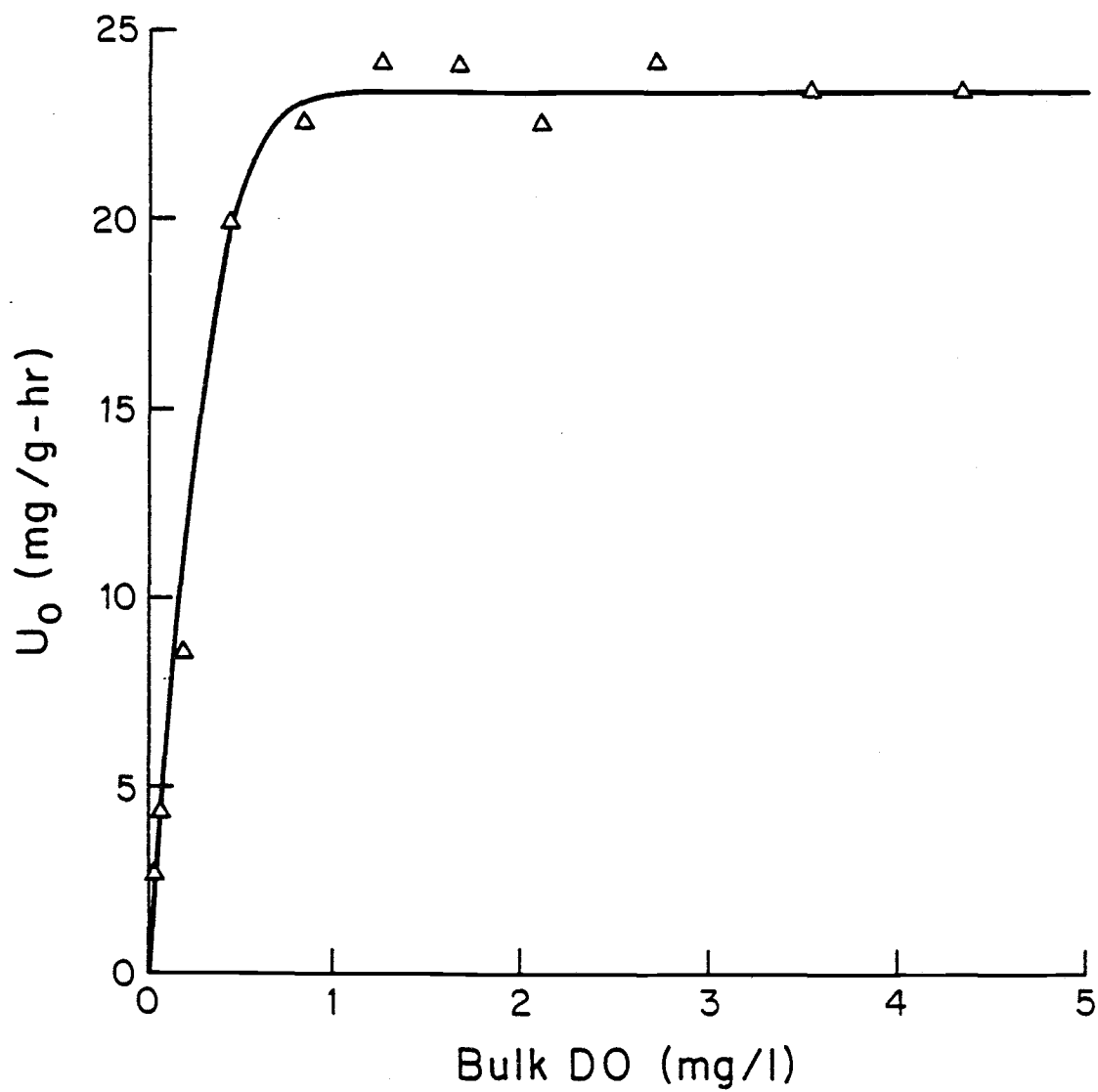
Figure 8-4. Specific Respiration Rate versus Bulk
DO at the Salem WWTP on 8/5/80.
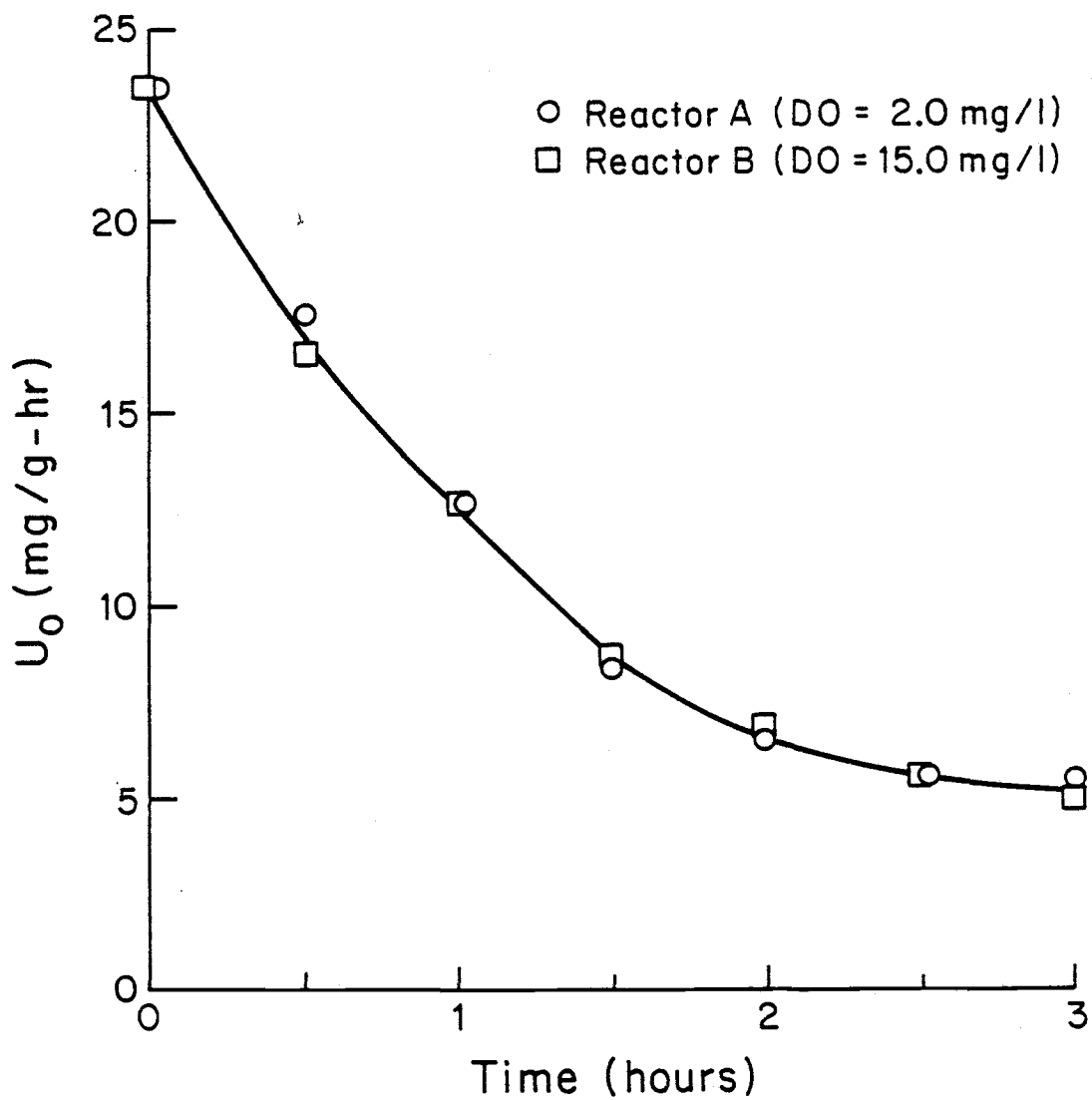
Figure 8-5. Specific Respiration Rate versus Time in the Batch
Reactor Simulation at the Salem WWTP on 8/5/80.
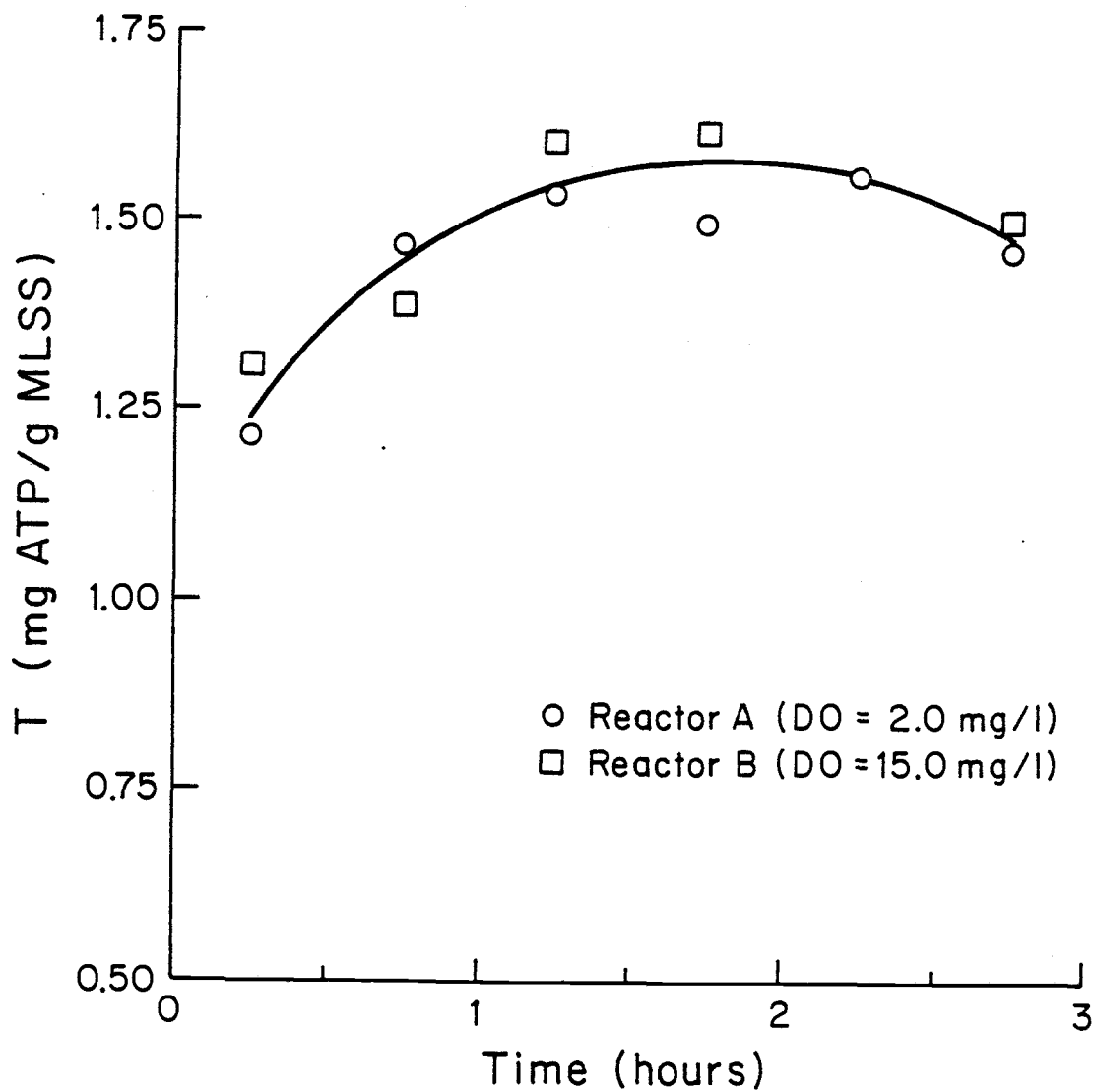
Figure 8-6. Specific ATP Concentration versus Time in the Batch Reactor Simulation at the Salem WWTP on 8/5/80.
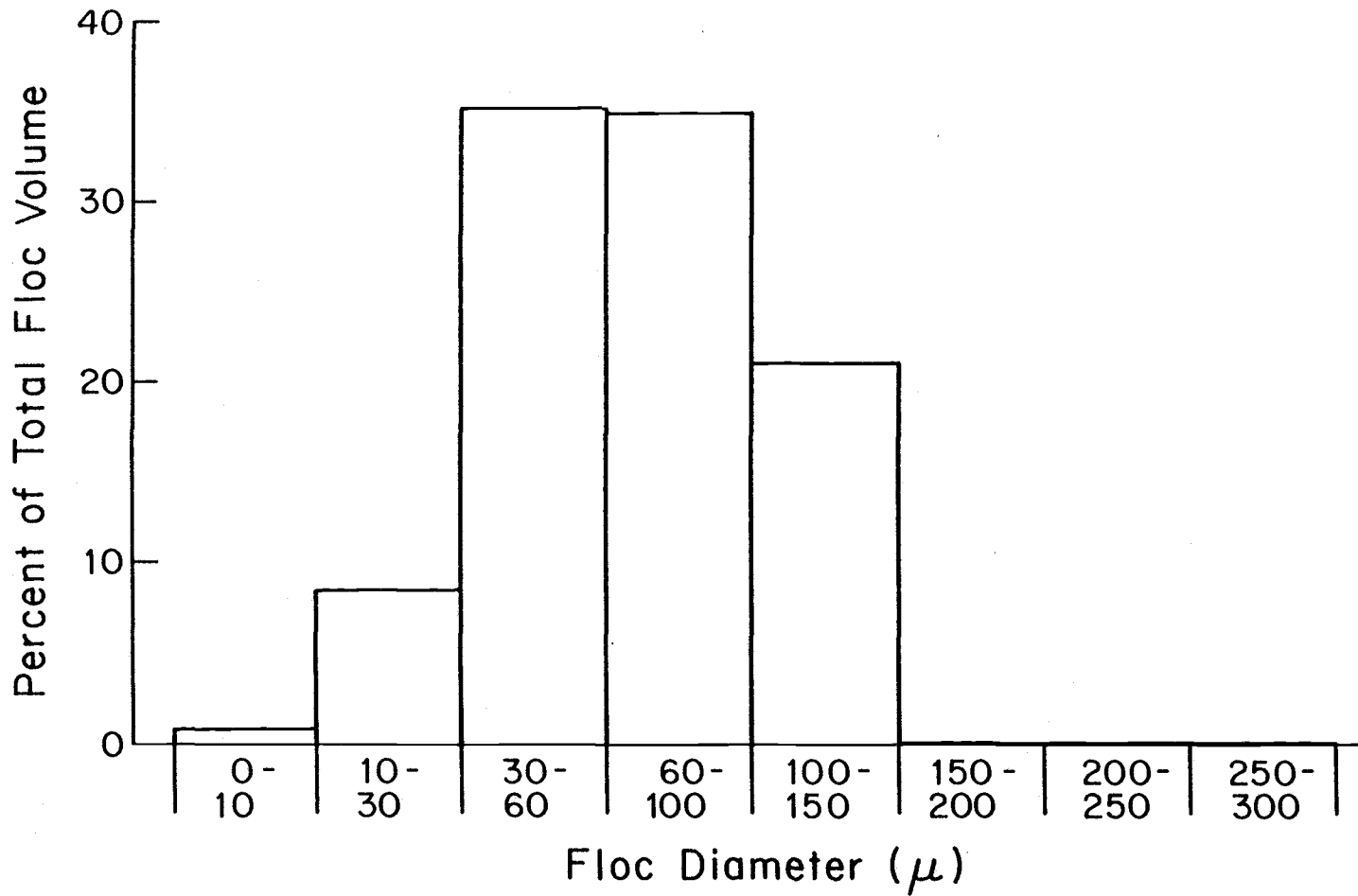
Figure 8-7.   Floc Size Distribution at the Salem WWTP on 8/6/80.

respiration rate was so rapid, in fact, that DO readings on the respirometer were difficult due to the rapidly falling DO concentration. Therefore, a second test was conducted with a diluted return sludge sample, which allowed easier and more accurate readings. Results of this second respirometric test are shown in Figure 8-8 as a plot of specific respiration rate versus the bulk DO. The values of C_o^c, $C_o^{0.9}$, and $C_o^{0.5}$ were about 1.4, 1.0, and 0.35 mg/ℓ, respectively, which were noticeably higher than previous days.

The floc sizes on August 7, 1980 at Salem were somewhat smaller than on previous days as shown in Figure 8-9. The maximum floc diameter was 100 μ and the median was in the 30-60 μ range. A heavy cannery load was again being received at the time the experimental tests were conducted and it was necessary to conduct the respirometric test on a diluted sample.

Results of the respirometric test on August 7, 1980 are shown in Figure 8-10. The values of C_o^c, $C_o^{0.9}$, and $C_o^{0.5}$ were about 1.2, 0.8, and 0.3 mg/ℓ, respectively, and the maximum respiration rate was 57.6 mg/g-hr. These values were similar to the previous day.

Batch reactor tests (plug flow simulations) were also performed on August 7, 1980. Conditions were the same as in the previous batch tests and the resulting plots of specific respiration rate and specific ATP content versus time are shown in Figures 8-11 and 8-12, respectively. As shown, there was no consistent difference in either respiration rate or ATP content between the batch reactor maintained at 2 mg/ℓ and the reactor maintained at 15 mg/ℓ.
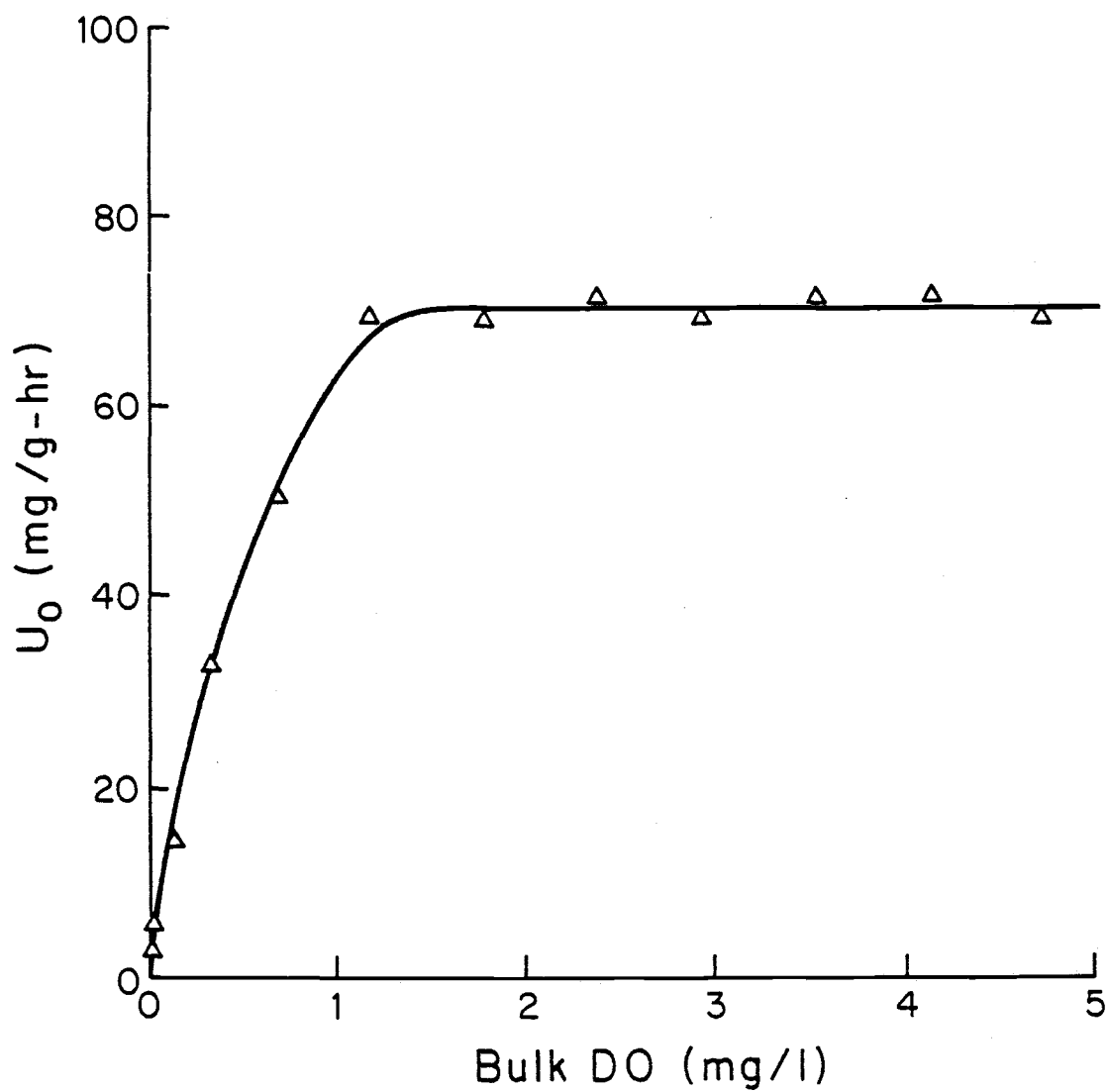
Figure 8-8. Specific Respiration Rate versus Bulk DO
for Test No. 2 at the Salem WWTP on 3/6/80.
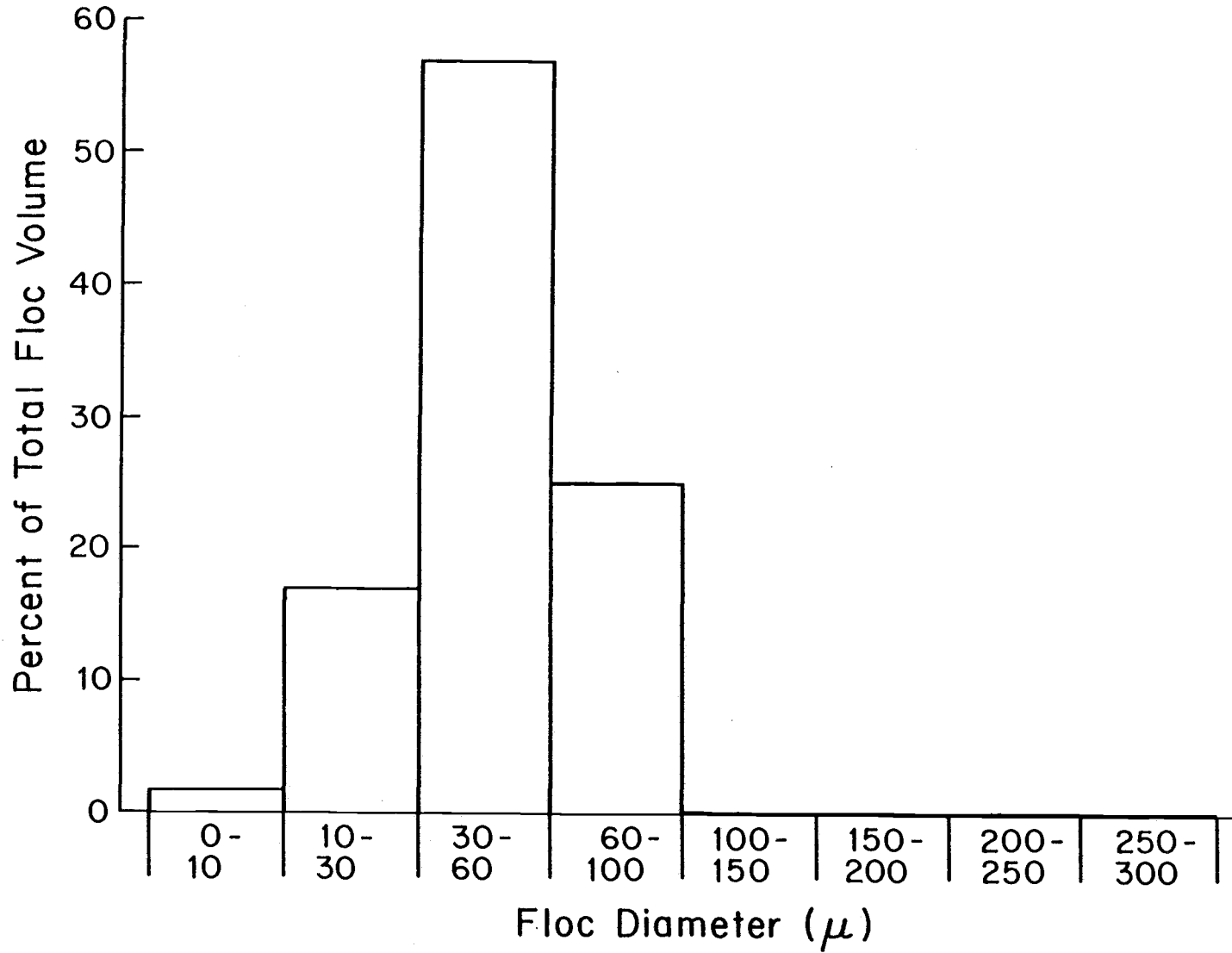
Figure 8-9.   Floc Size Distribution at the Salem WWTP on 8/7/80.
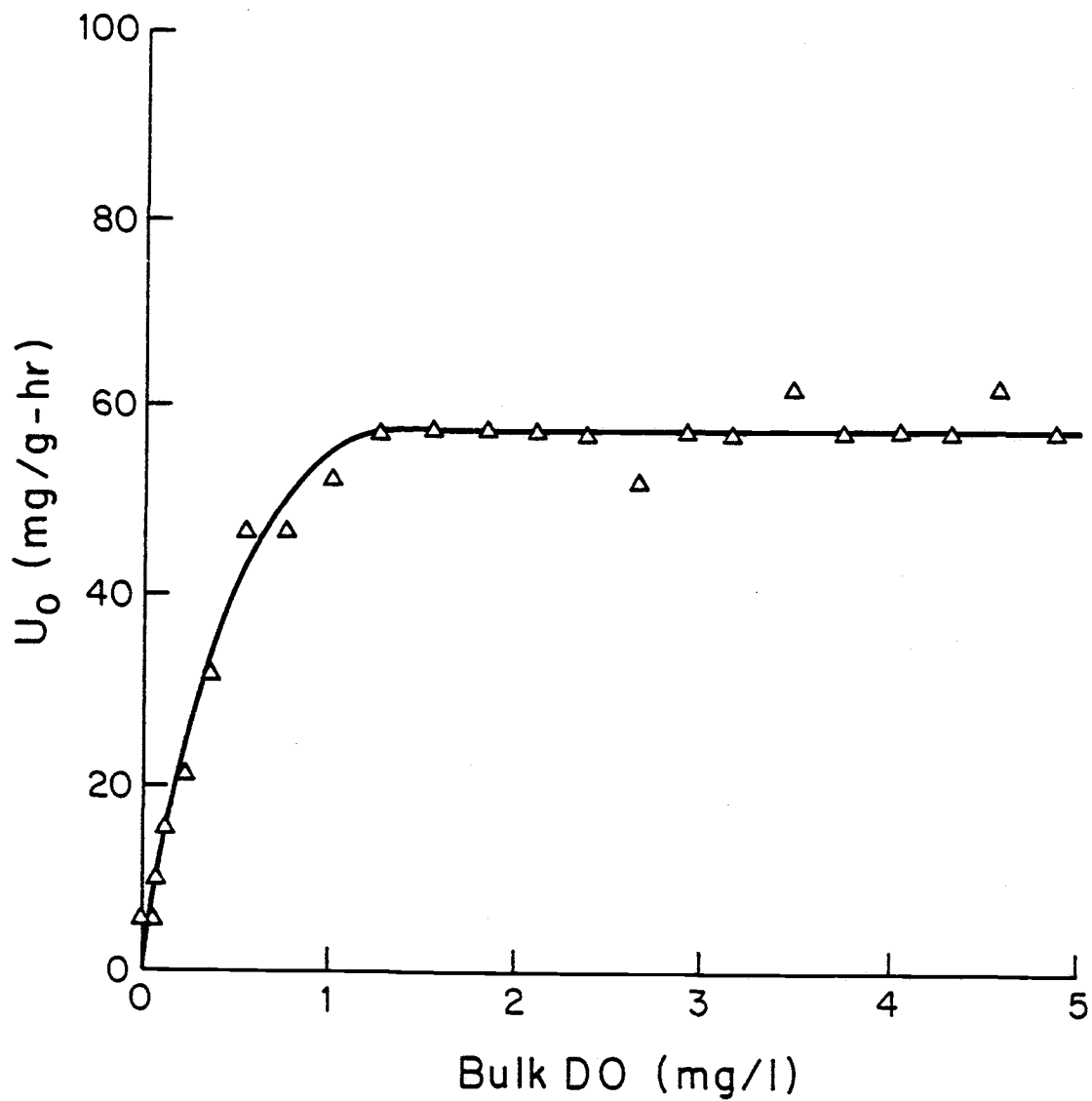
Figure 8-10. Specific Respiration Rate versus Bulk DO
for Test No. 2 at the Salem WWTP on 8/7/80.
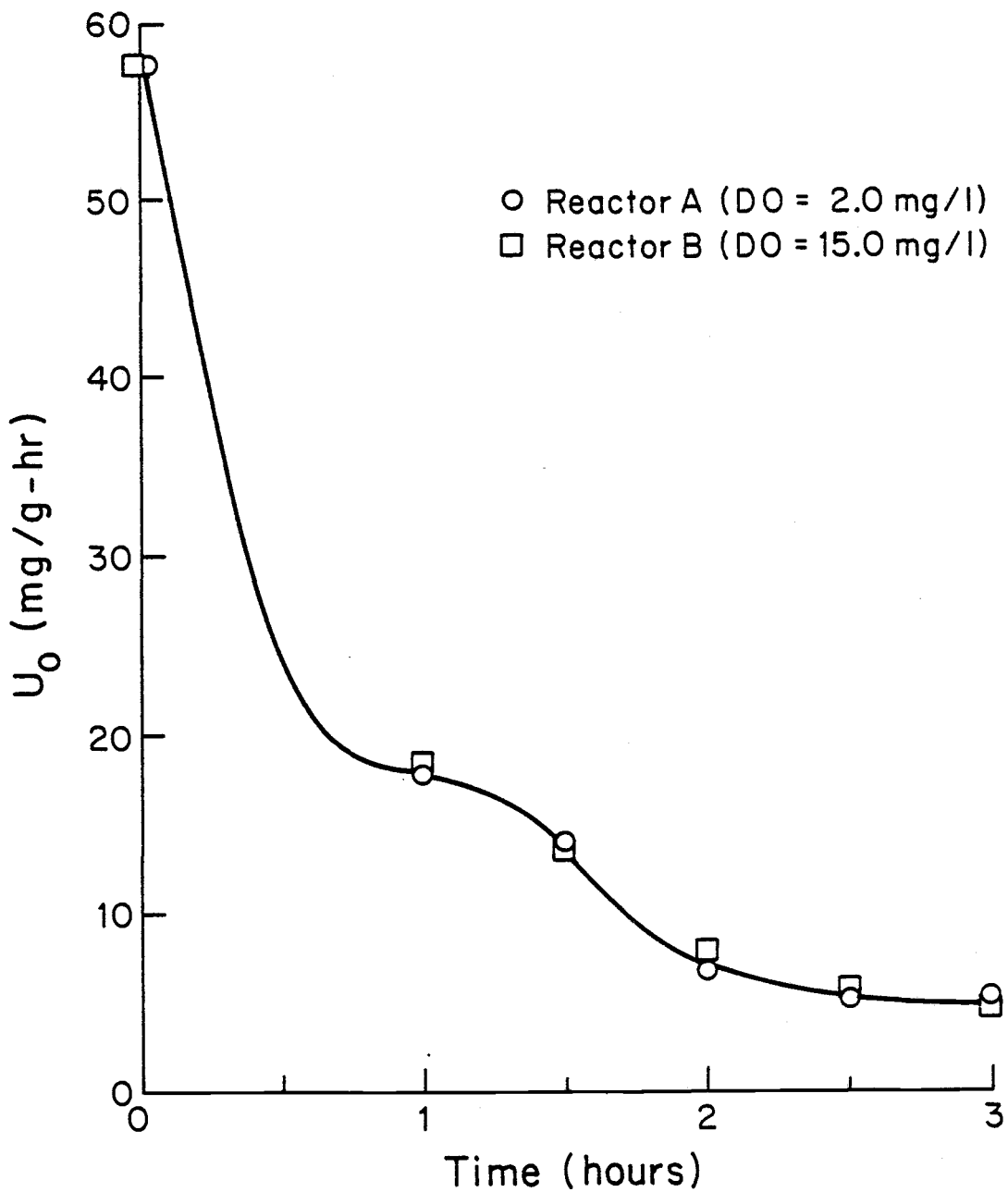
Figure 8-11. Specific Respiration Rate versus Time in the
Batch Reactor Simulation at the Salem WWTP
on 8/7/80.
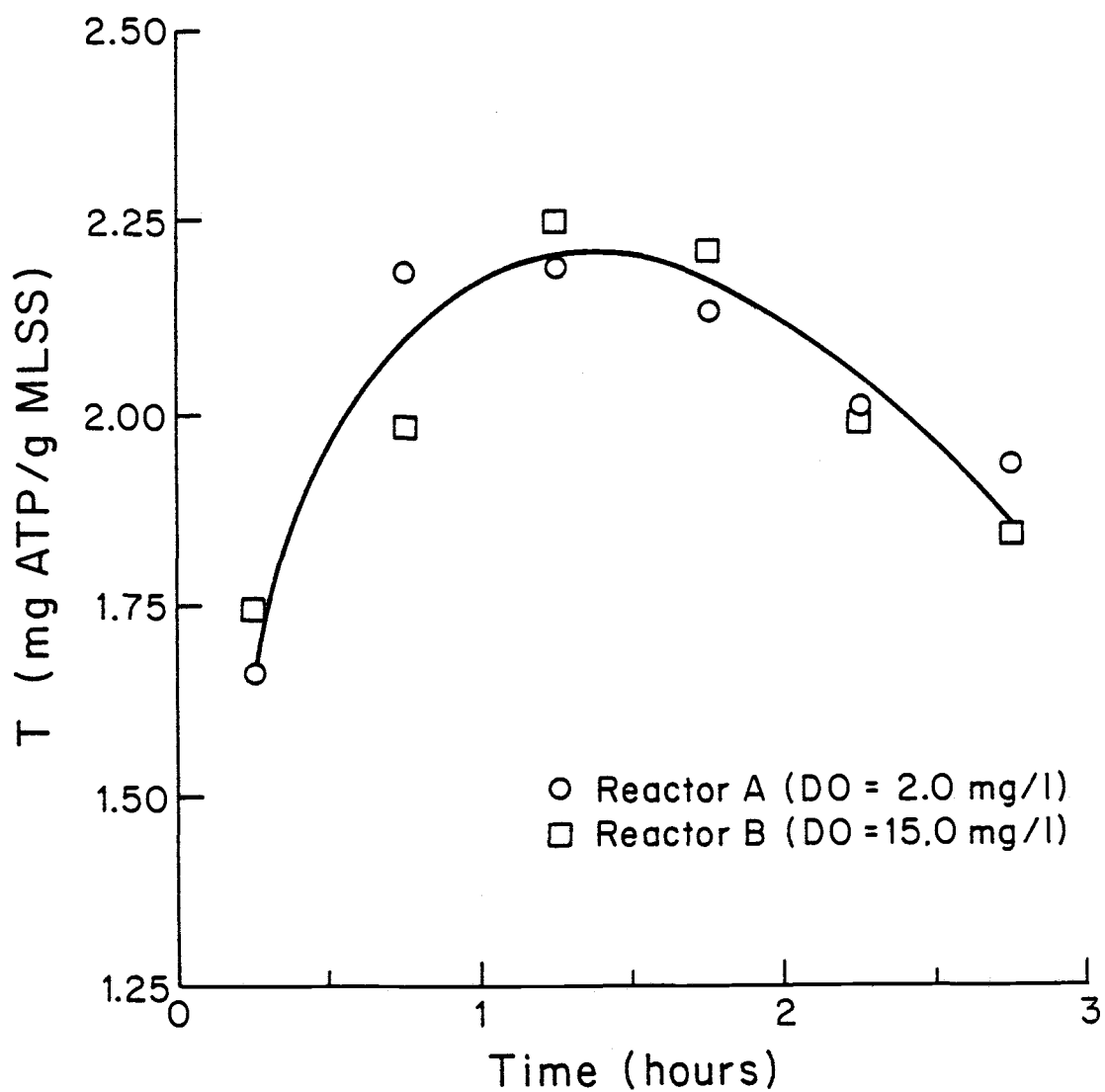
Figure 8-12.   Specific ATP Concentration versus Time in the
Batch Reactor Simulation at the Salem WWTP on
8/7/80.

ALBANY, OREGON WWTP

Description of Plant

The Albany, Oregon WWTP is a small, conventional activated sludge plant that treats primarily domestic wastewaters. The major liquid process unit operations are primary clarification followed by completely-mixed activated sludge with diffused air aeration.

Preliminary tests were conducted on samples obtained from this plant on December 28, 1980 and final tests on December 31, 1980. During this time the plant flow was about 8 MGD, the average primary effluent BOD_5 was about 80 mg/ℓ, and the wastewater temperature was 14°C. The plant was being operated at an F:M ratio of about 0.3, a return sludge flow of 20-25 percent, and a DO concentration in the aeration basins of 1-3 mg/ℓ. The operational performance of the plant was good during the testing period with an effluent TSS typically about 30 mg/ℓ, an SVI of about 100, and a return sludge concentration of about 1.0 percent. The activated sludge floc contained some filaments.

Description of Tests and Results

Batch respirometric tests and batch simulations of one pass through a plug-flow reactor system were performed on samples from the Albany WWTP similar to those performed at the Salem WWTP and previously described.

Final tests were conducted on December 31, 1980. Results of the microscopic floc size measurements on this date are illustrated in Figure 8-13. The median floc diameter on this day was in the 60 to 100 micron size range and the maximum floc size was 300 microns.
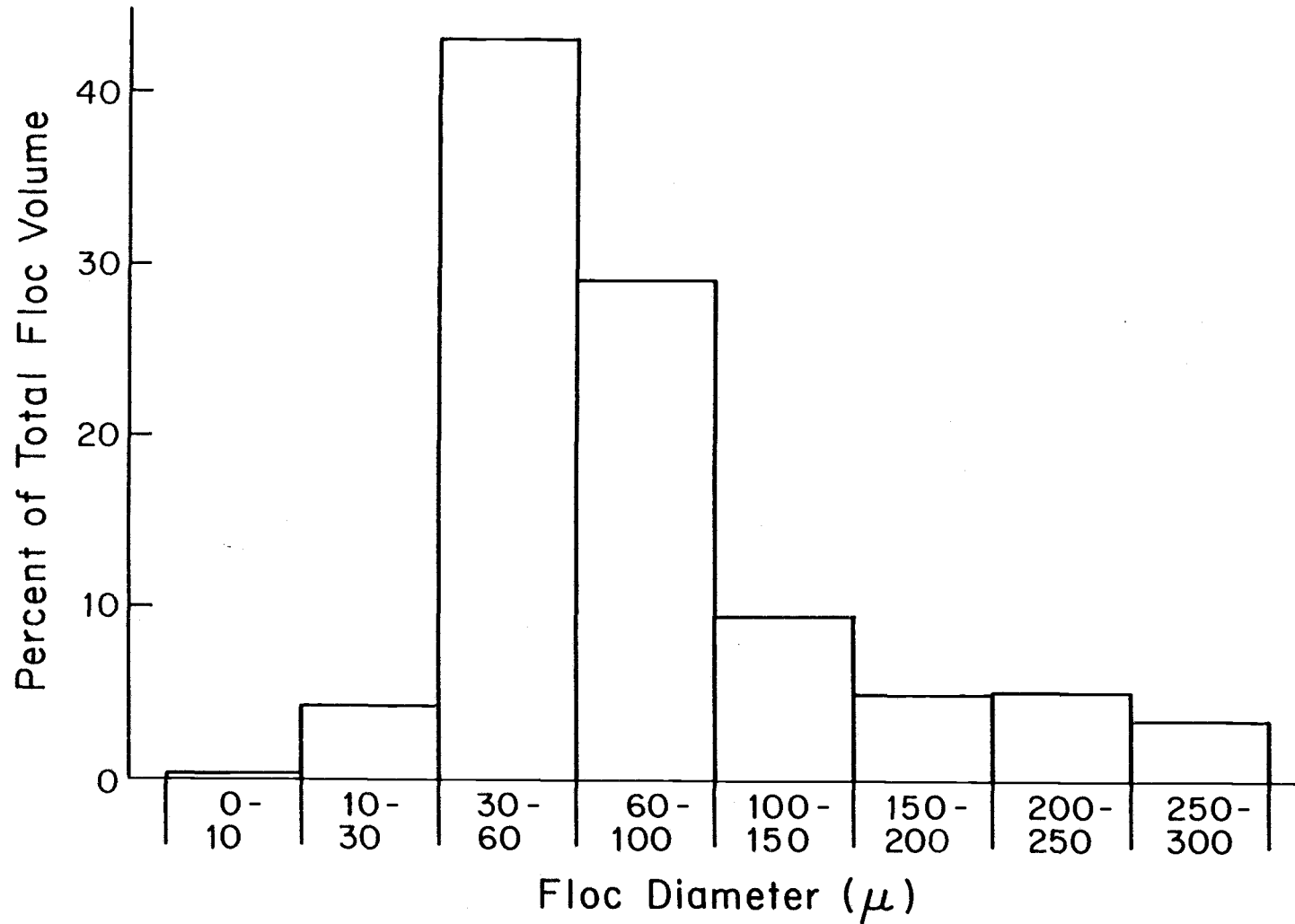
Figure 8-13. Floc Size Distribution at the Albany WWTP on 12/31/80.

Direct results of the respirometric test on December 31, 1980 using primary effluent as the feed are shown in Figure 8-14. An anticipated problem was encountered in that the DO versus time curve was not linear at higher DO levels as was the case at Salem (Table E-12 of Appendix E illustrates this phenomenon more directly). The problem was assessed to be due to substrate depletion over the time period of the respirometric test, which resulted in a decreasing respiration rate over time independent of the DO level. Starting the respirometric test at other DO levels resulted in a similar curvature, confirming that substrate depletion was the cause rather than the occurrence of oxygen diffusional limitations at high DO levels. Consequently, it was not possible to convert the DO versus time curve in Figure 8-14 to a specific respiration rate versus DO curve.

It was possible to approximately assess the critical DO level from this data, however, as the point at which the DO versus time function begins to curve at an increasing rate, as opposed to the relatively constant curvature that results at higher DO levels from substrate depletion. This analysis indicates a critical DO in Figure 8-14 of about 0.5 mg/ℓ.

Because the plant's primary effluent was too weak to support a constant respiration rate over the time period required to complete a respirometric test, the feeding of other substrates was attempted. Glucose was found, in preliminary experiments, to produce a good response. Therefore, the second test on December 31, 1980 utilized a 1000 mg/ℓ glucose solution as feed rather than primary effluent, still in proportion to the plant's recycle ratio. The mixed liquor with glucose feed
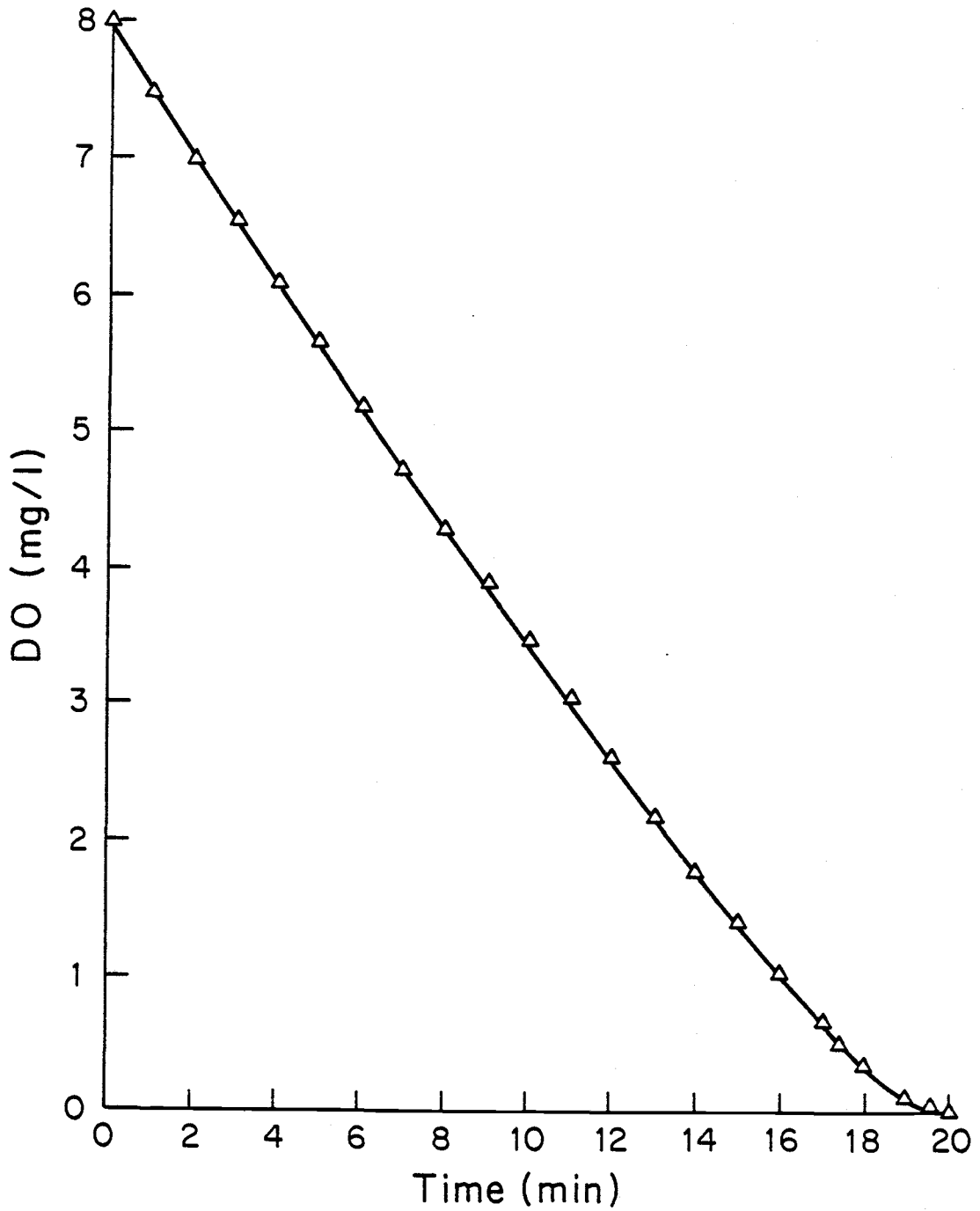
Figure 8-14. Dissolved Oxygen Concentration versus Time in Batch Respirometric Test No. 1 at the Albany WWTP on 12/31/80.

was aerated for one-half hour to allow acclimation to the glucose substrate.

Results of the respirometer test with the glucose feed indicated a constant respiration rate over time in the absence of oxygen diffusional limitations. Therefore, the specific respiration rate versus time data was converted into a plot of specific respiration rate versus the bulk DO as shown in Figure 8-15. The C_o^c, $C_o^{0.9}$, and $C_o^{0.5}$ values for this curve were approximately 0.6, 0.3, and 0.1 mg/ℓ, respectively. The maximum specific oxygen uptake rate of 12.2 mg/g-hr was slightly lower than the initial maximum respiration rate using the primary effluent as feed.

A batch reactor plug-flow simulation was also conducted on December 31, 1980 on samples from the Albany WWTP. Due to the findings that critical DO levels at full-scale plants tend to be quite low, one reactor (Reactor A) was maintained at a DO of 1.0 mg/ℓ and the other (Reactor B) at 8.0 mg/ℓ. Results are shown in Figures 8-16 and 8-17 for specific oxygen uptake rate and specific ATP concentration versus time.

These results indicate no identifiable difference in either specific respiration rate or specific ATP concentration in a simulated pass through a plug-flow aeration basin.

CORVALLIS, OREGON WWTP

Description of Plant

The Corvallis, Oregon WWTP is a small, combined trickling filter/ activated sludge plant that treats primarily domestic wastewaters. The activated sludge process utilizes diffused air aeration and consists of
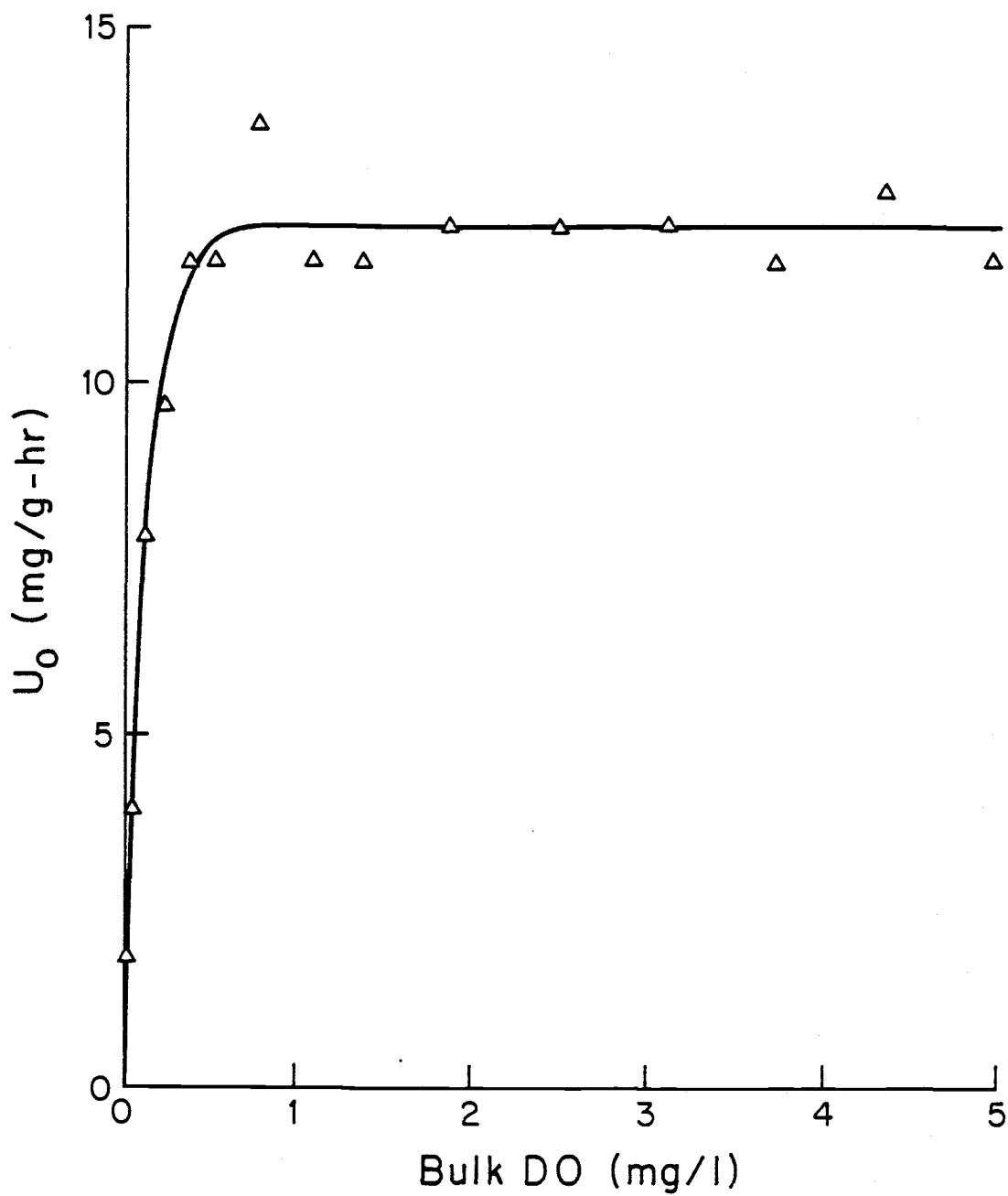
Figure 8-15.   Specific Respiration Rate versus Bulk DO
for Test No. 1-G at the Albany WWTP on
12/30/80.
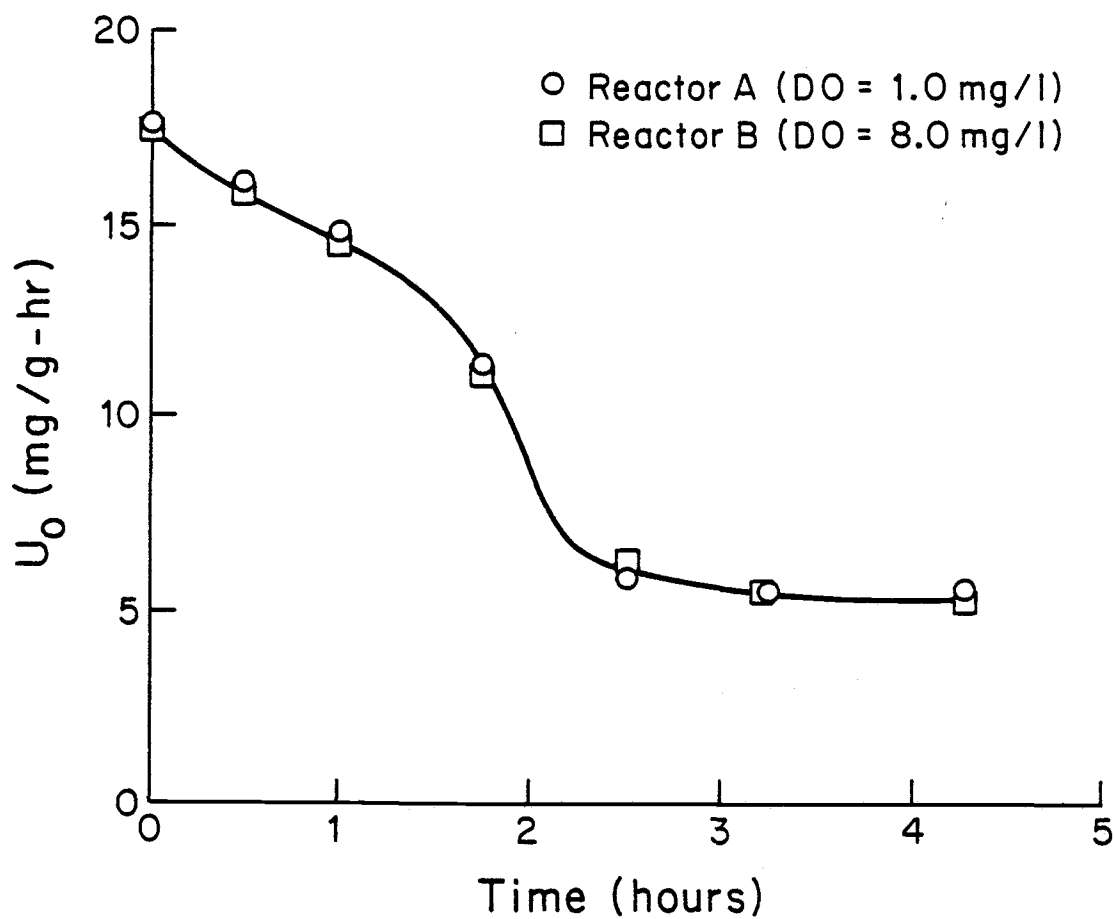
Figure 8-16. Specific Respiration Rate versus Time in the
Batch Reactor Simulation at the Albany WWTP
on 12/31/80.
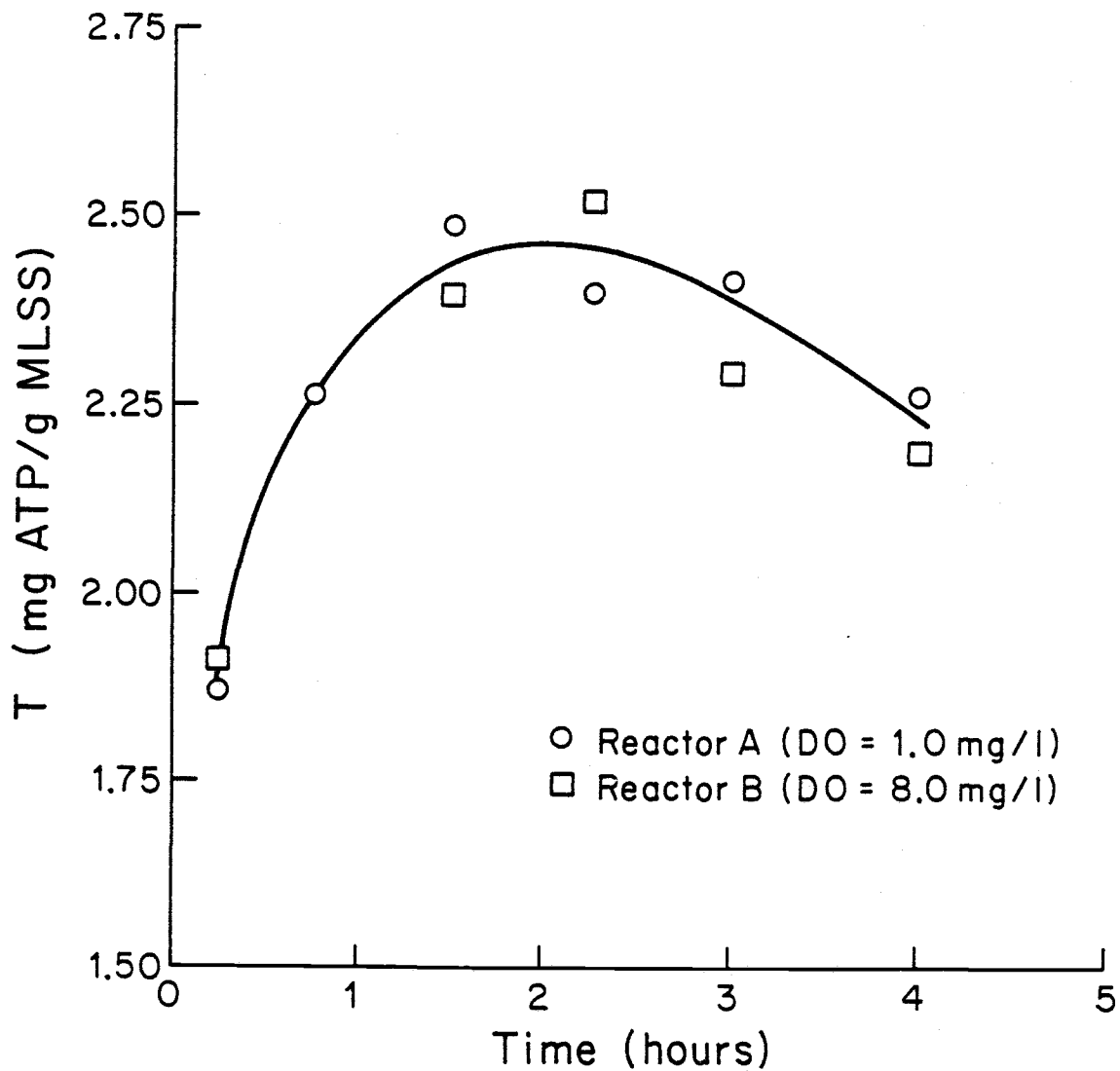
Figure 8-17. Specific ATP Concentration versus Time in the Batch Reactor Simulation at the Albany WWTP on 12/31/80.

four aeration basins that may be operated either in series or in parallel to achieve a plug-flow or completely-mixed operational mode. At the time tests were conducted at this plant (late December 1980), the process flow scheme consisted of primary clarification, trickling filter treatment (roughing), and activated sludge treatment with the four aeration basins in series. Trickling filter effluent was fed to the second aeration basin; the first aeration basin was used to reaerate return sludge.

The dry weather flow to the Corvallis WWTP is about 7.0 MGD. However, during the testing period (late December 1980) the plant flow was between 10 and 20 MGD due to wet weather and resulting infiltration and inflow. The primary effluent during the testing period typically exhibited a BOD_5 of between 50 and 100 mg/ℓ. The activated sludge unit was operated at an F:M ratio between 0.1 and 0.2 g BOD_5/g MLVSS-day and a sludge age of about six days. The effluent from the plant was of excellent quality with a BOD_5 and TSS typically less than 10 mg/ℓ.

The activated sludge culture was not filamentous, contained numerous higher organisms and exhibited excellent settling characteristics with an SVI of about 50 ml/g.

Description of Tests and Results

Batch respirometric tests were performed on samples from the Corvallis WWTP similar to those performed at the Salem WWTP and previously described.

Final tests were conducted on December 29, 1980 and January 1, 1981. The floc size distribution measured on December 29, 1980 is

shown in Figure 8-18. The median floc size on this day was in the 60 to 100 micron size range and the maximum floc size was 200 microns.

The respirometric test on December 29, 1980 combined primary effluent and return sludge in proportion to the plant's operating R/Q ratio. Results of this test are shown in Figure 8-19 as a plot of bulk DO versus time for two runs that were identical except that the second run was initiated at a higher DO level. Results of the first run indicate that the specific respiration rate was continually dropping throughout the test (Table E-15 of Appendix E illustrates this curvature more effectively). The relatively constant curvature of the bulk DO versus time function suggested that substrate depletion was the fundamental cause rather than oxygen diffusion limitations. Consequently, a second run was conducted beginning at a higher DO concentration and the results are also shown in Figure 8-19. The parallelism of these two curves down to a DO level of about 0.6 mg/ℓ in Run #1 illustrates the lack of oxygen effects above this DO level.

Other respirometric tests were conducted at the Corvallis WWTP using the synthetic feed (1000 mg/ℓ glucose) instead of primary effluent, similar to the tests at the Albany plant. However, the Corvallis activated sludge respired very poorly on glucose. This was attributed to the use of a roughing filter which removed most readily degradable materials such as sugars and caused the activated sludge culture to be unacclimated to a glucose feed.

DISCUSSION OF RESULTS

This phase of the research produced some very interesting and important results regarding floc sizes, respiration rates, and the
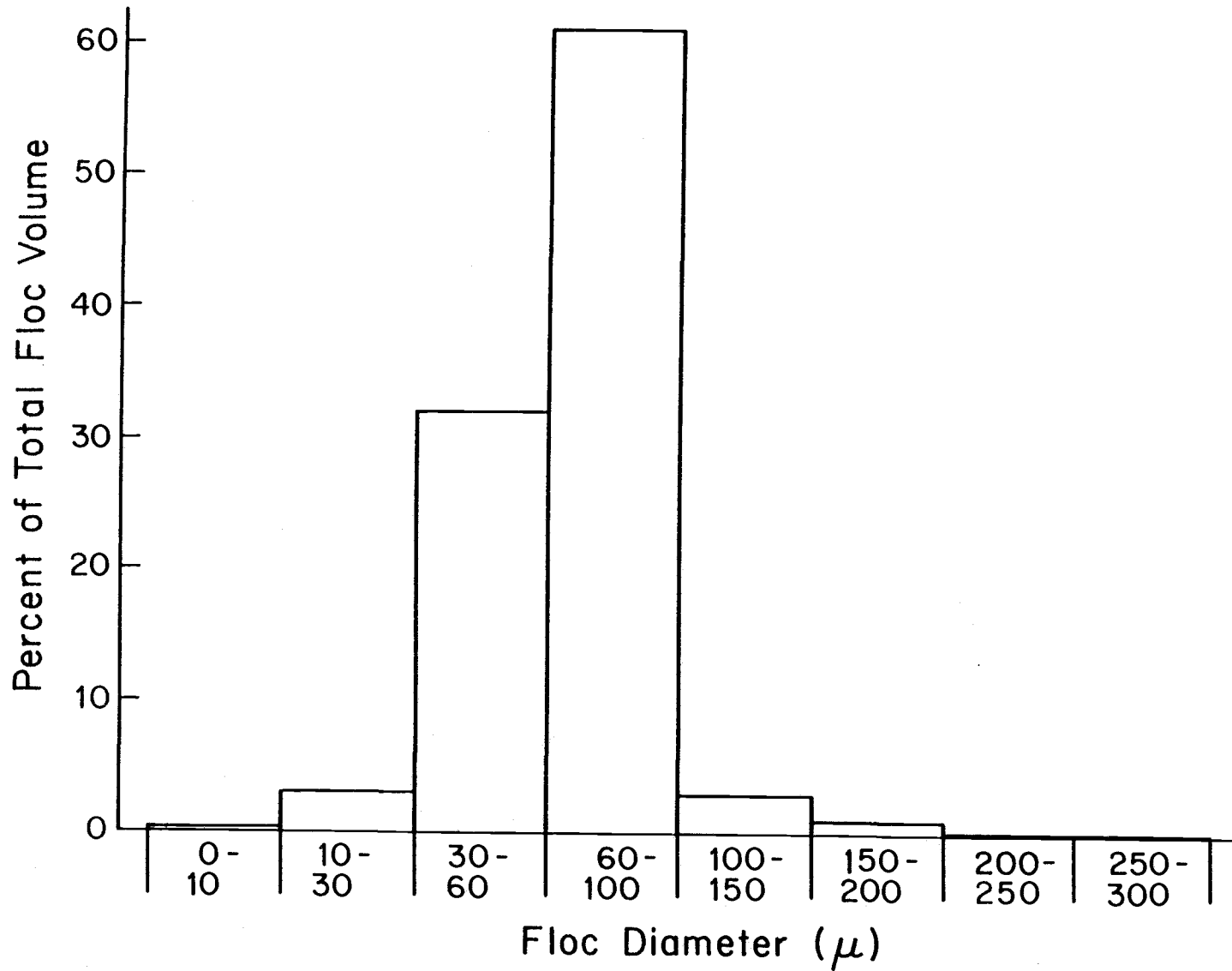
Figure 8-18. Floc Size Distribution at the Corvallis WWTP on 12/29/80.

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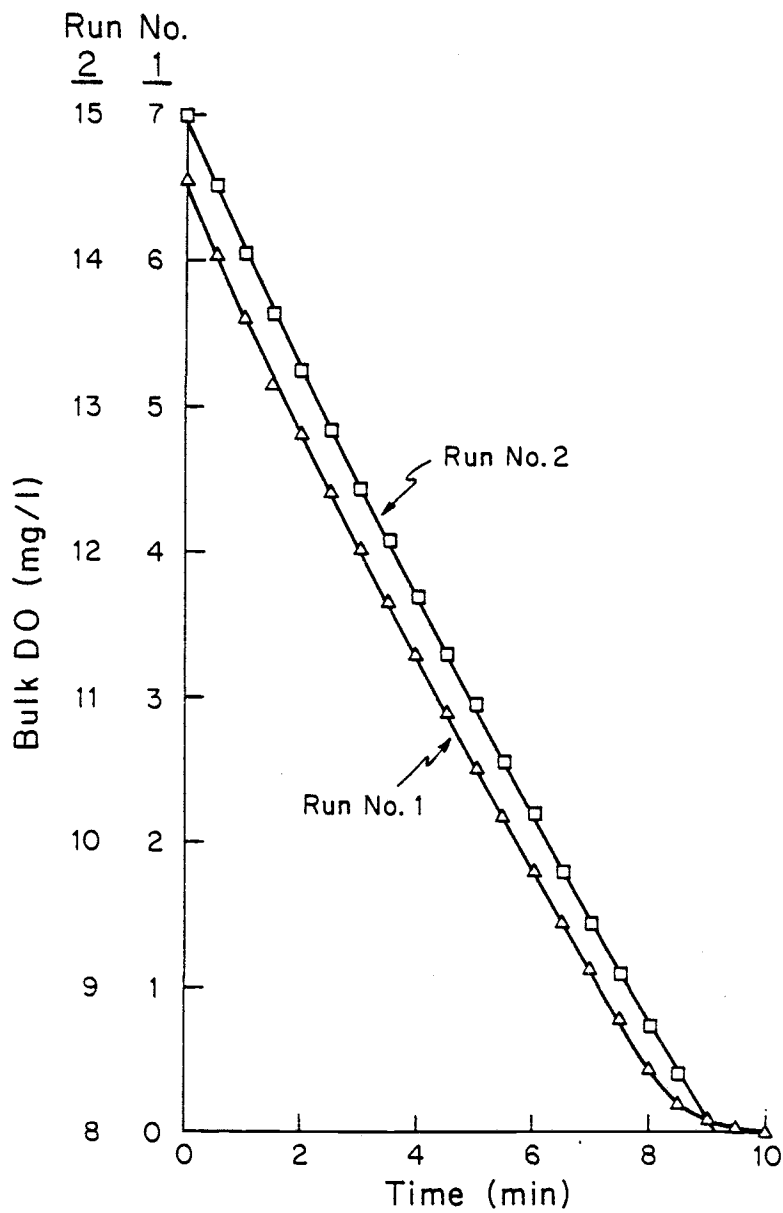
Figure 8-19. Bulk DO versus Time for the Respirometer
Test at the Corvallis WWTP on 12/29/80.

possible occurrence of oxygen diffusional limitations at full-scale activated sludge plants.

First, the floc size measurements at three full-scale activated sludge plants indicated that maximum floc diameters of 100 to 300 microns are typical. Although the number of plants surveyed was limited, these results agree with measurements of previous researchers at full-scale plants (see Table 4-2). However, these results also indicate that floc sizes in laboratory-scale activated sludge units may be substantially larger than those typical of full-scale plants. For example, the laboratory tests conducted within Phase 2 of this study resulted in activated sludge cultures with maximum floc diameters of up to 1200 microns. The floc size measurements of Parker, et al. (1971) and Knudsen, et al. (1980), wherein maximum floc diameters of 1000 microns or larger were measured in laboratory-scale and pilot-scale activated sludge units, respectively, further illustrates this discrepancy. This discrepancy in typical floc sizes between laboratory-scale and full-scale activated sludge units and the causes and implications of this finding are further discussed in Chapter IX.

A list of the mean and maximum floc diameters associated with each of the respirometric tests, as derived from the floc size distributions, is contained in Table 8-1. The mean (volume weighted) floc size was in the range of 50 to 100 microns at all three plants. A difference in the floc size distributions between the three plants that was very noticeable, however, was the maximum floc size, which ranged from 100 to 150 microns at the Salem pure oxygen plant to 300 microns at the Albany diffused air plant. In a related study of floc size distributions at full-scale activated sludge plants, Titus (1980) found that

the two pure oxygen plants surveyed exhibited a noticeably smaller max-
imum floc size as compared to conventional, diffused-air plants. A
second noticeable and related correlation observed within this phase
of the study was between the SVI of an activated sludge culture and the
maximum floc size. Higher SVI's at plants seemed to be directly cor-
related with larger maximum floc diameters.

The above-described correlations must be regarded as preliminary
due to the limited number of plants surveyed and in need of further
research for confirmation. However, these apparent correlations have
a logical basis for occurrence. Pure oxygen activated sludge plants
typically utilize high-speed mechanical (prop-type) aerators. These
mechanical aeration systems do not typically involve higher average
power inputs per unit volume than diffused-air systems. However, like
a blender, mechanical aeration systems, particularly those with prop-
type aerators, result in a very high shearing turbulence in the immedi-
ate vicinity of the blades. It is the author's belief that this in-
creased shearing action reduces the maximum floc size and, further,
that the reduced floc size results in a more compactible sludge (lower
SVI). The mechanism for this may be due to the greater compactibility
of smaller particles or as a result of encouraging the development of
smaller, higher density floc. These theories are discussed further in
Chapter IX.

Results of the respirometric tests within this phase were plotted
in terms of the specific respiration rate versus the bulk DO. The
anoxic core mathematical model was not applied to these data, as was
done in Phase 2, for two reasons: first, the extent of diffusional
limitations was so small in most cases that the zero-order assumption

between respiration rate and DO within the model was no longer valid;

second, because the critical DO was typically so small, the number of

data points below the critical DO was very limited and fitting of the

model to this limited data was not judged to produce meaningful results

in many cases. Consequently, the curve of best fit was drawn using

best engineering judgement and values for $C_o^{0.5}$, $C_o^{0.9}$, and C_o^c were

derived from this curve.

A summary of results from the respirometric tests is listed in

Table 8-1. As shown, the critical DO (C_o^c) ranged from 0.6 mg/ℓ at the

Albany plant to 1.4 mg/ℓ on one day at the Salem plant. The value of

$C_o^{0.9}$ similarly ranged from 0.3 to 1.0 mg/ℓ and $C_o^{0.5}$ from 0.1 to 0.35

mg/ℓ. The primary determinant of the critical DO in these tests was

the maximum specific respiration rate (U_o^{max}), which varied from 12.2

mg/g-hr in the test at the Albany plant to nearly 70 mg/g-hr in one

test at the Salem plant. Although the average floc size at the Albany

plant was larger than that at the Salem and Corvallis plants, there did

not seem to be noticeable effect of this on the occurrence of oxygen

diffusional limitations. Tests for which U_o^{max} was similar at the

Albany, Corvallis, and Salem plants yielded similar results in terms of

the specific respiration rate versus DO curve. This is probably the

result of the lower density of larger floc particles as discovered in

Phase 2 of this study (see Chapter VII). That is, the lower density of

the larger floc particles largely compensated for the increased diffu-

sional resistance that would normally result with a larger diameter

particle. An additional factor was that the differences in the floc

size distributions between the three plants were not of a major magni-

tude.

Table 8-1. Summary of Results from Respirometric Tests in Phase 3.

DATE	WWTP	FLOC DIAMETER (μ) MEAN[1]	MAX	U_o^{MAX} (mg/g-hr)	C_o^c (mg/ℓ)	$C_o^{0.9}$ (mg/ℓ)	$C_o^{0.5}$ (mg/ℓ)
8/04/80	Salem	83	150	16.6	0.9	0.5	0.2
8/05/80	Salem	$-^2$	$-^2$	23.4	1.0	0.5	0.2
8/06/80	Salem	76	150	69.9	1.4	1.0	0.35
8/07/80	Salem	54	100	57.6	1.2	0.8	0.3
12/31/80	Albany	90	300	12.2^3	0.6	0.3	0.1
12/29/80	Corvallis	74	200	14^4	0.7^4	-	-

[1] Volume-weighted basis.
[2] Assumed similar to values on 8/4/80 and 8/6/80.
[3] Synthetic glucose substrate used.
[4] Approximate values derived from DO versus time curve (Figure 8-19).

The major finding of this phase of the research study was that the critical DO in full-scale activated sludge plants is typically quite low and below the normal operational levels at air activated sludge plants. As a generalization, it can be stated that the critical DO for plants treating domestic wastewaters is typically below 1.0 mg/ℓ. For the worst possible case, that is, the initial point of a plug-flow aeration basin at a plant treating high-strength soluble industrial wastewaters, the critical DO may be higher than 1.0 mg/ℓ but the results of this study suggest that an extreme upper limit of about 2.0 mg/ℓ may be defined.

These findings contradict theories regarding improved performance in pure oxygen activated sludge plants because oxygen diffusional limitations are eliminated. The normal DO levels maintained in the aeration basins at air activated sludge plants (1-2 mg/ℓ) is sufficient to prevent the occurrence of oxygen diffusional limitations in bacterial flocs. Furthermore, the results of this phase identified alternative mechanisms for improved settling performance at full-scale pure oxygen plants and offered explanations for the widely-ranging and often contradictory results of numerous previous laboratory-scale studies on oxygen diffusional limitations in activated sludge cultures. These topics are discussed further in Chapter IX.

CONCLUSIONS

The following conclusions were derived from this phase of the research study:

1. The mean floc size at full-scale activated sludge plants
    is typically in the range of 50 to 100 microns and the
    maximum is in the range of 100 to 300 microns.

2. Laboratory-scale activated sludge systems do not neces-
    sarily develop floc size distributions typical of full-
    scale plants.

3. The critical DO for full-scale activated sludge systems
    is typically less than 1.0 mg/ℓ for plants treating do-
    mestic wastewaters and is less than 2.0 mg/ℓ even at
    rapid respiration rates resulting from treatment of
    high-strength industrial wastewaters.

4. The occurrence of oxygen diffusional limitations is
    not a reasonable basis for explaining observed differ-
    ences in performance between full-scale pure oxygen
    and conventional air activated sludge plants.

5. The development of smaller, more dense floc as a result
    of increased floc-shearing forces from mechanical aera-
    tors may be a fundamental explanation for the occurrence
    of improved compactibility of sludges at pure oxygen
    activated sludge plants.

CHAPTER IX

SIGNIFICANCE

The results of this study have important applications and significance to the design and operation of activated sludge systems. Those findings are primarily related to the effects of dissolved oxygen concentration (DO) on the activated sludge process. However, during the course of this study, several interesting findings were made regarding other aspects of the activated sludge process, including typical floc sizes and settleability, that were not directly related to DO. The significance of this study in these two areas is described below.

The Effect of DO

The claims of pure oxygen proponents that bacterial floc in activated sludge systems are frequently diffusion-limited with respect to oxygen at the operational DO levels typical of air-activated sludge plants were not substantiated by this study. Rather, this study indicated that the maintenance of high DO levels (above 2.0 mg/ℓ) in activated sludge aeration basins offers no particular advantage with respect to degradation of substrates, settleability of the activated sludge culture, or any other factors. The reason for this is that flocs within typical full-scale activated sludge plants are too small and specific respiration rates not rapid enough to create substantial oxygen diffusional limitations under typical operating conditions.

The general conclusion of this study was that an aeration basin DO of 1.0 mg/ℓ was sufficient for treatment of typical domestic wastewaters and that 2.0 mg/ℓ would ensure that oxygen diffusional limitations did

not occur even under the most severe conditions (i.e., initial point of a plug-flow aeration basin for a plant treating high-strength industrial wastewaters). This conclusion has important significance and application to the design and operation of activated sludge plants.

For the design engineer, this study illustrates that the ability to maintain a high DO level in the aeration basin of an activated sludge plant is not necessary to provide efficient treatment of either municipal or industrial wastewaters. If all other process parameters are equal, the incorporation of an air aeration system into an activated sludge plant that is capable of consistently maintaining a DO level of 1-2 mg/ℓ will provide equivalent system performance as compared to a pure oxygen aeration system that is capable of maintaining DO levels of 15 mg/ℓ or more. The fact that all other process parameters (besides aeration basin DO level) are not typically equivalent between air and pure oxygen plants is a matter for separate consideration.

In making the above statement, several related matters must be addressed. First, there are substantial practical advantages related to the maintenance of high DO levels, as is common in pure oxygen activated sludge systems. The maintenance of high DO levels provides a buffer against sudden drops in aeration basin DO levels that may result from shock loads of high-strength organic wastewaters. Such shock loads may be common at municipal plants that have a substantial industrial wastewater contribution or at purely industrial wastewater treatment facilities. Although the maintenance of a DO level of 1.0-2.0 mg/ℓ in the aeration basin of an activated sludge plant would prevent the occurrence of oxygen diffusional limitations, the fact is that very

few plants have the DO monitoring and control systems necessary to ensure that a minimum DO level is being maintained in all portions of an aeration basin at all times. This is a particular problem where plug-flow aeration basins are utilized because the specific respiration rate varies over the length of the aeration basin and initial respiration rates may be extremely high.

Second, the ability of pure oxygen systems to transfer a given amount of oxygen into solution at a lower aerator power requirement than an air system may have practical advantages in terms of aerator equipment selection and basin sizing. The typical pure oxygen activated sludge system utilizes a plug-flow hydraulic scheme (four completely-mixed aeration cells in series), a relatively short hydraulic retention time in the aeration basin (typically 2-3 hours), and a high biomass concentration in the mixed liquor. The volumetric respiration rates at the initial point in the aeration basin (first cell) may be extremely high under these conditions if high-strength industrial wastewaters are treated, as found within this study at the Salem plant. The pure oxygen system provides aeration gas with the highest oxygen content at this critical point, which allows the maintenance of high DO levels without diffuculty. Application of air aeration systems under similar conditions has frequently resulted in problems in maintaining minimum DO levels. The author believes that air aeration systems may be applied in these conditions if designed appropriately. However, smaller aeration cells and much higher aerator power inputs may be required, leading to increased costs.

Finally, pure oxygen activated sludge plants may perform better as a result of other factors not related to DO. These factors are discussed in the final section of this chapter.

For the operators of activated sludge systems, this study defines the approximate DO level that is necessary to maintain fully aerobic conditions in activated sludge systems. Previous studies have demonstrated that the development of an anoxic core in large bacterial floc may lead to reduced substrate utilization rates, increased sludge yields, and most important, to settleability problems as a result of filamentous or dispersed bacterial growth. While the results of this study may be applied directly to establish an approximate minimum DO level that should be maintained, an additional value of this study is that procedures were developed whereby the critical DO level at any plant may be evaluated. The batch respirometric test, which was conducted within the laboratory phases of this study and then adapted to direct testing of mixed liquor samples from full-scale activated sludge plants, is relatively simple and capable of defining the critical DO at a specific plant under the specific loading and operating conditions of that plant.

Other Findings

In the course of this study, several findings of significance were made not directly related to the effects of DO. These findings concern typical floc sizes, and the factors that affect floc size.

A very interesting finding of this study was that typical floc sizes at full-scale activated sludge plants are relatively small (maximum diameter of 100 to 300 microns) and, furthermore, that the floc

sizes in laboratory-scale activated sludge systems may be substantially larger (up to 1200 microns in diameter).

These findings regarding floc size at full-scale plants have two important applications. First, they illustrate that laboratory-scale systems may not effectively model full-scale activated sludge systems in terms of the floc size characteristics of the culture that develops. Consequently, the utilization of laboratory-scale activated sludge systems to model diffusional limitations, settleability and compactibility, or other characteristics of full-scale systems that depend on the floc-size characteristics of the culture may not yield valid results. The author believes that this discrepancy is a primary reason why numerous previous studies on diffusional limitations in activated sludge systems have often led to widely-varied and even contradictory results.

Second, this discrepancy illustrates the partial understanding that presently exists regarding factors that impact on the floc-size characteristics of an activated sludge culture. Parker, et al. (1972) have argued convincingly that mixing intensity is the primary variable affecting floc size through the shearing action of micro-scale eddies on large flocs. Parker, et al. verified that the mean floc size decreased as the power input into a standard mixing chamber increased, illustrating that increased power inputs result in increased micro-scale shearing forces. However, at present there exists no rational basis for comparing the micro-scale shearing forces that result from the different types of aerators used at activated sludge plants. The average power input per unit volume, a parameter that is widely used in assessing mixing intensity, does not reflect the distribution of micro-scale shearing forces that may result from an aerator or mixer. The

localized maximum power dissipation rate per unit volume may be more meaningful in assessing the impact of mixing intensity on the floc size distribution.

It is the author's conjecture that certain mechanical aerators function in a manner similar to a blender in providing very high micro-scale shearing forces in the immediate vicinity of the blades, resulting in a reduced maximum floc size and shearing of projecting filaments from floc. This phenomenon is the proposed reason why many pure oxygen plants develop much smaller maximum floc sizes and frequently demonstrate improved compactibility of the activated sludge as measured by the SVI.

CHAPTER X

SUMMARY

The purpose of this research study was to evaluate the effects of dissolved oxygen concentration (DO) on the activated sludge process. The impetus for this study was the reported improved performance that pure oxygen activated sludge systems often achieve as compared to air activated sludge systems.

A literature review revealed that dispersed microbial cells typically respond to DO, as measured by respiration rate or intracellular concentrations of metabolites, only at very low concentrations (less than about 0.1 mg/ℓ). Consequently, the occurrence of diffusional limitations in large bacterial aggregates (floc) was identified as the only reasonable basis for explaining effects of DO in the operational range of activated sludge plants. A specific model of diffusion and reaction within microbial aggregates (the anoxic core model) was adopted as a theoretical basis for this study. The overall model that was developed indicated that oxygen diffusional limitations in steady-state activated sludge systems could be assessed quantitatively in three ways: they could be predicted mathematically from the anoxic core model, measured as a reduction in the overall specific utilization rate, or measured as a reduction in specific ATP content.

Attempts to predict the extent of oxygen diffusional limitations directly from the anoxic core model were only partially successful. The major problem identified in this theoretical application was that typical values for activated sludge floc characteristics, including the

bacterial density (ρ), diffusivity (D_o), and equivalent diffusional radius could be defined only within a wide range.

The major purposes of the first experimental phase of this study were to: 1) confirm, for a dispersed bacterial culture, that the maintenance of DO levels above that typically maintained at air activated sludge plants (1-2 mg/ℓ) has no effect on measurable characteristics of the culture, and 2) to test and compare methods for the extraction of ATP. Tests on five different ATP extraction procedures indicated that direct injection of a culture sample into boiling Tris buffer (1:50 ratio of culture to buffer) was comparable to the more widely accepted perchloric acid extraction technique in terms of extraction efficiency and inactivation of ATP-hydrolyzing enzymes. Additionally, the method was judged to be simpler and more suitable to the requirements of this and similar studies.

To fulfill the second objective of the Phase 1 experiments, two laboratory-scale chemostats were fed a synthetic feed solution (phenol) and operated identically except that a high DO (15 mg/ℓ) was maintained in one and a low DO (2 mg/ℓ) in the other. The dispersed bacterial cultures that developed were very effective at degrading phenol and produced effluent phenol concentrations in the microgram-per-liter range. The chemostats were operated to steady-state at two hydraulic retention times and the following analyses performed: reactor TSS (indicative of net yield), effluent phenol concentration, specific ATP viability, and adenylate energy charge. Additionally, batch reactor studies on the cultures were performed to evaluate Monod half-velocity and maximum substrate utilization coefficients (K_s and k) using the infinite dilution procedure of Williamson and McCarty (1974). A

statistical analysis of results indicated no consistent difference between the two reactors in any of the parameters measured. Respirometric experiments indicated a half-velocity DO for phenol degradation by this dispersed culture of less than 0.1 mg/ℓ and a critical DO of less than 0.5 mg/ℓ. These results confirmed that differences in activated sludge characteristics which apparently result from the maintenance of high DO levels can only be attributed to diffusional limitations in large bacterial floc as described in the anoxic core theory.

The purpose of the second experimental phase of this study was to confirm the applicability of the anoxic core model to laboratory-grown flocculant bacterial cultures and then to assess the possible occurrence of oxygen diffusional limitations in typical full-scale activated sludge plants based on these results. The planned approach was to operate two laboratory-scale activated sludge systems to steady-state at different DO levels and to correlate differences in specific ATP viability and specific utilization rate between these two systems within the context of the anoxic core model. However, extreme difficulty was encountered in maintaining a stable, settleable activated sludge culture at a steady-state condition. This difficulty was attributed to the occurrence of major substrate diffusional limitations in highly-adapted activated sludge cultures that are fed a synthetic feed solution. Consequently, the operational mode of the laboratory activated sludge systems was changed to a pulse-feed mode and then to a fill-and-draw mode. The fill-and-draw operational mode was very successful in establishing a stable, settleable activated sludge culture; however, it was necessary to reformulate the experimental approach to concentrate

upon respirometric and other batch tests using the culture from the fill-and-draw reactor.

The major emphasis of the Phase 2 experiments was, therefore, batch respirometric studies, which were conducted under conditions of high substrate concentration to ensure that oxygen was the diffusion-limiting species and that all aerobic portions of floc were respiring at a uniform rate as assumed within the anoxic core model. Results of these batch respirometric tests were utilized to develop specific respiration rate versus bulk DO data, which was inserted into the model (Equations 3-30 and 3-31) and the parameter D_o/ρ used as a fitting parameter for the model. The resulting curve was also defined in terms of a half-velocity DO ($C_o^{0.5}$), a 90-percent velocity DO ($C_o^{0.9}$), and a critical DO (C_o^c) for comparison of results from various tests.

Results indicated that the critical DO for the laboratory-grown activated sludge cultures varied from about 2 mg/ℓ to over 10 mg/ℓ for maximum specific respiration rates in the range of 75 to 250 mg/g-hr and average (volume-weighted) floc diameters in the range of 150 to 1000 microns. Application of the anoxic core model to the data provided a very good fit; however, the optimum value of D_o/ρ varied widely and showed a strong correlation with floc size, indicating that larger flocs are typically of lower density. The variation of floc density with floc size was also a limiting factor in the fit of the anoxic core model to the respirometric data.

The results of the Phase 2 studies were analyzed in the context of the anoxic core model and the possible occurrence of oxygen diffusional limitations under conditions typical of full-scale activated sludge plants was evaluated. This evaluation indicated that oxygen diffusional

limitations did not occur within typical activated sludge plants at aeration basin DO levels of 1-2 mg/ℓ unless the flocs were extremely large and specific respiration rates very rapid.

The third phase of this research study was designed to determine directly whether oxygen diffusional limitations are important in full-scale activated sludge plants. Activated sludge cultures at three full-scale activated sludge plants were subjected to batch respirometric tests similar to those conducted during the Phase 2 experiments. Floc size distributions were measured and specific respiration rate versus DO curves were developed. Results indicated that the maximum floc size at full-scale activated sludge plants is typically between 100 and 300 microns, which is substantially smaller than that measured in many laboratory activated sludge studies. Values for the critical DO varied between about 0.5 and 1.5 mg/ℓ, the higher values occurring at one plant that received a substantial industrial wastewater contribution from canneries and exhibited much higher maximum specific respiration rates than the other two plants.

Other batch tests simulated one pass through a plug-flow aeration basin with mixed liquor samples at the plants. Results indicated no significant difference in specific respiration rate or specific ATP content throughout the simulated pass when reactors were operated at a low DO typical of air activated sludge (1 or 2 mg/ℓ) or at a high DO typical of pure oxygen activated sludge (8 or 15 mg/ℓ). It was con-cluded that the occurrence of oxygen diffusional limitations is not a reasonable basis for explaining differences in performance between full-scale pure oxygen and conventional air activated sludge plants. Results of this phase and a previous study by Titus (1980) indicated that the

development of smaller floc of higher density may occur at pure oxygen activated sludge plants as a result of increased micro-scale shearing forces from mechanical aerators and that this development may be a fundamental explanation for the frequently observed improved compactibility (lower SVI) of activated sludges at pure oxygen plants.

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APPENDIX A

Phase 1 Data

Table A-1.   Daily Data for Run 1-1[1]

DAY	θ (hrs)		pH		C_s^e [2] (mg/ℓ)		TSS (mg/ℓ)	
	A	B	A	B	A	B	A	B
1	119	111	7.2	7.3	0.7	0.5	670	655
2	118	112	7.0	7.2	0.4	0.5	610	595
3	110	113	7.0	7.4	0.5	0.4	580	545
4								
5	111	112	6.9	7.0	0.2	0.1	540	510
6	112	112	6.9	7.1	0.2	0.1	505	495
7	110	113	7.0	7.0	0.3	0.2	515	480
8								
9	113	113	6.85	6.9	0.1	<0.1	420	425
10	112	114	6.9	7.15	<0.1	<0.1	395	380
11	109	111	6.9	6.95	<0.1	<0.1	400	395
12								
13	108	112	6.85	7.0	<0.1	<0.1	370	400
14								
15	110	113	6.9	7.0	<0.1	<0.1	367	378
16	111	112	6.9	6.95	<0.1	<0.1	338	345
17	115	113	7.0	7.05	<0.1	<0.1	350	340
18	113	113	6.95	6.95	<0.1	<0.1	363	345
19[3]	113	113	7.0	7.0	0.04	0.01	344	338
20[3]	113	110	6.8	6.9	<0.01	0.05	367	325
21[3]	110	112	6.9	6.85	0.04	<0.01	338	346

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[1] Reactor A DO = 2 mg/ℓ
   Reactor B DO = 15 mg/ℓ

[2] The concentration of phenol in the effluent.

[3] Steady-state measurements during these days.

Table A-2.   Daily Data for Run 1-2[1]

DAY	θ (hrs)		pH		C_s^e (mg/ℓ)		TSS (mg/ℓ)	
	A	B	A	B	A	B	A	B
1	42.1	39.8	7.0	7.2	0.9	0.5	587	513
2	42.6	40.1						
3	38.7	39.8	7.2	7.1	0.4	0.4	600	510
4	38.9	40.0	7.0	7.1	0.2	0.6	553	493
5	39.6	39.7	6.85	7.1	0.1	0.3	530	485
6	38.9	39.2	6.9	7.05	<0.1	0.1	490	463
7	38.6	39.9	6.8	7.0	<0.1	<0.1	503	475
8	39.1	40.2	6.8	7.0	0.2	<0.1	465	455
9	39.7	38.8	6.55	6.85	<0.1	<0.1	458	448
10	38.9	38.5	7.05	7.0	<0.1	<0.1	463	465
11	39.6	38.9	6.9	7.0	<0.1	<0.1	433	445
12[2]	40.6	39.6	6.85	7.0	.015	.029	443	440
13[2]	38.5	38.1	6.9	6.85	.010	.006	427	460
14[2]	39.0	38.7	6.8	6.85	.011	.023	420	430

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[1] Reactor A DO = 2 mg/ℓ
    Reactor B DO = 15 mg/ℓ

[2] Steady-state measurements during these days.

Table A-3.   Respirometer Data for Sample from
             Reactor A, Run #1-1.

TIME (min)	DO (mg/ℓ)	$U_{v,o}$ (mg/ℓ-hr)	U_o (mg/g-hr)
0.00	1.95		
		24	56
0.50	1.75		
		27	56
1.00	1.525		
		24	56
1.5	1.325		
		24	56
2.0	1.125		
		24	56
2.5	0.925		
		24	56
3.0	0.725		
		24	56
3.25	0.625		
		24	56
3.5	0.525		
		24	56
3.75	0.425		
		24	56
4.00	0.325		
		18	42
4.25	0.25		
		18	42
4.50	0.175		
		18	42
4.75	0.10		
		12	28
5.00	0.05		
		6	14
5.25	0.025		
		6	14
5.50	0.00		
		0	0
6.00	0.00		

NOTE: Initial phenol concentration = 50 mg/ℓ.
       TSS = 427 mg/ℓ.

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Table A-4. Respirometer Data for Sample from Reactor B, Run #1-1.

TIME (min)	DO (mg/ℓ)	$U_{v,o}$ (mg/ℓ-hr)	U_o (mg/g-hr)
0.00	2.45		
		33	72
0.50	2.175		
		33	72
1.00	1.90		
		33	72
1.50	1.625		
		30	65
2.00	1.375		
		33	72
2.50	1.10		
		33	72
3.00	0.825		
		33	72
3.50	0.55		
		33	72
4.00	0.275		
		30	65
4.25	0.15		
		24	52
4.50	0.05		
		6	13
4.75	0.025		
		6	13
5.00	0.00		

NOTE: Initial phenol concentration = 50 mg/ℓ.
TSS = 460 mg/ℓ.

APPENDIX B

Phase 2 Data

Runs # 2-1 and 2-2

Table B-1. Daily Data for Run 2-1.

DAY	θ (hrs)		pH		θ_c (days)		C_s^c (mg/ℓ)		X^e (mg/ℓ)		X (mg/ℓ)		SVI (ml/g)	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
0	8.5	8.9	7.3	7.1	$-^1$	$-^1$	-	-	-	-	-	-	-	-
1	8.6	8.9	6.9	6.8	$-^1$	$-^1$	0.7	0.5	102	143	640	510	-	-
2	8.7	8.7	6.7	6.7	$-^1$	$-^1$	0.2	0.1	87	112	810	650	140^2	110^2
3	8.6	8.7	6.6	6.5	$-^1$	$-^1$	<0.1	<0.1	53	78	1030	840	120^2	100^2
4	8.7	8.7	6.7	6.2	2.0	2.0	<0.1	<0.1	57	62	1170	1060	90	85
5	8.7	8.8	6.9	6.1	2.0	2.0	<0.1	<0.1	38	47	1220	1130	100	90
6	8.8	8.8	7.1	6.2	2.0	2.0	<0.1	<0.1	62	52	1180	1240	130	160
7	8.8	8.9	6.7	6.3	2.0	1.2^1	<0.1	<0.1	123	280	970	910	240	350^2
8	8.6	8.8	6.7	6.7	2.0	0.84^1	<0.1	<0.1	90	363	1040	830	270	420^2
9	8.6	8.5	6.8	6.9	1.6^1	0.60^1	<0.1	<0.1	216	404	950	690	360^2	560^2
10	8.7	8.6	6.6	6.7	0.90^1	0.58^1	-	-	328	283	810	540	510^2	720^2
11	8.6	8.8	6.7	6.8	0.56^1	0.86^1	<0.1	<0.1	413	260	640	610	640^2	700^2
12	8.6	8.7	6.7	6.8	0.69^1	0.65^1	<0.1	<0.1	320	310	620	560	690^2	750^2

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[1] No sludge wastage.

[2] 100-ml graduated cylinder used for SVI test.

NOTE: Reactor A DO = 2.0 mg/ℓ, Reactor B DO = 15.0 mg/ℓ.

Table B-2. Daily Data for Run 2-2.

DAY	θ (hrs)		pH		θ_c (days)		X^e (mg/ℓ)		X (mg/ℓ)		SVI (ml/g)		COMMENTS
	A	B	A	B	A	B	A	B	A	B	A	B	
0	9.0	8.8	6.9	6.7	-[1]	2.0	-	128	-	1015	-	260	Reactor A failure (washout)
1		8.6		6.6		2.0		113		950		-	Reactor A restarted
2		8.4		6.4		2.0		82		900		180	
3		8.4		6.7		2.0		154		970		-	
4	8.9	8.7	6.8	6.6	-[1]	0.51[1]		540		760		580[2]	Filamentous bulking, Reactor B
5	9.0	8.9	6.5	6.4	1.8[1]	0.47[1]	180	462	870	590	-	-	
6	8.7	8.6	6.7	6.8	2.0	0.74[1]	123	360	920	745	125[2]	420[2]	
7	9.2	9.2	-	-	1.6[1]	0.75[1]	197	303	818	595	-	-	
8	9.0	8.9	6.8	6.7	0.56[1]	1.3[1]	462	187	690	640	460[2]	380[2]	Filamentous bulking, Reactor A
9	9.0	8.8	6.6	6.7	0.45[1]	0.91[1]	515	253	620	627	520[2]	390[2]	
10	8.8	8.9	6.6	6.8	0.50[1]	0.52[1]	378	380	512	533	580[2]	460[2]	
11	8.7	8.8	6.8	6.8	0.54[1]	0.92[1]	323	183	483	460	510[2]	410[2]	
12	8.9	9.0	6.7	6.8	0.81[1]	2.0	216	90	470	570	280[2]	260[2]	
13	9.0	9.0	6.6	6.7	1.9[1]	2.0	122	63	620	860	210[2]	210	
14	8.8	9.0	6.7	6.7	2.0	2.0	68	40	780	1170	165[2]	175	Filaments greatly reduced, both Reactors
15	-	8.9	-	6.8	-	1.6[1]	-	320	-	1380	-	290[2]	
16	-	8.8	-	6.7	-	0.60[1]	-	760	-	1240	-	430[2]	
17	-	8.9	-	6.7	-	0.94[1]	-	330	-	840	-	-	

[1] No sludge wastage.
[2] 100-ml graduated cylinder used for SVI test.

NOTE: DO (Reactor A) = 2.0 mg/ℓ, DO (Reactor B) = 8.0 mg/ℓ.

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Table B-3. Floc Size Distribution, Reactor B, Day 4, Run 2-2.

d^1 (μ)	Number Fields	Number Floc	P_N^2 (%)	P_V^3 (%)
0- 10	1	65	37.8	<0.1
10- 30	1	39	22.7	0.2
30- 60	1	34	19.8	1.6
60-100	1	11	6.4	2.6
100-150	1	12	7.0	10.0
150-200	1	4	2.3	8.7
200-250	1	2	1.2	9.0
250-300	1	1	0.6	8.1
300-350	1	3	1.7	39.6
350-400	1	1	0.6	20.3

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[1] Spherical equivalent floc diameter.

[2] Percent of total number.

[3] Percent of total volume.

Table B-4. Floc Size Distribution, Reactor A, Day 8, Run 2-2.

d (μ)	Number Fields	Number Floc	P_N (%)	P_V (%)
0- 10	1	50	24.4	<0.1
10- 30	1	51	24.9	0.2
30- 60	1	54	26.4	1.6
60-100	1	25	12.2	3.8
100-150	1	15	7.3	8.1
150-200	1	2	1.0	2.8
200-250	5	4	0.4	2.3
250-300	5	8	0.8	8.4
300-350	5	11	1.1	19.0
350-400	5	7	0.7	18.5
400-450	5	5	0.5	19.2
450-500	5	3	0.3	16.0

Table B-5. Floc Size Distribution, Reactor B, Day 8, Run 2-2.

d (μ)	Number Fields	Number Floc	P_N (%)	P_V (%)
0- 10	1	37	15.9	<0.1
10- 30	1	55	23.6	0.1
30- 60	1	60	25.8	1.4
60-100	1	45	19.3	5.2
100-150	1	14	6.0	5.8
150-200	1	7	3.0	7.5
200-250	5	24	2.1	10.7
250-300	5	29	2.5	23.5
300-350	5	8	0.7	10.6
350-400	5	6	0.5	12.2
400-450	5	5	0.4	14.7
450-500	5	2	0.2	8.2

Table B-6. Floc Size Distribution, Reactor A, Day 12, Run 2-2.

d (μ)	Number Fields	Number Floc	P_N (%)	P_V (%)
0- 10	1	110	46.4	0.2
10- 30	1	70	29.5	3.7
30- 60	1	45	19.0	20.4
60-100	1	8	3.4	18.2
100-150	10	29	1.2	23.7
150-200	10	8	0.3	17.0
200-250	10	2	0.1	8.8
250-300	10	1	<0.1	8.0

Table B-7. Floc Size Distribution, Reactor B, Day 12, Run 2-2.

d (μ)	Number Fields	Number Floc	P_N (%)	P_V (%)
0- 10	1	75	58.1	0.3
10- 30	1	46	35.6	5.4
30- 60	1	4	3.1	4.1
60-100	1	2	1.5	10.2
100-150	10	12	0.9	22.0
150-200	10	6	0.5	28.5
200-250	10	3	0.2	29.6

Table B-8. Respirometer Data for Sample from
Reactor B, Day 4, Run 2-2.

FLOC			ULTRASONICALLY DISPERSED		
time	DO	U_o	time	DO	U_o
(min)	(mg/ℓ)	(mg/g-hr)	(min)	(mg/ℓ)	(mg/g-hr)
0.0	20.0		0.0	8.0	
		228			219
0.5	18.7		0.5	6.75	
		228			219
1.0	17.4		1.0	5.5	
		246			211
1.5	16.0		1.5	4.3	
		246			211
2.0	14.6		2.0	3.1	
		246			219
2.5	13.2		2.5	1.85	
		246			193
3.0	11.8		2.75	1.3	
		263			175
3.5	10.3		3.0	0.8	
		246			149
4.0	8.9		3.25	0.375	
		263			88
4.5	7.4		3.5	0.125	
		254			35
5.0	5.95		3.75	0.025	
		246			9
5.5	4.55		4.0	0.00	
		237			0
6.0	3.2				
		237			
6.5	1.85				
		193			
6.75	1.3				
		176			
7.0	0.8				
		158			
7.25	0.35				
		70			
7.5	0.15				
		43			
7.75	0.025				
		9			
8.0	0.00				
		0			

$$U_o^{max} = 216$$

$$U_o^{max} = 251$$

NOTES: 1) Sample transferred to respirometer consisted of 1:9
proportions of 500 mg/ℓ feed solution and culture.

2) Reactor TSS = 760 mg/ℓ.

3) Ultrasonication was accomplished at the 60% power setting
(maximum) for two minutes.

Table B-9. Respirometer Data for Sample from
Reactor A, Day 5, Run 2-2.

	FLOC			BLENDED	
time (min)	DO (mg/ℓ)	U_o (mg/g-hr)	time (min)	DO (mg/ℓ)	U_o (mg/g-hr)
0.0	19.0		0.0	20.0	
		169			184
0.5	17.9		0.5	18.8	
		184			199
1.0	16.7		1.0	17.5	
		199			199
1.5	15.4		1.5	16.2	
		199			199
2.0	14.1		2.0	14.9	
		199			215
2.5	12.8		2.5	13.5	
		199			215
3.0	11.5		3.0	12.1	
		215			215
3.5	10.1		3.5	10.7	
		199			215
4.0	8.8		4.0	9.3	
		207			215
4.5	7.45		4.5	7.9	
		207			215
5.0	6.1		5.0	6.5	
		207			215
5.5	4.75		5.5	5.1	
		199			222
6.0	3.45		6.0	3.65	
		192			215
6.5	2.2		6.5	2.25	
		184			199
6.75	1.6		6.75	1.60	
		169			199
7.0	1.05		7.0	0.95	
		138			169
7.25	0.6		7.25	0.4	
		100			84
7.5	0.275		7.5	0.125	
		54			23
7.75	0.1		7.75	0.05	
		23			8
8.0	0.025		8.0	0.025	
		8			8
8.25	0.00		8.25	0.00	
		0			0

$$U_o^{max} = 204 \qquad\qquad U_o^{max} = 216$$

NOTES: 1) Sample transferred to respirometer consisted of 1:9 pro-
portions of 500 mg/ℓ feed solution and culture.

2) Reactor TSS = 870 mg/ℓ.

3) Blending was performed in a Waring blender for two minutes.

Table B-10. Respirometer Data for Sample from
Reactor B, Day 5, Run 2-2.

	FLOC			BLENDED	
time (min)	DO (mg/ℓ)	U_o (mg/g-hr)	time (min)	DO (mg/ℓ)	U_o (mg/g-hr)
0.0	20.0	249	0.0	20.0	203
0.5	18.9	249	0.5	19.1	249
1.0	17.8	249	1.0	18.0	249
1.5	16.7	271	1.5	16.9	249
2.0	15.5	271	2.0	15.8	249
2.5	14.3	271	2.5	14.7	271
3.0	13.1	271	3.0	13.5	249
3.5	11.9	271	3.5	12.4	271
4.0	10.7	271	4.0	11.2	271
4.5	9.5	271	4.5	10.0	260
5.0	8.3	282	5.0	8.85	260
5.5	7.05	271	5.5	7.7	260
6.0	5.85	271	6.0	6.55	271
6.5	4.65	282	6.5	5.35	260
7.0	3.4	271	7.0	4.2	260
7.25	2.8	249	7.5	3.05	260
7.5	2.25	249	8.0	1.9	237
7.75	1.7	226	8.5	0.85	181
8.0	1.2	181	8.75	0.45	124
8.25	0.8	158	9.0	.175	56
8.5	0.45	90	9.25	0.05	23
8.75	0.25	68	9.75	0.00	
9.0	0.10	23			
9.25	0.05	11			
9.5	0.025	11			
9.75	0.00	0			

$U_o^{max} = 273$ $U_o^{max} = 262$

NOTES: 1) Sample transferred to respirometer consisted of 1:9 proportion of 500 mg/ℓ feed solution and culture.

2) Reactor TSS = 590 mg/ℓ.

3) Blending was performed in a Waring blender for two minutes.

Table B-11. Respirometer Data for Samples from
Reactors A and B, Day 8, Run 2-2.

	REACTOR A		REACTOR B	
time	DO	U_o	DO	U_o
(min)	(mg/ℓ)	(mg/g-hr)	(mg/ℓ)	(mg/g-hr)
0.0	16.0		14.0	
		126		135
1.0	14.7		12.7	
		126		135
2.0	13.4		11.4	
		126		135
3.0	12.1		10.1	
		135		135
4.0	10.7		8.8	
		126		135
5.0	9.4		7.5	
		126		135
5.5	8.75		-	
		126		
6.0	8.1		6.2	
		126		135
6.5	7.45		-	
		126		
7.0	6.8		4.9	
		135		125
7.5	6.1		4.3	
		126		135
8.0	5.45		3.65	
		126		125
8.5	4.8		3.05	
		126		125
9.0	4.15		2.45	
		135		115
9.5	3.45		1.9	
		126		125
10.0	2.8		1.3	
		116		104
10.25	-		1.05	
				83
10.5	2.2		0.85	
		116		83
10.75	1.9		0.65	
		116		83
11.0	1.6		0.45	
		97		62
11.25	1.35		0.3	
		97		52
11.5	1.1		0.175	
		97		31
11.75	0.85		0.10	
		97		21
12.0	0.6		0.05	
		77		10
12.25	0.4		0.025	
		58		10
12.5	0.25		0.00	
		58		0
12.75	0.1			
		29		
13.0	0.025			
		5		
13.5	0.00			

$$U_o^{max} = 128 \qquad\qquad U_o^{max} = 135$$

NOTES: 1) Sample transferred to respirometer consisted of
1:9 proportions of 500 mg/ℓ feed solution and
culture.

2) Reactor A TSS = 690, Reactor B TSS = 640.

Table B-12. Respirometer Data for Samples from
Reactors A and B, Day 12, Run 2-2.

	REACTOR A		REACTOR B	
time	DO	U_o	DO	U_o
(min)	(mg/ℓ)	(mg/g-hr)	(mg/ℓ)	(mg/g-hr)
0.0	7.0		6.0	
		85		70
0.5	6.7		5.7	
		99		82
1.0	6.35		5.35	
		85		70
1.5	6.05		5.05	
		99		82
2.0	5.7		4.7	
		85		70
2.5	5.4		4.4	
		99		76
3.0	5.05		4.075	
		85		76
3.5	4.75		3.75	
		85		76
4.0	4.45		3.425	
		85		76
4.5	4.15		3.1	
		99		70
5.0	3.8		2.8	
		85		76
5.5	3.5		2.475	
		85		70
6.0	3.2		2.175	
		85		76
6.5	2.9		1.85	
		85		64
7.0	2.6		1.575	
		85		64
7.5	2.3		1.3	
		85		64
8.0	2.0		1.025	
		85		58
8.5	1.7		0.775	
		78		58
8.75	-		0.65	
				58
9.0	1.425		0.525	
		71		47
9.25	-		0.425	
				47
9.5	1.175		0.325	
		71		35
9.75	-		0.25	
				35
10.0	0.925		0.175	
		71		23
10.25	0.80		-	
		71		
10.5	0.675		0.075	
		57		12
10.75	0.575		-	
		57		
11.0	0.475		0.025	
		57		6
11.25	0.375		-	
		43		
11.5	0.30		0.00	
		43		
11.75	0.225			
		43		
12.0	0.15			
		21		
12.5	0.075			
		14		
13.25	0.00			

$$U_o^{max} = 90 \qquad\qquad U_o^{max} = 76$$

NOTES: 1) Sample transferred to respirometer consisted of
1:9 proportions of 500 mg/ℓ feed solution and
culture.

2) Reactor A TSS = 470, Reactor B TSS = 570.

Table B-13. Respirometer Data for Samples from
Reactor B, Day 14, Run 2-2.

UNDILUTED SAMPLE			DILUTED 1:9		
time	DO	U_o	time	DO	U_o
(min)	(mg/ℓ)	(mg/g-hr)	(min)	(mg/ℓ)	(mg/g-hr)
0.0	9.95	148	0.0	6.0	171
0.5	8.65	160	3.0	5.1	161
1.0	7.25	171	6.0	4.25	161
1.5	5.75	160	9.0	3.4	160
2.0	4.35	171	14.0	2.0	157
2.25	3.6	160	16.0	1.45	142
2.5	2.9	171	17.0	1.2	142
2.75	2.15	160	18.0	0.95	142
3.0	1.45	160	19.0	0.7	142
3.25	0.75	125	20.0	0.45	128
3.5	0.2	28	21.0	0.225	85
3.75	0.075	6	21.5	0.15	85
4.0	0.05	3	22.0	0.075	57
4.5	0.025	3	22.5	0.025	28
5.0	0.00		22.75	0.00	

$$U_o^{max} = 166 \qquad\qquad U_o^{max} = 163$$

NOTES: 1) Sample transferred to respirometer consisted of
1:9 proportions of 500 mg/ℓ feed solution and
culture.

2) Reactor TSS = 1170 mg/ℓ.

Table B-14. Respirometer Data for Samples from
Reactor B, Day 15, Run 2-2.

time	100 RPM		200 RPM		500 RPM	
	DO	U_o	DO	U_o	DO	U_o
(min)	(mg/ℓ)	(mg/g-hr)	(mg/ℓ)	(mg/g-hr)	(mg/ℓ)	(mg/g-hr)
0.0	6.0		6.0		6.0	
		141		152		152
0.5	5.35		5.3		5.3	
		152		147		152
1.0	4.65		4.625		4.6	
		152		152		147
1.5	3.95		3.925		3.925	
		147		147		152
2.0	3.275		3.25		3.225	
		147		152		152
2.25	2.95		2.9		2.875	
		152		152		141
2.5	2.6		2.55		2.55	
		141		141		152
2.75	2.275		2.225		2.2	
		141		141		141
3.0	1.95		1.9		1.875	
		130		130		141
3.25	1.65		1.6		1.55	
		120		130		130
3.5	1.375		1.3		1.25	
		120		120		130
3.75	1.1		1.025		0.95	
		109		120		109
4.0	0.85		0.75		0.7	
		109		109		109
4.25	0.6		0.5		0.45	
		98		87		98
4.5	0.375		0.3		0.225	
		76		76		65
4.75	0.2		0.125		0.075	
		43		33		33
5.0	0.1		0.05		0.0	
		22		22		
5.25	0.05		0.0			
		22		0		
5.5	0.0		0.0			
5.75						
6.0						

$$U_o^{max} = 148 \qquad U_o^{max} = 150 \qquad U_o^{max} = 151$$

NOTES: 1) Respirometer sample consisted of 40% culture from
the main reactor, 10% of 500 mg/ℓ feed solution,
and 50% distilled water.

2) Main reactor TSS = 1380 mg/ℓ.

APPENDIX C

Phase 2 Data

Run #2-3

Table C-1. Summary of Daily Data for Run 2-3.

DAY	pH	θ_c (days)	X (mg/ℓ)	X^e (mg/ℓ)	SVI (ml/g)	
1	6.7	–	345	68	–	Begin Fill and Draw Operation
2	6.8	2.0	250	60	160	Set θ_c = 2.0 days
3	6.8	2.0	203	56	–	
4	6.8	2.0	215	43	–	
5	–	2.0	203	47	–	
6	7.0	2.0	190	50	105	Substantial Filamentous Outgrowth
7	6.9	2.0	193	38	–	
8	6.9	2.0	195	36	–	
9	6.9	2.0	195	40	180	
10	6.9	2.0	207	53	–	
11	–	2.0	220	23	165	
12	6.9	2.0	213	30	–	
13	–	2.0	225	27	–	
14	6.9	2.0	233	123	236	
15	–	2.0	205	24	–	
16	6.9	2.0	197	39	–	
17	–	3.0	190	40	221	Set θ_c = 3.0 days
18	6.8	3.0	205	45	–	
19	–	3.0	227	51	245	
20	6.9	3.0	235	48	–	
21	–	3.0	280	43	–	
22	6.9	3.0	292	44	223	
23	–	3.0	283	25	–	
24	6.9	3.0	307	18	–	
25	–	3.0	276	18	217	
26	–	3.0	287	20	–	

Table C-2.   Floc Size Distribution on Day 2 of Run 2-3.

FLOC SIZE (μ)	NUMBER OF FIELDS	NUMBER OF FLOC	RELATIVE % BY NUMBER	RELATIVE % BY VOLUME
0- 10	1	45	43.9	<0.1
10- 30	1	24	23.4	0.2
30- 60	1	19	18.6	1.2
60-100	1	9	8.8	2.8
100-150	5	14	2.7	15.5
150-200	5	6	1.2	17.2
200-250	5	4	0.8	23.9
250-300	5	2	0.4	21.6
300-350	5	1	0.2	17.7
350-400	5			

NOTE: Dense, nonfilamentous floc.

Table C-3. Floc Size Distribution on Day 6 of Run 2-3.

FLOC SIZE (μ)	NUMBER OF FIELDS	NUMBER OF FLOC	RELATIVE % BY NUMBER	RELATIVE % BY VOLUME
0- 10	1	132	79.6	0.1
10- 30	1	14	8.4	0.2
30- 60	1	8	4.8	1.1
60-100	1	7	4.2	5.1
100-150	5	8	1.0	4.2
150-200	5	5	0.6	6.8
200-250	5	4	0.5	11.2
250-300	5	3	0.4	15.2
300-350	5	3	0.4	24.9
350-400	5	1	0.1	12.7
400-450	5	1	0.1	18.4
450-500	5	0	0	0.0

NOTE: Large flocs (7250 μ) with substantial filamentous outgrowth.

Table C-4. Floc Size Distribution on Day 9 of Run 2-3.

FLOC SIZE (µ)	NUMBER OF FIELDS	NUMBER OF FLOC	RELATIVE % BY NUMBER	RELATIVE % BY VOLUME
0- 10	1	75	64.9	<0.1
10- 30	1	24	20.8	0.4
30- 60	1	11	9.5	1.7
60-100	1	3	2.6	2.4
100-150	20	24	1.0	3.4
150-200	20	9	0.4	3.3
200-250	20	2	0.1	1.5
250-300	20	3	0.1	4.2
300-350	20	3	0.1	6.8
350-400	20	4	0.2	13.9
400-450	20	3	0.1	15.1
450-500	20	2	0.1	14.0
500-600	20	3	0.1	33.2

NOTE: Large flocs (>300 µ) with substantial filamentous outgrowth.

Table C-5. Floc Size Distribution on Day 14 of Run 2-3.

FLOC SIZE (µ)	NUMBER OF FIELDS	NUMBER OF FLOC	RELATIVE % BY NUMBER	RELATIVE % BY VOLUME
0- 10	1	68	61.7	<0.1
10- 30	1	28	25.4	0.3
30- 60	1	8	7.3	0.8
60-100	1	5	4.5	2.5
100-150	50	33	0.6	1.2
150-200	50	10	0.2	0.9
200-250	50	2	<0.1	0.4
250-300	50	1	<0.1	0.4
300-350	50	1	<0.1	0.6
350-400	50	0	0.0	0.0
400-450	50	2	<0.1	2.5
450-500	50	3	<0.1	5.3
500-600	50	4	0.1	11.2
600-700	50	1	<0.1	4.6
700-800	50	0	0.0	0.0
800-900	50	1	<0.1	10.2
900-1000	50	1	<0.1	14.2
1000-1200	50	2	<0.1	44.9

NOTE: Large flocs (>400 µ) with substantial filamentous growth.

Table C-6. Floc Size Distribution on Day 19 of Run 2-3.

FLOC SIZE (μ)	NUMBER OF FIELDS	NUMBER OF FLOC	RELATIVE % BY NUMBER	RELATIVE % BY VOLUME
0- 10	1	33	54.4	<0.1
10- 30	1	12	19.8	0.3
30- 60	1	7	11.6	1.5
60-100	1	6	9.9	6.5
100-150	25	45	3.0	7.0
150-200	25	11	0.7	4.5
200-250	25	3	0.2	2.5
250-300	25	2	0.1	3.0
300-350	200	4	<0.1	1.2
350-400	200	3	<0.1	1.4
400-450	200	1	<0.1	0.7
450-500	200	3	<0.1	2.9
500-600	200	4	<0.1	6.1
600-700	200	5	<0.1	12.5
700-800	200	2	<0.1	7.6
800-900	200	1	<0.1	5.5
900-1000	200	0	0.0	0.0
1000-1200	200	3	<0.1	36.5

Table C-7. Respirometric Data on Day 2 of
Run 2-3 (t = 0.5 hours).

TIME (min)	DO (mg/ℓ)	U_o (mg/g-hr)
0.0	6.0	84
1.0	5.65	84
2.0	5.3	96
3.0	4.9	84
4.0	4.55	84
5.0	4.2	84
6.0	3.85	84
7.0	3.5	84
8.0	3.15	84
9.0	2.8	84
10.0	2.45	84
11.0	2.1	78
12.0	1.775	78
13.0	1.45	78
14.0	1.125	72
15.0	0.825	72
15.5	0.675	72
16.0	0.525	72
16.5	0.375	60
17.0	0.25	48
17.5	0.15	36
18.0	0.075	24
18.5	0.025	12
19.0	0.0	0

$$U_o^{max} = 85$$

NOTES: 1) Reactor TSS = 250 mg/ℓ.

Table C-8. Respirometric Data on Day 6 of
Run 2-3 (t = 0.5 hours)

	FLOC			BLENDED	
TIME (min)	DO (mg/ℓ)	U_o (mg/g-hr)	TIME (min)	DO (mg/ℓ)	U_o (mg/g-hr)
0.0	6.0		0.0	6.0	
1.0	5.6	126	1.0	5.6	126
2.0	5.25	111	2.0	5.25	111
3.0	4.9	111	3.0	4.9	111
4.0	4.55	111	4.0	4.5	126
5.0	4.2	111	5.0	4.15	111
6.0	3.85	111	6.0	3.8	111
7.0	3.525	103	7.0	3.4	126
8.0	3.175	111	8.0	3.05	111
9.0	2.85	103	9.0	2.7	111
10.0	2.525	103	10.0	2.35	111
11.0	2.225	95	11.0	2.0	111
12.0	1.925	95	12.0	1.675	103
13.0	1.65	87	13.0	1.325	111
14.0	1.4	79	14.0	1.0	103
15.0	1.15	79	15.0	0.675	103
16.0	0.925	71	16.0	0.375	95
17.0	0.7	71	16.5	0.25	79
18.0	0.5	63	17.0	0.125	79
19.0	0.325	55	17.5	0.025	63
20.0	0.175	47	18.0	0.0	16
21.0	0.10	24			0
21.5	0.05	32			
22.0	0.025	16			
23.0	0.00	8			

$$U_o^{max} = 111 \qquad\qquad U_o^{max} = 116$$

NOTES: 1) Reactor TSS = 190 mg/ℓ.

Table C-9. Respirometric Data on Day 9 of Run 2-3.

t = 0.5 hours[1]			t = 1.5 hours[2]			t = 3.0 hours[3]		
TIME	DO	U_o	TIME	DO	U_o	TIME	DO	U_o
(min)	(mg/ℓ)	(mg/g-hr)	(min)	(mg/ℓ)	(mg/g-hr)	(min)	(mg/ℓ)	(mg/g-hr)
0.0	7.1		0.0	7.0		0.0	6.9	
2.0	6.6	75	2.0	6.25	109	1.0	6.3	158
4.0	6.1	75	4.0	5.5	109	2.0	5.75	145
6.0	5.65	68	6.0	4.75	109	3.0	5.2	145
8.0	5.15	75	8.0	4.05	101	4.0	4.675	139
10.0	4.7	68	10.0	3.35	101	5.0	4.15	139
12.0	4.25	68	12.0	2.7	94	6.0	3.65	132
14.0	3.8	68	14.0	2.075	91	7.0	3.15	132
16.0	3.35	68	15.0	1.775	87	8.0	2.65	132
18.0	2.925	64	16.0	1.5	80	9.0	2.175	126
20.0	2.5	64	17.0	1.225	80	10.0	1.725	119
22.0	2.1	60	18.0	0.975	72	10.5	1.525	106
24.0	1.725	57	19.0	0.725	72	11.0	1.325	106
26.0	1.35	57	20.0	0.525	58	11.5	1.15	93
27.0	1.175	53	21.0	0.35	51	12.0	0.975	93
28.0	1.025	45	22.0	0.2	43	12.5	0.80	93
29.0	0.875	45	23.0	0.1	29	13.0	0.65	79
30.0	0.725	45	24.0	0.05	14	13.5	0.5	79
31.0	0.6	38	26.0	0.0	7	14.0	0.375	66
32.0	0.475	38			0	14.5	0.275	53
33.0	0.35	38				15.0	0.175	53
34.0	0.25	30				15.5	0.125	26
35.0	0.175	23		U_o^{max} = 109		16.0	0.075	26
36.0	0.125	15				16.5	0.05	13
37.0	0.075	15				17.0	0.025	13
38.0	0.025	15				18.0	0.0	7
39.0	0.0	8						0
	U_o^{max} = 73						U_o^{max} = 151	

[1] C_s = 93 mg/ℓ, X = 199 mg/ℓ.

[2] C_s = 80 mg/ℓ, X = 207 mg/ℓ.

[3] C_s = 47 mg/ℓ, X = 227 mg/ℓ.

Table C-10. Respirometric Data on Day 14 of Run 2-3.

t = 1.0 hours[1]			t = 3.0 hours[2]			t = 4.0 hours[3]		
TIME	DO	U_o	TIME	DO	U_o	TIME	DO	U_o
(min)	(mg/ℓ)	(mg/g-hr)	(min)	(mg/ℓ)	(mg/g-hr)	(min)	(mg/ℓ)	(mg/g-hr)
0.0	10.0		0.0	10.0		0.0	10.0	
2.0	9.35	81	2.0	9.05	109	2.0	8.75	135
4.0	8.7	81	4.0	8.0	120	4.0	7.45	141
6.0	8.0	87	6.0	7.0	115	6.0	6.15	141
8.0	7.3	87	8.0	6.0	115	7.0	5.5	141
10.0	6.6	87	10.0	5.0	115	8.0	4.9	130
12.0	5.95	81	11.0	4.525	109	9.0	4.3	130
14.0	5.25	87	12.0	4.075	103	10.0	3.725	125
16.0	4.6	81	13.0	3.625	103	11.0	3.175	119
18.0	3.975	78	14.0	3.2	97	12.0	2.65	114
20.0	3.35	78	15.0	2.8	92	13.0	2.15	108
22.0	2.75	75	16.0	2.4	92	14.0	1.675	103
23.0	2.45	75	17.0	2.0	92	15.0	1.225	97
24.0	2.175	68	18.0	1.65	80	16.0	0.85	81
25.0	1.9	68	19.0	1.325	74	17.0	0.55	65
26.0	1.65	62	20.0	1.025	69	18.0	0.3	54
27.0	1.4	62	21.0	0.75	63	19.0	0.125	38
28.0	1.175	56	22.0	0.525	52	21.0	0.0	14
29.0	0.975	50	23.0	0.325	46			0
30.0	0.775	50	24.0	0.175	34			
31.0	0.6	44	25.0	0.075	23			$U_o^{max} = 141$
32.0	0.45	37	26.0	0.025	11			
33.0	0.3	37	27.0	0.0	6			
34.0	0.175	31			0			
35.0	0.10	19						
36.0	0.05	12			$U_o^{max} = 115$			
38.0	0.0	6						
		$U_o^{max} = 85$						

[1] $C_s = 86$ mg/ℓ, X = 241.

[2] $C_s = 52$ mg/ℓ, X = 262

[3] $C_s = 26$ mg/ℓ, X = 277

Table C-11. Respirometric Data on Day 19 of Run 2-3.

| t = 1.0 hours[1] | | | t = 3.0 hours[2] | | | t = 5.0 hours[3] | | |
TIME (min)	DO (mg/ℓ)	U_o (mg/g-hr)	TIME (min)	DO (mg/ℓ)	U_o (mg/g-hr)	TIME (min)	DO (mg/ℓ)	U_o (mg/g-hr)
0.0	8.0	72	0.0	8.5	102	0.0	9.8	143
2.0	7.45	72	2.0	7.65	102	1.0	9.15	143
4.0	6.9	72	4.0	6.8	102	2.0	8.5	154
6.0	6.35	72	6.0	5.95	96	3.0	7.8	132
8.0	5.8	72	8.0	5.15	96	4.0	7.2	132
10.0	5.25	65	9.0	4.75	90	5.0	6.6	143
12.0	4.75	65	10.0	4.375	84	6.0	5.95	132
14.0	4.25	65	11.0	4.025	90	7.0	5.35	132
16.0	3.75	65	12.0	3.65	84	8.0	4.75	126
18.0	3.25	62	13.0	3.3	84	9.0	4.175	126
20.0	2.775	62	14.0	2.95	84	10.0	3.6	121
22.0	2.3	55	15.0	2.6	78	11.0	3.05	115
24.0	1.875	55	16.0	2.275	78	12.0	2.525	110
26.0	1.45	46	17.0	1.95	72	13.0	2.025	110
28.0	1.1	46	18.0	1.65	66	14.0	1.525	99
30.0	0.75	39	19.0	1.375	60	15.0	1.075	77
31.0	0.6	33	20.0	1.125	60	16.0	0.725	77
32.0	0.475	33	21.0	0.875	54	16.5	0.55	66
33.0	0.35	26	22.0	0.65	54	17.0	0.4	44
34.0	0.25	26	23.0	0.425	48	17.5	0.3	44
35.0	0.15	20	24.0	0.225	24	18.0	0.2	33
36.0	0.075	13	25.0	0.125	18	16.5	0.125	22
37.0	0.025	7	26.0	0.05	6	17.0	0.075	7
38.0	0.0	0	28.0	0.00	0	18.5	0.025	5
						19.5	0.0	0

$$U_o^{max} = 72 \qquad U_o^{max} = 102 \qquad U_o^{max} = 143$$

[1] $C_s = 95$ mg/ℓ, $X = 233$ mg/ℓ.

[2] $C_s = 67$ mg/ℓ, $X = 250$ mg/ℓ.

[3] $C_s = 29$ mg/ℓ, $X = 273$ mg/ℓ.

Table C-12. Respirometric Data on Day 23 of Run 2-3.

TIME (min)	t = 0.5 hours[1] DO (mg/ℓ)	U_o (mg/g-hr)	t = 1.5 hours[2] DO (mg/ℓ)	U_o (mg/g-hr)	t = 2.5 hours[3] DO (mg/ℓ)	U_o (mg/g-hr)
0.0	7.5		10.0		9.7	
1.0	7.0	106	9.45	111	8.85	162
2.0	6.5	106	8.9	111	8.05	152
3.0	6.05	95	8.3	122	7.25	152
4.0	5.65	85	7.75	111	6.45	152
5.0	5.2	95	7.15	122	5.7	143
6.0	4.775	90	6.6	111	4.9	152
7.0	4.35	90	6.05	111	4.15	143
8.0	3.925	90	5.45	122	3.4	143
9.0	3.5	90	4.9	111	2.675	138
10.0	3.1	85	4.35	111	2.0	129
11.0	2.7	85	3.825	106	1.4	114
12.0	2.325	80	3.275	111	0.875	100
13.0	1.975	74	2.775	101	0.425	86
14.0	1.625	74	2.25	106	0.15	52
15.0	1.3	69	1.775	96	0.025	24
16.0	1.0	64	1.35	86	0.00	5
17.0	0.7	64	0.95	81		
18.0	0.425	58	0.575	76	U_o^{max} = 162	
19.0	0.275	32	0.3	56		
20.0	0.125	32	0.1	41		
21.0	0.05	16	0.025	15		
22.0	0.025	5	0.00	5		
23.0	0.00	5				
24.0		U_o^{max} = 91	U_o^{max} = 115			

[1] C_s = 97 mg/ℓ, X = 285

[2] C_s = 78 mg/ℓ, X = 296

[3] C_s = 46 mg/ℓ, X = 315

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APPENDIX D

Application of the Anoxic Core

Model to Respirometric Data

The anoxic core mathematical model of oxygen diffusion and reaction within microbial aggregates was developed in Chapters II and III. The final model incorporates two basic equations that must be solved simultaneously:

$$\frac{6}{U_o^{max}} \frac{D_o}{\rho} \frac{C_{b,o}}{R^2} = 2 \left(\frac{r_a}{R}\right)^3 - 3 \left(\frac{r_a}{R}\right)^2 + 1 \qquad \text{(D-1)}$$

$$U_o = U_o^{max} \left[1 - \left(\frac{r_a}{R}\right)^3\right] \qquad \text{(D-2)}$$

with the following restriction on the domain for $\frac{r_a}{R}$

$$0 \leq \frac{r_a}{R} \leq 1 \qquad \text{(D-3)}$$

These equations (D-1 through D-3) specify the value of the overall specific respiration rate, U_o, for a spherical microbial aggregate with a maximum specific respiration rate of U_o^{max}, a diffusivity of D_o, a density ρ, and a radius R. The assumptions utilized in the development of these equations were discussed in Chapters II and III.

If we let

$$\alpha = 1 - \frac{6}{U_o^{max}} \frac{D_o}{\rho} \frac{C_{b,o}}{R^2} \qquad \text{(D-4)}$$

and

$$y = \frac{r_a}{R} \qquad \text{(D-5)}$$

Then Equations D-1 through D-3 can be rewritten:

$$y^3 - \frac{3}{2} y^2 + \frac{\alpha}{2} = 0 \qquad \text{(D-6)}$$

$$U_o = U_o^{max} \left[1 - y^3\right] \qquad \text{(D-7)}$$

$$0 \leq y \leq 1 \qquad \text{(D-8)}$$

Equation D-6 is a simple cubic equation that can readily be solved by numerical techniques to yield the value of y for any given value of α. This value for y may then be substituted into Equation D-7 to obtain the value of U_o. The domain for α is restricted to the interval

$$0 \leq \alpha \leq 1 \qquad\qquad\qquad \text{(D-9)}$$

As noted in Chapter III, the major difficulty involved in application of this model is the measurement of the diffusivity, density, and diffusional radius of bacterial aggregates such as occur in the activated sludge process. Within this study, the diameters of activated sludge floc were measured microscopically. However, neither the density nor the diffusional radius of the flocs were measured due to the lack of an appropriate and accurate technique. Therefore, application of the model to the respirometric data obtained within this study requires the use of the parameters D_o and ρ as "fitting" parameters. As can be seen from Equation D-4, these two parameters can be combined into a single "fitting" parameter of $\dfrac{D_o}{\rho}$.

Because the mixed cultures utilized within this study exhibited a broad distribution of floc sizes and because each respirometric experiment yielded a set of values of U_o versus $C_{b,o}$, application of the model to these data require an expansion of Equations D-4, D-6, and D-7 as follows:

$$\alpha_{i,j} = 1 - \frac{6}{U_o^{max}} \frac{D_o}{\rho} \frac{C_j}{R_i^2} \qquad\qquad \text{(D-10)}$$

$$y_{i,j}^3 - \frac{3}{2} y_{i,j}^2 + \frac{\alpha_{i,j}}{2} = 0 \qquad\qquad \text{(D-11)}$$

$$U_j = \sum_{i=1}^{m} f_i U_o^{max} [1 - y_{i,j}^3] \qquad\qquad \text{(D-12)}$$

where f_i is the percent of bacterial floc volume that is present in floc size range i with average (volume-weighted) radius R_i, $\alpha_{i,j}$ is the value of α for a floc with radius R_i at a bulk DO ($C_{b,o}$) of C_j, $y_{i,j}$ is the corresponding value of y, and U_j is the value of U_o at a bulk DO of C_j.

A computer program was developed to solve the previous three equations and to determine the optimum value of $\frac{D_o}{\rho}$, that is, the value that produced the best curve fit to the respirometric data. The computer program functioned in the following manner:

1) For a given respirometric experiment, the values of U_o versus $C_{b,o}$ (U_j versus C_j) and of f_i versus R_i, and the value of U_o^{max} were read in.

2) An initial value of $\frac{D_o}{\rho}$ was assumed and the values of $\alpha_{i,j}$ were calculated from Equation D-10 for each R_i and C_j.

3) The values of $y_{i,j}$ were determined from Equation D-11 by Newton's Method.

4) The value of $\hat{U}_j$ was determined from Equation D-12 for each C_j.

5) The value of $\frac{D_o}{\rho}$ was optimized by minimizing the sum of squared errors (SSE) according to the method of Coggins (1974)

$$SSE = \sum_{j=1}^{N} (U_j - \hat{U}_j)^2$$

6) The optimum value of $\frac{D_o}{\rho}$ was then utilized to calculate predicted values of U_o.

Two techniques were utilized to reduce the amount of data obtained from the respirometric experiments so that plots of U_o versus $C_{b,o}$ were

clear and so that computer time was minimized. First, because the charge in $C_{b,o}$ within intervals was only several times the readability of the DO meter, the same value of U_o was often repeated for adjacent points in the U_o versus $C_{b,o}$ data. The value of $C_{b,o}$ was averaged for up to three adjacent data points containing the same value of U_o and only a single point was plotted. Second, when the value of U_o increased and then decreased for successive data points with increasing values of $C_{b,o}$, the two points were averaged and a single point reflecting the average U_o at the average $C_{b,o}$ was plotted. It was found that these two techniques resulted in a clearer graphical presentation of the data. Furthermore, it was found that these two averaging techniques had only a very small impact on the optimum value of $\dfrac{D_o}{\rho}$ and resulted in a considerable savings in computer time.

APPENDIX E

PHASE 3 DATA

Table E-1. Plant Data from the Salem, Oregon WWTP.

DATE	PRIMARY EFFLUENT FLOW (MGD)	PRIMARY EFFLUENT BOD$_5$ (mg/ℓ)	AERATION BASIN pH	AERATION BASIN MLSS[1] (mg/ℓ)	AERATION BASIN θ (hrs)	ACTIVATED SLUDGE OPERATION F:M[2] (g/g-day)	ACTIVATED SLUDGE OPERATION SRT (days)	ACTIVATED SLUDGE OPERATION SVI[3] (ml/g)	ACTIVATED SLUDGE OPERATION RAS (% TSS)	R/Q[4] (%)
8/01	13.34	136	5.7	4859	2.52	0.25	39.9	62	1.84	35.0
02	16.43	129	5.6	3819	3.19	0.24	42.7	61	1.75	26.4
03	14.81	105	5.4	4000	3.47	0.17	64.7	60	1.70	31.0
04	17.19	154	5.5	4009	3.04	0.28	34.8	59	1.74	25.9
05	17.66	146	5.5	4386	2.87	0.26	39.2	56	1.76	27.9
06	16.96	143	5.5	4729	2.94	0.23	60.9	60	1.84	36.4
07	16.79	143	5.6	4898	2.82	0.21	77.6	58	1.90	34.7
08	18.08	278	5.6	5274	2.64	0.42	32.4	55	1.86	42.7
09	16.27	123	5.5	5971	2.85	0.15	49.2	59	1.90	46.8
10	14.24	104	5.2	5621	3.37	0.12	12.4	61	1.93	41.9

[1] MLVSS ≃ 0.80 x MLSS.
[2] F:M ratio expressed as grams BOD$_5$ removed per gram mixed liquor suspended solids per day.
[3] Thirty-minute settling test.
[4] Ratio of return activated sludge flow to primary effluent flow.

NOTE: Plant microscopic exams reported the following microbial composition: absence of filamentous bacteria, nematodes, and rotifers; few free swimming ciliates and flagellates; numerous stalked ciliates.

Table E-2. Floc Size Distribution of the
Salem WWTP on August 4, 1980.

FLOC DIAMETER (μ)	NUMBER OF FLOC	PERCENT OF TOTAL NUMBER	PERCENT OF TOTAL VOLUME
0- 10	123	49.8	0.4
10- 30	75	30.4	6.5
30- 60	37	15.0	27.9
60-100	10	4.0	37.8
100-150	2	0.8	27.3
150-200	0	0.0	0.0

Table E-3. Respirometric Tests at Salem WWTP on August 4, 1980.

TEST NO. 1			TEST NO. 2		
TIME	DO	U_O	TIME	DO	U_O
(min)	(mg/ℓ)	(mg/g-hr)	(min)	(mg/ℓ)	(mg/g-hr)
0.0	20.0		0.0	5.0	
2.0	17.7	17.4	0.5	4.45	16.7
4.0	15.3	18.2	1.0	3.925	14.4
6.0	13.0	17.4	1.5	3.4	15.9
8.0	10.6	18.2	2.0	2.825	17.4
10.0	8.25	17.8	2.25	2.55	16.7
11.0	7.0	18.9	2.5	2.275	16.7
12.0	5.8	18.2	2.75	2.0	16.7
13.0	4.625	17.8	3.0	1.7	18.2
14.0	3.425	18.2	3.25	1.425	16.7
15.0	2.25	17.8	3.5	1.175	15.2
16.0	1.1	17.4	3.75	0.9	16.7
16.5	0.6	15.2	4.0	0.65	15.2
17.0	0.275	9.8	4.25	0.40	15.2
17.5	0.1	5.3	4.5	0.2	12.0
18.0	0.0	3.0	4.75	0.075	7.6
			5.0	0.025	3.0
			5.25	0.0	1.5

$$U_O^{MAX} = 17.9$$

$$U_O^{MAX} = 16.6$$

NOTES: 1) MLSS = 3960 mg/ℓ.
        2) R/Q = 29.2%
        3) Primary Effluent COD = 324 mg/ℓ, TSS = 88 mg/ℓ.
        4) T = 22°C.

Table E-4. Batch Respirometic Test at the
Salem WWTP on August 5, 1980.

TIME (min)	DO (mg/ℓ)	U_o (mg/g-hr)
0.0	8.0	22.0
1.0	6.45	23.8
2.0	4.775	23.4
2.5	3.95	23.4
3.0	3.125	24.1
3.5	2.275	22.7
3.75	1.875	24.1
4.0	1.45	24.1
4.25	1.025	22.7
4.5	0.625	20.0
4.75	0.275	8.5
5.0	0.125	4.3
5.5	0.05	2.8
6.0	0.0	

$$U_o^{MAX} = 23.4 \text{ mg/g-hr}$$

NOTES; 1) MLSS = 4230 mg/ℓ.
2) R/Q = 33.1%
3) Primary Effluent COD = 396 mg/ℓ,
TSS = 84 mg/ℓ.

Table E-5. Batch Reactor Simulation at the
Salem WWTP on August 5, 1980.

TIME	U_o^1 (mg/g-hr)		T^2 (mg/g)	
(hours)	A^3	B^3	A	B
0	23.4	23.4	-	-
0.25	-	-	1.22	1.31
0.5	17.7	16.6	-	-
0.75	-	-	1.47	1.39
1.0	12.8	12.8	-	-
1.25	-	-	1.53	1.60
1.5	8.4	8.6	-	-
1.75	-	-	1.49	1.62
2.0	6.5	6.8	-	-
2.25	-	-	1.56	-
2.5	5.7	5.7	-	-
2.75	-	-	1.46	1.50
3.0	5.4	5.0	-	-

[1] Specific respiration rate in milligrams of oxygen utilized per gram of mixed liquor suspended solids per hour.

[2] Specific ATP content in milligrams ATP per gram of mixed liquor suspended solids.

[3] Batch Reactor A was maintained at a DO concentration of 2.0 mg/ℓ, Reactor B at 15.0 mg/ℓ.

Table E-6. Floc Size Distribution of the
Salem WWTP on August 6, 1980.

FLOC SIZE (μ)	NUMBER OF FLOC	PERCENT OF TOTAL NUMBER	PERCENT OF TOTAL VOLUME
0- 10	177	64.1	0.9
10- 30	62	22.5	8.3
30- 60	30	10.9	35.1
60-100	6	2.2	34.7
100-150	1	0.4	21.1
150-200	0	0.0	0.0

Table E-7. Batch Respirometric Tests at the Salem WWTP on August 6, 1980.

TEST NO. 1 (not diluted)			TEST NO. 2 (RAS diluted 1:1)		
TIME (min)	DO (mg/ℓ)	U_o (mg/g-hr)	TIME (min)	DO (mg/ℓ)	U_o (mg/g-hr)
0.0	9.0		0.0	5.0	
0.5	6.6	71.6	0.25	4.425	68.7
0.75	5.5	65.7	0.5	3.825	71.6
1.0	4.3	71.6	0.75	3.225	71.6
1.25	3.1	71.6	1.0	2.65	68.7
1.5	1.9	71.6	1.25	2.05	71.6
1.75	0.8	65.7	1.5	1.475	68.7
2.0	0.15	38.8	1.75	0.9	68.7
2.25	0.05	6.0	2.0	0.475	50.7
2.75	0.0	1.5	2.25	0.2	32.8
3.0	0.0	0.0	2.5	0.075	14.9
			2.75	0.025	6.0
			3.0	0.0	3.0

$U_o^{MAX} = 69.6$ (Test No. 1)

$U_o^{MAX} = 69.9$ (Test No. 2)

NOTES: 1) MLSS = 4020 mg/ℓ (undiluted), 2010 mg/ℓ (1:1 dilution).
2) R/Q = 27.9%
3) Primary Effluent COD = 486 mg/ℓ, TSS = 77 mg/ℓ.

Table E-8. Floc Size Distribution of the
Salem WWTP on August 7, 1980.

FLOC SIZE (μ)	NUMBER OF FLOC	PERCENT OF TOTAL NUMBER	PERCENT OF TOTAL VOLUME
0- 10	212	63.5	1.5
10- 30	85	25.4	16.4
30- 60	34	10.2	57.0
60-100	3	0.9	25.1
100-150	0	0.0	0.0

Table E-9. Batch Respirometric Tests at the Salem WWTP on August 7, 1980.

TEST NO. 1 (not diluted)			TEST NO. 2 (RAS diluted 1:3)		
TIME (min)	DO (mg/ℓ)	U_o (mg/g-hr)	TIME (min)	DO (mg/ℓ)	U_o (mg/g-hr)
0.0	15.0		0.0	5.0	
		51.9			57.1
0.25	14.0		0.25	4.725	
		57.1			62.3
0.5	12.9		0.5	4.425	
		57.1			57.1
0.75	11.8		0.75	4.15	
		57.1			57.1
1.0	10.7		1.0	3.875	
		57.1			57.1
1.25	9.6		1.25	3.6	
		62.3			62.3
1.5	8.4		1.5	3.3	
		57.1			57.1
1.75	7.3		1.75	3.025	
		62.3			57.1
2.0	6.1		2.0	2.75	
		62.3			52.0
2.25	4.9		2.25	2.5	
		57.1			57.1
2.5	3.8		2.5	2.225	
		62.3			57.1
2.75	2.6		2.75	1.95	
		51.9			57.1
3.0	1.6		3.0	1.675	
		57.1			57.1
3.25	0.5		3.25	1.4	
		15.6			57.1
3.5	0.2		3.5	1.125	
		5.2			52.0
3.75	0.1		3.75	0.875	
		2.6			46.8
4.0	0.05		4.0	0.65	
		1.3			46.8
4.25	0.025		4.25	0.425	
		1.3			31.2
4.5	0.0		4.5	0.275	
					20.8
			4.75	0.175	
					15.6
			5.0	0.1	
					10.4
			5.25	0.05	
					5.2
			5.5	0.025	
					5.2
			5.75	0.0	

$$U_o^{MAX} = 58.2 \text{ mg/g-hr}$$

$$U_o^{MAX} = 57.6 \text{ mg/g-hr}$$

NOTES:
1) MLSS = 4620 mg/ℓ (undiluted), 1155 mg/ℓ (diluted 1:3).
2) R/Q = 31.2%
3) Primary Effluent COD = 512 mg/ℓ, TSS = 69 mg/ℓ.

Table E-10. Batch Reactor Simulation at the Salem WWTP on August 7, 1980.

TIME	U_o (mg/g-hr)		T (mg/g)	
(hours)	A[1]	B[2]	A	B
0.0	57.6	57.6	-	-
0.25			1.66	1.74
0.5	-	-		
0.75			2.18	1.98
1.0	17.8	18.2		
1.25			2.19	2.25
1.5	14.0	13.6		
1.75			2.13	2.22
2.0	6.7	7.9		
2.25			2.01	1.98
2.5	5.2	5.6		
2.75			1.93	1.84
3.0	5.1	4.8		

[1] Reactor A DO = 2 mg/ℓ.

[2] Reactor B DO = 15 mg/ℓ.

Table E-11. Floc Size Distribution at the
Albany WWTP on December 31, 1980.

FLOC SIZE (μ)	NUMBER OF FIELDS	NUMBER OF FLOC	RELATIVE % BY NUMBER	RELATIVE % BY VOLUME
0- 10	1	152	48.2	0.3
10- 30	1	70	22.2	4.3
30- 60	1	81	25.7	42.9
60-100	1	11	3.5	29.1
100-150	10	10	0.3	9.6
150-200	10	2	<0.1	5.0
200-250	10	1	<0.1	5.1
250-300	50	2	<0.1	3.7

Table E-12. Respirometric Tests at the Albany
WWTP on December 31, 1980.

TEST NO. 1			TEST NO. 1-G		
TIME (min)	DO (mg/ℓ)	U_o (mg/g-hr)	TIME (min)	DO (mg/ℓ)	U_o (mg/g-hr)
0.0	8.0	19.5	0.0	7.9	13.6
1.0	7.5	19.5	2.0	7.2	12.7
2.0	7.0	17.5	4.0	6.55	12.7
3.0	6.55	17.5	6.0	5.9	12.7
4.0	6.1	17.5	8.0	5.25	11.7
5.0	5.65	17.5	10.0	4.65	12.7
6.0	5.2	17.5	12.0	4.0	11.7
7.0	4.75	17.5	14.0	3.4	12.2
8.0	4.3	15.6	16.0	2.775	12.2
9.0	3.9	16.6	18.0	2.15	12.2
10.0	3.475	16.6	20.0	1.525	11.7
11.0	3.05	16.6	21.0	1.225	11.7
12.0	2.625	16.6	22.0	0.925	13.6
13.0	2.2	15.6	23.0	0.6	11.7
14.0	1.8	14.6	23.5	0.45	11.7
15.0	1.425	14.6	24.0	0.3	9.7
16.0	1.05	14.6	24.5	0.175	7.8
17.0	0.675	13.6	25.0	0.075	3.9
17.5	0.5	11.7	25.5	0.025	1.9
18.0	0.35	11.7	26.0	0.0	
18.5	0.2	9.7			
19.0	0.075	3.9			
19.5	0.025	1.9			
20.0	0.0				

$$U_o^{MAX} = 12.2$$

NOTES:
1) MLSS = 1540 mg/ℓ.
2) R/Q = 22%
3) Primary Effluent COD = 176 mg/ℓ, TSS = 74 mg/ℓ.
4) For Run No. 1-G, 1000 mg/ℓ glucose was substituted for primary effluent in the respirometric test.

Table E-13. Batch Reactor Simulation at the Albany WWTP on December 31, 1980.

TIME	U_o (mg/g-hr)		T (mg/g)	
(hours)	A[1]	B[2]	A	B
0.0	17.5	17.4		
0.25			1.87	1.91
0.5	16.0	15.7		
0.75			2.26	-
1.0	14.8	14.5		
1.5			2.48	2.39
1.75	11.3	11.0		
2.25			2.40	2.52
2.5	5.8	6.2		
3.0			2.42	2.29
3.25	5.5	5.5		
4.0			2.27	2.18
4.25	5.5	5.2		

[1] Reactor A DO = 1.0 mg/ℓ.

[2] Reactor B DO = 8.0 mg/ℓ.

Table E-14. Floc Size Distribution at the Corvallis
WWTP on December 29, 1980.

FLOC SIZE (μ)	NUMBER OF FIELDS	NUMBER OF FLOC	RELATIVE % BY NUMBER	RELATIVE % BY VOLUME
0- 10	1	105	26.3	0.1
10- 30	1	123	30.8	2.9
30- 60	1	156	39.1	31.8
60-100	1	60	15.0	61.3
100-150	10	8	2.0	2.9
150-200	10	1	<0.1	1.0
200-250	10	0	0.0	0.0

Table E-15. Respirometric Data for Corvallis WWTP on December 29, 1980.

RUN NO. 1			RUN NO. 2		
TIME (min)	DO (mg/ℓ)	U_o (mg/g-hr)	TIME (min)	DO (mg/ℓ)	U_o (mg/g-hr)
0.0	6.55		0.0	15.0	
0.5	6.05	21.3	0.5	14.5	21.3
1.0	5.6	19.1	1.0	14.05	19.1
1.5	5.15	19.1	1.5	13.65	17.0
2.0	4.8	14.9	2.0	13.25	17.0
2.5	4.4	17.0	2.5	12.85	17.0
3.0	4.025	16.0	3.0	12.45	17.0
3.5	3.65	16.0	3.5	12.1	14.9
4.0	3.275	16.0	4.0	11.7	17.0
4.5	2.9	16.0	4.5	11.3	17.0
5.0	2.525	16.0	5.0	10.95	14.9
5.5	2.175	14.9	5.5	10.55	17.0
6.0	1.8	16.0	6.0	10.2	14.9
6.5	1.45	14.9	6.5	9.8	17.0
7.0	1.125	13.8	7.0	9.45	14.9
7.5	0.775	14.9	7.5	9.1	14.9
8.0	0.45	13.8	8.0	8.75	14.9
8.5	0.2	10.6	8.5	8.4	14.9
9.0	0.075	5.3	9.0	8.1	12.8
9.5	0.025	2.1	9.5	7.75	14.9
10.0	0.00	1.1	10.0	7.45	12.8

NOTE: R/Q = 0.39
MLSS = 2820
Primary Effluent COD = 114 mg/ℓ, TSS = 57 mg/ℓ.