Respirometric measurement of kinetic parameters: effect of activated sludge floc size

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Abstract The variation of activated sludge floc size with the mixing intensity of a mechanically stirred respirometer, expressed in terms of the mean energy dissipation rate, was characterized using a photometric dispersion analyzer. The floc size decreased rapidly when the energy dissipation rate was increased from $1.33 \times 10^{-3}$ to $2.68 \times 10^{-3}$ W/kg. Experiments were performed to investigate the effect of floc size on the oxygen saturation coefficient measured under the condition of acetate oxidation. The respirometric data were interpreted by considering only the kinetics of biochemical reactions. The variation of the oxygen saturation coefficient with mixing intensity was found to correlate with the variation of floc size with mixing intensity. The oxygen saturation coefficient was found to decrease from 0.23 to 0.08 mg/L when the mean energy dissipation rate was increased from $1.33 \times 10^{-3}$ to $2.68 \times 10^{-3}$ W/kg. The dependence of the oxygen saturation coefficient on floc size or mixing intensity suggests the presence of mass transfer resistances in large flocs.

Keywords Activated sludge; floc size; kinetic parameter; mixing intensity; respirometry

Introduction
In recent years respirometric techniques have been employed to characterize various aspects of aerobic wastewater treatment processes. Recent developments in instrumentation have made respirometric techniques a useful tool for the studies of toxicity and inhibition kinetics, process operation and design, and measurement of biokinetic parameters (Rozich and Gaudy, 1992; Ros, 1993; Ellis et al., 1996; Kong et al., 1996; Lukasse et al., 1997; Brouwer et al., 1998; Goudar and Ellis, 2001; Orupold et al., 2001). Although the use of respirometry for kinetic parameter measurement is gaining importance, a number of issues pertaining to the measurement procedures need to be addressed. Grady et al. (1996) highlighted several complicating factors associated with the estimation of kinetic parameters using respirometry and other techniques. Differences in factors such as culture history, parameter identifiability, and the nature of the assay procedure appear to be responsible for the considerable variability in the reported values of kinetic parameters.

This study addresses another important factor in the form of mass transfer limitations that could potentially affect the values of kinetic parameters measured using respirometry. In respirometric measurement a sample of microorganisms is placed in a batch reactor where the oxygen consumption rates are measured. It is well known that oxygen transport limitations should be avoided when oxygen is supplied to the microorganisms by aeration. As a result, vigorous mixing is often employed to enhance the oxygen transfer process from the gas to the liquid phase. The conditions under which gas-to-liquid oxygen transfer limitations exist in a respirometer have been analyzed in detail by Li and Zhang (1996). Although vigorous mixing can overcome oxygen transport limitations from the gas to the liquid phase, it has an important side effect which is often not recognized in respirometric experiments. Intense agitation can cause microbial flocs to break into small fragments.
Kinetic parameters measured under such conditions may therefore reflect the intrinsic biological reaction kinetics because mass transfer limitations do not exist in these small flocs. However, a number of studies have indicated that oxygen and substrate diffusion limitations can be significant in full-scale activated sludge processes which usually contain several thousands mg/L of mixed liquor suspended solids. At these concentrations, the microorganisms do not exist as dispersed cells in the suspension but as relatively large flocs, especially in quiescent areas and regions with low levels of turbulence.

Stenstrom and Song (1991) demonstrated the adverse effects of oxygen transport limitation on nitrification in the activated sludge process. Haas (1981) theoretically showed that activated sludge flocs with a size range of 10–100 μm exhibited substrate or oxygen diffusion limitations. Elionsov et al. (1996) demonstrated significant effects of diffusion limitations on the performance of continuous flow suspended growth reactors under varying floc characteristics. Beccari et al. (1992) showed that the effect of intrafloc diffusion resistances cannot be neglected in evaluating the overall kinetics when the floc size is greater than 100 μm. Table 1 lists some reported floc size distributions in laboratory as well as full-scale activated sludge processes. It is obvious that large flocs are routinely present in full-scale treatment plants, indicating that mass transfer limitations, especially intraparticle diffusion, could be present in these large floc particles. In light of these findings, the question arises as to whether it is appropriate to use kinetic parameters measured under the condition of vigorous mixing in respirometers to describe the behavior of full-scale activated sludge processes where metabolic reactions are sometimes coupled with mass transfer within the floc matrix. The objectives of this study are to (1) establish the relationship between activated sludge floc size and the mixing intensity of a mechanically stirred respirometer and (2) measure the oxygen saturation coefficient as a function of floc size which is largely governed by the mixing intensity in the respirometer.

### Materials and methods

The respirometer used in this study consisted of a baffled vessel equipped with a mechanical stirrer. Two identical Rushton impellers were mounted on the shaft of the stirrer. The dimensions of the vessel and impeller are given in Table 2.

### Table 1  Floc size distributions in laboratory and full-scale activated sludge processes

<table>
<thead>
<tr>
<th>Floc size distribution</th>
<th>Process</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;85% of flocs in the range 10–70 μm</td>
<td>Laboratory-scale</td>
<td>Andreadakis (1993)</td>
</tr>
<tr>
<td>&gt;90% of flocs in the range 300–1,500 μm</td>
<td>Laboratory-scale</td>
<td>Galil et al. (1991)</td>
</tr>
<tr>
<td>30–1,200 μm</td>
<td>Full-scale</td>
<td>Lee et al. (1996)</td>
</tr>
<tr>
<td>189–1,231 μm</td>
<td>Laboratory-scale</td>
<td>Li and Ganczarczyk (1990)</td>
</tr>
<tr>
<td>425–587 μm</td>
<td>Full-scale</td>
<td>Li and Ganczarczyk (1990)</td>
</tr>
<tr>
<td>0.5–500 μm</td>
<td>Full-scale</td>
<td>Li and Ganczarczyk (1990)</td>
</tr>
<tr>
<td>&lt; 1 μm to &gt; 1,000 μm</td>
<td>Full-scale</td>
<td>Li and Ganczarczyk (1990)</td>
</tr>
</tbody>
</table>

### Table 2  Dimensions of vessel and impeller

| Curved bottom vessel | | |
|----------------------|------------------|
| Diameter             | 1.27 × 10⁻¹ m    |
| Height               | 2.4 × 10⁻¹ m     |
| Working volume       | 3.0 × 10⁻³ m³    |
| Number of baffles    | 3                |
| Impeller             | Six-blade Rushton turbine |
| Diameter             | 4.5 × 10⁻² m     |
**Characterization of mixing intensity**

In this study we characterize the mixing intensity of a mechanically stirred respirometer in terms of the mean energy dissipation rate per unit mass ($\varepsilon$):

\[ \varepsilon = \frac{P_o N^3 D^5}{V} \]  

(1)

where $P_o$ is the dimensionless power number, $N$ is the rotating speed, $D$ is the impeller diameter, and $V$ is the volume of the liquid phase. Eq. (1) indicates that the mean energy dissipation rate for a given mixing system can be calculated provided the power number is known. The power number is usually determined experimentally since it is a function of the impeller design, impeller Reynolds number, and whether the vessel is baffled or not. In this work we carried out torque measurements in order to estimate the power number. The respirometer was completely filled with water and torque measurements were carried out at a range of impeller rotational speeds (1.33–5.0 s\(^{-1}\) or 80–300 rpm) using a strain gauge whose output signal was transmitted to a torque meter for data processing. The measured torque is related to $P_o$ by the following equation:

\[ P_o = \frac{2 \pi T}{\rho N^2 D^5} \]  

(2)

where $T$ is the torque and $\rho$ is the liquid density. The mean energy dissipation rates corresponding to a given range of impeller rotational speeds can now be estimated from Eqs (1) and (2).

**Floc size measurement**

The variation of activated sludge floc size with mixing intensity was monitored using an indirect technique. A photometric dispersion analyzer (PDA) (Rank Brothers Ltd., Cambridge, England) was employed to monitor the change in floc size at different mixing intensities. The respirometer was completely filled with an activated sludge suspension obtained from the wastewater treatment plant of TNO (Delft, The Netherlands) and mechanically stirred at different rotational speeds (1.33–5.0 s\(^{-1}\)). The floc size at a given rotational speed was monitored by pumping the suspension through a capillary tube placed between a light source and a photodiode of the PDA. Laminar flow conditions were maintained in the tube according to the criteria developed by van Hamersveld (1996) in order to prevent floc aggregation or breakup in the measuring device.

The PDA measures two properties of the floc suspension flowing through the tube; the turbidity of the suspension and the turbidity fluctuations caused by the flowing suspension (Gregory, 1985). The output of the PDA consists of two components; an alternating voltage and a steady voltage. The ratio of these two signals is defined as follows (Gregory, 1985):

\[ \text{PDA signal ratio} = \left( \frac{N_f L}{A} \right)^{0.5} \frac{\pi}{4} d_f^2 Q_s \]  

(3)

where $N_f$ is the number of flocs per unit volume, $L$ is the length of light path, $A$ is the cross-sectional area of light beam, $d_f$ is the floc diameter, and $Q_s$ is a scattering coefficient. If the microbial flocs break up the floc diameter decreases whereas the floc number increases, resulting in a net decrease in the signal ratio, according to Eq. (3). Conversely, if the flocs aggregate the floc diameter increases whereas the floc number decreases, resulting in a net increase in the signal ratio. Therefore, the signal ratio of the PDA decreases with decreasing floc size and increases with increasing floc size. The PDA signal ratio can be regarded as an indirect indicator of the variation of floc size with mixing intensity in the respirometer. It was thus not necessary to determine the actual size of the activated sludge flocs.
Respirometric measurement

Oxygen consumption rates under the condition of acetate oxidation were measured using the respirometer described above. An activated sludge suspension with a suspended solids concentration of 3.3 g/L was first aerated overnight to remove any residual substrates. The respirometer was completely filled with the activated sludge suspension to exclude any gas space and was mechanically stirred. Nitrogen gas was then bubbled through the suspension. The temperature was maintained at 25°C while the pH was in the range 7.1–7.4. A small amount of a concentrated acetate solution was injected into the sealed vessel through a diaphragm at the vessel top to give an acetate concentration in excess of the theoretical amount that could be oxidized by the activated sludge. Hydrogen peroxide as an oxygen source was then injected into the vessel, and the resulting dissolved oxygen consumption rate due to acetate oxidation was continuously recorded. Hydrogen peroxide was used to eliminate oxygen transfer from the gas to the liquid phase which is associated with conventional methods of oxygen supply by aeration. The presence of catalase, a peroxide decomposing catalyst, in the activated sludge suspension makes it possible to use hydrogen peroxide as an oxygen source (Schlegel, 1977). Oxygen consumption rates were measured at different impeller rotational speeds (1.33–5.0 s⁻¹). Each experiment was conducted in triplicate to ensure accuracy of results.

Results and discussion

The mixing intensity of a mechanically stirred respirometer was measured at different impeller rotational speeds. These rotational speeds were used to calculate the corresponding mean energy dissipation rates using Eq. (1), as shown in Table 3. The mean energy dissipation rate permits a more meaningful comparison of the mixing intensity of respirometers equipped with different impellers.

The variation of activated sludge floc size with mixing intensity was monitored using a photometric dispersion analyzer (PDA). As mentioned previously, the PDA signal ratio, which decreases with decreasing floc size and increases with increasing floc size, can be regarded as an indirect indicator of the variation of floc size with mixing intensity. Figure 1 shows the PDA signal ratio as a function of the mean energy dissipation rates listed in Table 3. The signal ratio decreased rapidly when the energy dissipation rate was increased from $1.33 \times 10^{-3}$ to $2.68 \times 10^{-3}$ W/kg, implying that the average floc size of the activated sludge decreased with increasing mixing intensity. Beyond a mean energy dissipation rate of $22.9 \times 10^{-3}$ W/kg only a slight decrease in the signal ratio was detected.

Several mechanisms may account for the breakup of the flocs. Hydrodynamic forces, collisions between the flocs, collisions between the flocs and the vessel wall or between the flocs and the stirrer device and probes can all cause breakup of the flocs. If the density difference between the flocs and fluid is low, floc breakup via collisions is relatively insignificant (Ayazi Shamlou et al., 1994). Since activated sludge flocs are highly porous, the density difference between the flocs and the surrounding liquid is rather small. Activated sludge densities are usually < 1,100 kg/m³. For example, the reported values range from

<table>
<thead>
<tr>
<th>Rotational speed (s⁻¹)</th>
<th>Mean energy dissipation rate × 10³ (W/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.33 (80 rpm)</td>
<td>1.33</td>
</tr>
<tr>
<td>1.67 (100 rpm)</td>
<td>2.68</td>
</tr>
<tr>
<td>3.33 (200 rpm)</td>
<td>22.9</td>
</tr>
<tr>
<td>5.00 (300 rpm)</td>
<td>78.9</td>
</tr>
</tbody>
</table>
1,015 to 1,034 kg/m$^3$ (Andreadakis, 1993), 1,003 to 1,029 kg/m$^3$ (Chen et al., 1996), and 1,020 to 1,060 kg/m$^3$ (Dammel and Schroeder, 1991). It can be concluded that collisions of the flocs with each other and with the vessel are unlikely to cause breakage and that hydrodynamic forces are largely responsible for the breakup of the flocs.

Breakup of flocs by hydrodynamic forces in the turbulent regime can occur via two mechanisms; splitting of flocs into two or more parts and erosion of individual cells from floc surface (van Hamersveld, 1996). Both phenomena lead to a decrease in floc size. Floc splitting is a result of velocity fluctuations in the fluid medium, causing pressure differences on opposite sides of a floc. The velocity fluctuations are proportional to the shear rate which can be calculated from the power input. Erosion of cells from floc surface is caused by fluid velocity gradients near the floc surface. The velocity gradients can also be calculated from the power input. The two breakup mechanisms therefore depend on the power input or mean energy dissipation rate. With increasing mixing intensity in the respirometer, greater forces are placed on the flocs leading to a smaller floc size as a result of breakage. It is not possible to determine the relative importance of these two mechanisms in the breakup of the flocs observed in this study due to limited experimental data. Although the PDA results clearly show the effect of mixing intensity on floc size (Figure 1), they are not sufficient for further analysis unless the data are converted to actual floc size.

Having determined the effect of mixing intensity on floc size, respirometric experiments were carried out at the same range of mean energy dissipation rates ($1.33 \times 10^{-3}$ to $2.68 \times 10^{-3}$ W/kg) to investigate the effect of mixing intensity on the oxygen consumption rate of the activated sludge. Oxygen consumption rates were measured using the respirometer which was completely filled with a suspension of activated sludge. The oxygen concentration decay profile measured at the lowest mean energy dissipation rate tested ($1.33 \times 10^{-3}$ W/kg) is presented in Figure 2. Similar profiles obtained at higher energy dissipation rates are not shown here. In the absence of a gas phase in the respirometer, hydrogen peroxide was injected into the sealed vessel as a source of dissolved oxygen for acetate oxidation. The carbon substrate was present in excess while oxygen was the limiting substrate. Under such conditions and neglecting endogenous respiration, the oxygen consumption rate ($R_o$) can be described by the following Monod kinetics expression:

$$R_o = \frac{dC_o}{dt} = \frac{V_o C_o}{K_o + C_o}$$

where $C_o$ is the oxygen concentration at time $t$, $K_o$ is the oxygen saturation coefficient, and $V_m$ is defined as follows:

$$V_m = \mu_m X \frac{C_s}{S + C_s}$$

where $\mu_m$ is the maximum specific growth rate, $X$ is the biomass concentration, $Y$ is the

![Figure 1](https://iwaponline.com/wst/article-pdf/48/8/61/423861/61.pdf)

**Figure 1** Variation of PDA signal ratio with the mean energy dissipation rate

![Figure 2](https://iwaponline.com/wst/article-pdf/48/8/61/423861/61.pdf)

**Figure 2** Oxygen concentration decay profile due to acetate oxidation measured at a mean energy dissipation rate of $1.33 \times 10^{-3}$ W/kg
yield coefficient (g biomass/g oxygen), \( C_s \) is the acetate concentration at time \( t \), and \( K_s \) is the substrate saturation coefficient. Both \( C_s \) and \( X \) can be considered constant since acetate was present in excess in the respirometric experiments, and under oxygen limiting conditions cell growth in the respirometer was minimal. \( V_m \) is thus treated as a constant in the following analysis. After integration and linearization of Eq. (4) we obtain

\[
\frac{C_i - C_o}{\ln C_i/C_o} = \frac{t}{V_m} - K_o
\]

where \( C_i \) is the initial oxygen concentration. By plotting \( (C_i - C_o)/\ln(C_i/C_o) \) versus \( t/\ln(C_i/C_o) \) one obtains a straight line whose intersection with the ordinate gives the value of \( K_o \). Eq. (6) indicates that the biomass concentration in the respirometer is not required for the determination of \( K_o \).

The data in Figure 2 are transformed and plotted according to Eq. (6) to extract \( K_o \), as shown in Figure 3. The intercept of the straight line in Figure 3 gives the value of \( K_o \). Data not shown here were given the same treatment. The values of \( K_o \) obtained by the graphical method are listed in Table 4 and plotted as a function of the mean energy dissipation rate in Figure 4. It is evident that \( K_o \) decreased rapidly when the mean energy dissipation rate was increased from \( 1.33 \times 10^{-3} \) to \( 2.68 \times 10^{-3} \) W/kg and it remained unchanged at mean energy dissipation rates beyond \( 2.68 \times 10^{-3} \) W/kg.

The observed effect of the mean energy dissipation rate on \( K_o \) leads to the conclusion that \( K_o \) is affected by the mixing condition in the respirometer. A comparison of Figures 1 and 4 indicates a remarkably similar pattern. After an initial period of rapid decrease, both the floc size (PDA signal ratio) and the oxygen saturation coefficient reached a plateau. This similarity indicates that mass transfer limitations are present in large flocs which exist under the condition of low mixing intensity, resulting in large \( K_o \) values. \( K_o \) measured under such conditions is actually the lumped or apparent oxygen saturation coefficient whose value is dependent upon the hydrodynamic conditions of the measuring device. With increasing mixing intensity in the respirometer, the flocs break into smaller fragments in which mass transfer resistances, especially intraparticle diffusion, become insignificant, leading to relatively constant \( K_o \) values which reflect the intrinsic oxygen uptake process.

![Figure 3](https://iwaponline.com/wst/article-pdf/48/8/61/423861/61.pdf)  
**Figure 3** Linearization of the data in Figure 2 according to Eq. (6)

![Figure 4](https://iwaponline.com/wst/article-pdf/48/8/61/423861/61.pdf)  
**Figure 4** Variation of the oxygen saturation coefficient \((K_o)\) with the mean energy dissipation rate under the condition of acetate oxidation

<table>
<thead>
<tr>
<th>Mean energy dissipation rate (\times 10^3) (W/kg)</th>
<th>(K_o) (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.33</td>
<td>0.23</td>
</tr>
<tr>
<td>2.68</td>
<td>0.08</td>
</tr>
<tr>
<td>22.9</td>
<td>0.07</td>
</tr>
<tr>
<td>78.9</td>
<td>0.07</td>
</tr>
</tbody>
</table>

*Table 4* Variation of the oxygen saturation coefficient with the mean energy dissipation rate
It is known that the oxygen saturation coefficient for carbon oxidation is small but varies significantly. For example, the $K_o$ value was observed to change from 0.01 mg/L for a filamentous bacterium to 0.15 mg/L for a floc-forming bacterium (Orhon and Artan, 1994). The large range in the reported $K_o$ values for carbon oxidation may be attributed to the fact that $K_o$ will be influenced by a number of factors including variations in the measuring techniques and activated sludge conditions. This study suggests that part of the large variability in the reported $K_o$ values could be the consequence of ignoring the existence of mass transfer limitations within the floc phase.

The pronounced dependence of the oxygen saturation coefficient on the mixing intensity of the respirometer shows that mass transfer limitations in the floc phase do play an important role and must be accounted for in suspended growth systems such as the activated sludge process. Shieh (1980) theoretically demonstrated the significance of mass transfer resistances in the floc phase on the interpretation of kinetic data of suspended growth systems. He observed that larger saturation coefficient values would be obtained in the presence of significant mass transfer resistances in the floc phase. Andrews (1991) also postulated that the effect of mass transfer resistances is not to invalidate the form of the Monod kinetics equation but to increase the apparent value of the saturation coefficient. Indeed, a theoretical analysis (Hamdi, 1995) clearly showed that with mass transfer limitations consideration the saturation coefficient increased with increasing diameter of the floc. All these theoretical analyses have been confirmed experimentally in this study. Note that mass transfer limitations from the gas to the liquid phase can also influence the value of the oxygen saturation coefficient in a similar manner. However, the observed change in the oxygen saturation coefficient with mixing intensity cannot be attributed to gas-to-liquid mass transfer limitations because hydrogen peroxide was used as a source of dissolved oxygen in this study, resulting in elimination of the gas-to-liquid oxygen transport step owing to the absence of a gas phase in the respirometer.

**Conclusions**

The mixing intensity of a mechanically stirred respirometer was characterized in terms of the mean energy dissipation rate which incorporates the effects of both the impeller rotational speed and impeller diameter. Using a photometric dispersion analyzer it was found that the floc size of an activated sludge suspension decreased with increasing mean energy dissipation rate, indicating that intense agitation can cause the activated sludge flocs to break into smaller fragments. The oxygen saturation coefficient measured under the condition of acetate oxidation was significantly affected by activated sludge floc size. The oxygen saturation coefficient measured at low mean energy dissipation rates (large flocs) was found to be much bigger than that measured at high mean energy dissipation rates (small flocs) due to the presence of mass transfer resistances within the large flocs. The use of kinetic parameters measured with small flocs, as is the case in respirometers operated under vigorous mixing, for modeling and optimization of full-scale treatment plants where much larger flocs are often present in areas of low turbulence may lead to errors in the evaluation of substrate oxidation rates.

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**References**


