Mixing Estimation of a Laboratory-Scale External-loop Air-lift Bioreactor for Fungal Culture Using pH Tracer Method

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Abstract. Mixing has been one of the important parameters in characterizing and designing external air-lift bioreactors for the biological processes that require intensive mixing for mass transfer. In this study, pH tracer method was employed to characterize the mixing quality of a three-phase laboratory-scale external-loop air-lift bioreactor (EALR) used for cultivation of a pelletized filamentous fungal culture. By labeling fluid elements using pH tracer, the response curves can be obtained, which were then used to estimate the mixing characteristics in the individual bioreactor sections expressed by the Bodenstein numbers (Bo). When applied to a 1.25-L EALR with culture medium solution only, the technique yielded reasonable results. Results showed that the riser behaved as a CSTR and the downcomer as a plug-flow reactor. When applied to fungal pellet culture, however, the technique virtually failed to provide quality results due to the presence of heterogeneous phase.

Keywords. Mixing, Bodenstein number, air-lift bioreactor, fungal pellet culture.
**Introduction**

Filamentous fungi are well known as producers of commercially important products such as antibiotics, chemicals, and enzymes. The difficulties associated with fungal cultures are that the morphological changes throughout their life cycles result in a highly viscous non-Newtonian broth, which has a considerable negative impact on the bioreactor performance, especially on the mixing, thus the nutrient transfer. Cultivating filamentous fungal culture in a discrete pellet form avoids the aforesaid problem because pelleted growth of filamentous fungi exhibits low viscosity and approaches Newtonian flow behavior. Using the external-loop air-lift bioreactors also provides the efficacy of effective mixing, well-defined liquid flow pattern at relatively low power requirements, and efficient solid fluidization, which partially overcome the poor mass and heat transfer in the highly viscous filamentous fungal broth. The authors have reported a study on a continuous-flow external-loop air-lift bioreactor (EALR) for production of secreted enzymes through a perfusion fungal pellet culture (Su and He, 1997). Characterizations of the bioreactor system were also conducted to understand the behavior of the fungal pellet system, among them is the mixing phenomenon.

Mixing is an important factor in bioreactor characterization. It heavily influences the performance of the bioreactor such as nutrient distribution and heat transfer. Mixing itself is affected by many different parameters such as flow pattern, turbulence, and momentum transfer behaviors between liquid-gas-solid phases. Rapid convective transport and dispersion of nutrients and acid or alkaline injected into the reactor for the purpose of pH control are essential to prevent regions of high local concentrations over excessively long periods. Mixing in EALRs is usually characterized in two ways: mixing time and mixing intensity. A wide variety of mixing measurement methods can be found in the literature, most of them are pulse response methods. The pH tracer method (Danckwerts and Sharma, 1966; Kennard and Janekeh, 1991; Kawase et al., 1994; Rueffer et al., 1995), conductivity method, coloring/decoloring method (Shah et al., 1978), radioactive tracer method (Field and Davidson, 1980), heat-pulse (Sisak et al., 2000; Shamlou et al., 1998), and flow follower method (Fields et al., 1984; Klein et al., 2000, 2001), are among the examples.

The pH tracer method is the most often used technique for large and small scale mixing measurement. With this method, an acid is injected into the reactor and the response is detected by one or more pH probes. Cautions have to be given to the following two aspects. The response signals have to be converted to acid concentrations because pH signal is nonlinear with proton concentration. Danckwerts and Sharma (1966) pointed out that only acid solution could be used as an input tracer signal because of the effects of carbon dioxide and carbonates.

Bodenstein (Bo) number is a dimensionless parameter used to describe degree of axial mixing:

\[
Bo = \frac{vL}{D}
\]  

(1)

A mass balance over liquid phase in the reactor, neglecting radial concentration gradient, gives:

\[
\frac{\partial c}{\partial \theta} = \frac{1}{Bo} \frac{\partial^2 c}{\partial x^2} - \frac{\partial c}{\partial x}
\]  

(2)

Where \( c \) is the normalized dimensionless concentration defined as:

\[
c = \frac{C - C_0}{C_m - C_0}
\]  

(3)

Solution to Equation (2) for an ideal initial Dirac pulse in ALR, taking the circulation flow into account, was represented by (Levenspiel, 1972):
However, since it is impossible to create an ideal Dirac pulse, an experimental error is already included when using the solution. A method involving the time domain analysis was proposed by Verlaan et al. (1989a) for an arbitrary input signal. Using the dispersion model as the transfer function and convoluting with the input function in the frequency domain, an output function in Fourier domain can be obtained. This function is transformed back to the time domain by inverse Fourier Transformation (IFFT). The calculated output function is then compared with the experimental output function to determine the Bo number which appears in the transfer functions. This procedure is illustrated in figure 1 (Verlaan et al., 1989a).

![Figure 1. Schematic illustration of the parameter estimation method.](image)

The transfer function in the Fourier domain is:

\[ H(i\omega) = \text{Re}(i\omega) + i \text{Im}(i\omega) \]  

with the following specifications:

\[ \text{Re}(i\omega) = \exp\left[\frac{Bo}{2}(1 - \sqrt{z}\cos\frac{\phi}{2})\right] \cos\left[\frac{Bo}{2}\sqrt{z}\sin\frac{\phi}{2}\right] \]  

\[ \text{Im}(i\omega) = \exp\left[\frac{Bo}{2}(1 - \sqrt{z}\cos\frac{\phi}{2})\right] \sin\left[\frac{Bo}{2}\sqrt{z}\sin\frac{\phi}{2}\right] \]  

\[ \phi = \arctg\left(\frac{4\pi\omega}{Bo}\right) \]  

\[ z = \sqrt{1 + \left(\frac{4\pi\omega}{Bo}\right)^2} \]  

This paper reports the results of the mixing characterization in individual sections in a 3-phase EALR system, i.e., gas-liquid-solid, for cultivation of a fungal pellet culture using pH tracer method.
Materials and Methods

The External Air-lift Bioreactor

Mixing phenomena was characterized on a laboratory-scale external-loop air-lift reactor (EALR), which was used for secreted enzyme production by pelleted fungal culture (Su and He, 1997). Schematic of the EALR and the experimental set-up are shown in figure 2. The inner diameter of the riser is 50 mm, and downcomer and joint arms 25 mm, with a working volume of 1.25 liters. There is a cell settling/air-liquid separation zone integrated with the bioreactor, where a 45-mm inside diameter cylinder is split into two channels. One channel is a part of the downcomer, and the other is a cell settling zone from which the spent medium was continuously withdrawn. A porous glass sparger, 10 mm in diameter with a mean pore size of about 140 µm, was used for introducing air into the riser.

![Figure 2. External-loop air-lift bioreactor and the experimental set-up for mixing characterization using pH tracer.](image)

Working Media

The working media used in pH tracer method included tap water and filamentous fungal pellet cultures with cell loading rates of 0%, 20%, 40%, and 60% of cell settling volume of the riser.
capacity, which are corresponding to dry-basis biomass concentrations of 0., 0.7, 1.4, and 2.1 g/L, respectively. A unique temperature-sensitive colonial mutant strain cot of filamentous fungus *Neurospora crassa* was provided by courtesy of Dr. Dorsey Stuart, Department of Genetics of the University of Hawaii at Manoa. This mutant strain forms pellets when cultivated at temperature of 32°C. The fungal pellets, about 3 mm in diameter, were obtained by cultivating the fungal culture in the EALR for four days after inoculation (He, 1996).

**Tracer Response Curve Measurement and Bo Estimation**

Danckwerts and Sharma (1966) pointed out that only acid solution could be used as input tracer signal because of the effects of carbon dioxide and carbonates. In this study, the operating pH range was chosen from 3.5 to 5.5. The starting pH value was adjusted to 5.3 using 1N sodium hydroxide before each measurement. The input tracer was 0.1 N or 1 N hydrochloric acid solutions, depending on the working medium used, and was injected into the system between the riser and downcomer. The dimensionless proton concentration is obtained by:

$$c = \frac{[H^+] - [H_0^+]}{[H_\infty^+] - [H_0^+]}$$  \hspace{1cm} (10)

The proton concentration changes versus the voltage signals in the system were calibrated through a correlation:

$$c = \frac{10^{0.02882(S_0 - S_\infty)}}{10^{0.02882(S_0 - S_\infty)}} - 1$$  \hspace{1cm} (11)

Equation (11) was used for calculating the input and output signal concentrations, which was based on a pH calibration curve constructed beforehand (figure 3).

![Figure 3. Calibration curve of pH tracer method for Bo estimation.](image)

Two glass Calomel (Hg/Hg₂Cl₂) pH electrodes (Cole-Parmer, Chicago, IL) were used as the signal sensors. A micro dataloger (Campbell Scientific, Logan, Utah) was used to perform data acquisition. The Bo numbers were estimated by least-square fitting of predicted output signals to the experimental output signals. The least-square method involves the minimization of
summed squares (ss, dimensionless) of the difference between the calculated data from the dispersion model and the experimental output data:

\[ ss = \sum [(\text{experimental data}) - (\text{predicted data})]^2 \]  

(12)

MathCAD (MathSoft, Inc. Cambridge, MA) was employed as a mathematical tool for estimating Bo numbers based on previously mentioned axial dispersion model by Verlaan et al. (1989a).

Some of other hydrodynamic parameters were also investigated. Fractional gas holdup in the riser was determined by means of hydrostatic differential pressure measurement with an inverted U-tube (Chisti, 1989). Liquid circulation time was measured with two methods, the flow follower method and tracer response technique. A single fungal pellet, which has very close density to the working medium solution, was used as a flow tracer. The circulation time of the liquid was estimated by the flow tracer’s average circulation time of 50 or more revolutions in the bioreactor. Tracer response technique was associated with the estimation of Bo number. The time difference between the adjacent peaks of the decaying sinusoidal tracer profile was recognized as the liquid circulation time. The mixing time was determined by the time when the tracer profile decayed to 5% of initial mixing intensity.

**Results and Discussions**

Bo number is a parameter that measures the extent of axial dispersion. It is inversely proportional to the axial dispersion coefficient. When Bo approaches zero, there is an intense axial dispersion, then the mixed flow prevails. When Bo reaches infinity the flow has negligible axial dispersion, hence becomes plug flow. This model is usually more satisfactory for describing flow patterns that deviate not too much from plug flow.

**Bo Estimation without Cell Loading**

In the experiments, tracer amount and concentration have to be adjusted carefully in order to operate the system in the desired pH range of 3.5 - 5.5, which is considered very important by Dankckwerts and Sharma (1966). The tracer profiles typically showed only two to three cycles due to large dispersion in the riser. If the tracer strength (combing the tracer amount and concentration) were too weak, the output profiles became very flat. On the other hand, if it were too strong, the first output profile would overshoot and exceed the optimum pH range. Without cell loading, a tracer strength of 0.1 ml of 0.1 N hydrochloric acid worked properly.

Tracer injecting position was also very important for tracer profile quality. The position should not be too close to the pH probe because the flow may not have well developed radially when it crosses the section where the probe locates. If the position was too far away from the probe, the input tracer profile may be markedly dampened. Another parameter affecting the tracer response profile is the data acquisition interval. In this study, the data acquisition interval of 0.5 s was found proper, compared to 0.25 s, without loosing much information.

Examples of tracer input and output profiles without cell loading are shown in figure 4. The input tracer was injected into the riser before the upper pH probe. Comparisons of the experimental and calculated output profiles (based upon the best estimate of Bo number) are shown in figure 5. In the absence of cell particles, the calculated output profiles generally fit the experimental data well. The Bo numbers thus displayed an upward trend with increased superficial velocities, \( U_g \), from 0.21 cm/s to 1.3 cm/s, although the data are somewhat scattering as shown in figure 6. The large Bo number of 50 to 120 indicates that the flow in downcomer without cell loading was close to plug flows. There was little axial dispersion occurred. Results showed that the least-
square parameter fitting criterion was not very sensitive to the changes of \( Bo \) numbers, which may in part cause the scattering data.

![Figure 4. tracer input and output profiles in the downcomer in the absence of fungal pellets.](image)

![Figure 5. Comparisons of experimental and calculated output profiles in the downcomer in the absence of fungal pellets.](image)

In the riser, the tracer input and output profiles showed that the output profiles did not resemble the input profiles. The input signals dissipated quickly before it was detected, which resulted in a poor parameter fitting. The \( Bo \) numbers were evidently unreliable. This problem is believed due to the high degree of back mixing caused by the air bubbles in the riser. The dispersion model is a one-parameter model which describes well in the situation where plug flow is prevailed. The unsatisfactory fit in the riser showed that the flow pattern in the riser was more towards mixed flow. A typical set of tracer profiles and parameter fitting for \( Bo \) estimation in the riser are shown in figures 7 and 8.
Several parameters affected the flow in the riser. Sparger position was found heavily influencing the flow pattern. A porous glass sparger was located at the bottom of the bioreactor. If the sparger was at the very bottom of the riser, the air distribution and liquid flow at the entrance from downcomer to riser were markedly interfered (figure 9a).

The air bubble stream was forced aside by the recirculated liquid flow from the downcomer. The uneven air bubble distribution, in turn, created a vertex above the sparger. The flow in the riser deviated from well-defined upward-directed flow, towards a mixed flow pattern as occurred in bubble columns. To improve this problem, the location of the sparger was lifted to the position just above the liquid stream from downcomer. Uniform air bubble distribution and better liquid flow pattern were observed after this modification (figure 9b).
When a pH sensitive phenolphthalein dye solution was used as a tracer to visualize the flow in the riser, high degree of back mixing existed in spite of the correction of sparger position. The flow was relatively smooth and steady at low superficial velocities, e.g., 0.42 cm/s to 0.64 cm/s (aeration rates 0.2 to 0.4 vvm, based on the bioreactor volume). The overall mixing time ranged from 40 to 60 seconds. The weighted average circulation time at $U_{g,r}$ of 0.42, 0.85, and 1.3 cm/s were 19, 16, and 13 seconds, respectively. It took 2 to 3 circulations for the system to completely mix. Liquid circulation time distribution measured by tracer particle method showed that the skewness of the distribution increased with increased superficial velocity in the riser ($U_{g,r}$). The higher the $U_{g,r}$, the more the circulation time deviated from normal distribution (figure 10). This is another indication of high-degree back mixing in the riser.
**Bo Estimation with Cell Loading**

When the fungal pellets were present, the tracer profiles were significantly dampened compared to those without cell loading. This was due primarily to the adsorption of the tracer by the fungal pellets. A higher tracer intensity had to be injected to obtain a similar pH range and tracer output profile, as shown in table 1.

### Table 1. Tracer strength on resulting pH range at different cell loading rates.

<table>
<thead>
<tr>
<th>Tracer vol./conc.</th>
<th>pH range at different cell loading rate</th>
<th>0%</th>
<th>20%</th>
<th>40%</th>
<th>60%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 ml / 0.1 N</td>
<td>5.32 - 4.41</td>
<td>5.31 - 5.22</td>
<td>5.31 - 5.26</td>
<td>5.31 - 5.26</td>
<td></td>
</tr>
<tr>
<td>0.8 ml / 0.1 N</td>
<td>---</td>
<td>5.31 - 4.48</td>
<td>5.33 - 5.06</td>
<td>5.31 - 5.17</td>
<td></td>
</tr>
<tr>
<td>0.4 ml / 1.0 N</td>
<td>---</td>
<td>---</td>
<td>5.33 - 4.42</td>
<td>5.32 - 4.51</td>
<td></td>
</tr>
<tr>
<td>0.6 ml / 1.0 N</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>5.32 - 4.34</td>
<td></td>
</tr>
</tbody>
</table>

Figures 11 and 12 show the examples of tracer input and output profiles and model fitting for the downcomer with 40% cell loading (1.4 g/L dry basis). Results showed that the fitting curves deviated significantly from the output profile. In other words, the single parameter dispersion model could not adequately describe the tracer response. Tracer adsorption onto the fungal pellets could be one of the causes. In order to account for tracer adsorption, the single parameter dispersion model needs to be modified to include intra-particle diffusion. By including the intra-particle diffusion term, one more mass balance that describes tracer diffusion into the pellets would be needed, which substantially complicates the calculation (Thompson and Worden, 1992).
The flow pattern in the downcomer remained plug flow in the presence of cell loading from 20% to 60% cell loading rates, even at low aeration rates (circulation rates). On the other hand, the flow pattern in the riser changed significantly as the cell loading increased. Formation of circulation elements (due to high back mixing) and radial mixing were apparent in the riser. The higher the cell loading, and the higher aeration rates, the more turbulent flow pattern resulted in the riser. This is believed owing to the increase of the friction between the cell particles and the liquid. This phenomenon is more likely to happen in small-scale airlift bioreactor (Verlaan et al., 1989b), as in the case of this study.

Results demonstrated that that the riser behaved as a CSTR and the downcomer as a plug-flow reactor. When applied to fungal pellet culture, however, the technique virtually failed to provide quality results due to the presence of the heterogeneous phase. No quantitative descriptions were obtained for the cases with fungal pellet loading.
In summary, the laboratory-scale EALR in this study encompassed the features of (1) substantial back mixing in the riser and akin to plug flow in downcomer, which led to a high oxygen mass transfer rate, (2) short mixing time and Low gas holdup. In addition, the bioreactor may be modeled as three inter-linked compartments with different mixing strength: well-mixed riser, plug flow downcomer, and a settler with essentially no mixing.

Conclusion
The pH tracer method provided a reasonable estimation for mixing described by Bodenstein numbers in a laboratory-scale external-loop air-lift bioreactor without cell loading. Results demonstrated that that the riser behaved as a CSTR and the downcomer as a plug-flow reactor. When applied to fungal pellet culture, however, the technique virtually failed to provide quality results due to the presence of a heterogeneous phase. No quantitative descriptions were obtained for the cases with fungal pellet loading. Alternative techniques have to be explored to effectively determine the mixing phenomena of a fungal culture in situ.

References


**Nomenclature**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bo</td>
<td>Bodenstein number, dimensionless</td>
</tr>
<tr>
<td>c</td>
<td>normalized concentration, dimensionless,</td>
</tr>
<tr>
<td>C</td>
<td>concentration at any time, mol/L</td>
</tr>
<tr>
<td>C₀</td>
<td>concentration at time zero, mol/L</td>
</tr>
<tr>
<td>C∞</td>
<td>concentration at time infinity, mol/L</td>
</tr>
<tr>
<td>D</td>
<td>axial dispersion coefficient, m²/s</td>
</tr>
<tr>
<td>[H⁺]</td>
<td>concentration of the working media in pH tracer, mol/L</td>
</tr>
<tr>
<td>[H⁺₀]</td>
<td>initial concentration of the working media in pH tracer measurement, mol/L</td>
</tr>
<tr>
<td>[H⁺∞]</td>
<td>concentration after tracer completely dispersed and reaching a plateau, mol/L</td>
</tr>
<tr>
<td>H</td>
<td>transfer function in Fourier domain</td>
</tr>
<tr>
<td>i</td>
<td>i² = -1</td>
</tr>
<tr>
<td>Im</td>
<td>imaginary part of the transfer function in Fourier domain</td>
</tr>
<tr>
<td>Re</td>
<td>real part of the transfer function in Fourier domain</td>
</tr>
<tr>
<td>S</td>
<td>voltage signal in pH tracer measurement, mV</td>
</tr>
<tr>
<td>S₀</td>
<td>initial voltage signal corresponding to initial pH, mV</td>
</tr>
<tr>
<td>S∞</td>
<td>voltage signal corresponding to the pH after tracer completely dispersed, mV</td>
</tr>
<tr>
<td>t</td>
<td>time, s</td>
</tr>
<tr>
<td>U₉,r</td>
<td>superficial air velocity in the riser, m/s</td>
</tr>
<tr>
<td>v</td>
<td>liquid velocity, m/s</td>
</tr>
<tr>
<td>x</td>
<td>dimensionless length of the measured section,</td>
</tr>
</tbody>
</table>

**Greek Symbols**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>τ</td>
<td>dimensionless residence time (= residence time/circulation time)</td>
</tr>
<tr>
<td>ω</td>
<td>frequency</td>
</tr>
<tr>
<td>φ</td>
<td>phase angle</td>
</tr>
<tr>
<td>θ</td>
<td>dimensionless time, θ = t/t_c.</td>
</tr>
</tbody>
</table>