Setting-up and characterisation of the new Perspex block based BioScope

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Thanks to Dirk Geerts and Rob Kerst for his technical support in this project.

Finally, I would to give special thanks to Ko Vinke for his help, advises, and technical support in the lab.
Summary

The BioScope is a plug flow satellite reactor with a working volume of few millilitres that can be used to perform pulse response experiments. This report presents the physical characteristics of the design parameters of an improved Perspex block BioScope, such as:

1. Pressure drop in channel
2. Oxygen transfer rate and carbon dioxide removal rate
3. Residence time
4. Peclet number

Also, the glucose uptake rate during steady state between the fermentor and the BioScope is described in this report. Knowledge of the precise residual substrate concentration is imported for substrate mass balance during pulse response experiments during which the residual substrate is increased and the uptake rate followed over a short time window (60-300 seconds).

The glucose uptake follows this function $q_s = 1.1563 \times 10^{-3} \cdot C_{GH} \left( \frac{mg_{GH} \cdot C_{GH}}{g_{bio} \cdot s} \right)$; this equation is useful to estimate the glucose concentration that gets into the BioScope coming from the fermentor broth. This equation is only valid for low concentrations of glucose, and it was determined under the grow conditions given in Appendix I.

The maximum pressure drop achieved through the channel was 0.3 and 1.45 bar for flow rates of 2 and 6 mL/min respectively. These results are in agreement with the basic requirements of the BioScope, since at pressure drop above 2 bar, the pump fails to deliver a constant flow.

The oxygen transfer rate was found to be given by the overall mass transfer coefficient $K_{overall} = 3 \times 10^{-5} \text{ mL s}^{-1}$ using a flow rate around 2mL/min at 30°C. This value is enough to ensure a good oxygenation during a pulse response experiments. The overall mass transfer coefficient for the carbon dioxide was $K_{overall} = 8.07 \times 10^{-6} \text{ m/s}$, for each case, however it is still remains to be evaluated seen if this value is sufficient to keep a low concentration of CO$_2$ during the pulse experiment.
The residence time ranges from 4.8 s for the first sampling port after the pulse to 114 s for the last port using 2 mL/min as a flow rate. These residence time values are double of the desired ones.

The Peclet number was around 35-40, but in some cases it was lower than 30. This imported parameter, which characterises the mixing characteristics of the Bioscope, should to be studied further since a Peclet number > 30 is required for plug flow. Plug flow is a key requirement for the BioScope.
1. Introduction

1.1 General

Metabolic engineering aims at the directed improvement of product formation or certain properties of microorganisms, using recombinant DNA technology (Bailey 1991). A desired change often requires modification of levels or properties of multiple enzymes, but metabolic networks have complex interactions such as allosteric effects, cofactor coupling and compartmentation. To understand how a cell carries out and regulates these reaction sequences is highly necessary to build a mathematical model, which describes the kinetic behaviour in a cell. The construction of these models requires a thorough understanding of the in vivo kinetics. These can be studied in dynamic experiments during which a steady-state chemostat culture is perturbed and the subsequent response of intracellular metabolites is measured (Theobald et al, 1993 and 1997).

These perturbation experiments can be carried out in a fermentor itself (Lange, 2002). The sampling frequency must be sufficiently high to follow the rapid intracellular changes in intracellular metabolite concentrations. Furthermore, it is important to rapidly inactivate all cellular enzymes to prevent any turnover of metabolites after sampling.

The conventional rapid sampling technique developed by Theobald et al (1993) has a several number of disadvantages (Visser 2002):

1. After addition of the glucose pulse, the steady state in the fermentor is lost.
2. The approach cannot be applied to fed-batch systems and large-scale reactors.
3. Due to the high sampling frequency, the amount of sample that can be withdrawn is small. Hence, the number of metabolites that can be analysed in one sample is limited.
4. In one experiment, only one quenching/extraction method can be used. Since it is not possible to use the same methods for all metabolites of interest, the pulse experiment needs to be repeated several times.
5. Large amounts of perturbing agent are required. When expensive agents are used, this is a significant contribution to the overall costs.
6. The high sampling frequency requires a well-trained and coordinated rapid sampling team of 3-4 people.

The BioScope (Visser 2002) has been developed in order to overcome the problems mentioned above.
1.2 The BioScope Concept

The BioScope concept is based on the continuous flow method as illustrated in Figure 1.1. Instead of perturbing the fermentor itself, as in the conventional approach, only a small flow of broth is perturbed outside the fermentor. The perturbation starts when this flow is mixed with a flow containing the perturbing agent. Samples can be taken from the flowing broth at different locations. At constant flow rates, the distance between the point of mixing and the point of sampling determines the time (or residence time) during which the sampled cells have been exposed to the perturbation.

Successful application of this concept demands that several requirements are met. There is a need for plug flow, to eliminate the effects of dispersion. Oxygen transfer must be sufficient to sustain the oxygen consumption during the pulse response experiments carried out under aerobic conditions. Similarly, transport of carbon dioxide must be sufficient to remove the carbon dioxide that is produced. Mixing of broth and perturbing agent must be fast (within seconds). The sample must be immediately quenched to stop any metabolic activity.

BioScope is a more suitable approach for investigation of \textit{in vivo} kinetics, compared to conventional approaches, in which the perturbation is carried out in the fermentor; the BioScope offers a number of advantages (Visser 2002):

1. A large number of different perturbation experiments can be carried out on the same day, because the physiological state of the fermentor is not perturbed.

2. \textit{In vivo} kinetics during fed-batch experiments and in larger-scale reactors can be investigated.
3. All metabolites of interest can be measured using samples obtained in a single experiment, because the volume of the samples is 'unlimited'.

4. The amount of perturbing agent spent is minimal, since only a small volume of broth is perturbed.

1.3 Materials & Methods

The original BioScope consisted of oxygen permeable silicone tubing. The tubing had a special geometry (serpentine geometry, Figure 1.2) to ensure plug flow. After leaving the fermentor, the broth is mixed with a flow of perturbing agent, in the same way as it can be seen in Figure 1.1.

![Figure 1.2 A serpentine unit of the original BioScope](image)

The original BioScope had some disadvantages: each serpentine unit had to be tested separately, the system was difficult to reproduce and it was not robust enough. An improved BioScope was designed by Visser (2002). It consists of a circular channel milled in Perspex, which is configured according to a two dimensional serpentine geometry (Figure 1.3 and 1.4A). The channel consists of two parts, a gas and a liquid channel, which are separated by an oxygen permeable silicone membrane, as can be seen in Figure 1.4B. The airflow serves to deliver the oxygen and remove the carbon dioxide.
The device has 10 ports including one before the pulse, the diameter of the coils is ($d_c = 2e^{-3}$ m), the channel internal diameter is ($d_i = 1.2e^{-3}$ m), the silicone membrane
thickness is $D = 1.2 \times 10^{-3}$ m). The tubes used in the sample ports have an internal diameter = $1.2 \times 10^{-3}$ m, and the Y-piece used in the sample ports have an internal diameter = 1/16 inch.

Figure 1.4 (A) 2D serpentine channel geometry, (B) Cross-section of the incubation channel

The BioScope device should meet the following requirements:

1. A minimal sampling interval of several seconds, it is determined by the liquid velocity and the channel length.
2. A maximal sampling period of 100-200 s
3. A sufficient high value of the Peclet number to reduce the effects of dispersion as much as possible ($Pe_L > 30$) (Visser).
4. Sufficient transport of oxygen and carbon dioxide. The oxygen is essential for aerobic fermentation, and the carbon dioxide should be removed.
5. A limited pressure drop of a several bar ($\Delta P < 1-2$ bar), since at a pressure drop over 2 bars, the pump cannot offer a constant flow rate.

The scope of this report is the set-up and characterization of the new prototype BioScope. Some of the requirements mentioned above are discussed in this report, such as the pressure drop, the oxygen transfer and the residence time distribution.
2. Residual glucose determination

2.1 Introduction

The main substrate of the fermenter is glucose, so it is important to know how much residual glucose is in the fermenter during the steady state. It is also important to know how the residence time, from the fermenter to the sampling tube, affects the amount of residual glucose. The stainless steel beads method of residual substrate determination (Mashego et al, 2003) was adapted to quenching the activity of the yeast when it was collected into the acceptor (sampling tube or syringe).

2.2 Materials & Methods

2.2.1 Sampling without a pump

This kind of sampling was done using a sample port of the reactor. A valve was adjusted to get a flow rate = 2mL/min. The schematic overview of the experiment set-up is shown in Figure 2.1. The required materials and equipments are listed in Table 2.1.

<table>
<thead>
<tr>
<th>Component</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fermenter</td>
<td>Applicon</td>
</tr>
<tr>
<td>Valve</td>
<td></td>
</tr>
<tr>
<td>Valve</td>
<td></td>
</tr>
<tr>
<td>Stainless steel beads</td>
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<tr>
<td>Stop watch</td>
<td></td>
</tr>
<tr>
<td>Tubes</td>
<td></td>
</tr>
<tr>
<td>Syringes</td>
<td></td>
</tr>
<tr>
<td>Filters</td>
<td></td>
</tr>
</tbody>
</table>
Experimental steps

1. The flow rate was adjusted with a valve in order to get a liquid flow rate of approximately 2mL/min.
2. Stainless steel beads (30g) were weighted for each sampling tube, and kept them in the cryostat at 1°C.
3. As a control, 62g of stainless steel beads were also weighted in a 60mL syringe, and cooled to -18°C in freezer.
4. Each sampling tube was filled with approximately 2mL fermentation broth at 30°C and kept in the cryostat (1°C).
5. Two syringes containing stainless steel beads at -18°C were also filled with approximately 5mL of broth, and immediately filtered (0.45 Dm filters Gelman).
6. All the contents of the sampling tubes kept in the cryostat at 1°C were filtered using syringes connected to filters (0.45 Dm filters Gelman).
7. All the filtrates were kept into the freezer at -20°C until analysis.
8. The residual glucose concentration was determined according to the glucose/cuvette method according to the Boehringer kit no. 716251.

![Diagram](image)

Figure 2.1 Experimental set-up for residual glucose without pump

2.2.2 Sampling with a pump

This kind of sampling was done using a sample port of the reactor connected to silicone tubing. A rotary pump was used in order to get a flow rate of approximately 2mL/min.

This experiment was repeated three times to insure the reproducibility of the results.

The schematic overview of the experimental set-up is shown in Figure 2.2. The required materials and equipments are listed in Table 2.2
Table 2.2 Materials and equipments

<table>
<thead>
<tr>
<th>Component</th>
<th>Name</th>
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<td>Fermenter</td>
<td>Applicon</td>
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<tr>
<td>Pump</td>
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<tr>
<td>Silicon tubing</td>
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<td>Stainless steel beads</td>
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<tr>
<td>Syringes</td>
<td></td>
</tr>
<tr>
<td>Filters</td>
<td></td>
</tr>
</tbody>
</table>

**Experimental steps**

1. The flow rate was adjusted using the pump in order to get 2mL/min approx.
2. Around 30g of stainless steel beads were weighted for each sampling tube, and kept them in the cryostat (1°C).
3. Around 62g of stainless steel beads were weighted for each syringe, and kept in the freezer (-18°C). For the rapid sampling.
4. Each sampling tube was filled with broth approximately 2mL, and kept in the cryostat (1°C).
5. Two syringes containing stainless steel beads at -18°C were filled with 5mL of broth, and immediately filtered (0.45 μm filters Gelman).
6. The samples of the sampling tubes were filtered using syringes with filters.
7. All the filtrates were kept in the freezer at -20°C until analysis.
8. The residual glucose concentration was determined according to the glucose/cuvett method according to the Boehringer kit no. 716251.

Figure 2.2 Experimental set-up for residual glucose with pump.
2.3 Results & Discussions

The samples 1 and 2 of each plot in Figure 2.3 were taken using rapid sampling technique (Mashego et al, 2003), so the average of these values was assumed as the residual glucose concentration in the fermenter = 21.62 mg/L.

The average of residual glucose after the sampling port without pump was 14.5 mg/L.

The average of residual glucose after the sampling with pump was 11.31 mg/L.

These values seem good because the residual glucose decreased when the residence time from the fermenter to the acceptor was increased.

1. Specific glucose up-take rate in the fermenter is calculated to be = 0.025

\[ q_s = \frac{D \cdot (C_m - C_{out})}{X_{bio}} = \frac{0.05 \cdot (27.100 - 21.62)}{15} = 0.025 \text{ mgGlu/} g_{bio} \cdot s \]

D = dilution rate = 0.05 h⁻¹.

\[ C_m = \text{Residual glucose in the medium} = 27.1 \text{ g/L}. \]

\[ C_{out} = \text{residual glucose in the fermenter} = 21.62 \text{ mg/L}. \]

\[ X_{bio} = \text{Biomass concentration} = 15 \text{ g/L}. \]

It was supposed that the glucose up-take rate, in this concentration of substrate, was a linear function of concentration of glucose; \( q_s = k \cdot C_{Glu} \) so:

\[ q_s = 1.1563 \times 10^{-3} \times C_{Glu} \]

2. Residence time between the fermenter and the sample tube (with pump) was calculated to be = 34.4 s, based on the equation below.

\[ q_s \cdot X_{bio} \cdot \tau_{tot} = C_{out} - C_{accept \ with \ pump} \]

\[ q_s = 1.1563 \times 10^{-3} \times \left( \frac{C_{out} + C_{accept \ with \ pump}}{2} \right) = 0.019 \ \text{mgGlu/} g_{bio} \cdot s \]

\[ 0.019 \times 15 \times 3.162 - 11.3 \rightarrow \ \tau_{tt} = 34.4 \text{ s} \]

\[ q_s = \text{Average of glucose up-take between } C_{out} \text{ and } C_{accept \ with \ pump}. \]

\( \tau_{tt} = \text{Residence time between the fermenter and the acceptor using the pump}. \)

\( C_{accept \ with \ pump} = \text{Residual glucose in the acceptor using the pump} = 11.31 \text{ mg/L} \)
3. Residence time of the sample port without pump = \textbf{22.73} s based on the equation below

\[ q_s = 1.1563 \times 10^{-3} \times \left( \frac{C_{\text{out}} + C_{\text{accept, Without pump}}}{2} \right) = 0.0212 \ \text{mg}_{\text{Glu}}/g_{\text{Bio}} \times s \]

\[ q_s \times x_{\text{Bio}} \times \tau_{\text{port}} = C_{\text{out}} - C_{\text{accept, Without pump}} \]

\[ 0.0212 \times 15 \times \tau_{\text{port}} = 21.62 - 14.5 \implies \tau_{\text{port}} = 22.73 \text{ s} \]

\( C_{\text{accept, Without pump}} \) = Residual glucose in the acceptor without using the pump = 14.5 mg/L.

4. Residence time of the silicon tubing = \textbf{11.2} s

\[ V = L \times \pi \times \frac{d^2}{4} = 6.0 \times \pi \times \frac{0.0089^2}{4} = 3.7 \times 10^{-4} \text{ L} \]

\[ \tau_{\text{tub}} = \frac{V}{F} = \frac{3.732 \times 10^{-4}}{2 \times 10^{-3}} = 0.1462 \text{ min} = 11.2 \text{ s} \]

\( V \) = Tubing's volume (L or dm³)

\( L \) = length of the tubing = 6 dm.

\( D_i \) = Internal diameter of the tubing = 0.0089 dm.

\( \tau_{\text{tub}} \) = residence time of the tubing (s).

\( F \) = Flow rate inside the tubing = 2 \times 10^{-3} \text{ L/min}.

5. Estimation of the residual glucose in the acceptor (with pump) using the residence time (\( \tau_{\text{tub}} = 11.2 \) s) was calculated to be = \textbf{11.93} mg/L, according to the equation below

\[ q_s \times x_{\text{Bio}} \times \tau_{\text{tub}} = C_{\text{accept without pump}} - C_{\text{accept With pump}} \]

\( C_{\text{accept With pump}} = 11.31 \) is used for the first iteration.

\[ q_s = 1.1563 \times 10^{-3} \times \left( \frac{C_{\text{accept without pump}} + C_{\text{accept With pump}}}{2} \right) = 0.015 \ \text{mg}_{\text{Glu}}/g_{\text{Bio}} \times s \]

1st. iteration : \( 0.01524 \times 15 \times 11.2 = 14.5 - C_{\text{accept With pump}} \implies C_{\text{accept With pump}} = 11.93 \text{ mg/L} \)

2nd. iteration : \( 0.01528 \times 15 \times 11.2 = 14.5 - C_{\text{accept With pump}} \implies C_{\text{accept With pump}} = 11.93 \text{ mg/L} \)

These values are quite good, because the estimation of the residual glucose in the acceptor (with pump) using the residence time \( \tau_{\text{tub}} = 11.2 \) s gives a residual glucose quite similar to the value that was found experimentally. Anyway, using the BioScope
it will be possible to determine the amount of the residual glucose just before the pulse, and the important thing is to get an approximation.

\[ C_{\text{accept, with pump}} = 11.93 \text{ mg/L. Estimated using } \tau_{\text{mix}} = 11.2 \text{ s, and the glucose up-take} \]

\[ (q_s = k' C_{\text{in}}) \]

\[ C_{\text{accept, with pump}} = 11.31 \text{ mg/L. That was found experimentally.} \]
Figure 2.3. Residual glucose. (On the top) without pump. (Below) using pump. ■ Done in 3/4/03. ● Done in 7/4/03.
3. Pressure drop

3.1 Introduction

One of the requirements of the Bioscope design is a limited maximum pressure drop, because the pump cannot deliver a constant flow rate for a pressure drop above 2 bar.

The pressure inside the Bioscope is also important, because too much pressure could increase the volume of the channel due to the possible expansion of the silicone membrane separating the liquid channel from the gas channel. This will undoubtedly result in the increase of the residence time in the BioScope.

3.2 Theory

In order to get a good approximation of the pressure drop is important get a good friction factor of the serpentine channels and then add the pressure drop of the connectors between the ports.

3.2.1 Pressure drop in regular helical coil tube

The pressure drop can be expressed by Fanning equation (Ali 2001):

\[ \Delta P = f \cdot 0.5 \cdot \rho \cdot v^2 \cdot \frac{S \cdot L}{A} \]  

(3.1)

Where:
\( \Delta P \) = Pressure drop (Pa)
\( f \) = The friction factor (-)
\( \rho \) = Density (Kg/m\(^3\))
\( v \) = Average velocity (m/s)
\( S \) = Circumference (m)
\( L \) = Length (m)
\( A \) = Cross section (m\(^2\))

In order to calculate the friction factor used in a correlation for flow through regular helical coil tube described in (Ali 2001).
Chapter 3

Pressure drop

\[ f_x = 5.22 \times (Re \times \frac{d_c}{d_i})^{0.6} \]  \hspace{1cm} (3.2)

\[ Re = \frac{\rho \times v \times D_h}{\eta} \]  \hspace{1cm} (3.3)

\[ D_h = \frac{4 \times A}{S} = \frac{4 \pi \times d_i^3}{\pi^2 \cdot d_i + d_i} \]  \hspace{1cm} (3.4)

Where:

d_c = Diameter of the coil = 2 \times 10^{-3} \text{ m}.
d_i = internal diameter of the BioScope channels = 1.2 \times 10^{-3} \text{ m}.
Re = Reynolds number (\text{-}).
D_h = Hydraulic diameter (\text{m}).

Tables 3.1, 3.2, and 3.3 show the results of these estimations using equations 3.4, 3.3, 3.2, 3.1 in succession:

Table 3.1 Hydraulic diameter.

<table>
<thead>
<tr>
<th>Dh (m)</th>
</tr>
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<tr>
<td>7.33E-04</td>
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</table>

Table 3.2 Intermediate results.

<table>
<thead>
<tr>
<th>V (m/s)</th>
<th>Re</th>
<th>Fc</th>
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<tbody>
<tr>
<td>0.000</td>
<td>0.00</td>
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<tr>
<td>0.059</td>
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<td>0.147</td>
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</tr>
<tr>
<td>0.177</td>
<td>129.17</td>
<td>0.218</td>
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</table>
Table 3.3 Estimated pressure drop in the serpentine channels

<table>
<thead>
<tr>
<th>Sample port</th>
<th>Flow rate (mL/min)</th>
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<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.00</td>
<td>0.00</td>
<td>0</td>
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<tr>
<td>1</td>
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<td>0.021</td>
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<tr>
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</tr>
<tr>
<td>4</td>
<td></td>
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<td>0.042</td>
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</tr>
<tr>
<td>5</td>
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<td>0.093</td>
<td>0.127</td>
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<tr>
<td>7</td>
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<tr>
<td>10</td>
<td></td>
<td>0.212</td>
<td>0.374</td>
<td>0.560</td>
<td>0.765</td>
<td>0.988</td>
</tr>
</tbody>
</table>

3.3 Materials & Methods

In previous experiments (Beek & Muttzall), it has been shown that the airflow rate does not affect the flexibility of the membrane neither the pressure drop in the liquid channel. In this experiment the airflow rate was 0.

The schematic overview of the experiment set-up is shown in Figure 3.1. The required materials and equipments are listed in Table 3.4

Table 3.4 Materials and equipments

<table>
<thead>
<tr>
<th>Component</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Distilled water</td>
</tr>
<tr>
<td>Pump</td>
<td>Ismatec, IPC</td>
</tr>
<tr>
<td>Pressure meter</td>
<td>Wika</td>
</tr>
<tr>
<td>Tubes</td>
<td></td>
</tr>
<tr>
<td>Stop watch</td>
<td></td>
</tr>
</tbody>
</table>

Experimental steps

1. The pump was calibrated to deliver a constant desired flow rate.
2. Water was flushed through the system. At the beginning, the first port was used.
3. The pressure drop was measured for the different flow rates (2,3,4,5,6) mL/min, using one port at a time.
4. Silicone tubing in a form of a bend was used as a connector between the sampling ports in order to use the port other sampling ports in sequence.
5. The pressure drop in the second port was measured as mentioned before, using the same flows rates.
6. The pressure drop in the rest of the ports was measured in the same way.

![Diagram of experiment set-up](image)

Figure 3.1 The experiment set-up for measure the pressure drop.

### 1.4 Results & Discussions

As can be seen the measured pressure drop (Table 3.6) is always higher than the calculated one, but this is reasonable because the calculated pressure drop in the channels did not include the connectors and was assumed to be the total pressure drop, and some things like little changes of diameter were not taken into account. Anyway, the difference is not very large, and the main aim of this experiment was to ensure that the pressure drop is always below 2 bars, and this is the case at least for the flow rates lesser than 7mL/min.

As can be seen in Figures 3.2 and 3.3, under a constant flow, the pressure drop is proportional to the channel length; this observation is according with the equation 3.1 and in general the results are satisfactory.
Table 3.5. Estimated pressure drop in the serpentine channels

<table>
<thead>
<tr>
<th>Sample port</th>
<th>Flow rate (mL/min)</th>
</tr>
</thead>
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<td>5</td>
<td>0.10</td>
</tr>
<tr>
<td>6</td>
<td>0.11</td>
</tr>
<tr>
<td>7</td>
<td>0.14</td>
</tr>
<tr>
<td>8</td>
<td>0.19</td>
</tr>
<tr>
<td>9</td>
<td>0.25</td>
</tr>
<tr>
<td>10</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Figure 3.2 Pressure drop using 2mL/min of flow rate. (■) Calculated. (♦) Experimental
Figure 3.3 (A) Pressure drop using 4mL/min. (B) Pressure drop using 6mL/min. (■) Experimental. (♦) Calculated, in both plots
4. Mass Transfer

4.1 Introduction

Oxygen is essential for aerobic respiration, and most of microorganisms can only utilize dissolved oxygen. It is therefore crucial, to ensure sufficient oxygen concentration in the liquid phase. On the other hand, the carbon dioxide production should be removed in order to prevent any unwanted side effects during the perturbation.

The mass transfer must be only diffusion transfer, because the convection transfer can form bubbles in the liquid phase and these bubbles can effect the residence time and alter the plug flow characteristics of the BioScope.

This chapter contains the experimental estimation of the oxygen transfer coefficient, and the carbon dioxide transfer coefficient, derived from the oxygen transfer.

4.2 Oxygen Transfer

The oxygen comes from the gas phase to the liquid phase, by diffusion phenomena.

4.2.1 Theory

The oxygen transfer rate can be calculated by the following equation derived from the mass balance:

\[
\text{OTR} = \frac{\partial C_{o2L}}{\partial t} = K_{\text{overall}} \cdot A \cdot (C^*_{o2L} - C_{o2L}) - \text{OUR}
\]

Where:

- \( \text{OTR} \) = Oxygen transfer rate (mol·m\(^{-3}\)·s\(^{-1}\))
- \( C_{o2L} \) = Oxygen concentration in liquid phase (mol/m\(^3\))
- \( C^*_{o2L} \) = Saturated oxygen concentration in liquid phase = 0.213 \(^{\circ}\) (mol/m\(^3\))
- \( K_{\text{overall}} \) = Global transfer coefficient (m/s)
- \( A \) = Specific area (m\(^{2}\)/m\(^{3}\))
- \( \text{OUR} \) = Oxygen uptake rate (mol·m\(^{3}\)·s\(^{-1}\))
- \(^{\circ} \) at 30 \(^{\circ}\)C.
Chapter 4 Mass transfer

Equation (4.1) can be expressed in:

\[ OTR = \frac{dC_{O_2L}}{dt} = \frac{8 \cdot K_{overall}}{\pi \cdot d_{in} \cdot v} \cdot (C^*_{O_2L} - C_{O_2L}) - OUR \]  \hspace{1cm} (4.2)

Where:
\[ d_{in} = \text{Internal diameter of the tubes} = 1.2 \cdot 10^{-3} \text{m} \]
\[ v = \text{Velocity of the liquid (m/s)} \]
\[ OUR = \text{Oxygen uptake rate} \]

Finally, equation (4.2) can be integrated, given:

\[ \frac{\pi \cdot d_{in} \cdot v}{8} \cdot \ln(C^*_{L} - C_{L}) = ct - K_{overall} \cdot x \]  \hspace{1cm} (4.3)

Where:
\[ ct = \text{is a constant value (m}^2/\text{s)} \]
\[ \text{In this experiment OUR is equal to 0.} \]

The aim of this chapter is to estimate \( K_{overall} \), and equation (4.3) seems to be the most useful, because it can be express as a linear function: \( y = ct - K_{overall} \cdot x \)

On the BioScope \( K_{overall} \) is given by:

\[ \frac{1}{K_{overall}} = \frac{1}{m \cdot k_g} + \frac{1}{m \cdot k_M} + \frac{1}{k_L} \]  \hspace{1cm} (4.4)

Where:
\[ m = \text{Partition coefficient (\text{-})} \]
\[ k_g = \text{Transfer coefficient in gas phase (m/s)} \]
\[ k_M = \text{Transfer coefficient in liquid phase (m/s)} \]
\[ k_M = \text{Membrane transfer coefficient (m/s)} \]

The resistance through the membrane and in the gas phase can be assumed as negligible (Huang 2002) and Visser 2002).
4.2.2 Materials & Methods

Water in the reactor was flushed with nitrogen gas to remove all dissolved oxygen. The deoxygenated water (30°C) was fed to the BioScope at three different flow rates: 0.5, 2 and 4mL/min approximately. The dissolved oxygen concentration (DO) was measured using DO probe Mettler Toledo, at all the sample ports. The air flow rate was set to 131.23mL/min.

The overall oxygen transfer coefficient was estimated from these measurements using the equation (4.3).

The schematic overview of the experimental set-up is shown in Figure 4.1. The required materials and equipments are listed in Table 4.1.

<table>
<thead>
<tr>
<th>Component</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Tap water</td>
</tr>
<tr>
<td>Pump</td>
<td>Ismatec, IPC</td>
</tr>
<tr>
<td>Nitrogen supply</td>
<td>Compressed N₂</td>
</tr>
<tr>
<td>Air supply</td>
<td>Compressed air</td>
</tr>
<tr>
<td>Fermentor</td>
<td>Applikon</td>
</tr>
<tr>
<td>Stirrer</td>
<td>Applikon, AD1012</td>
</tr>
<tr>
<td>Biocontroller</td>
<td>Applikon, AD1030</td>
</tr>
<tr>
<td>Mass flow controller</td>
<td></td>
</tr>
<tr>
<td>Computer</td>
<td></td>
</tr>
<tr>
<td>Thermostat</td>
<td></td>
</tr>
<tr>
<td>2 DO probes</td>
<td>Mettler Toledo</td>
</tr>
<tr>
<td>Flow cell</td>
<td></td>
</tr>
</tbody>
</table>

Experimental steps

1. The pump was calibrated, and the water flow rate was set at approximately 2mL/min.
2. The temperature of the water in the fermentor was controlled at 30°C.
3. The DO probes were calibrated in the fermentor. During the DO probe calibration the stirrer speed was set to 600 rpm. The 0% was set by flushing N₂ gas and the 100% saturation was set by flushing with air.
4. The fermentor was flushed with nitrogen (N₂) and set to 0%.
5. One DO probe was placed into the flow cell and this was connected to the first sample port, as shown in Figure 4.1.
6. The BioScope temperature was controlled at 30°C.
7. The pump was started. The DO signal was recorded at all times. The DO signal can take more than one hour to get stable.
8. The DO level was measured for all the ports.

![BioSCOPE Diagram](image)

Figure 4.1 Experimental set up for DO measurements

**4.2.3 Results & Discussions**

During the oxygenation experiment, as can be seen in Figures 4.1 and in the simulations (Figures 4.4 and 4.5), the oxygen increases very rapidly during the first 1 or 2 meters of the BioScope and stabilises around 0.213 mol/m$^3$, which is the saturation concentration of oxygen in water at 30°C.

For 2mL/min a $K^i$ equal to 3e-5 m/s was found. This value is similar to that reported by Visser of 2.9e-5 m/s (Visser 2002).

The value of $K^o$ is estimated using the equation (4.3) as can be seen in the Figure 4.2, when using this method the last points are useless, only the points where there is a big change of the DO have information about the transfer velocity. Thus, the experiment with the flow rate approximately 0.5mL/min is not very accurate.

The values of the overall mass transfer coefficients are shown in Figure 4.3. An important observation is that the mass transfer increases with the increase in flow rate.
rate but shows a saturation behaviour, which is expected. Therefore, the oxygen transfer in the BioScope has two opposite contributions according to the velocity: the transfer velocity decreases with a lower liquid flow rate, but if the liquid spends more time in the silicone channels, it will have more exposure time to get more oxygen. Even so, during the oxygenation experiment a slow velocity increases the dissolved oxygen in each port.

As can be seen in Figure 4.5 using a normal oxygen uptake rate equal to 7.78e-3 mol/(m$^3$s) and an initial concentration of 0.15 mol/m$^3$, the profile decreases a little bit at the beginning, but it gets rapidly stabilizes around 0.14 mol/m$^3$. This value is enough to insure non-oxygen limited situation, so the oxygen transfer appears to be good enough.
Figure 4.1 Experimental data during the oxygenation. (■) Flow rate of 0.5 mL/min. (●) Flow rate of 2 mL/min. (▲) Flow rate of 4 mL/min.

Figure 4.2 Estimation of $K_{O_{2}}$ for 4 mL/min.
Chapter 4

Mass transfer

Figure 4.3 $K_{\text{overall}}$ estimations vs flow rate (A) in plot format (B) in table format.

<table>
<thead>
<tr>
<th>Flow rate (mL/min)</th>
<th>$K_{\text{overall}}$ (m/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.535</td>
<td>1.5e-05</td>
</tr>
<tr>
<td>2.00</td>
<td>3e-05</td>
</tr>
<tr>
<td>4.02</td>
<td>4e-05</td>
</tr>
</tbody>
</table>

Figure 4.4 Results of oxygen dissolved for 0.535mL/min. (Circles) experimental points. (Line) Simulation
Figure 4.5 Results of oxygen dissolved. (Top) For 0.2mL/min. (Bottom) For 4mL/min. (Circles) Experimental points (Line) Simulation
4.3 Carbon Dioxide Transfer

The carbon dioxide coming from the liquid phase and is removed by airflow.

4.3.1 Theory

The carbon dioxide transfer coefficient can be estimated from the oxygen transfer coefficient, using the relations shown below (Huang 2002).

\[ \text{Sh} = \frac{k_{i,L} \cdot d}{D_{L,L}} \]  \hspace{1cm} (4.5)

Where:

- \text{Sh} = \text{Sherwood number}
Chapter 4 Mass transfer

\( k_{l,l} = \text{Transfer coefficient in liquid phase} \quad \text{(m/s)} \)
\( d = \text{Diameter} \quad \text{(m)} \)
\( D_{l,l} = \text{Diffusion coefficient in liquid phase} \quad \text{(m}^2/\text{s)} \)

The Sherwood number can also be calculated from the Reynolds number and the Schmidt number:

\[
Sh = c \cdot Re^n \cdot Sc^{1/3}
\]

\[
Sc = \frac{\eta}{\rho \cdot D_{l,l}}
\]

Where:
\( c = \text{is a constant} \)
\( \eta = \text{Liquid viscosity} = 1e-3 \text{ kg/(m}^s) \)
\( \rho = \text{Liquid density} = 998.23 \text{ kg/m}^3 \)

These equations can be combined to give:

\[
k_{l,l} = \frac{c \cdot Re^n \sqrt[3]{\frac{\eta}{\rho}} \cdot D_{l,l}^{1/3}}{d}
\]

In this equation, everything is independent of the component except the diffusion coefficient; all the other parameters are properties of the fluid (water) and its dynamics. Therefore, \( k_{l,l} \) can be described as a function of the diffusion coefficient:

\[
k_{l,l} = C \cdot D_{l,l}^{1/3}
\]

Where:
\( C = \text{is a constant} \)

For a flow rate of 2mL/min the oxygen transfer coefficient in liquid phase is 3e-3 m/s, and the diffusion coefficient for oxygen in water at 30°C is 2.54e-9 m²/s [7].
4.3.2 Results & Discussions

1. \[3 \times 10^{-5} = C \cdot (2.54 \times 10^{-9})^{1/2} \Rightarrow C = 16.115\]

2. \[k_{CO_{2,L}} = 16.115 \cdot D_{CO_{2,L}}^{1/2}, \text{ the diffusion coefficient for carbon dioxide is } 1.99 \times 10^{-9} \text{ m}^2\text{s}^{-1} \text{ (Perry 1997)}; \text{ so }\]

3. \[k_{CO_{2}} = 2.55 \times 10^{-5} (\text{m/s})\]

Table 4.2 Transfer coefficients for carbon dioxide

<table>
<thead>
<tr>
<th>Flow (mL/min)</th>
<th>(k_{CO_{2,L}} \text{ (m/s)})</th>
<th>(K_{\text{overall}} \text{ (m/s)})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>1.27E-05</td>
<td>6.12E-06</td>
</tr>
<tr>
<td>2</td>
<td>2.55E-05</td>
<td>8.07E-06</td>
</tr>
<tr>
<td>4</td>
<td>3.40E-05</td>
<td>8.76E-06</td>
</tr>
</tbody>
</table>

It is important to have an estimation of the carbon dioxide removal rate (CTR), and if the approximate production of carbon dioxide during a pulse experiment is known, a prediction or a simulation of the carbon dioxide concentration in the BioScope will be estimated using equation (4.10).

\[
CTR = K_{\text{CO_{2,overall}}} \cdot a \cdot (C_{CO_{2,L}} - C_{CO_{2}}^*)
\]

(4.10)

Where:

- CTR = Carbon dioxide transfer rate \((\text{mol/(m}^3\text{*s)})\)
- \(C_{CO_{2}} = \text{Carbon dioxide concentration in liquid phase (mol/m}^3\))
- \(C_{CO_{2}}^* = \text{Carbon dioxide concentration in interphase (mol/m}^3\))

In a normal glucose pulse experiments the carbon dioxide production increases from 24 mmol/(L*h) to 54 mmol/(L*h) (Visser 2002), and a normal value of carbon dioxide concentration in a fermentor is 0.3 mol*m\(^3\). The simulation profile shown in Figure 4.6 is based on these values.

As can be seen in Figure 4.6 the carbon dioxide concentration is rising until 0.8 mol*m\(^3\) approximately. This fact has to be studied and if it could be a problem in each case. It important to note that using a higher flow rate, it is possible to reduce the CO\(_2\) concentration but the residence time is reduced also to approximately 60 seconds at the last sampling port of the BioScope. So in each case it has to be known if the CO\(_2\) produced will detrimentally affect the results of the pulse experiment.
Figure 4.6 Simulation of CO₂ concentration using 15e-3 mol·m⁻³·s⁻¹ as the CO₂ production (A) 2mL/min flow rate (B) 0.5mL/min and 4mL/min flow rates.
5. Residence time

5.1 Introduction

In pulse response experiments, the residence time is one of the most important aspects, because one of the main objectives in this kind of experiments is to see how both the extracellular and intracellular metabolites concentration changes over the time.

This chapter studies the residence time from the mixing point (between the broth and the pulse substrate) to each sample port, and thus the experimental length of the BioScope channels according to this residence time.

The effect of dispersion expressed by the Peclet number, is also studied here, since it an important parameter of the BioScope to ensure the plug flow.

5.2 Volume Determination

After the mounting of the BioScope in the cabinet, the volume of the water in all the channels, the tubes and the connectors was determined.

5.2.1 Materials & Methods

This experiment was done using 2mL/min as a flow rate, although the licking test performed with 2 and 4mL/min, in order to ensure that there was no lick or weak connection. The whole the system was dried flushing ethanol and air, and filled with water. Then this amount of water can be measured gravimetrically.

The schematic overview of the experimental set-up is shown in Figure 5.1. The required materials and equipments are listed in Table 5.1

<table>
<thead>
<tr>
<th>Component</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Distilled water</td>
</tr>
<tr>
<td>Pump</td>
<td>ISMATIC, IPC</td>
</tr>
<tr>
<td>Acceptor</td>
<td></td>
</tr>
<tr>
<td>Balance</td>
<td></td>
</tr>
</tbody>
</table>
Experimental steps

1. The pump was calibrated and set at 2mL/min.
2. Ethanol was flushed through whole the system, followed by flushing with air in order to dry it.
3. The flask with water and the acceptor were weighed.
4. When the system was dry, water was flushed through whole system.
5. When this part of the BioScope was filled; the pump was stopped. The acceptor with water and the flask with the remaining water were weighed. The difference between the weights was water inside the system and thus the total volume of the Bioscope including the connectors.

![Figure 5.1 The experiment set-up for measure the volume determination.](image)

5.2.2 Results & Discussions

During this experiment, the time that water took to fill the system was also recorded, this can gives a rough estimation of the residence time.

The water volume occupying the connecting tube from the water reservoir to the entrance of the BioScope was about 0.32mL.

The water volume occupying the whole system from the water reservoir to the last port (10") was about 4.09mL.

Therefore, the total volume of the BioScope, including the connectors, was approximately 3.76mL.

It is useful to introduce the concept of equivalent length, because the bends, the ports and other tubes, have different internal diameters, and almost all the key parameters are function of the residence time. It is therefore assumed that all the
tubes have the same cross-area (5.65e-7 m$^2$), and the equivalent length is used in order to keep their real volume.

The equivalent length of whole the BioScope (from the mixing point to the last port) is 6.66 m.

The results of the volume determination and its equivalent length are shown below, in Table 5.2. The residence time in Table 5.2 is for liquid flow rate of 2mL/min.

As can be seen in Figure 5.2 all the sample ports, do not have the same length. Ports numbers 1, 2, 3 and 4 have a length of approximately 0.3m; ports number 5 and 6 have a length of approximately 0.43m; ports number 7 and 8 have length of approximately of 0.7m; and finally ports number 9 and 10 have a length of approximately 1.61m.

Table 5.2 Volume of the BioScope and its equivalent length

<table>
<thead>
<tr>
<th>S. port</th>
<th>Volume (mL)</th>
<th>Length (m)</th>
<th>Residence time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>1</td>
<td>0.16</td>
<td>0.28</td>
<td>4.8</td>
</tr>
<tr>
<td>2</td>
<td>0.33</td>
<td>0.58</td>
<td>10.0</td>
</tr>
<tr>
<td>3</td>
<td>0.48</td>
<td>0.87</td>
<td>14.6</td>
</tr>
<tr>
<td>4</td>
<td>0.67</td>
<td>1.19</td>
<td>20.2</td>
</tr>
<tr>
<td>5</td>
<td>0.91</td>
<td>1.61</td>
<td>27.3</td>
</tr>
<tr>
<td>6</td>
<td>1.16</td>
<td>2.05</td>
<td>34.8</td>
</tr>
<tr>
<td>7</td>
<td>1.55</td>
<td>2.75</td>
<td>46.7</td>
</tr>
<tr>
<td>8</td>
<td>1.94</td>
<td>3.45</td>
<td>58.5</td>
</tr>
<tr>
<td>9</td>
<td>2.85</td>
<td>5.05</td>
<td>85.7</td>
</tr>
<tr>
<td>10</td>
<td>3.76</td>
<td>6.66</td>
<td>113.0</td>
</tr>
</tbody>
</table>

Figure 5.2 Length (by volumetric determination) of the BioScope at each sample port.
5.3 Residence Time Distribution (RTD)

The residence time is the time average that a molecule spends inside the reactor. In an idealized plug-flow, all the particles spend the same time, but in reality there is a distribution like Gauss bell type.

RTD can be determined experimentally by injecting an inert chemical, called tracer, into the reactor at time 0, then the concentration of the tracer has to be measured at the effluent stream as a function of time, or in a plug flow, as a function of the length. Usually there are two kinds of experiments in order to find the residence time distribution, using a tracer: pulse input and step input. In a pulse input, an amount of tracer should be injected instantaneously in a short time as possible. In a step input, the tracer concentration is suddenly increased from 0 (at time < 0) to a constant value at the entrance (at time > 0).

The step input was chosen because this method is very similar to the glucose pulse experiments that the BioScope is designed (Visser 2002).

5.3.1 Theory

In a step input, the tracer concentration is suddenly increased from 0 to a constant value at the entrance.

\[ t < 0 \quad \Rightarrow \quad C_{\text{tracer, entrance}} = 0. \]
\[ t > 0 \quad \Rightarrow \quad C_{\text{tracer, entrance}} = \text{Constant.} = C_0 \]

The residence time distribution is defined as:

\[ E(t) = \frac{C(t)}{\int_0^t C(t)dt} \quad (4.11) \]

Where:

- \( E(t) \) = residence time distribution
- \( C(t) \) = Tracer concentration at time \( t \).

The degree of dispersion is expressed with the dimensionless Peclet number (4.12)
Chapter 5

Residence time

\[ Pe = \frac{u \cdot x}{D} \]  
(4.12)

Where:

- \( D \) = Dispersion effect \( (m^2/s) \)
- \( u \) = Velocity \( (m/s) \)
- \( x \) = Length from the mixing point to the sample point \( (m) \)

For Peclet number larger than 30, the error introduced by dispersion effects is negligible (Visser 2002).

5.3.2 Materials & Methods

The residence time distribution was determined from step experiment for each sampling port. Two reservoirs, containing water and 1mM KCl solution respectively were connected to the BioScope. A peristaltic pump was used to feed both solutions to the BioScope at constant flow rates. A flow cell was connected to one of the sampling port of interest of the BioScope to be measured. The conductivity of the liquid that flows through this cell was measured using a conductivity meter (ED40 Electrochemical detector, Dionex Sunnyvale, CA, USA). The KCl solution flow was approximately 10% of the total flow rate. The residence time and the Peclet number were estimated using a program developed by Visser and based on Matlab (see appendix 4).

The schematic overview of the experimental set-up is shown in Figure 5.3. The required materials and equipments are listed in table 5.3.

<table>
<thead>
<tr>
<th>Component</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Distilled water</td>
</tr>
<tr>
<td>KCl solution</td>
<td>1 mM</td>
</tr>
<tr>
<td>Pump</td>
<td>Ismatec, IPC</td>
</tr>
<tr>
<td>Conductivity detector</td>
<td>Dionex ED 40</td>
</tr>
<tr>
<td>Computer</td>
<td>Bioview program</td>
</tr>
</tbody>
</table>

Table 5.3 Materials and equipments.
Experimental steps

1. The pump was calibrated to deliver a flow rate of 1.85mL/min 1.67mL/min of water + 0.18mL/min of 1mM KCL solution using tubing 2.79 and 0.89mm internal diameter respectively. The pump speed was set at 5.
2. Water was flushed through the system. When the detector reached a constant value, the reading was of the conductivity meter was set to zero.
3. The time was also set to zero and the KCL was flushed into the BioScope, at the same moment. The tube from the KCL reservoir to the mixing point was already filled.
4. The water and the solution were running until it got a stable reading.

Figure 5.3 Experimental set-up for residence time distribution
5.3.3 Results & Discussions

As can be seen in Table 5.4 and Figure 5.4 the residence times found using the step input experiment are pretty close to the ones found using volumetric determination. The correlation between the residence time and the channel length is supposed to be linear, and it is but the volumetric estimation seems to be more accurate.

For a flow rate approximately to 2mL/min the minimum residence time is around 4.8 seconds, for some experiments this is large; this problem could be solved by increasing the liquid flow rate. The maximum residence time is around 114 seconds; this value could be increased using a lower flow rate. This flow changes have to be studied in each case.

The Peclet number, in these results, is not always larger than 30, and one of the requirements of the BioScope is that the Peclet number has to be higher than 30 to insure plug flow characteristics. In other experiments like Visser 2002 and Huang 2002, the Peclet number found was larger than 30. This results need to be rechecked in further experiments. However, it is only one or two ports that gave a Peclet number lower than 20.

The Peclet number does not seem to show the theoretical behaviour of increasing with the residence time/ channel length.

Table 5.4 Mean residence time and Peclet number for flow rate 1.85 mL/min.

<table>
<thead>
<tr>
<th>Sample port</th>
<th>Residence time (s)</th>
<th>Residence time (s)</th>
<th>Peclet number</th>
<th>Peclet number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.8</td>
<td>4.8</td>
<td>55.0</td>
<td>56.0</td>
</tr>
<tr>
<td>2</td>
<td>11.2</td>
<td>11.2</td>
<td>8.9</td>
<td>9.0</td>
</tr>
<tr>
<td>3</td>
<td>14.5</td>
<td>14.7</td>
<td>25.3</td>
<td>24.0</td>
</tr>
<tr>
<td>4</td>
<td>21.7</td>
<td>22.0</td>
<td>18.1</td>
<td>18.3</td>
</tr>
<tr>
<td>5</td>
<td>29.2</td>
<td>29.2</td>
<td>25.0</td>
<td>25.0</td>
</tr>
<tr>
<td>6</td>
<td>39.7</td>
<td>39.7</td>
<td>40.8</td>
<td>39.0</td>
</tr>
<tr>
<td>7</td>
<td>61.7</td>
<td>61.8</td>
<td>23.8</td>
<td>23.9</td>
</tr>
<tr>
<td>8</td>
<td>63.0</td>
<td>62.9</td>
<td>185.9</td>
<td>193.0</td>
</tr>
<tr>
<td>9</td>
<td>74.0</td>
<td>87.6</td>
<td>71.3</td>
<td>26.5</td>
</tr>
<tr>
<td>10</td>
<td>113.9</td>
<td>114.5</td>
<td>59.5</td>
<td>54.8</td>
</tr>
</tbody>
</table>
Figure 5.4 (A) Residence times of the BioScope ports. (■) Residence time estimated using volumetric determination. (♦) Residence time estimated using step input experiment. (B) Peclet number of the BioScope ports.
Conclusions & Discussion

Generally, the overall behaviour of the new BioScope characteristics, are in agreement with the basic requirements. The measured pressure drop is not too large, always lower than 2 bars even with 6mL/min, so it can be concluded that the pressure drop will not give any problem for the flow rates tested.

Oxygen transfer is sufficient to ensure a good oxygenation, during a pulse experiment, but if the experiment requires a deoxygenated medium, the silicone connectors should be changed or the whole Bioscope should cabinet should be flushed with nitrogen gas.

Also, the carbon dioxide removal, is quite good although during the pulse, the CO$_2$ concentration can increase above to 0.8 mol/m$^3$, this value doesn’t seem to be a problem in general but is not negligible, thus during a pulse experiment this value has to be taken into account.

The length of the BioScope is larger that it was expect, largely because in the designing stage, the connecters were not taken into account, with the real length, the Bioscope can works in a residence time range between 4.8 seconds and 114 seconds for liquid flow rate of 2mL/min. However, the residence time can be adjusted by changing the flow rate.

The flow rate has also a problem; the tubes inside the peristaltic pump change a little bit their diameter in the course of time, thus the flow rate has to be check before the start of an experiment. This change is not very big, but a pulse experiment, usually, is strongly dependent of the residence time, and this change of diameter could alter the incubation time and the ratio of pulsing solution to the steady state sample.

The Peclet number is the other major parameter that should to be checked. In the previous experiments, such as Visser 2002 and Huang 2002, this parameter did not give any problem, but in our case, sometimes the Peclet number was lower than 30 although never lower than 10. In most-cases, this parameter is larger than 30.
References


Beek W.J. & Muttzall K.M.K, 1986, Transport Phenomena

Huang, 2002, Development of an improved BioScope for short time pulse response experiments, Graduation Thesis, Delft University of Technology


Appendix I

Growth conditions

The yeast *Saccharomyces cerevisiae* was cultivated aerobically in continuous mode (D = 0.05 h⁻¹) at 30 °C and pH of 5 in a 7 L fermentor (Applicon, the Netherlands) with a working volume of 4 L. The stirrer speed was 600 rpm and the overpressure was 0.3 bar. Experiment code: SC-CC28.

The medium composition designed to obtain a biomass concentration approximately 15 g DW[h⁻¹], the glucose concentration was 27.1 g/L and 1.42 g/l of ethanol was added. The air flow was approximately 230 L/h.

The batch phase took 30 h, and the system was assumed to be in steady state 5 residence times after inoculation.

The biomass concentration was approximately 14.6 g DW[h⁻¹] and the DO was around 85% saturation.
Appendix II
Example of Oxygen simulation using MATLAB

Oxyl2.m
[t,Co]=ode45('df1length2',[0 7],[0.15]);
hold on
plot(t-0.2,Co);

df1length2.m
function yprima=df1(t,Co)
%K=overall mass transfer coefficient;
%di=internal diameter;
%Asec=cross section;
%Cg=oxygen concentration in gas phase;
%m=partition coefficient;
%Co=oxygen concentration in liquid phase in time t;
%OUR=oxygen uptake rate;v=velocity;
yprima=zeros(1,1);v=0.059;
K=5e-5;di=1.2e-3;
Asec=5.655e7;OUR=7.78e-3;
yprima=K*di/v/Asec*(0.213-Co)-OUR/v;
Appendix III

Example of Carbon Dioxide simulation using MATLAB

Carb.m

\[ \text{[t,Co]}=\text{ode45('dfcarb',}[0 \ 7],[0.3]); \]
\[ \text{hold on} \]
\[ \text{plot(t-0.2,Co);} \]

dfcarb

function yprima=df1(t,Co)

%K=overall mass transfer coefficient;
%di=internal diameter;
%Asec=cross section;
%Cg=oxygen concentration in gas phase;
%m=partition coefficient;
%Co=oxygen concentration in liquid phase in time t;
%OUR=oxygen uptake rate;v=velocity;

yprima=zeros(1,1); v=0.118;
K=8.76e-6; di=1.2e-3;
Asec=5.655e-7; OUR=15e-3;

yprima=-K*di/v/Asec*(Co-0)+OUR/v;
Appendix VI

Procedure: HOW TO WORK WITH MATLAB TO RETRIEVE RESIDENCE TIME BIOSCOPE

1) Before opening the MATLAB program.
First copy your Bioview datafiles to the directory.
H:\STM-BIO2\Groups\Rapid Sampling\Bioscope\Residence times\measurements\name file.
Copy from H:\STM-BIO2\Groups\Rapid Sampling\Bioscope\Residence times\calculation files-
all the files to your name.file. (files are E.fun, F.fun, tau.fun, var.fun, test and RTD)
In calculation files – open RTD.XLS files and minimize the file.
Close calculation files folder.

2) Open MATLAB 5.3
[screen MATLAB Command Windows]
Go to Path Browser
Select Browse
Go to H:\STM-BIO2\Groups\Rapid Sampling\Bioscope\Residence times\measurements\name file.
Select name file ENTER (OK).
Minimize the folder (don’t close it).

3) Go to MATLAB Command Windows.
Type by >> RTD
If everything is ok, the measurements files from Bioview will show.
Example: LBI031A.TXT LBI031C.TXT
LBI031B.TXT LBI031D.TXT etc.

Datafile?
Type or copy, after the datafile? ‘LBIO31A.TXT’ ENTER
(use single quote character)

After ENTER, Figure No. 1 will show.
1) Select two points, one at the very beginning and one before the curve goes up.
2) Select two point, one at the top of the curve where it’s stable and one at the end of the curve.

When the last point (of 4 points) is selected, the figure No. 1 is changed. (smooth curve occure).
4)  

In MATLAB Command Window:

location steepest point? Type a number ENTER

After ENTER: two curves will show.

In MATLAB Command Windows:

file = 'LBO31A.TXT'
ans =
  1.0000
 16.6087
 5.7120
569.1729
17.7838
52.7912

»

Copy the numbers:

1.0000
16.6087
5.7120
569.1729
17.7838
52.7912

Go to Rtd.xls (Excel file) and copy by p(I)

Sampling ports

<table>
<thead>
<tr>
<th>Datafile</th>
<th>LBO31A.TXT</th>
<th>Typing in</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>3</td>
<td>Sample</td>
</tr>
<tr>
<td>p(1)</td>
<td>1.0000</td>
<td>Calc.</td>
</tr>
<tr>
<td>p(2)</td>
<td>16.6087</td>
<td>Calc.</td>
</tr>
<tr>
<td>p(3)</td>
<td>5.7120</td>
<td>Calc.</td>
</tr>
<tr>
<td>p(4)</td>
<td>569.1729</td>
<td>Calc.</td>
</tr>
<tr>
<td>tau</td>
<td>17.7838</td>
<td>Calc.</td>
</tr>
<tr>
<td>var</td>
<td>52.7912</td>
<td>Calc.</td>
</tr>
<tr>
<td>corr. tau</td>
<td>16.16</td>
<td></td>
</tr>
<tr>
<td>corr. var</td>
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<td></td>
</tr>
<tr>
<td>dim.less var</td>
<td>0.202</td>
<td></td>
</tr>
<tr>
<td>Pe</td>
<td>13.0</td>
<td></td>
</tr>
</tbody>
</table>

Two graph: 1 = Residence time, 2 = Peclet number.
Data will be correct for the conductivity cell.
Do this for all the data files.
Change the name RTD.