

The precultivation in shake flasks for the execution of bioreactor cultivations

by Prof. Dr.-Ing. Reiner Luttmann et al., Research and Transfer Center of Bioprocess Engineering and Analytical Techniques Hamburg University of Applied Sciences

For the execution of bioreactor cultivations the precultivation in shake flasks is of great interest. The cells should be in good condition to avoid a long adaptation phase at the beginning. To ensure vital cells in the preculture, no substrate and no oxygen limitation should occur during cultivation, and cells should be in exponential growth.

Shaking flask experiments have been carried out for the optimization of preculture conditions. Therefore, a 1 l glass Erlenmeyer flask was equipped with the BlueSens Sensors BCP-O₂ and BCP-CO₂ for the measurement of oxygen and carbon dioxide in the gas phase. For comparison, an optical oxygen microsensor was also used.

A recombinant *Escherichia coli* strain was cultivated. The experiments were conducted in a shaking flask cabinet at 200 rpm and 37 °C.

In figure 1, the course of the percentage molar fraction of oxygen x_{O_2} and carbon dioxide x_{CO_2} is shown. The signal from the BlueSens

O₂-sensor (BS) corresponds very well to the signal of the optical sensor. The BlueSens signal, however, is much noisier compared to the other.

In the beginning, x_{O_2} starts at a value around 21% which is equal to the oxygen fraction of air (20.94%). With increasing cell growth, the oxygen demand is increasing proportionally, so that the x_{O_2} is decreasing to a value around 15.7% at $t = 6.5$ h. The signal of x_{CO_2} is correspondingly

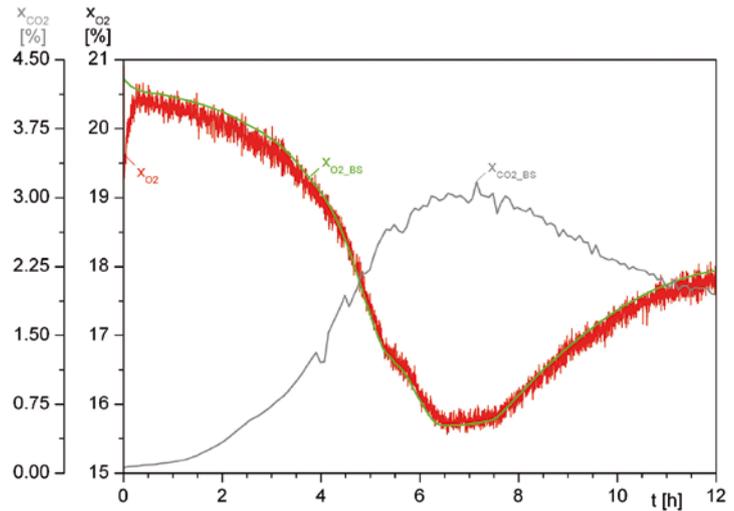


Figure 1: Course x_{O_2} and x_{CO_2} signals of shaking flask experiment

proportional to x_{O_2} .

Another experiment was conducted with additional measurement of the dissolved oxygen tension pO_2 in the liquid phase (figure 2). This gives the opportunity for a better identification of oxygen limitation and verification of the data from the gas phase.

The signals of the x_{O_2} signals are corresponding still very

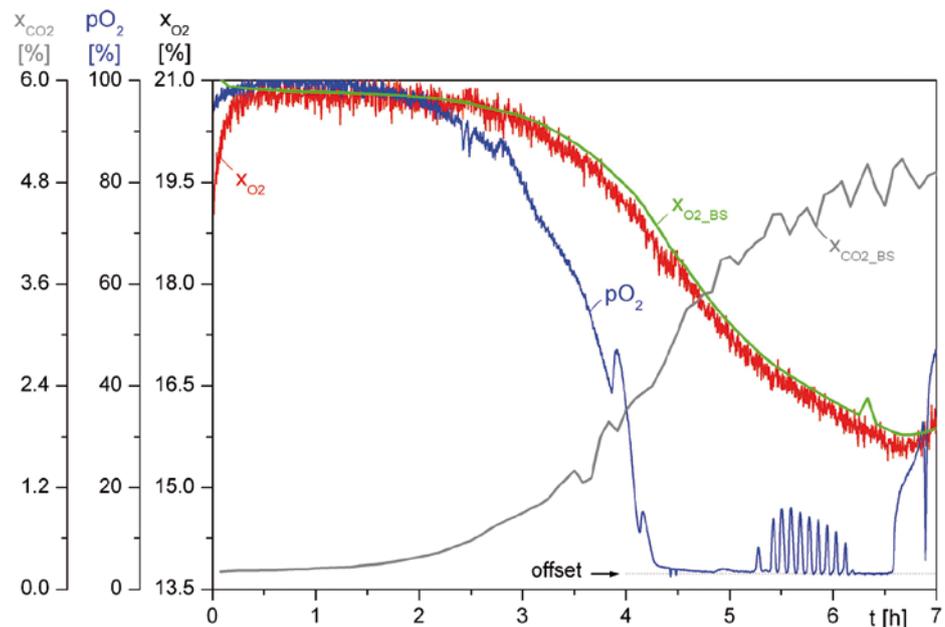
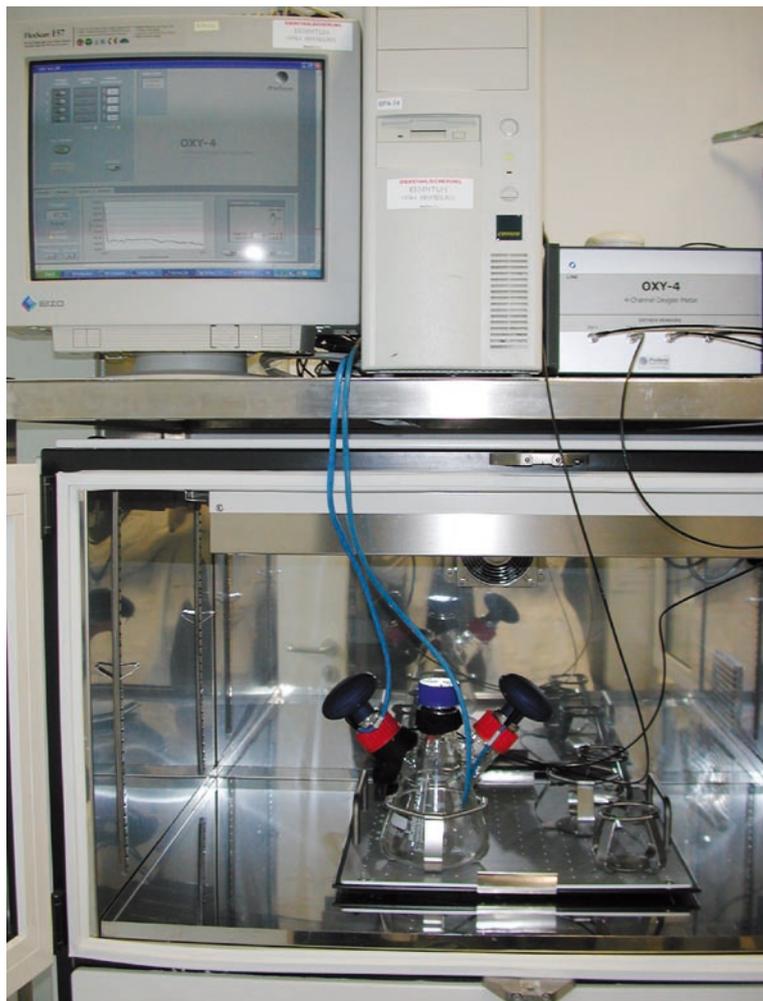


Figure 2: oxygen measurement in gas and liquid phase of shaking flask

Application Report

well in this experiment. The pO_2 is decreasing exponentially with increasing cell growth. After 4.2 hours oxygen limitation occurs. This can be seen also in the x_{O_2} signal in a decreasing slope of the curve. At $t = 6.5$ h the substrate is exhausted and substrate limitation begins. The x_{O_2} graph is at the lowest point at this time.

As mentioned in the beginning, the cells should be in exponential growth and limitations should be avoided. Therefore the duration of the preculture should not exceed 3.5 hours. With an optimized preculture consistent initial conditions for bioreactor cultivations can be realized. Thus a better reproducibility and robust cultivation conditions can be achieved.



Shaking flask experiments with BlueSens Sensors