Respiration Activity MOnitoring System (RAMOS)

AACHENER VERFAHRENSTECHNIK

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Excellence in Lab Scale Technology

AVT-Bioverfahrenstechnik – Biochemical Engineering

Introduction

Shaken bioreactors are outstanding because of their easy handling and their low price. Due to these advantages in comparison to stirred bioreactors, they are irreplaceable in applications that require a high number of experiments, e.g. screening for efficient strains or optimisation of media.
Although shaken bioreactors are frequently used methods that enable online measurement are very limited. This problem is solved by **RAMOS** (Fig. 1), which determindes the oxygen transfer rate (OTR), the carbondioxide transfer rate (CTR) and the respiratory quotient (RQ) of a biological system online. The respiration rates (OTR, CTR) are the most suitable measureable variables to quantify the physiological state of a culture of aerobic microorganisms.

Principle of Measurement

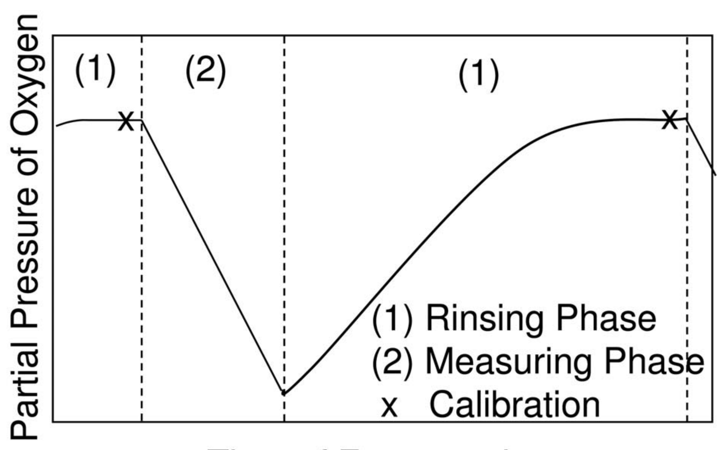

Fig. 1: Measuring-Cycle showing the O2-measurement

During fermentation a measuring cycle is continuously repeated. This measuring cycle is separated into a measuring and a rinsing phase (Fig.1). During the rinsing phase "fresh" gas flows through the measuring flask. At the beginning of the measuring phase the valves for the inlet and the outlet stream are closed.
The respiration activities of the microorganisms now lead to a change of the gas composition in the headspace of the measuring flask. The changes of the partial pressures can be detected by sensors. From the gradient of the sensor signals during the measuring phase the oxygen transfer rate and the carbon dioxide transfer rate can be calculated. The drift of the sensors is compensated by a calibration at the end of each rinsing phase.

Typical OTR-Courses

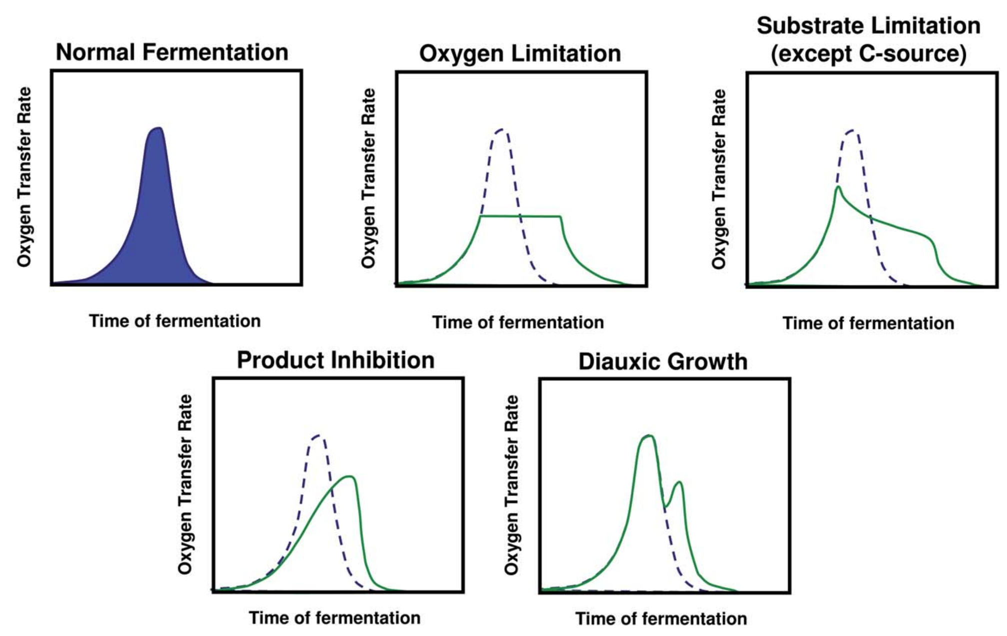

Fig. 2: Examples for OTR-Courses

Fig. 2 shows typical biological phenomena and their effects on the oxygen transfer rate during fermentations. With these basic types of OTR-courses important conclusions on problems with cultivation conditions or composition of media in real fermentations can be drawn.

Main Characteristics

- Online-measurement of OTR, CTR and RQ in shaking flasks
- Operation with microbial cultures, animal and plant cell cultures is possible
- Non-invasive measuring principle
- Measurement is quasi-continously
- Transferability of results from conventional shaking flasks is ensured
- 8 experiments in parallel

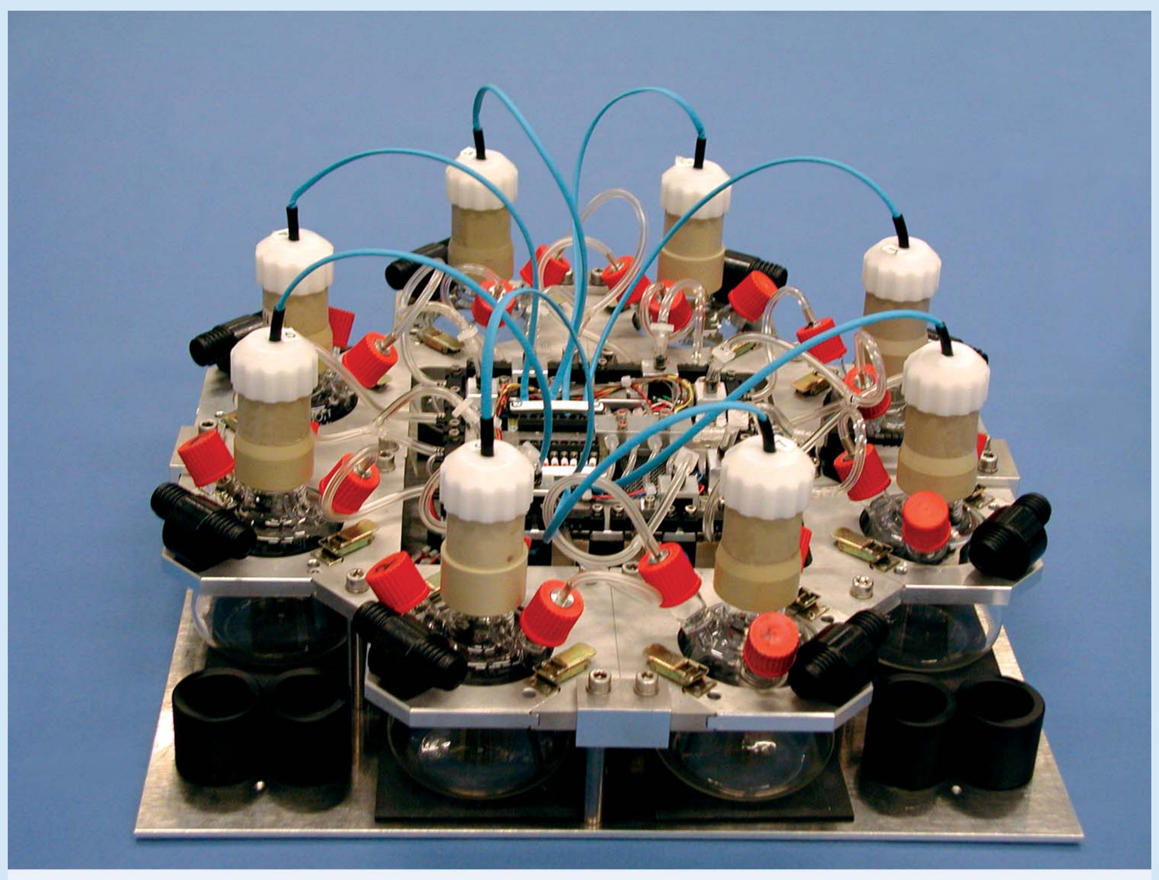
Fig. 3: The Ramos-Device

Fields of application

- Detection and prevention of oxygen limitation
- Optimization of operating conditions and duration for conventional screening
- Optimization of media
- Access of important information for scale-up of processes in early stages of process development
- ...

Example 1: Optimization of media

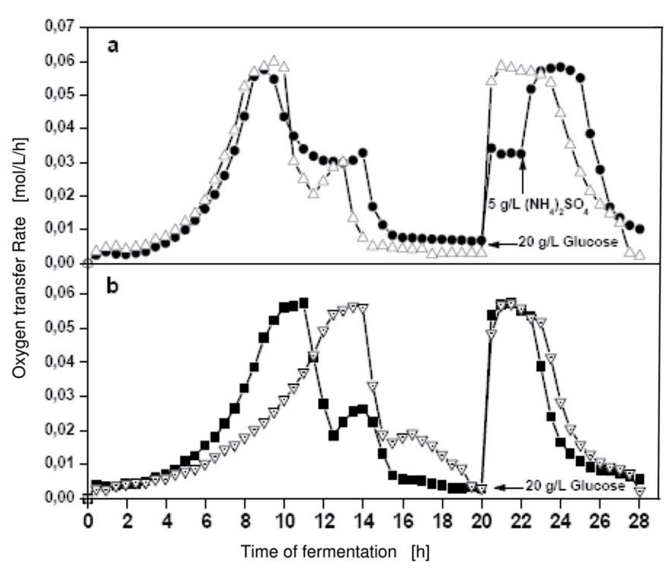

Fig. 4: Optimzation of media for *E. coli* BL21 pLys pRSET eYFP-IL-6 Media: Wilms-MOPS-Meda with 20 g/L glucose. Initial ammoniumsulfate conzentration: **a:** 2,68 g/L (●) and 5 g/L (Δ); **b:** 8,5 g/L (■) and 12 g/L (▽). Experimantal conditions: T = 37°C; n = 420 rpm; d_0 = 50 mm; V_L = 12,5 mL; pHα = 7,5; OD600,α = 0,5.

Fig. 4 illustrates an experiment, testing a medium with respect to a nitrogen limitation. After the complete glucose has been exhausted, 20 g/L glucose were added again. This results in an increase of the OTR in the standard media (2.68 g/L $(NH_4)_2SO_4$) up to the level of limitation of around 0.03 mol/L/h. Only after addition of 5 g/L ammoniumsulfate (upper arrow) the OTR increases to a maximum again. Hence a nitrogen limitation of the media was proved. By means of the strong OTR peak after the addition, the enhancement of the media became considerable.

Example 2: Characterization of reactors

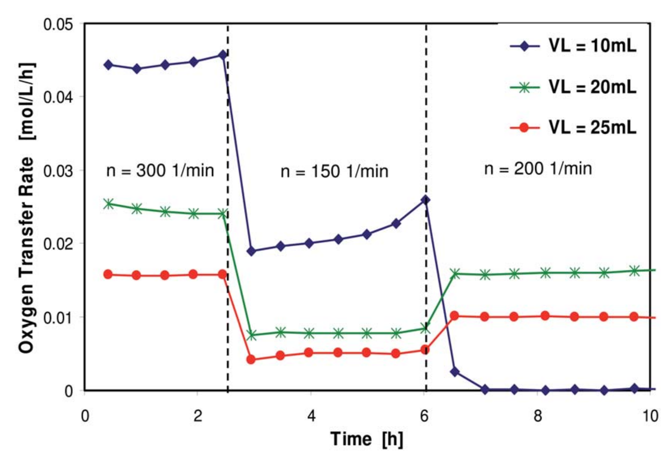

Fig. 5: Measurement of OTR for 0,5M sulfitesystem for characterisation of the maxium Oxygen transfer capacity of 250mL Erlenmeyer flasks with variation of operating conditions. d_0 = 50mm

Fig. 5 illustrates exemplary how to use the Ramos device for characterization of the maximum oxygen transfer capacity of shaking flask. With the help of a chemical model system (with clearly defined physical and chemical properties) as O_2-consumer the OTR is measured during the variation of operating conditions. In the presented case the filling volume V_L and the rotational speed of shaking are varied. By correlation of the obtained OTR values and the operating conditions a prediction of the OTR for other operating conditions is possible. Also the transferability of the results obtained on this way to biological experiments is possible without much effort.

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