Biochemical Engineering
RWTH Aachen University
Prof. Dr.-Ing. Jochen Büchs
Worringerweg 1
D-52056 Aachen, Germany

BIOVERFAHRENSTECHNIK AACHEN

The Oxygen Transfer Rate (OTR) as the Key Parameter for the Characterisation of *Hansenula polymorpha* Screening Cultures

Christoph Stöckmann*, Ulrike Maier*, Tibor Anderlei*, Gerd Gellissen$, Jochen Büchs*
*Biochemical Engineering, Aachen University of Technology (Germany), $Rhein Biotech GmbH, Düsseldorf (Germany)
Contact: Dipl.-Biol. Christoph Stöckmann, Phone: +49 (0)241 807179, E-mail: stoeckmann@biovt.rwth-aachen.de

Introduction

The company Rhein Biotech develops production strains for pharmaceutical proteins based on the yeast *Hansenula polymorpha*. In batch test tube and shaking flask cultures production strains have to be selected out of a large pool of transformands. Due to an accurate strain selection, defined culture conditions are absolutely vital.

Method

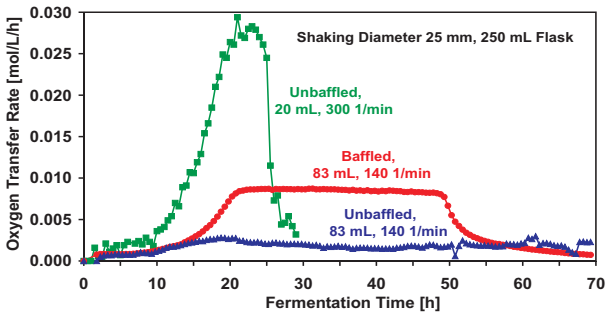
The oxygen transfer rate (OTR) is the most convenient, detectable parameter for the characterisation of the physiological activity of the aerobic *Hansenula polymorpha* screening cultures. Therefore the devices for the online measurement of the oxygen transfer rate in shaken cultures[1] were applied as the central measuring technique.

Results

Shaking Conditions

Low shaking frequencies and large filling volumes lead to limited culture respirations due to low oxygen transfer capacities (fig. 1, red, blue). Increasing the shaking frequency and lowering the filling volume, the oxygen transfer capacity is raised above the maximum respiration rate of the cultures, as shown in fig. 1, green. Thus, unlimited and reproducible oxygen supplies are guaranteed.

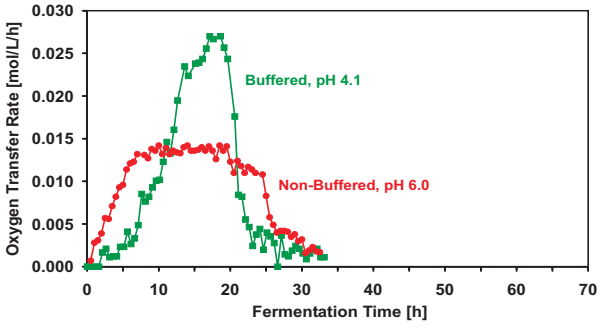
Fig. 1



pH and Buffering

One of the screening media caused limited OTR courses, although the oxygen supply was found to be sufficient (fig. 2, red). This could be traced back to the omitted pH stabilisation of this medium. Adjusting the pH and buffering of the medium, unlimited OTR courses as in fig. 1, green, were achieved (fig. 2, green).

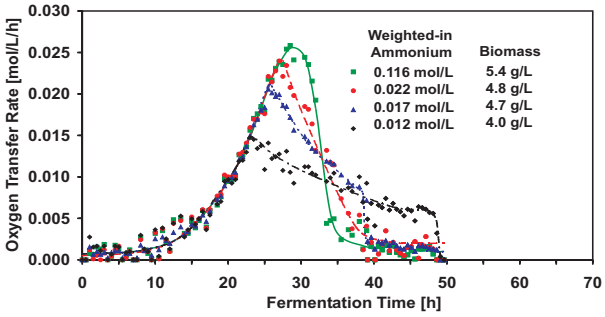
Fig. 2



Ammonium Supply

Insufficient ammonium concentrations in the screening cultures lead to a sudden decline of the culture respiration at the time of ammonium exhaustion and to reduced biomass formation (fig. 3, red, blue, black). By means of fixed stochiometric relations between the oxygen, ammonium and glycerol consumption the ammonium requirement was balanced with 2.6 mmol ammonium per gram glycerol. Thereby ammonium-unlimited culture courses can be achieved (fig. 3, green).

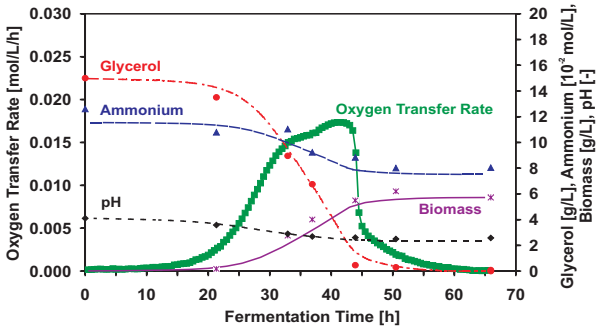
Fig. 3



Modelling of Culture Parameters

The use of mineral salt media with glycerol as carbon source and the stable stochiometric metabolism of *Hansenula polymorpha* on glycerol offer an ideal basis for balancing culture parameters. Therefore the courses of glycerol, ammonium, biomass concentration and pH of a recombinant strain could be derived from the OTR (fig. 4, lines). The calculations were validated by taking independent fermentation samples (fig. 4, symbols). Consequently the OTR reflected the courses of several important fermentation parameters.

Fig. 4



Conclusion

Limiting culture conditions were detected and characterised by only few measurements of the OTR. Important culture parameters could be derived from the OTR. The OTR was proved to be the key parameter for the behaviour of *Hansenula polymorpha* screening cultures.

ANDERLEI, T., BÜCHS, J., 2001, *Device for sterile online measurement of the oxygen transfer rate in shaking flasks*, Proceedings of the International Symposium on Applied Shaking Technology, Basel, 22.-23.4.1999, Biochemical Engineering Journal, 7(2): 157-162, RAMOS Respiration Activity Monitoring System: HiTec Zang GmbH, Ebertstrasse 30, 52134 Herzogenrath