

Investigation and control of fermentation processes using fermenter-calorimetry

M. Meier-Schneiders, U. Grosshans, C. Busch, and G. Eigenberger
Institut fuer Chemische Verfahrenstechnik der Universitaet Stuttgart,
Boebling Str. 72, D-7000 Stuttgart 1.

Introduction

It is well known, that heat generation and total metabolic activity in biological systems are strongly correlated. Thus the heat production can provide important information for the study, supervision and control of biological fermentations. Microcalorimeters are widely used for studies concerning physiology, ecology, and pharmacology /1, 2/. They are very sensitive, but the measuring principle exhibits methodical disadvantages for its application in biotechnology (direct control of the fermentation inside of the measuring cell and sampling is impossible) /4/. Reaction calorimeters used in chemistry are too insensitive for the detection of small changes in heat generation. Therefore, we developed a new isothermal heat-flux calorimeter-fermenter with a working volume of 2 l /3/. The detection level for heat generating processes is 10 mW/l under standard fermenting conditions including agitation, aeration and pH-control. In situ sterilization of the apparatus can be handled easily. The calorimeter is thought to be a versatile tool for laboratory optimization of a fermentation. The aim of the presented work is to discuss what results have been obtained so far and how the heat signal can be used advantageously in biotechnology.

Measuring principle

The temperature inside of the fermenter is detected with a high degree of accuracy by a thermistor (5×10^{-4} K) and controlled to a chosen constant value by the compensation heater (Fig. 1). Between fermenter and cooling jacket a constant temperature difference is adjusted. This temperature difference, which is measured integrally by a thermopile in the airspace, leads to a constant heat-flux from the fermenter to the jacket. To prevent undefined heat losses from the top of the vessel, the temperature of the top is controlled accurately to the reactor temperature. The measuring probe of this controlling circuit is also a thermopile. Because of the constant reactor temperature, each heat generation in the fermenter leads to a reduced power input of the compensation heater. The change of heating power therefore represents the measuring signal. The feeds, also the gas feed, are adjusted to reactor temperature before they enter the vessel. Additional substrate feed control and level control allow an operation mode for continuous cultivation.

Sensitivity

A long-term stability in the base-line signal of 10 mW/l can be achieved. A heating power of 10 mW/l generated for 5 min can be clearly distinguished from the background noise (Fig. 2). Using the signal filter for the detection level of 10 mW/l the response time of the calorimeter on a step input of 1 W/l is 8 min. Accepting a detection level of 20 mW/l the response time can be shortened to 30 s (data not shown).

Results and Discussion

Aerobic and anaerobic cultivations of different pure cultures in batch-, fed-batch and continuous mode showed, that the profile of the thermograms correlated well with the metabolic activities within the suspension as measured by dry weight, optical density, educt and product concentrations (Figs. 3 - 7, correlation shown for *Zymomonas mobilis* in Fig. 4). Thus, the heat production can be used as a sensitive, fast and reproducible signal for the present state of a culture. If correlated with parameters as mentioned above, specific information of the fermentation may be drawn from the time-course of the thermogram. If the fermentation is run under standardized conditions, estimations of educts as well as of products will be possible based upon the heat signal, which is detected with a higher accuracy than any analytical measurement. Thus, time-consuming and approximate analytical measurements of metabolites may be replaced or complemented by fermenter-calorimetry to enhance the optimization of fermentations in laboratory scale.

Under the constant conditions of continuous cultivation, heat production is affected immediately by nutrient (glucose) addition when growth is $< \mu_{max}$ (visualized for *Z. mobilis* in Fig. 6. a+b). Inhibitory substances may be tested or detected in the same way by watching the heat production (Fig. 5). Therefore, fermenter-calorimetry exhibits a great potential for the optimization of growth and production processes under continuous cultivation.

During the aerobic growth of *Saccharomyces cerevisiae* on glucose (concentration > 100 mg/l) in batch culture the consumption of glucose is accompanied by the release and metabolization of ethanol. The transition between respiro-fermentative (Crabtree-effect) and respirative metabolism is clearly related to the heat production (see Fig. 7). This example shows, that fermenter-calorimetry may be used as tool for the study of metabolic regulation. It is expected, that especially the activity of genetically engineered microorganisms can be monitored easily by fermenter-calorimetry.

Concluding, the heat-signal may be used for the monitoring of both aerobic and anaerobic processes on a laboratory scale. If the experiments are carried out under well defined conditions, the heat signal may be used instead of analytical measurements for the estimation of educts and products during a fermentation. Once established, it should be possible, especially for aerobic cultivations, to use the results of the heat measurements from the laboratory scale to develop control strategies for aerobic large scale fermenters; contrary to laboratory scale fermenters the heat evolved from large scale fermenters is so big that it can be easily detected by standard process measurements /5/.

Literature

- /1/ Calorimetry and thermodynamics of biological processes. Proc. 8th ISBC Conference, Göteborg - Gullmarsstrand, Sweden, May 15 - 19, 1992. L. Gustafsson (ed.).
- /2/ Larsson, C., Blomberg, A., Gustafsson, L. Biotech. Bioeng. (1991), 38, 447 - 458.
- /3/ Ott, J., Meier, M., Eigenberger, G. (1991), 352 - 356. In: Biochemical Engineering - Stuttgart. M. Reuss, H. Chmiel, E.D. Gilles, H.-J. Knackmuss (eds.). G. Fischer press, Stuttgart.
- /4/ Stockar, U., von, Marison, I.W. Adv. Biochem. Eng. Biotechnol. (1989), 40, 93 - 136.
- /5/ Stockar, U., von, Marison, I.W. Thermochim. Acta (1991), 193, 215 - 242.

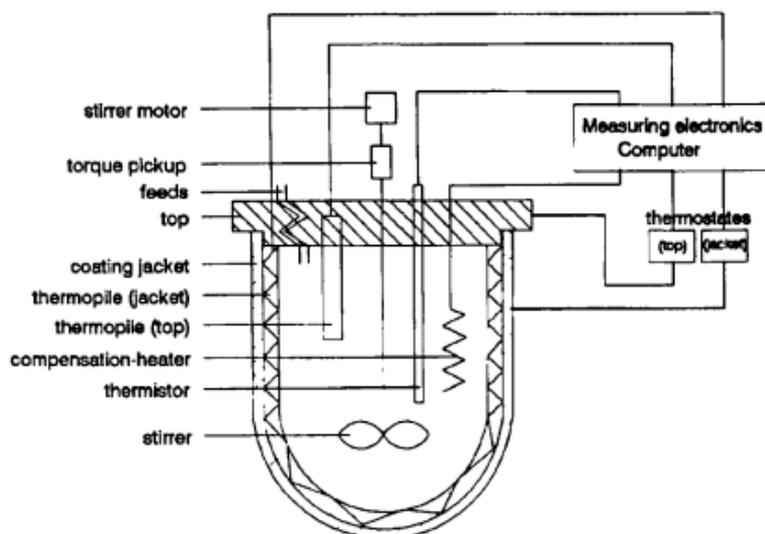


Fig. 1 Schematic diagram of the fermenter-calorimeter (working volume 2 l)

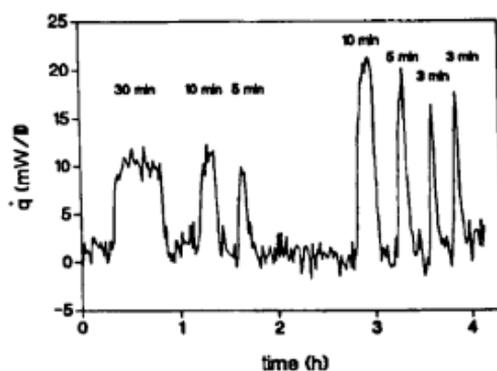


Fig. 2 Detection level and resolution (filter $f_{im} = 0.0015$ Hz, temperature 30°C , agitation 500 rpm)

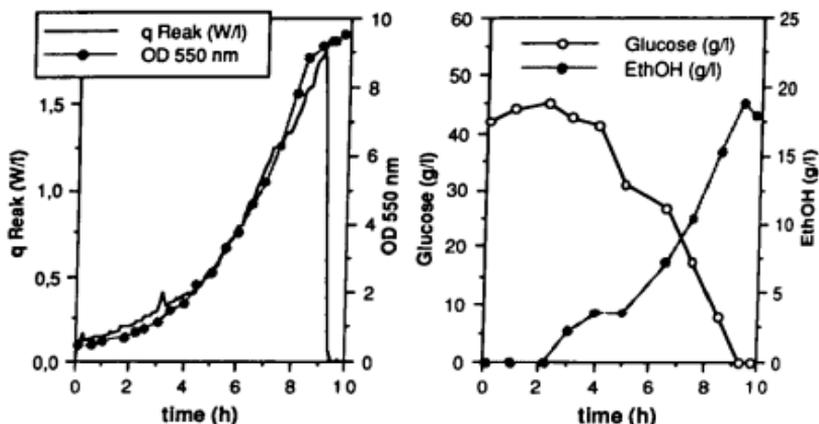


Fig. 3 Anaerobic batch fermentation of *Zymomonas mobilis* in complex medium (pH 5.5, 30 °C, agitation 500 rpm)

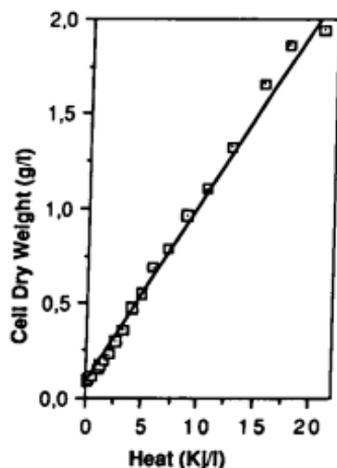


Fig. 4 Correlation of heat production and biomass production for the experiment in Fig. 3

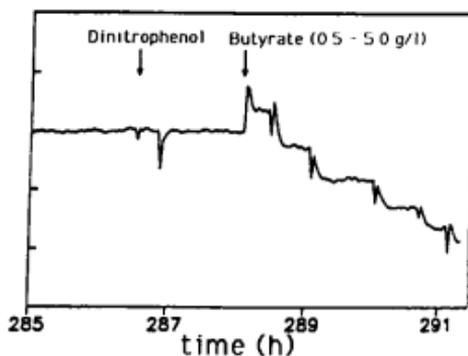


Fig. 5 Influence of toxic substances on the heat production of *Z. mobilis* in continuous culture (complex medium, pH 5.5, 30 °C, agitation 500 rpm, $D = 0.25 \text{ h}^{-1}$)

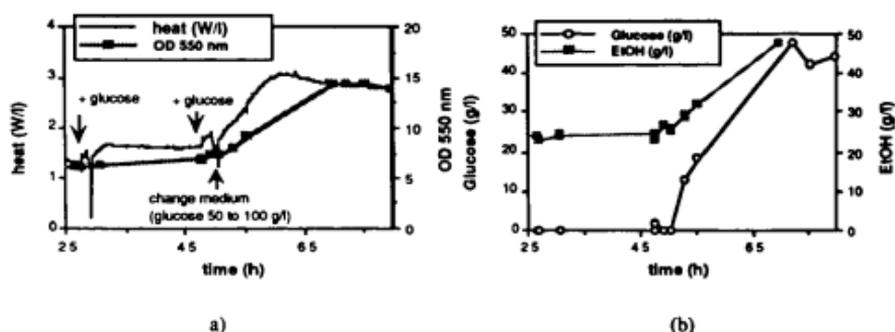


Fig.6 Influence of nutrient variation on the heat production, biomass concentration (a) and the glucose and ethanol concentration (b) of *Z. mobilis* in continuous culture (complex medium, pH 5.5, 30 °C, agitation 550 rpm, $D = 0.25 \text{ h}^{-1}$)

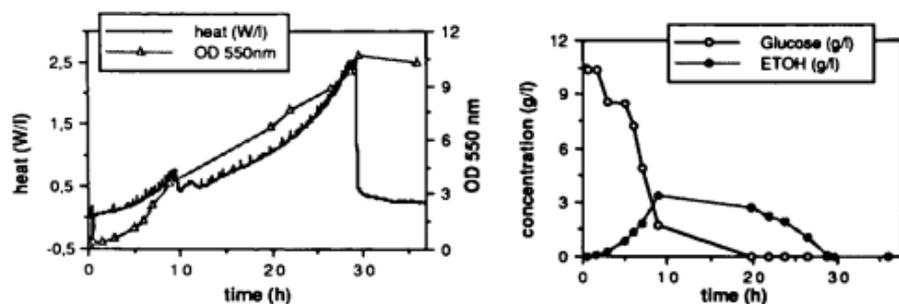


Fig. 7 Aerobic batch culture of *Saccharomyces cerevisiae* CBS 426 in defined medium showing the Crabtree-effect (pH 4.5, 30°C, agitation 500 rpm, $0.21 \text{ l O}_2 / \text{l x min}$)