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ANAEROBIC CALORIMETRY OF THE GROWTH OF *LACTOBACILLUS HELVETICUS* USING A HIGHLY SENSITIVE BIO-RC1

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Abstract

To meet the need for studies of anaerobic microbial and animal cell cultures involving much lower heat effects as compared to aerobic microbial cultures, a bench scale calorimeter, Bio-RC1, has been improved for achieving a higher long-term sensitivity. This newly improved Bio-RC1 was used for heat measurement of anaerobic growth of *Lactobacillus helveticus*. The results showed that the bench-scale calorimetry has powerful potential for on-line monitoring and control of anaerobic bioprocesses as well as fundamental studies, such as stoichiometry, thermodynamics and kinetics of cellular growth.

Keywords: anaerobic culture, bench scale calorimetry, bio-calorimeter, monitoring

Introduction

Cellular growth processes are thermodynamically irreversible, in which cells invariably and continuously dissipate Gibbs energy, as a result, always exchange heat with the environment. The heat generation can be monitored in a calorimeter. Thus the calorimetric measurement is clearly a key factor in understanding thermodynamics of life processes. Moreover, it is also of great practical importance to have sufficient quantitative information on microbial heat exchanges when designing cooling facilities for biotechnological processes at large scale. The on-line measured heat signals could also be used together with other on-line data for bioprocess monitoring, optimization and control.

In order to yield such quantitatively significant results, as opposed to qualitative 'calorimetric curves', heat measurements must be carried out in a bench/large scale or reaction calorimeter, which allows for on-line measurements and a tight control of all culture parameters such as pH, pO_2, nutrient concentrations, etc. However, the bench scale/reaction calorimeters suffer traditionally from significant lower sensitivity, as compared to microcalorimeters, therefore, in the past two decades, great attempts have been made to development and/or improvement of calorimetric systems at bench scale [1–3]. Amongst others, the Bio-RC1, which was modified from RC1 reaction calorimeter (Mettler-Toledo AG, Switzerland) for biological process operation in our laboratory, has been widely used for the studies of aerobic and some anaerobic bioprocesses [4–6], and demonstrated to be a powerful tool for monitoring

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and control of bioprocesses. The Bio-RC1 as well as some newly developed bench-scale calorimetric systems have achieved considerably high sensitivity, ca. 20~50 mW L^{-1} under optimal conditions. But most anaerobic microbial and animal cell cultures are poorly exothermic or even endothermic processes, therefore, require the development of even higher resolution calorimetric systems.

Lactobacillus helveticus is a key strain in food industry. In the homofermentative degradation of sugar by *L. helveticus*, no on-line signal is normally available for process monitoring as this growth process does not release CO_2. Monitoring heat production is thus an obvious solution for on-line assessment of the metabolic activities of cellular cultures. In this work, anaerobic growth of *L. helveticus* in a semi-defined medium was studied using an improved Bio-RC1.

Experimental

The improved Bio-RC1 calorimeter

The work principle of the RC1 and its modification for biological purposes have previously presented in details [4]. After extensively studying the factors which determine the Bio-RC1's detection limit (longer term sensitivity), the resolution of the A/D convert for the signal of jacket temperature (T_j) was increased to the same order of that for reactor temperature (T_r), i.e. 0.2 mK; the heating power range of the 'hot' oil circuit was largely reduced to 100~500 W; a more precise PI controller for T_r was implemented in place of the previous P controller. The details of technical measures have been described in a recent article [7]. In order to minimize the external influence, mainly from the fluctuation of the ambient temperature, a thermostat housing was built around the reactor, which has been proved to be very helpful for achievement of a high long-term sensitivity, up to 5 mW L^{-1}.

Microorganism and medium

The strain *L. helveticus G* used in this study was supplied by Forschungsanstalt für Milchwirtschaft (FAM) (Bern, Switzerland). It was grown on a semi-defined medium of the following composition (in g L^{-1}): glucose 20; yeast extract 30; $MgSO_4$ 0.6; $FeSO_4$ 0.03; $MnSO_4$ 0.03; sodium acetate 1.0; KH_2PO_4 0.5; K_2HPO_4 0.5.

Culture condition

Fermentation experiments were conducted in this newly improved Bio-RC1 calorimeter at 42°C and an agitation speed of 400 rpm. The pH was maintained at 5.9 with automatical addition of NaOH (ca 6 N). The homofermentation of *L. helveticus* is not strictly anaerobic, and there is not gaseous product to be measured, therefore, the cultures in this reported work were just flushed with N_2 before inoculation and nitrogen blankets were maintained over the cultures in the reactor. Sparging continuously with N_2 was unnecessary for experiments in this work. Under the operating condition in this work, the detection limit of the Bio-RC1 was about 10 mW L^{-1}, for at least 48 h.

On-line and off-line data

A data acquisition system (LABVIEW software with Macintosh) was used to store all the on-line measured signals (heat, addition of NaOH, pH, etc.). Samples were regularly taken from the broth cultures for off-line analysis. Growth was determined by measuring the absorbance of the culture spectrophotometrically (U-3210 spectophotometer) at 620 nm and relating to a previously calibrated curve of cell dry mass *vs.* absorbance. Concentrations of glucose and lactic acid were determined using standard enzymatic assays (Bochringer Mannheim, Germany). The composition ($CH_{1.62}O_{0.38}N_{0.23}$) and heat of combustion (536.1 kJ C-mol^{-1}) of the cells of *L. helveticus* were determined previously [8].

Results and discussion

On-line calorimetric measurement and correlations of heat generation to other process variables

Growth curves from on-line and off-line measurements are given in Fig. 1. We can see that cells grew exponentially until the depletion of carbon/energy source, glucose, which was signified by the on-line measured heat production rate. Figure 2 depicts a correlation between the generated heat and the formed biomass, from which the heat yields $Y_{Q/X}$ was calculated to be 324.8 kJ C-mol^{-1}. Similarly, we could also determine the product yield $Y_{P/S}$ and the biomass yield $Y_{X/S}$ to be 0.856 and 0.110, respectively, which result in a carbon recovery of 0.97. However, the measured heat yield was notably higher than the calculated value (252.2 kJ C-mol^{-1}) based on heat balance of the growth reaction. This discrepancy may be attributed to the side-reactions, mainly the neutralization, during the anaerobic growth. In homofermentation of *L. helveticus,* the product was lactic acid which would result in a

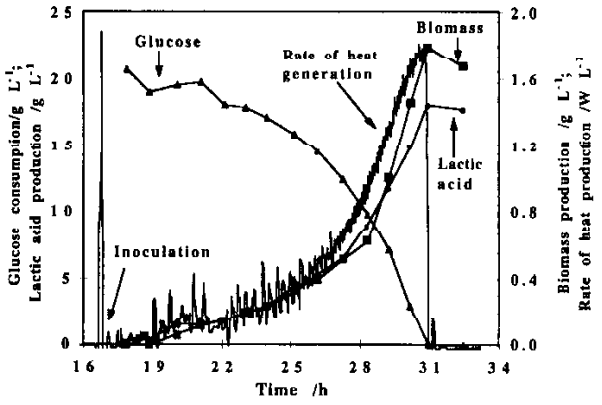

Fig. 1 Anaerobic growth of *Lactobacillus helveticus* in a glucose-limited semi-defined medium. — – rate of heat production; ▲ – glucose concentration; ■– biomass dry mass; • – lactic acid concentration

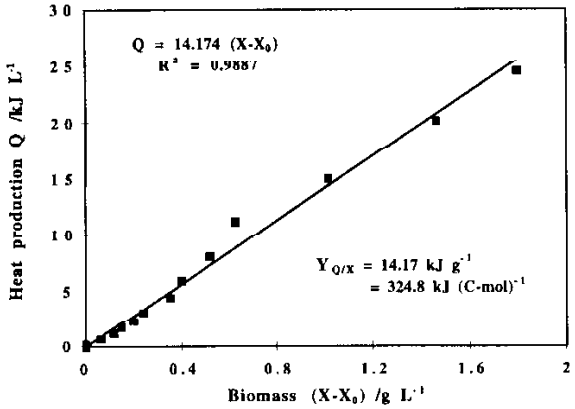

Fig. 2 Determination of heat yield $Y_{Q/X}$ for anaerobic growth of *Lactobacillus helveticus* in a glucose-limited semi-defined medium

considerable decrease of pH in the culture, thus a much larger amount of base, as opposed to normal aerobic growth, had to be added automatically to the culture in order to maintain the pH constant. Although this side-reaction is kinetically in conjunction with the growth reaction, it has to be taken into account in heat balancing, particularly in designing the cooling facilities in industry. The importance of the influence of side-reactions on enthalpy balance of anaerobic growth has been addressed in [9].

Determination of kinetic parameter by means of calorimetric measurements

The exponential growth kinetics is described by $dX/dt=\mu X$, where X and μ are the concentration of biomass and the specific growth rate, respectively. μ is generally

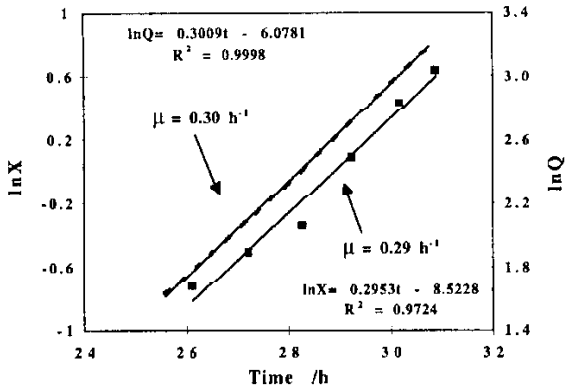

Fig. 3 Determination of specific growth rate of anaerobic growth of *Lactobacillus helveticus* in a glucose-limited semi-defined medium by off-line biomass measurement (■) and by on-line heat measurement (thick broken line)

determined by the measuement of biomass, i.e. plot of $\ln X$ *vs.* time t. If the stoichiometry of growth reaction is constant, then the rate and the amount of biomass formation, (dX/dt) and $(X-X_0)$, are proportional to the rate and the amount of heat production, (dQ/dt) and Q, respectively. Provided that the initial concentration of biomass X_0 after inoculation is very low, i.e. $X \approx X-X_0$, then μ can be determined by means of calorimetric measurement, i.e. plot of $\ln Q$ *vs.* time t. This is particularly true when the data point are taken from the later part of the exponential growth phase. As shown in Fig. 3, data from the anaerobic culture of *L. helveticus* in the Bio-RC1 were used for determination of the specific growth rate, by plot of $\ln X$ and $\ln Q$ against time t, which result in μ of 0.29 and 0.30 h^{-1}, respectively, and they are in agreement with the reported value under the same growth condition, 0.31 h^{-1} [10]. Therefore calorimetry provides an alternative to determine the specific growth rate. Since much more on-line data points are available from calorimetric measurement, this proposed alternative is believed to be of advantages of precise and ease, as compared to the off-line biomass measurement.

Conclusions

Calorimetric studies of anaerobic growth of *L. helveticus* showed that the Bio-RC1 is a powerful tool for studies of stoichiometry, thermodynamics and kinetics of cellular growth. Moreover, heat signal has been demonstrated as a very useful on-line variable for assessment of the metabolic activities of anaerobic cultures, and thus can be used for on-line medium optimization, monitoring and control of anaerobic cultures. Further application of the improved Bio-RC1 in these subjects will be reported elsewhere.

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