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A NOVEL BENCH-SCALE CALORIMETER FOR BIOLOGICAL PROCESS DEVELOPMENT WORK

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ABSTRACT

The application of microcalorimetry for measuring the heat generated during the growth of microorganisms in submerged culture is limited due to, among other things, difficulties in aerating and mixing of its contents. Therefore, a novel bench-scale calorimeter has been modified for biological work. The modified calorimeter is shown to be sensitive enough to permit accurate and easy monitoring of the heat dissipated by a weakly exothermic culture of E. coli under stirred and aerobic conditions.

INTRODUCTION

Given the fact that all biological reactions resulting from the metabolic activity of living systems are exothermic, calorimetry should prove just as useful a tool for monitoring and analyzing fermentation processes as thermal analysis and calorimetry are for chemical reactions. A calorimeter suitable for microbial reactions would lend itself to easy determination of important process parameters such as cooling requirements. Also, it should be ideal for monitoring fermentations on the bench-scale and should thus greatly enhance process development work. Moreover, possible existing correlations between the rate of heat generation and other variables such as the growth rate and the rate of oxygen uptake could be elucidated and studied quantitatively which would in turn be very useful for process control purposes on production scale.

EXISTING CALORIMETRIC EQUIPMENT

The low level of heat generation observed in biological reactions, which only rarely exceeds 20 W/l of culture fluid, but more often is on the order of a few W/l or less, constitutes the main reason why the bulk of the research so far has been carried out using microcalorimeters (refs. 1,2,3,4) affording excellent sensitivity in the range 1-10 μW, corresponding to about 0.01W/l of

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culture. However, due to difficulties in (1) aerating and mixing the contents of such calorimeters (2), controlling the pH and other environmental factors and (3) sampling the culture for analyses of biomass and medium components, microcalorimetry has generally been confined to studies of anaerobic fermentations under conditions which are not typical of technical processes. Several experimental systems have been proposed to obviate these problems.(i) Flow calorimetry enables the study of aerobic growth by continuously pumping fermentation broth from a standard laboratory fermenter through an external flow-through microcalorimeter (refs. 5,6). However, due to the time lag in the flow-lines between fermenter and measuring cell, nutrients and oxygen are often depleted, resulting in errors in the recorded thermal data. (ii) "Dynamic Calorimetry" involves an energy balance around a completely insulated standard fermenter vessel. The rate of temperature increase is measured over a period of 5-15 min after the fermenter cooling system is switched off (refs. 7,8). In "Continuous Calorimetry" (iii), the well insulated fermenter is continuously overcooled, the heat loss being compensated for by an immersion heater (refs. 9,10). By measuring the power dissipation by the heater which is necessary to maintain the set temperature, the exothermy can be deduced from a heat balance. Since both methods require a complete heat balance around the fermenter, they are overly affected by heat losses through the insulation, which are impossible to suppress. The result is a poor sensitivity (about 0.5 W/l) even when working with cumbersomely large fermenters (14 l), as suggested by the authors.

The technique proposed in this paper permits microbial cultures to be grown under standard laboratory fermenter conditions of temperature, aeration, agitation, pH, and allows a continuous in-situ heat measurement. The calorimetric principle does not rely on a heat balance and consequently affords a better sensitivity than methods (ii) and (iii), while making insulation of the apparatus unnecessary.

DESCRIPTION OF THE MODIFIED BSC-81 SYSTEM

A novel heat-flux calorimeter (model BSC-81) - developed by Ciba-Geigy AG, Basel, Switzerland (ref. 11) for studies of the thermodynamics of chemical reaction systems - has been modified for microbiological work (Figure 1). The reaction temperature T_R in the 2 litre glass culture vessel is controlled by pumping thermostating oil through the glass jacket. A sophisticated thermostat controls the temperature T_J of the oil in the jacket. By using a computer (LSI 11) and an accurate measurement of T_R by a Pt 100, it is possible to continuously adjust the temperature T_J in the jacket in such a way that the temperature of the culture T_R stays at its set point even if heat is generated or absorbed by the reaction mixture. Whenever heat is gene-

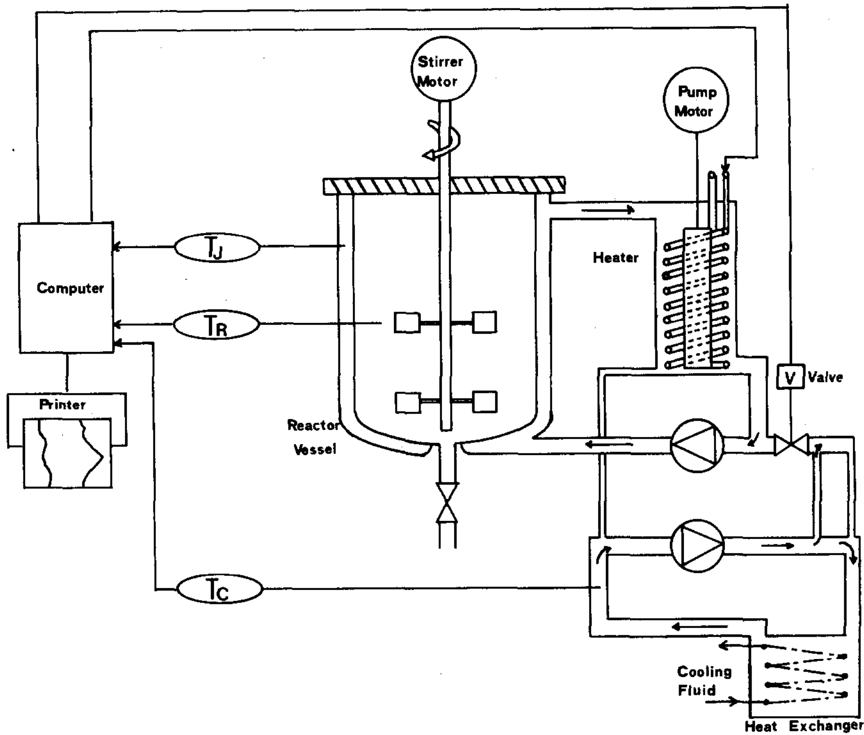

Fig. 1: Schematic representation of the heat-flux calorimeter (BSC 81)

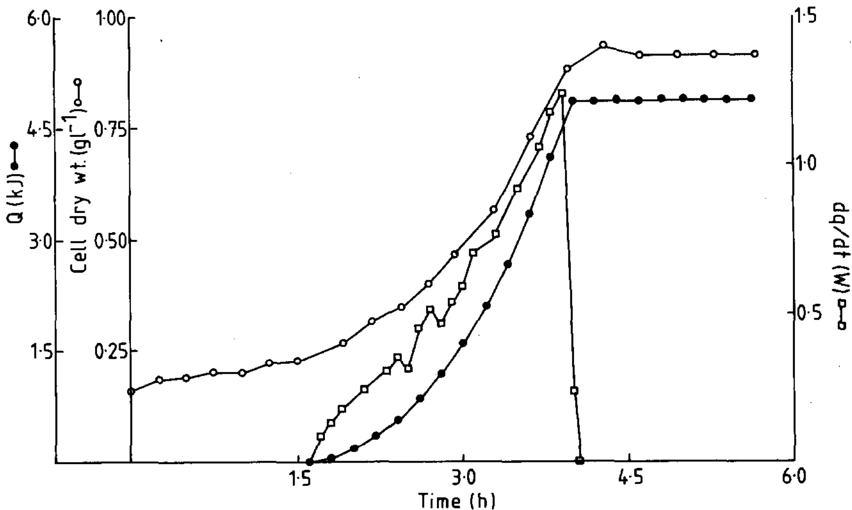

Fig. 2: Thermogram showing the relationship between heat evolution rate (dQ/dt), total heat evolved and cell dry weight for E. coli grown aerobically. From this plot, a constant ratio of the heat evolved to biomass formed of 7.41 kJ/g could be derived

rated in the vessel, T_J is decreased in order to cool the reactor accordingly. The temperature difference (T_R - T_J) therefore provides a measure of the heat evolution rate, which may be calculated according to eq. 1.:

$$q = U \cdot A \cdot (T_R - T_J) \quad (1)$$

where q is the heat generated (W); A is the heat transfer area of the vessel (m^2); U is the heat transfer coefficient (Wm^{-2} K^{-1}). An in-built electrical calibration heater (15 W) allows calibration of the heat signal and determination of the heat transfer coefficient (U) at any stage during the experiment. The culture is agitated by two 6-blade Rushton-turbines and aerated by sterile, pre-saturated and thermostated air.

RESULTS AND CONCLUSIONS

Escherichia coli strain W was grown in batch culture on a defined medium comprising (gl^{-1}): K_2HPO_4, 7.0; KH_2PO_4, 3.0; tri-sodium citrate•$3H_2O$, 0.5;Mg $SO_4 \cdot 7H_2O$, 0.1; $(NH_4)_2$ SO_4, 1.0; glucose, 1.39. The growth temperature (T_R) and pH were maintained at 37 °C and 7.0 respectively. The air flow rate was a constant 2 $l \cdot min^{-1}$. A typical thermogram is shown in Figure 2.

These results demonstrate that the sensitivity (about 0.1 W/l) attained by the modified BSC 81, enables accurate monitoring of the heat dissipation rate during aerobic growth of even a weakly exothermic culture. Heat-flux calorimetry could thus play an important role in monitoring and controlling the growth of microorganisms in submerged culture.

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