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USE OF A NOVEL HEAT-FLUX CALORIMETER FOR THE MEASUREMENT OF HEAT GENERATED DURING GROWTH OF K. FRAGILIS ON LACTOSE

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ABSTRACT

A novel bench-scale calorimeter, which is based on a heat-flux principle and has been modified to accommodate biological reactions, has been used to investigate the heat evolution rate of an aerobic culture of the yeast K. fragilis grown on lactose. The instantaneous heat evolution rate was an accurate indicator for the instantaneous overall metabolic activity. On the other hand, the integrated heat production increased in parallel to the accumulated cell dry weight. The ratios of heat generated per gram biomass produced and of heat generated per mole of consumed oxygen were found to be constant throughout the experiment despite changing growth rates and metabolism.

INTRODUCTION

All living organisms invariably dissipate heat as long as there is any metabolic activity taking place. This is obviously also the case in microorganisms, and the phenomenon of microbial heat generation has been extensively studied in microcalorimeters (ref.1-3).

A renewed interest in microbial heat evolution arose in the past ten years stemming from a point of view which is closer to Chemical Engineering and Biological Process Technology than to fundamental science (ref.4-10). This line of investigation has originally been stimulated by the engineering problem of predicting the cooling requirements of technical fermentation processes, since experience has shown that the removal of the heat generated by the microorganisms may well become the rate limiting factor in large scale processing equipment. Other aims pursued by these studies included the question of whether the rate of heat evolution can be used to monitor technical fermentation processes and the necessity to gain some insight into the enthalpy balances of such processes. The prediction of heat generation rates and the possibility of monitoring technical processes by continuous measurement of heat depend, of course, on the fundamental question of whether heat evolution rates can be

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correlated to other relevent variables, such as growth rate, oxygen uptake and CO_2 evolution rates, and of just how general such correlations are. There is an obvious need for more research, comprising a broad spectrum of substrates and organisms, in order to settle these questions.

As shown by Marison and von Stockar (ref.11), the conventional microcalorimeters are often unsuited for this kind of research. In consequence, they propose to use a novel bench-scale calorimeter based on a heat-flux principle for such studies. This calorimeter is a new version of the BSC 81 developped by CIBA-GEIGY for chemical work, which has been modified to accommodate biological reactions. The purpose of this paper is to show its application to the investigation of the correlations mentioned above in the case of one specific process example.

EXPERIMENTAL METHODS

Kluyveromyces fragilis NRRL 1109 was grown aerobically on a minimal medium with the composition (g l^{-1}):$(NH_4)_2\ SO_4$, 2.5; $(NH_4)_2\ HPO_4$, 2.5; $MgSO_4 \cdot 7H_2O$, 2.5; $CaCl_2 \cdot H_2O$, 2.5; yeast extract, 4.0; antifoam agent (polypropyleneglycol P2000), 0.4; lactose, 45.

The culture (1.32 liters) was grown at 30 °C and aerated with $2.50 \cdot 10^{-3}$ Nm^3 min^{-1} of presaturated air. The pH was automatically maintained at 5.5.

Off-gas analysis was accomplished using paramagnetic oxygen and infra-red carbon dioxide analyzers.

The growth experiments took place directly in the heat-flux calorimeter. The measuring principle and details of the structure of the calorimeter have been explained by Marison and von Stockar (ref.11).

RESULTS AND DISCUSSION

The results of the growth experiments on lactose are shown in figures 1 and 2. A closer analysis of the biomass concentration curve revealed that K. fragilis grew exponentially at a specific growth rate of 0.435 h^{-1} during the first 7-8 hours. After that, it continued to grow in a roughly exponential fashion for a few hours, but the growth rate had dropped to about 0.2 h^{-1}.

The heat evolution rate, which is shown for the entire culture of 1.32 liters in Watts in figure 2, obviously rose in parallel to the exponential development of biomass production rate, the maximum coinciding with the highest absolute volumetric biomass productivity as measured in g l^{-1} h^{-1}. After this peak, the heat evolution rate fell sharply since growth came to a halt due to the depletion of the main substrate (lactose).

On the other hand, the accumulated total heat production, shown as Q in kJ in figure 2, increases in parallel to the accumulated biomass (cell dry weight). This is an indication of correlations existing between heat and bio-

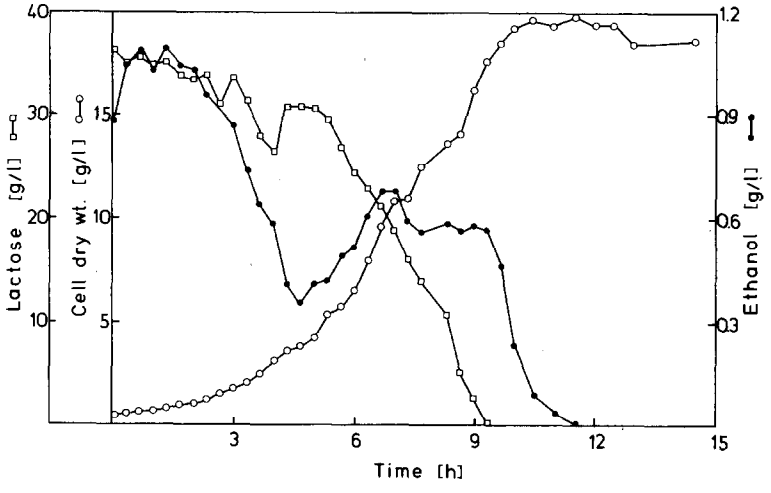

Fig. 1. Aerobic growth of K. fragilis on a minimal salts medium containing lactose as the main carbon substrate.

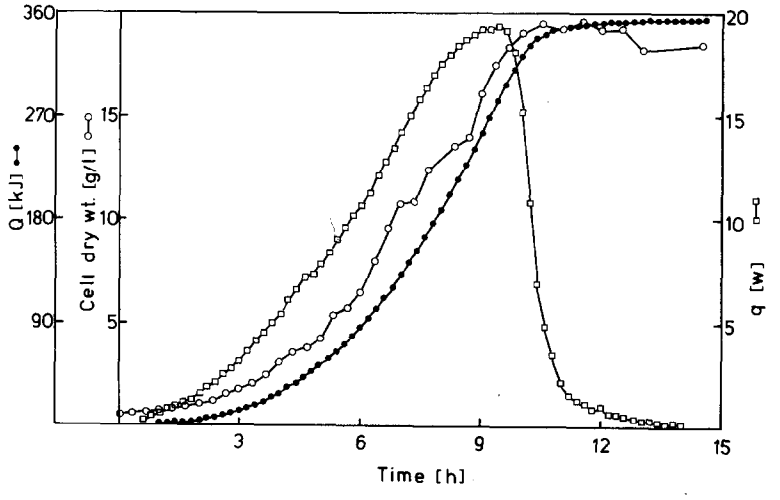

Fig. 2. Instantaneous rate of heat evolution q (W), total generated heat Q (kJ), and biomass produced (g/l) by 1.32 l of a culture of K. fragilis grown on lactose.

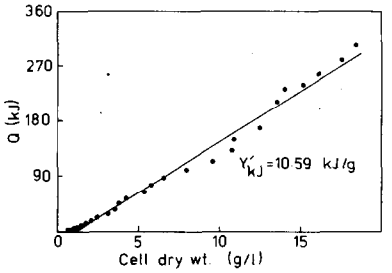

Fig. 3. Correlation between total heat production Q and cell dry weight.

mass production.

Figure 3 shows that the ratio between heat and biomass production of 10.59 kJ/g of cell dry weight remained constant despite the change of growth rate during the experiment. A similar correlation between heat production and oxygen consumption revealed a constant ratio of 574 kJ evolved per mole of oxygen consumed. This is considerably higher than the 444 kJ/mol that had been predicted on theoretical grounds (refs.5,12).

CONCLUSIONS

The correlations that could be established between heat evolution, growth, and oxygen up-take demonstrate the usefulness of calorimetry in monitoring technical fermentation processes eg as a means of measuring biomass concentration continuously. They also suggest easy estimation procedures to predict the cooling requirements of large scale equipment. It remains to be seen, in how far these correlations break down or are modified when other substrates and/or other organisms are employed.

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