Introduction

Calorimetry is an excellent tool for monitoring cell cultures in situ. Cellular growth is always accompanied by production of heat thus calorimetry is a universal technique. The amount of heat produced from the culture is related to the rate of substrate consumption and product formation. Using titration mode of the μRC the direct effect of inhibitor (or substrate) addition to the culture can be measured.

In the following application note bacterial growth in semi-skimmed milk, inoculated with yoghurt, was monitored in the μRC.

Experimental

The μRC was set to 35°C. 1.25ml of semi-skimmed milk inoculated with 25ul yoghurt was placed in the sample vial. An equal volume of water was placed in the reference position. Data collection mode was used to record the heat produced over a 19 hour period.

Further experiments were run with the same amounts of milk and yoghurt, but with the addition of 3% peroxide; with a bacteriostatic - Oxytetracycline and finally with water. The heat versus time response was recorded for a 16 hours after the injection.

Results - Milk & Yoghurt

Milk & Yoghurt with 50ul 3% peroxide

Milk & Yoghurt with 50ul Oxytetracycline

Milk & Yoghurt with 50ul water
Discussion and Conclusions

When nothing is added the Milk + Yoghurt sample it follows a typical Gaussian growth curve with a maximum heat output of 0.9mW. The sample was contained in a sealed HPLC vial, as the Oxygen is used up; the bacteria begin to die indicated by the decrease in heat signal.

Hydrogen peroxide is known to kill bacteria. Upon addition of peroxide, the heat output does not continue to rise, but remains constant for a period, then continues to increase.

Oxytetracycline is a bacteriostatic (i.e inhibiting or retarding bacterial growth) and is thought to exert its antimicrobial effect by the inhibition of protein synthesis. Adding 50µl 0.6mM Oxytetracycline instantly stops any further increase in heat production, indicating no further bacterial growth is taking place.

As a control experiment 50µl of water was added, the heat output continued to rise, showing bacterial growth was unaffected.

The ability of the µRC to detect the heat associated with bacterial growth demonstrates the suitability of the instrument to study microorganism metabolism.