
**Studies of fermentation and processes to optimize the effectiveness of starter cultures**

**Instruments to which this note applies**: Biocal 2000, Biocal 4000

**Target use**: Research and Quality Control related to fermentation of foods including dairy products, beer, wine and spirits and other fermentation processes.

## Introduction

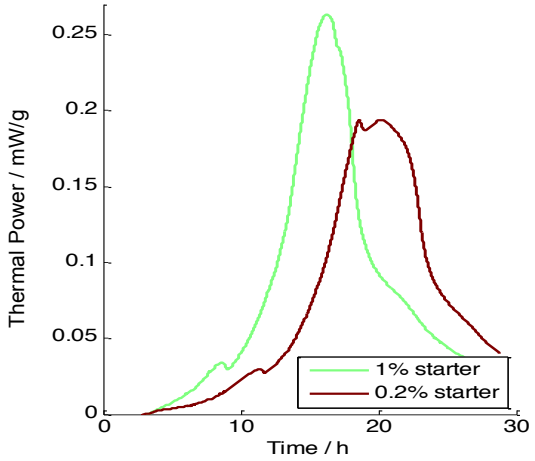
Fermentation has been used since prehistoric times to process solid food or liquids rich in carbohydrates either for preservation or for taste. Fermentation can produce a combination of organic acids, alcohols and gas in an exothermal reaction that is easy to measure continuously in an isothermal conduction calorimeter.
The effectiveness of fermentation is determined by factors such as strain selection, their concentration, temperature, etc. The effect of these factors on the fermentation reaction is very visible in an isothermal calorimetry curve and can be monitored without external intervention. Results can be retrieved on the click of a button. This makes isothermal calorimetry a convenient and cost effective tool to assess how fermentation develops in different food items, and it is easy to quickly compare fermentation rates as a function of temperature, starter concentrations etc. simply by comparing their isothermal calorimetry curves. Using a large sample cell calorimeter such as the Calmetrix Biocal, with 125 ml sample vials, increases the range of applications by making it possible to study food items such as pieces of vegetables, meat or cheese and other larger samples of solid and liquid foods. .
This Application Note shows the thermal activity in a sample of pasteurized milk treated with two different concentrations of starter cultures at 23 °C.

## Test Protocol

A small amount of starter culture (the fermented product) was added to milk, mixed and loaded into 125 ml sample vials that were placed in the calorimeter. The two samples only differed in the concentration of starter culture that was used (0.2% and 1% by mass of fermented product.

## Results and Interpretation

Both samples show the typical behavior during milk fermentation. First one exponential phase that ends with a sudden drop in activity, then the second main phase. The sample with lower starter concentration is slower.




The curves above look similar to published calorimetric milk fermentation results [1-3].

The sample with less culture lags about 3 h behind the other sample as the microorganisms with lower initial concentration take longer time to multiply to reach a certain number of bacteria (and a certain thermal power). As the ratio between the initial concentrations was 5 and it took 3 h for the lower concentration to reach the level of the higher one, the time constant of the exponential growth can be inferred easily from solving the equations for exponential growth:

$$k = \frac{\ln(5)}{3} \approx 0.54 \quad \mathrm{h^{-1}}$$

This corresponds to a doubling time of about 1.3 h, i.e., the bacteria divide every 1.3 h.

**Conclusion**

Isothermal calorimeters such as Calmetrix's Biocal models are an effective and easy-to-use tool for the study of fermentation processes, especially such processes for which it is difficult to follow the reaction with pH-sensors, like non-acidifying cultures or solid foodstuffs. One example of such applications is to study the effectiveness of starter cultures, as shown in this Note. After only a few hours, or even earlier at higher concentration, the activity of the starter culture can be measured effectively through a simple visual interpretation of the calorimetry graph. A closely related use may also be to check the content of active microorganisms in pro-biotic foods.

**References**

[1] Stulova, I., et al., Fermentation of reconstituted milk by *Streptococcus thermophilus*: Effect of irradiation on skim milk powder. Int. Dairy J., 31 (2013) 139-149.

[2] Wadsö, L. and F. Gómez Galindo, "Isothermal calorimetry for biological applications in food science and technology." Food Control 20(10) (2009) 956-961.

[3] Riva, M., et al., Growth and fermentation activity of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in milk: a calorimetric investigation. Annali di Microbiologia ed Enzimologia, 47 (1997) 199-211 (in English).