Microcalorimetric Monitoring of Anaerobic Waste Treatment Processes

A. Menert
Tallinn University of Technology
Department of Chemistry
Chair of Biotechnology
19086 Tallinn Estonia

INTRODUCTION

Microcalorimetric monitoring of anaerobic waste treatment processes uses the general advantages of calorimetry, i.e. low specificity, good reproducibility, non-destructive analysis, continuous registration of processes and the possibility to analyze turbid or coloured samples. Measurement of different growth parameters together with heat production rate has made clear that the shape of the power-time curve is influenced by the type of metabolic activity and can be related to the different physiological states of bacteria [1,2], The enthalpy change is the sum of the energetic contributions of all reactions that occur during metabolic processes [3].

As anaerobic digestion is dependent on the interactions of several microbial populations guaranteeing the ecological equilibrium is most important for achieving the stability of the process. The balance should exist between the formation of acidification products (acetate, H_2/CO_2) and the subsequent formal ion of methane. With soluble carbohydrate containing wastewaters (e.g. from food industry) the hydrolysis/ acidification phase proceeds much more rapidly than methanogenesis. As the following steps are sensitive to inhibition, in the case of overloading with carbohydrates, accumulation of H_2 and acidic end products occurs most often. Thus determination of VFAs is often used for establishing the danger of operational instability as acetic acid is a weak acid and the increase of VFAs is noticeable before extensive decrease of pH. Detection of H_2 concentration can be also used.

Calorimetry is a very promising method for studying the anaerobic processes, however, not sufficiently exploited for this purpose. Microcalorimetry can be used for monitoring anaerobic digestion processes of waste as well as for determination of growth characteristics of microorganisms isolated from there. At different stages of anaerobic digestion complex power-time curves can be obtained depending on the waste and reactor parameters (Figure 1). Microcalorimetry has been used for monitoring of anaerobic digestion processes of heavily polluted industrial wastewaters from food industry (cheese industry, distilleries, yeast plant) [4, 5] and of residual sludge. In these bioreactors growth of many bacterial species is proceeding simultaneously, also different substrates can be used (multiauxic growth). Therefore, for interpretation the resulting power-line curves additional analyses (HPLC, outplating, etc.) are inevitable (Figure 2).

EXPERIMENTAL

For monitoring the process, samples were taken anaerobically from the reactors

and placed immediately into the 2277 Thermal Activity Monitor. Batch experiments were run in standard mode with 3 mL glass ampoules at +35ºC. To initiate experiment, fresh substrate was added to sample taken from the working anaerobic reactor. For determination of metabolic products, four parallel experiments, run simultaneously on the microcalorimeter were stopped at different time moments by removing the ampoule from the calorimeter and adding 2-propanol [4, 5]. The content of ampoules can be analyzed off-line by HPLC or chemical analyses. Power-time curves were dynamically corrected and registered using the dedicated program Digitam 4.1.

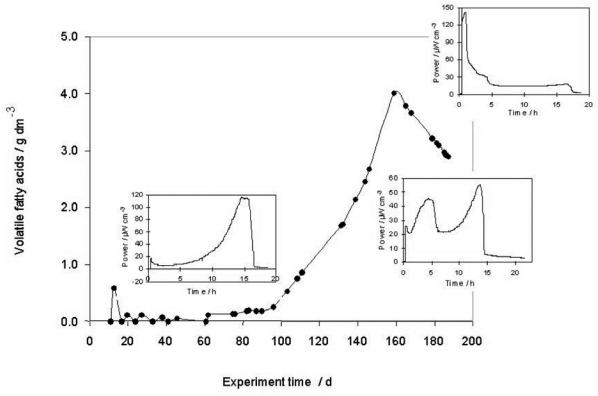

Figure 1. Different types of power time curves depending on the content of volatile fatty acids. a) VFAs 50-100 mg L^{-1}; b) VFAs 100-500 mg L^{-1}; c) VFAs up to 4000 mg L^{-1}.

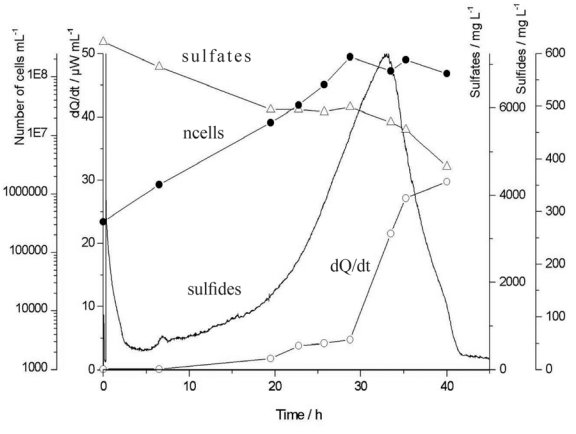

Figure 2. Cultivation of sulfate reducing bacteria (SRB) from yeast waste treatment plant in batch culture. Thermal power (dQ/dt), number of cells (ncells) and concentration of metabolites - sulfates (sulfates) and sulfides (sulfides)

RESULTS

From microcalorimetric data the thermodynamic (ΔH) as well as kinetic (μ=dX/(X·dt) parameters of a process can be calculated **(Figure 3)**. As in biological systems reactions take place in solutions at constant volume and pressure, ΔH corresponds to the experimentally measured heat production Q [3]. In 1988 Chang-Li *et al*. presented a thorough methodology for calculating specific growth rates μ [6] that was later confirmed by the studies of von Stockar group [7].

In exponential growth phase the relationship between the concentration of biomass X and specific growth rate μ is described by the first order kinetic equation dX/dt = μX. μ is usually determined from the measurement of biomass, i.e. from the plot of ln$X$ *vs*. time t. If the stoichiometry of biomass growth does not change during the growth, the rate and amount of biomass formation, (dX/dt) and (X-X_0), are proportional to the rate and amount of heat production, dQ/dt and Q. Assuming that the initial concentration of biomass is low, $X \cong X$-X_0, μ can be determined from calorimetric measurements, i.e. plot of ln$Q$ *vs*. time t [7], **Figure 4.** The rate of biomass increase dX/dt is proportional to the rate of increase in the heat production dQ/dt where Y_Q – yield of biomass per evolved heat amount - is the proportionality factor:

$$dX/dt = Y_Q \cdot dQ/dt \quad (1)$$

The direct relationship between heat production rate and specific growth rate un-

derlying the proportionality between these two parameters can be expressed as

$$\ln (dQ/dt) = \ln (dQ/dt)_{t=0} + \mu t \quad (2)$$

where

$$\ln (dQ/dt)_{t=0} = \ln (1/Y_Q \,.\, \mu \,.\, X_0 \,.\, e^{\mu})$$

Eq. (2) is the simplest equation for calculating specific growth rate. More sophisticated methods recommend considering also microbial death rate constant or time constant of calorimeter. However, automatic dynamic correction of the instrument with Tian equation, used in Digitam 4.1 excludes the need for additional calculation of the latter.

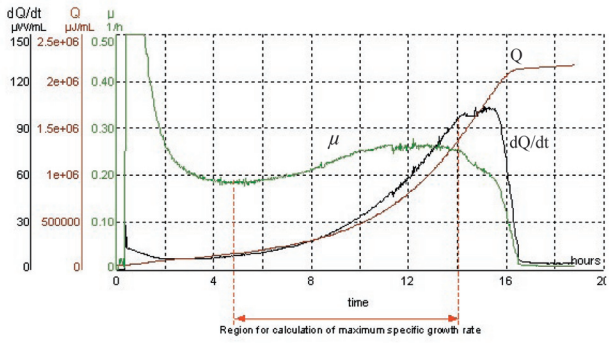

Figure 3. Calorimetric growth curve of a consortium from methanogenic reactor. Rate of heat production (dQ/dt); heat production (Q) and specific growth rate of microorganisms (μ)

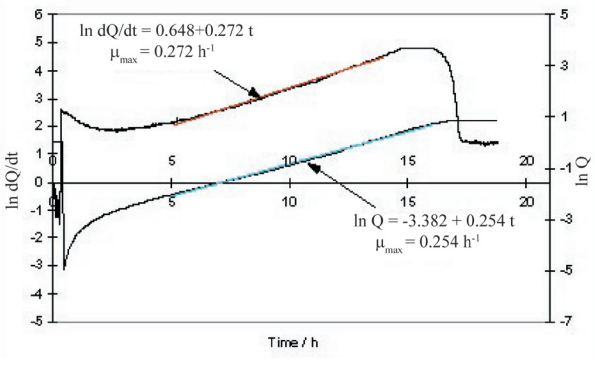

Figure 4. Growth of consortium from methanogenic reactor: semilogarithmic curves of heat production rate (ln dQ/dt) and heat production (ln Q). Calculation of maximum specific growth rate (μmax.).

CONCLUSIONS

Microcalorimetry even in its classical setup based on the use of sealed glass ampoules turns out to be a quite suitable method for studying anaerobic processes. During the exponential phase of growth the heat evolved per unit mass of bacteria versus time is constant. A linear correlation between the rates of heat production and biomass production has been reported for several microorganisms that makes microcalorimetry a prospective method for monitoring anaerobic bacterial growth processes. On-line data from calorimetric measurements are more precise and easier to obtain as compared to biomass measurements.

REFERENCES

1. Gustafsson, L. Thermochim. Acta 193. **(1991)** 145-171
2. Wadso, 1. Thermochim, Acta 294, **(1997)** 1-11.
3. Vandenhove, H. Microcalorimetric characterization of bacterial inocula. In: Advanced instrumentation, data interpretation and control of biotechnological processes, Chapter 5. (J. F. M. Van Impe, Vanrolleghem, P.A, Iserentant, D.M., eds,), **(1998)**, pp. 121-158.
4. Menert, A., Liiders, M., Kurissoo, T., Vilu, R. J. Therm, Anal. 64, **(2001)** 281-291.
5. Blonskaja. V., Menert, A., Vilu, R. Adv. Environ. Res. 7(3), **(2003)** 671-678.
6. Chang-Li. X., Hou-Kuan, T., Zhau-Hua, S., Song-Sheng, Q. Thermochim. Acta, 123 **(1988)** 33-41.
7. Liu, J.-S., Marison, I.W., van Stockar, U. J. Thermal Anal. 56, **(1999)** 1191-1195.