ELSEVIER

Thermochimica Acta 251 (1995) 85–97

thermochimica acta

Impact of carbon dioxide evolution on the calorimetric monitoring of fermentations ☆

M. Meier-Schneiders *, F. Schäfer, U. Grosshans, C. Busch

Institut für Chemische Verfahrenstechnik der Universität Stuttgart, Böblinger Str. 72, D-70199 Stuttgart, Germany

Received 29 August 1994; accepted 30 August 1994

Abstract

During fermentation, carbon dioxide formation usually takes place. The absorption of carbon dioxide in water is an exothermic reaction; its release from the medium is endothermic. These effects were first analysed in a fermenter–calorimeter using model media and then monitored during anaerobic batch and continuous cultivation of *Zymomonas mobilis* cells. The heat effects proved to be functions of the carbon dioxide partial pressure, the pH value of the medium, the gas flow rate through the liquid, and the pump rate of the medium through the fermenter (continuous culture). The heat of biological growth is usually diminished by carbon dioxide desorption. The maximum error introduced in the on-line measurement was calculated to be 48% for anaerobic conditions; the average measured was 35%. The worst case calculated for aerobic conditions was 4%. In the case of a pH-controlled fermentation, the neutralization heat from alkaline feeding contributes to the total heat production measured. When the carbon dioxide partial pressure was maintained constant and the volume of the exhaust gas produced and the alkaline consumption were known, monitoring of the small biological heat production rates under anaerobic conditions was possible with reasonable accuracy.

Keywords: Aerobic; Anaerobic; Carbon dioxide; Fermentation; pH; *Zymomonas*

☆ Presented at the ninth Conference of the International Society for Biological Calorimetry, Berlin-Schmerwitz, 27–31 May 1994.

* Corresponding author.

SSDI 0040-6031(94)02092-2

1. Introduction

The heat production of a metabolic process is widely used for on-line monitoring of a fermentation [1,2]. Even if the technical function of a calorimetric system is ensured, the heat rate measured is not necessarily the heat rate liberated by biological growth, because the latter may be accompanied by heat caused by non-biological reactions. In this paper the impact of carbon dioxide evolution and the impact of related reactions on the calorimetric monitoring of fermentations are discussed.

In general [3], CO_2 dissolves in water in an exothermic reaction, reaction (1), below. Depending on the partial pressure of CO_2 and the pH value, significant amounts of $CO_2(l)$ and hydrogen carbonate are formed, reaction (2). The equilibrium is shifted to hydrogen carbonate for high pH values. Most fermentations are run between pH 5 and 7. Because carbonate formation is only dominant above pH 7, CO_3^{2-} is negligible. HCO_3^- can be neglected below pH 5. The CO_2 dissolved in aqueous media in a fermenter is in contact with the gas phase, and desorption may take place if the $CO_2(l)$ concentration is above the CO_2 partial pressure in the head space. The release of carbon dioxide from the medium is an endothermic reaction

$$CO_2(g) \rightleftharpoons CO_2(l) \qquad \Delta H_R = -20.3 \text{ kJ mol}^{-1} \ (25°C, p_{CO_2} = 1 \text{ bar}) \tag{1}$$

$$H_2O + CO_2(l) \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H_3O^+ \qquad \Delta H_R = +7.5 \text{ kJ mol}^{-1} \tag{2}$$

Other medium conditions (salt concentration, the character and concentration of buffers) may also influence carbon dioxide heat effects [4,5]. What consequences arise from these considerations for calorimetrically monitored fermentations?

Most fermentations are accompanied by carbon dioxide formation. Carbon dioxide is believed to be released from the cells into the solution in its liquid form [6]. There is no heat effect expected until the equilibrium concentration determined by pH and CO_2 partial pressure is reached. When the CO_2-saturation capacity of the liquid is exceeded, desorption of carbon dioxide into the gas phase takes place. The heat signal measured is now influenced by this non-biological endothermic reaction. Yield calculations derived from the heat measurements may also be affected.

In order to differentiate these non-biological from biological heat effects, a newly developed, sensitive fermenter–calorimeter capable of all fermenter functions was applied for anaerobic batch and flow-through experiments run with model medium and a cell suspension of *Zymomonas mobilis*.

2. Experimental

2.1. Fermenter–calorimeter

The fermenter–calorimeter, described elsewhere in detail [7,8], consists of a glass vessel with a working volume of 2 l, surrounded by a double-wall glass jacket (Fig.

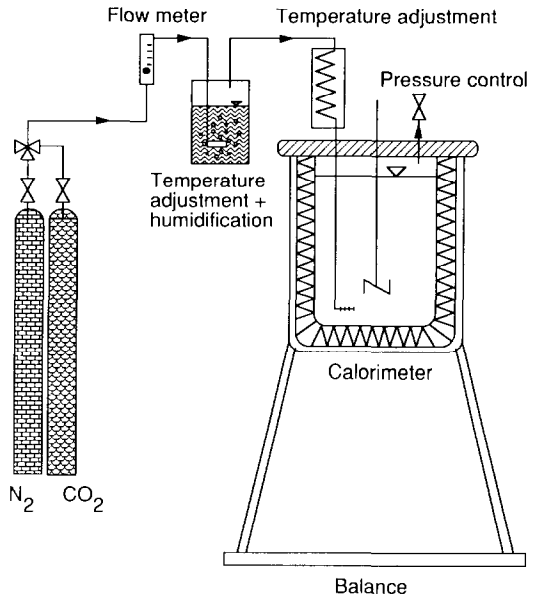

Fig. 1. Schematic diagram of the experimental setup of the fermenter–calorimeter for the gassing experiments with model media or with culture suspensions of *Zymomonas mobilis*.

1). The measuring principle is based on heat-flux calorimetry with power compensation. The temperatures of the liquid feeds are adjusted to reactor temperature before they enter the vessel. The gas feed is saturated at the fermenter temperature to avoid heat losses through evaporation. Exhaust gas is vented from the calorimeter through a pressure control valve. The pH value can be controlled to a constant value by alkaline feeding. Because the reactions of carbon dioxide are fast, the unfiltered heat signal with a time delay of a few seconds was monitored. Under gassed conditions, the noise of this signal is 50 mW l^{-1}.

For the experiments, the CO_2 partial pressure was varied between 0 and 130 hPa. CO_2 and N_2 were supplied from gas storage tanks. The gas flow was controlled to 0.3 l (1 min)$^{-1}$. Gassing experiments without cell suspension were conducted in demineralized water as well as in water containing 2–50 g NaCl per l, in water containing 2–50 g NaOH per l, and in a typical fermentation medium containing (in g l^{-1}): KH_2PO_4, 3.48; $MgSO_4$, 1.0; $(NH_4)_2SO_4$, 1.98 [9]. The medium was agitated (500 rpm) and the calorimeter was started. When constant conditions of the preset parameters were established, a zero offset calibration was done, the base line was monitored and the experiments were performed. Carbon dioxide solution and release were induced by changing from N_2 gassing to CO_2 and vice versa and the heat of reaction was measured. Experiments were run in batch and in continuous mode. When carbon dioxide equilibrium was reached, samples were withdrawn from the fermenter in order to analyse dissolved CO_x (see below). All gassing

experiments were performed at least in triplicate. The graphs show the results of representative single experiments.

2.2. Organism and culture conditions

Zymomonas mobilis ATCC 29191 was used for anaerobic batch and flow-through experiments ($D = 0.24\ h^{-1}$). The experiments were run at 30°C. The medium was agitated at 500 rpm. The growth medium consisted of (in g l^{-1}): KH_2PO_4, 1.0; $(NH_4)_2SO_4$, 1.0; $MgSO_4 \cdot 7H_2O$, 0.5; yeast extract, 5.0; glucose, 50.0 [10]. The pH was adjusted with 1 N NaOH prior to sterilization (20 min at 121°C) to 6.0. A stock solution of glucose was sterilized separately. Glucose was added to the medium immediately before inoculation. The suspension from an overnight pre-culture was centrifuged (10 min at 20°C, 3500 g), the pellet was resuspended in 20 ml of fresh medium and immediately transferred to the fermenter using a syringe to give an initial optical density (OD) of 0.5. During fermentations, the CO_2 partial pressure during external gassing was varied between 0 and 130 hPa (flow rate 0.3 l (l min)$^{-1}$. The pH of the medium was either non-controlled and therefore decreased during the fermentation due to ammonium consumption and hydrogen carbonate formation, or it was kept constant by alkaline feeding (1 N NaOH).

2.3. Analyses

The optical density (OD) of the suspension was determined at 550 nm by a photometer. The dry weight was measured at the end of the experiments. Glucose, ethanol, lactic and acetic acid were analysed simultaneously by HPLC using a refractive index detector [8]. CO_x dissolved in the model media was precipitated as $BaCO_3$ and the optical density of the suspension measured at 300 nm [11]. CO_2 in the exhaust gas was measured by infrared gas analysis [11].

3. Results and discussion

3.1. Pressure, pH, buffer and salt effects in model medium

The first part of the experiments was performed in order to evaluate the importance of non-biological heat effects at batch conditions. The results are shown for only one experiment (Fig. 2), which is representative for all the other data described later in this section. Demineralized water was used for this experiment; cells were not present. The medium was exposed to nitrogen flux. N_2 was then replaced by CO_2 gassing (phase 1 in Fig. 2). The expected exothermic reaction took place immediately. When the water was saturated with CO_2, indicated by zero heat production and constant pH value, a new equilibrium was reached and samples for CO_x analysis were withdrawn. As shown in Fig. 2, phase 2, the fermenter was then again exposed to N_2, followed by endothermic stripping of dissolved CO_2. Both reactions proved to be reproducible and close to the theoretical values (average difference for CO_x and heat enthalpy, 5%).

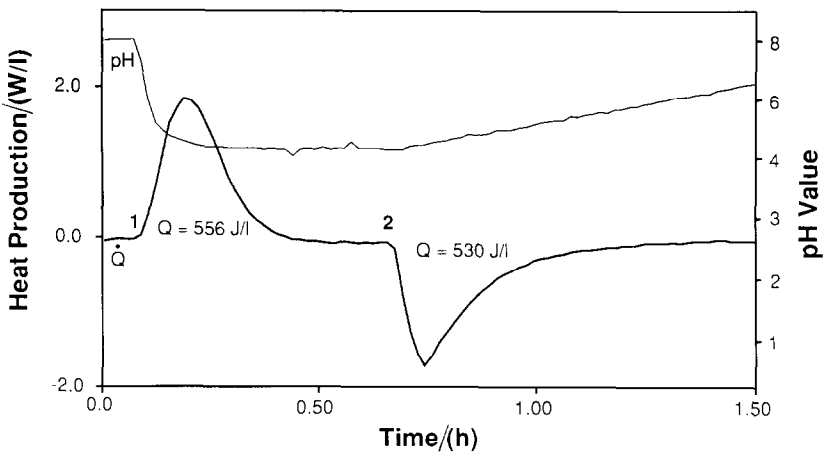

Fig. 2. Exothermic and endothermic reactions of CO_2 in demineralized water in a batch experiment. Cells were not present. 1, stop N_2, start CO_2 flow; 2, stop CO_2, start N_2 flow. (30°C, agitation 500 rpm, gas flow 0.3 l (1 min)$^{-1}$, heat signal unfiltered.)

Similar heat signals were obtained from further experiments studying the impact of salt concentration (NaCl). Salt lowered the heat enthalpy of the reaction to some extent (data not shown), as expected from Schumpe et al. [4]. However, the decrease in carbon dioxide solubility was less than 10% for salt concentrations commonly present in fermentation media (< 10 g l^{-1}).

When the pH of the medium during the solution of CO_2 was kept constant by alkaline feeding, more carbon dioxide was dissolved, which was related to higher heat production. The heat of the CO_2 reaction was also increased by the neutralization reaction (Eq. (3)). The heat production measured was in good accordance with the theory (data not shown)

$$OH^- + H^+ \rightarrow H_2O \qquad \Delta H_R = -55.9 \text{ kJ mol}^{-1} \text{ [12]} \tag{3}$$

Increased buffer concentrations stabilized the pH of the medium and led to increased heat production due to the enlarged CO_2-absorption of the medium. Increased CO_2 partial pressure of the gas phase led to the expected increase in the heat from enhanced carbon dioxide absorption, because the carbon dioxide equilibrium concentration is a function of the partial pressure. The results of CO_x equilibrium concentration and heat generation measured for varying pH values and carbon dioxide partial pressures were in good agreement with the theoretical expectations (data not shown, see Ref. [11]).

3.2. Non-biological heat effects during batch fermentations

The experiments carried out were aimed at analysing the impact of the heat effects of CO_2 on the heat production rates obtained from anaerobic batch

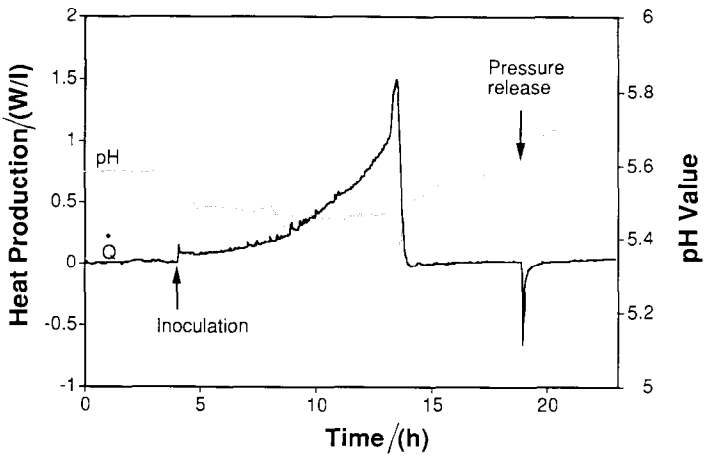

Fig. 3. Anaerobic batch fermentation with *Zymomonas mobilis* cells. Continuous pressure increase by carbon dioxide formation. Pressure was maintained during the fermentation. Pressure release led to an endothermic reaction. (Complex medium with 20 g glucose per l, pH controlled to 5.5, 30°C agitation 500 rpm.)

fermentations with *Zymomonas mobilis*. It converts glucose anaerobically via the Entner-Doudoroff pathway to ethanol [13]

$$1\ \text{glucose} \rightarrow 2\ \text{ethanol} + 2CO_2 \qquad \Delta H_R = -84\ \text{kJ mol}^{-1} \tag{4}$$

Due to the small enthalpy of reaction (-84 kJ mol^{-1}) the maximum heat production expected was low compared to the aerobic oxidation of glucose ($\Delta H_R = -2816$ kJ mol^{-1} [14]). Inoculation at $t = 4.5$ h was followed by a calorimetric curve exhibiting the profile of a typical growth curve (Fig. 3). Excess gas could only escape from the reactor at pressures higher than approximately 200 hPa due to a pressure control. At $t = 18.5$ h, this pressure was released. The endothermic effect of decreasing liquid carbon dioxide concentration could be clearly identified. As for most fermentations, the pH was maintained constant by alkaline feeding, which caused neutralization heat, contributing to the total heat production. The impact of CO_2 release and alkaline feeding on the heat production can be eliminated from the energy balance at the end of a fermentation, if the CO_2 partial pressure is maintained constant and the volume of exhaust gas and the alkaline consumption are known.

3.3. Flow-through experiments with model medium

Flow-through experiments using the fermenter–calorimeter were carried out in order to identify and to quantify non-biological heat effects, which are difficult to differentiate from biological activity in batch cultures. Fig. 4 shows CO_2 heat effects in demineralized water. The experimental pattern of alternated gassing (Fig. 4,

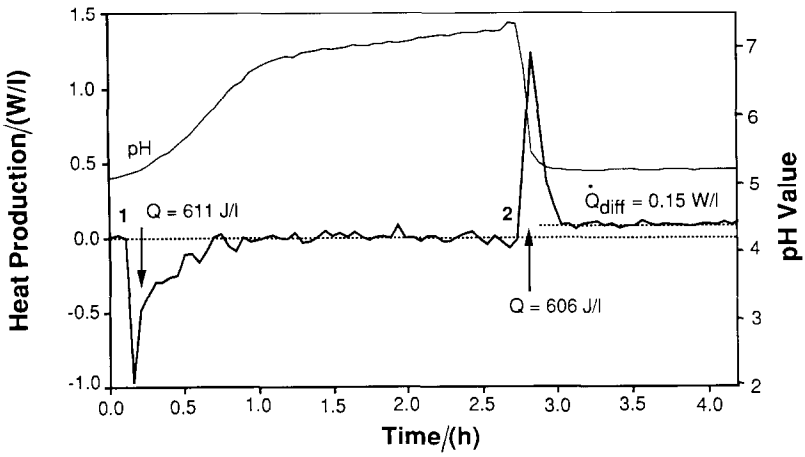

Fig. 4. Exothermic and endothermic reactions of CO_2 in water in continuous mode of operation. Cells were not present. Initially gas phase 100% CO_2, no flow. 1, start N_2 flow; 2, stop N_2, start CO_2 flow. ($D = 0.33$ h $^{-1}$, 30°C, agitation 500 rpm, gas flow 0.3 l (1 min) $^{-1}$, heat signal unfiltered.)

phases 1 and 2) was the same as for the batch experiments. Similar endothermic (start of N_2 gassing at $t = 0.15$ h) and exothermic reactions were measured (switch from N_2 to CO_2 gassing at $t = 2.7$ h). For the continuous equilibrium, N_2 flow did not affect the heat signal. The results were different for the CO_2 atmosphere. After a fall in the expected heat of reaction caused by switching from N_2 to CO_2 gassing, the heat signal remained at a constant, but significantly higher level (Fig. 4, $t > 3.1$ h). This was due to continuous absorption of CO_2 in non-saturated fresh medium flowing into the fermenter. In other words, this non-biological heat production is a function of the pump rate. As a consequence, the total heat production measured during a continuous fermentation has to be corrected. This was analysed during the following series of biological experiments.

3.4. Non-biological heat effects during continuous cultivations

Flow-through experiments using the fermenter–calorimeter were carried out in order to quantify non-biological effects during a continuous cultivation with *Zymomonas mobilis* (Fig. 5). The cell suspension was gassed by N_2 ($t < 0.45$ h). Carbon dioxide was continuously produced by the cells and stripped from the fermenter broth by external nitrogen gassing, diminishing the heat signal. The N_2 flow mixed with the CO_2 from biological production, and the exhaust gas composition was 87% N_2 and 13% CO_2 in continuous equilibrium. The biological heat production was calculated to be diminished by 30% (based on the biological carbon dioxide production of 2.75 l (1 h)$^{-1}$ measured). When the N_2 supply was stopped ($t = 0.45$ h), a change in the gas phase towards a carbon dioxide atmosphere was initiated. The heat production increased rapidly within seconds to a rate 35% above

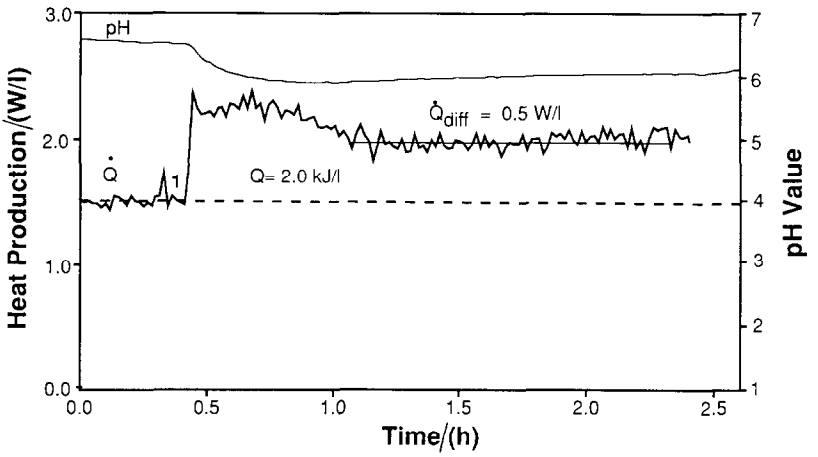

Fig. 5. CO_2 effects in continuous culture with anaerobically growing cells of *Zymomonas mobilis*. N_2 flow, gas phase 87% N_2, 13% CO_2 content from fermentative activity. 1, stop N_2 flow. (Complex medium with 50 g glucose per l, $D = 0.24\ h^{-1}$, 30°C, agitation 500 rpm, gas flow 0.3 l (1 min)$^{-1}$, heat signal unfiltered.)

the former level, due to the interruption of the endothermic reaction. Thus, calculation and measurement of the endothermic CO_2 effect proved to be in good agreement. The immediately declining pH was considered to be related to the increased carbon dioxide concentration of the liquid phase. This caused additional neutralization heat from alkaline feeding by the pH control. At $t > 1.2$ h, a constant but higher level of heat production was established. The higher level was calculated to be related to both exothermic CO_2 saturation of the medium pumped through the reactor and the alkaline reaction. These errors of biological heat measurements can be easily eliminated, if the feeding rate, the volume of exhaust gas produced and the alkaline consumption are known.

Yield calculations for the continuous cultivation showed good agreement with the heat production (1.55 W l^{-1} expected from the enthalpy of combustion calculated for the glucose feed, 1.51 W l^{-1} measured) as well as with the glucose consumption and ethanol production (12.46 g glucose per l reactor volume per h, 0.069 mol (1 h)$^{-1}$; 5.93 g ethanol per l per h, 0.069 mol (1 h)$^{-1}$). However, the carbon dioxide exhaust gas rate under N_2 gassing conditions was 13% lower than the expected production. There are three reasons for this: desorption depends on (1) the carbon dioxide gradient between liquid and gas phase; (2) the phase transfer area; and (3) the CO_2 partial pressure in the gas phase. The CO_2 partial pressure measured during N_2 gassing depends on biologically produced CO_2, which remains to a certain extent in the liquid phase. In the state of dynamic equilibrium, the carbon dioxide partial pressure in the fermenter was still 13 hPa. Therefore, the deficit in carbon dioxide exhaust gas must equal the portion of the carbon dioxide that leaves the fermenter as "oversaturation" of the medium.

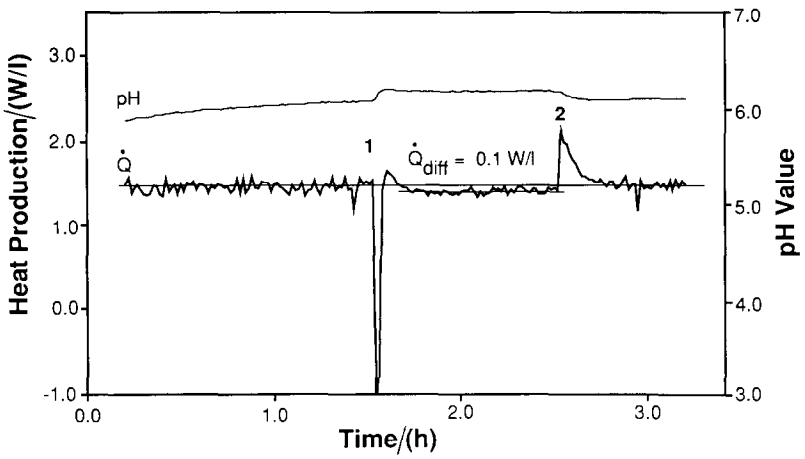

Fig. 6. CO_2 phase transfer effects in continuous culture with anaerobically growing *Zymomonas mobilis* cells. Gas phase maintained for 2 retention times at 100% CO_2 from fermentative activity. 1, start external CO_2 flow; 2, stop CO_2. (Complex medium with 50 g glucose per l, $D = 0.24\ h^{-1}$, 30°C, agitation 500 rpm, gas flow 0.3 l (1 min)$^{-1}$, heat signal unfiltered.)

In order to estimate the carbon dioxide oversaturation under N_2 gassing conditions, the kinetics of carbon dioxide release in batch experiments with model media was calculated [11]. The carbon dioxide release from the liquid is a reaction of first order. This was verified using the heat signal of carbon dioxide stripping by nitrogen gassing shown in Fig. 2, phase 2. The logarithm of the heat signal is a straight line and its slope equals the rate of phase transfer k_R. For continuous fermentations, the carbon dioxide release rate into the gas phase can be calculated by the equation

$$F(t) = k_l a(c^*_{CO_2} - c_{CO_2}(t)) \tag{5}$$

where $F(t)$ is the carbon dioxide release rate, $k_l a$ is the characteristic value of phase transfer, $c^*_{CO_2}$ the CO_2 equilibrium concentration, and $c_{CO_2}(t)$ the actual CO_2 concentration. During the N_2 gassing (phase 1 in Fig. 5), the total exhaust gas flow rate and the exhaust gas composition were measured. Based on these data, the carbon dioxide release rate $F(t)$ was calculated. The CO_2 equilibrium concentration $c^*_{CO_2}$ was calculated using Eq. (1). The characteristic value for CO_2 phase transfer $k_l a$ was assumed to equal the rate of phase transfer k_R of the experiment in batch mode without metabolic activity, because the gassing conditions were the same. Using these values, the actual carbon dioxide equilibrium concentration of the medium during the fermentation under N_2 gassing conditions (Fig. 5, phase 1) was calculated to be $c_{CO_2}(t) = 0.023$ mol l^{-1}. This high carbon dioxide concentration calculated in the liquid is in good accordance with the deficit of the CO_2 yield measured.

Without external gassing, this oversaturation is postulated to be larger because of the very low interfacial area between the gas and liquid phase. The differences in

carbon dioxide equilibrium concentrations in the continuous mode caused by varying interfacial area were analysed in the following experiment (Fig. 6). Continuous cultivation was maintained for more than 2 retention times without external gassing to ensure 100% CO_2 in the gas phase produced by *Zymomonas mobilis*. Then an external CO_2 gas flux was applied (Fig. 6, phase 1), immediately causing a transient endothermic reaction. Afterwards the level of total heat production was lower compared to the former situation. The difference was small but proved to be reliable. When the CO_2 flux was interrupted (Fig. 6, phase 2), an exothermic reaction took place. After approximately 5 min, the heat release declined to the level of heat production at the beginning of the experiment. The changes in the heat signal were closely related to pH changes, suggesting a different CO_2 saturation status of the liquid due to different phase transfer areas. Without external CO_2 supply, the CO_2(l) concentration is higher than with gassing. The transient endothermic and exothermic reactions are believed to give information about the change in phase transfer conditions. This could not, however, be evaluated because the CO_x analysis of the broth necessary for the evaluation of this assumption is not yet possible, because of medium-based colour interference during the photometric measurement.

4. Conclusions

It has been shown that the impact of carbon dioxide evolution on the calorimetric monitoring of fermentations strongly depends on the fermentation conditions. The errors identified are related to the presence of a gas head space phase and the aerobicity or anaerobicity of a fermentation. During an aerobic cultivation, the fermenter is typically exposed to excess air. Under these circumstances, the CO_2 concentration of the gas phase remains low (1–2%). In other words, CO_2 is continuously stripped from the medium, constantly diminishing the heat signal measured. Compared to the high energy production, e.g. from glucose (heat of combustion 2816 kJ mol^{-1} [14], CO_2 heat effects (6 mol CO_2 formed from 1 mol glucose consumed) account for a negligible maximum error of 4% (122 kJ). For anaerobic fermentations accompanied by CO_2 evolution, carbon dioxide effects may become much more important because the amount of biological heat production is often low, e.g. 84 kJ per mol glucose converted to ethanol. Therefore the biological heat production is in the same range as the interfering effect from CO_2 evolution (2 mol CO_2 formed from 1 mol glucose and stripped from the broth account for a maximum error of 48% (40.6 kJ)). The average difference measured was 35%. To eliminate disturbances of carbon dioxide reactions from the heat rate measured by fermenter-like calorimeters, two approaches can be made. The experimental conditions could be altered in order to prevent carbon dioxide desorption or to establish a known and constant carbon dioxide release rate. However, with respect to conditions desired to be close to a fermentation process, the prevention of heat effects is impracticable because fermentations are usually not run with very low substrate concentrations (in this case the CO_2 saturation capacity of the medium is

not exceeded), at high partial pressure (no desorption occurs) or at unphysiologically high pH values (carbon dioxide is precipitated as carbonate).

Alternatively, pressure and pH could be kept exactly constant. Under these conditions a constant carbon dioxide desorption rate is provided, diminishing the biological heat rate measured as described above. This means for anaerobic fermentations that presaturation of the medium with carbon dioxide is advisable prior to calorimetric measurements. There will still be CO_2 heat effects, which cannot be eliminated because they are directly caused by transitions of metabolic activity, e.g. after the start and stop of substrate feeding in continuous culture. But the effects can be explained, and inaccurate conclusions from the calorimetric signals can be prevented.

Considering the potential of biocalorimetry for the monitoring of animal cell cultures [15–17], CO_2 heat effects may become of great importance. Usually the media contain $NaHCO_3$ (1–3 g l^{-1}). In addition, they are gassed by air containing up to 5% CO_2 in order to keep the pH constant [18]. Hydrogen carbonate acts as a buffer, which is simultaneously a function of the pH. As a consequence, CO_2 heat effects from changing conditions are very likely. With respect to the proliferation rates of the cells ($> 1d$) and therefore low heat production, calorimetric measurements have to be carried out thoroughly.

The results of heat measurements described here for fermenters may also be important for heat measurements in microcalorimeters [19–21]. When used in flow-through mode, the cell suspension is passed through a heat exchanger into the measuring cell. The residence time varies between 1 and 5 min depending on the specific experimental conditions [2,20,22]. Most studies published have involved gassed fermentations. When the cell suspension enters the heat exchanger, the situation is very similar to that described in Fig. 5, when N_2 supply was stopped. It is predicted that oxygen concentration declines immediately, whereas carbon dioxide in the liquid phase increases. Oxygen depletion is related to a declining biological heat production depending on the substrate concentration, the growth phase of the organism and the pump rate of the suspension through the calorimetric unit [18]. Non-biological heat reactions are to be expected when a gaseous phase is formed enabling endothermic carbon dioxide desorption.

Because the heat of biological growth is usually diminished by carbon dioxide desorption, the question arises: what is the real heat of biological growth? Carbon dioxide is released from cells into the medium in its liquid form. All further reactions of CO_2 occur outside the cells. If, as considered by von Stockar et al. [23], for instance, only the cells excluding medium and gas phase are defined as the 'system', the heat of carbon dioxide desorption is not part of the heat of biological growth. If the system boundaries are drawn around the whole fermenter, stripping of carbon dioxide is of course part of the enthalpy balance. Only considering the cells as the 'system' will give information about the real biological heat production, but this can never be measured experimentally. Choosing the fermenter as the 'system' boundary simplifies heat calculations, because the heat of carbon dioxide release is part of the heat measured in the experiment.

Acknowledgements

We thank C. Fleischer and Prof. G. Eigenberger for fruitful discussions. This work was partly carried out during the first period of the Central Project Biotechnology Stuttgart and supported financially by the Bundesministerium für Forschung und Technologie, Germany, and the Land Baden-Württemberg.

References

[1] E.H. Battley, Energetics of Microbial Growth, John Wiley & Sons, New York, 1987.
[2] U. von Stockar and I.W. Marison, The use of calorimetry in biotechnology, Adv. Biochem. Eng., 40 (1989) 92–136.
[3] S. Topham, Carbon dioxide, in W. Gerhartz (Ed.), Ullmann's Encyclopedia of Industrial Chemistry, Vol. A5, VCH publishers, Weinheim, 1994, pp. 165–183.
[4] A. Schumpe, G. Quicker and W.D. Deckwer, Gas solubilities in microbial culture media, Adv. Biochem. Bioeng., 24 (1982) 1–38.
[5] U. Onken and E. Liefke, Effect of total and partial pressure (oxygen and carbon dioxide) on aerobic microbial processes, Adv. Biochem. Bioeng., 40 (1989) 137–170.
[6] P.N.C. Royce and N.F. Thornhill, Estimation of dissolved carbon dioxide concentrations in aerobic fermentations, AIChE J., 37 (1991) 1680–1686.
[7] M. Meier-Schneiders and G. Eigenberger, Biokalorimetrie—Methoden und Nutzungspotential. Biotec, 4 (1993) 6–9.
[8] M. Meier-Schneiders, U. Grosshans, C. Busch and G. Eigenberger, Biocalorimetric supported analysis of fermentation processes, Appl. Microbiol. Biotechnol., (1994) accepted for publication.
[9] J.E. Fein, R.C. Charley, K.A. Hopkins, B. Lavers and H. Lawford, Development of a simple defined medium for continuous ethanol production by *Zymomonas mobilis*, Biotechnol. Lett., 5 (1985) 1–6.
[10] S. Bringer-Meyer, M. Scollar and H. Sahm, *Zymomonas mobilis* mutants blocked in fructose utilization, Appl. Microbiol. Biotechnol., 23 (1985) 134–139.
[11] F. Schäfer, Kalorimetrisch gestützte Analyse der lonengleichgewichte von CO_2 in wäßrigen Lösungen und die Bedeutung von CO_2-Störeffekten für die Biokalorimetrie, Diploma thesis, Institut für Chemische Verfahrenstechnik der Universität Stuttgart, 1994.
[12] H.R. Christen, Grundlagen der allgemeinen und anorganischen Chemie, Sauerländer, Aarau, 1973.
[13] H. Doelle, W. Kirk, R. Crittenden and H. Toh, *Zymomonas mobilis*—science and industrial application, Crit. Rev. Biotechnol., 13(1) (1993) 57–98.
[14] J.H. Luong and B. Volesky, Heat evolution during the microbial process—estimation, measurement, applications, Adv. Biochem. Eng. Biotechnol., 28 (1983) 1–40.
[15] T. Randolph, I.W. Marison, C. Berney and U. von Stockar, Bench-scale calorimetry of hybridomas in suspension culture, Biotechnol. Tech., 3(6) (1989) 369–374.
[16] I. Wadsö, Progress and problems in microcalorimetric work on mammalian cell systems, Thermochim. Acta, 137 (1988) 1–10.
[17] R.B. Kemp, Calorimetric studies of heat flux in animal cells, Thermochim. Acta, 193 (1991) 253–268.
[18] R.I. Freshney, Culture of Animal Cells—A Manual of Basic Technique, 2nd ed., A.R. Riss Inc., New York, 1987.
[19] A. Beaubien and C. Jolicoeur, The toxicity of various heavy metal salts, alcohols and surfactants to microorganisms in biodegradation process: a flow microcalorimetry investigation, Drug Chem. Toxicol., 1 (1984) 261–281.
[20] I.H.D. Lamprecht, B. Schaarschmidt and J. Siemens, Microcalorimetric investigations on the metabolism of yeasts. X. The occurrence of a heat production peak late after the termination of growth in low glucose media, Thermochim. Acta, 94 (1985) 129–138.

[21] L. Gustafsson, Microbiological calorimetry, Thermochim. Acta, 193 (1991) 145–171.
[22] R. Hölzel, C. Motzkus and I. Lamprecht, Kinetische Untersuchungen zum mikrobiellen Stoffwechsel an Flow-Kalorimetern, Poster presented at the Eurostar Conference in Munich, 1993.
[23] U. von Stockar, L. Gustafsson, C. Larsson, I. Marison, P. Tissot and E. Gnaiger, Thermodynamic considerations in constructing energy balances for cellular growth, Biochim. Biophys. Acta, 1183 (1993) 221–230.