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Microcalorimetric study of growth of *Lactococcus lactis* IL1403 at different glucose concentrations in broth

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ABSTRACT

Growth of bacteria *Lactococcus lactis* IL1403 in broth was studied using microcalorimeter in combination with HPLC, *plate count*, pH and OD (optical density) measurements. On the basis of the calorimetric data maximum specific growth rate (μ_{max}, W h^{-1}), yield coefficients (Y_{XS}, g g^{-1}; Y_Q, J cfu^{-1}), produced heat (Q, J), lag–phase duration (λ, h), etc. were calculated for the bacteria. Power–time curves measured allowed to analyze growth curves as consisting of two phases—exponential phase and "deceleration phase". Active growth of biomass/active metabolic processes are taking place during the exponential phase of growth, and the second, "deceleration phase" combines the stationary phase of bacterial growth curve together with the deceleration of the metabolism of bacteria. The numerical data obtained for the analysis of the bacterial growth in broth form a necessary basis for further studies of *L. lactis* using calorimetric method.

1. Introduction

Due to their irreversible nature, life processes invariably and continuously dissipate Gibbs energy. As this is virtually always coupled with the continuous production and exchange of heat, calorimeters could be readily used for the monitoring of life processes. It has been shown that generation of heat by microbial cultures can be used for *on-line* monitoring of growth and metabolism of cells [1–5]. Calorimetry is especially helpful in the studies of growth in opaque media, for example solid media where agar, gelatine, etc. are used as jellifying agents [6], but it could be successfully applied also for the studies of spoilage processes and shelf-life determination [5,7–9]—everywhere where the possibilities of optical and other physical methods are limited due to the properties of non-transparent media [10,11]. But as heat effects are unspecific, they should be combined for more rich interpretation with the results of parallel experiments using other methods like measurements of concentration of substrates and products, outplating, for the determination of the number of bacteria, etc. [12,13]. In fact these parallel experiments allow to give a special meaning to the results of calorimetric measurements. Taking into account these circumstances a "rich use" of calorimetric method is possible after power–time curves have been complemented with the data of parallel experiments mentioned above. Knowledge of necessary conversion, yield, etc. coefficients allows to derive a number of characteristics of the growth of bacteria from the power–time curves. Carrying out of this type of preparatory study was one of the aims of the present work.

Microcalorimetry has been used for the experimental study of bacterial growth during the last 50 years. The first experiments were carried out with *Streptococcus faecalis* and it was shown that the rate of heat production was coupled with the rate of biomass growth [14]. Later growth of other bacteria was studied—growth of *Escherichia coli* in anaerobic conditions in case of low substrate and high inoculation concentrations [12], growth of *Lactobacillus helveticus* in different environmental conditions [15], etc. Further studies carried out have elaborated the coupling of kinetic and thermodynamic processes [16–22]. It has been shown that calorimetry could provide information about different metabolic changes such as shift from one substrate/type of catabolism to another, occurrence of limitations and inhibitions [1,9,23,24]. The enthalpy balance elaborated using calorimetric measurements can reveal the formation of unknown, unexpected by-products [19,24], or can be used in studying the synthesis of intermediate products of metabolism, and their effect on bacterial growth [25]. Production of ethanol by *Saccharomyces cerevisiae* has been studied [2], effect of added environmental toxicants on bacterial growth has been investigated [26], etc. Calorimetric power–time curves could be used also as imprints of (complicated) microbial processes, as the shape and the number of phases of the curves depend on the composition of the bacterial consortia [3] and reflect complicated patterns of multi-stage growth processes.

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Despite the noted advantages use of microcalorimetry was until recently limited in practice because of the absence of multichannel instruments. Carrying out experiments which take days, weeks or even months in 2–4 channel instruments was not a very attractive possibility, especially in the age of wide use of high throughput methods. However, the situation was changed with the appearance of the multichannel TAM III on the market—since the introduction of multichannel instrument it had been feasible to carry out microcalorimetric studies as regular investigations.

Growth of *L. lactis* in CRM broth media (CRM—*carbohydrate restricted medium*) with glucose as a main carbon source was studied in the present work using a multichannel thermal activity monitor TAM III. Optical density, concentrations of glucose and lactic acid in the culture media, *pH* of the culture media and *plate counts* of the bacteria were measured in parallel to the calorimetric measurements to obtain information, necessary for the quantitative interpretation of the calorimetric power–time curves in terms of peculiarities of growth processes of the bacteria. The methods were applied for the study of growth of *L. lactis* in liquid cultures on different concentrations of glucose (2–100 g L^{-1}). Growth of the bacteria during the exponential phase of growth and during the deceleration phase (the phase that combines the very short stationary phase and death phase of cells together with the decrease (deceleration) of metabolic activities) were analyzed separately. It was shown that the exponential growth of the bacteria was stopped due to the exhaustion of glucose at low concentration of glucose (regarding the growth of *L. lactis* at glucose concentration 2 g L^{-1}), whereas at higher glucose concentrations—up to 50–100 g L^{-1}, exponential growth of the bacteria was terminated by pH 4.3. A number of conversion coefficients were determined which have been used in the interpretation of the obtained power–time curves. These conversion coefficients could be used also in further studies of *L. lactis* in other experimental conditions including solid-state growth.

2. Materials and methods

2.1. Inoculum preparation

The cultivated strain *Lactococcus lactis* subsp. *lactis* IL1403 was kindly provided by Dr. Ogier from INRA (French National Institute for Agricultural Research, Jouy-en-Josas, France). Frozen storage cultures of *L. lactis* IL1403 were thawn and pregrown on Petri dishes with M17 Agar (LAB M, UK) for 24 h at 35 °C. One colony from a pregrown Petri dish was used as an inoculum for a 10 mL culture in liquid M17 Broth at 35 °C. 1 mL of bacterial suspension grown overnight (exactly 24 h) was used as inoculum for the next liquid 10 mL M17 Broth (FLUKA) medium and grown 14–16 h which allowed the *L. lactis* IL1403 bacteria to reach the middle of the exponential growth phase. The number of bacteria was determined by plating on M17 Agar and incubating for 3 days at 35 °C, and average number of bacteria in the mid-exponential culture was $(4.16 \pm 0.1601)\ 10^8$ cfu mL^{-1}. The inoculum needed for the experiments was prepared by diluting of samples of the mid-exponential culture in peptone water. The calculated inoculation was in calorimetric and in parallel growth experiments 10^2 cfu mL^{-1}.

2.2. Growth experiments

CRM broth for growth media was prepared in a 800 mL Erlenmeyer flasks, sterilized by autoclaving at 121 °C for 15 min and after that shared to the 200 mL screw capped glass bottles where the glucose was added according to the desired media compositions—2, 20, 50, 100 g L^{-1}. A definite amount (1%, v/v) of the inoculum was added to the bottles with media solutions to get an inoculation rate of 10^2 cfu mL^{-1}, and after the inoculation the suspensions were intensively stirred. 2 mL of the culture from each bottle was put into 3 mL calorimetric ampoules and run at +35 °C. All the calorimetric growth experiments were carried out in three parallels.

The 200 mL bottles with bacterial suspensions were placed into thermostat at +35 °C. Glucose and lactic acid concentrations, pH, OD were determined in 4 mL samples, *outplating* was carried out using 100 μL samples at regular time intervals determined by bacterial calorimetric growth curves.

2.3. Analytical methods

HPLC (High Performance Liquid Chromatography) Separations Module Waters 2695 Alliance was used with a Refractive Index Detector 2414 and column BioRad HPX-87H Organic Acid Analysis Aminex Ion Exclusion Column 300 × 7.8 mm for measuring glucose and lactate concentrations (solvent—sulfuric acid solution (2.2 mL per 1 L MilliQ water), flow rate 0.6 mL min^{-1}, measuring temperature 35 °C). Biomass was calculated from optical density data measured at 540 nm using a spectrophotometer (PHOTOMETR KFK-3, USSR). The pH was measured with pH meter (S20 Seven Easy Mettler Toledo with InLab 413 Mettler Toledo electrode).

2.4. Compositions of the media used

The composition of the balanced M17 Agar (LAB M, UK) was as follows: peptone 5.0 g L^{-1}, soya peptone 5.0 g L^{-1}, yeast extract 2.5 g L^{-1}, beef extract 5.0 g L^{-1}, lactose 5.0 g L^{-1}, sodium glycerophosphate 19.0 g L^{-1}, magnesium sulfate 0.25 g L^{-1}, ascorbic acid 0.5 g L^{-1}, agar No. 2 15 g L^{-1}.

The composition of the balanced M17 Broth (FLUKA) was as follows: tryptone 2.5 g L^{-1}, meat peptone (peptic) 2.5 g L^{-1}, soya peptone (papainic) 5.0 g L^{-1}, yeast extract 2.5 g L^{-1}, meat extract 5.0 g L^{-1}, glucose 20.0 g L^{-1}, sodium glycerophosphate 19.0 g L^{-1}, magnesium sulfate 0.25 g L^{-1}, Tween 80 1.08 g L^{-1}, ascorbic acid 0.5 g L^{-1}, lactose 5.0 g L^{-1}).

The composition of the peptone water used for the dilution of the inoculum was as follows: NaCl 8.5 g L^{-1}, bacteriological peptone (LABM, UK) 1 g L^{-1}.

CRM broth growth medium of the following composition was used: casitone (Difco) 20 g L^{-1}, Tween 80 1.08 g L^{-1}, sodium chloride (MERCK) 25 g L^{-1}, magnesium sulfate 0.58 g L^{-1}, manganese sulfate 0.3 g L^{-1}, ferric sulfate 0.06 g L^{-1}, B-vitamin solution (SIGMA) 10 ml L^{-1}, MES (2-[N-morpholino]ethanesulfonic acid, SIGMA) 8.5 g L^{-1}.

2.5. TAM III [27]

The TAM III, which is a type of heat conduction multi-channel microcalorimeter was used to determine the growth of cells [28]. TAM III is designed to monitor continuously a wide variety of processes and complex systems over the temperature range 15–150 °C. TAM III is a multichannel instrument with maximum 48 channels. We used 24-channel instrument in our experiments. A heat flow calorimeter works by channeling the heat produced or consumed by a reaction in the sample through heat flow sensors comprised of thermoelectric modules. When a temperature gradient is imposed (or formed) across the thermoelectric module, a voltage is created in accordance with the Seebeck effect. This voltage is proportional to the heat flow through the thermoelectric module and hence proportional to the rate of heat production or consumption by the sample. One side of the thermoelectric module is in contact with the sample and the other is kept isothermal by a heat sink which is in contact with the TAM III thermostat (a liquid-based system). TAM III maintains outstanding sensitivity because of the excellent stability of the thermostat (<±100 μK/24 h) even over long periods of time (*TA Instruments* [27]). The detection limit of 0.5 μW and the

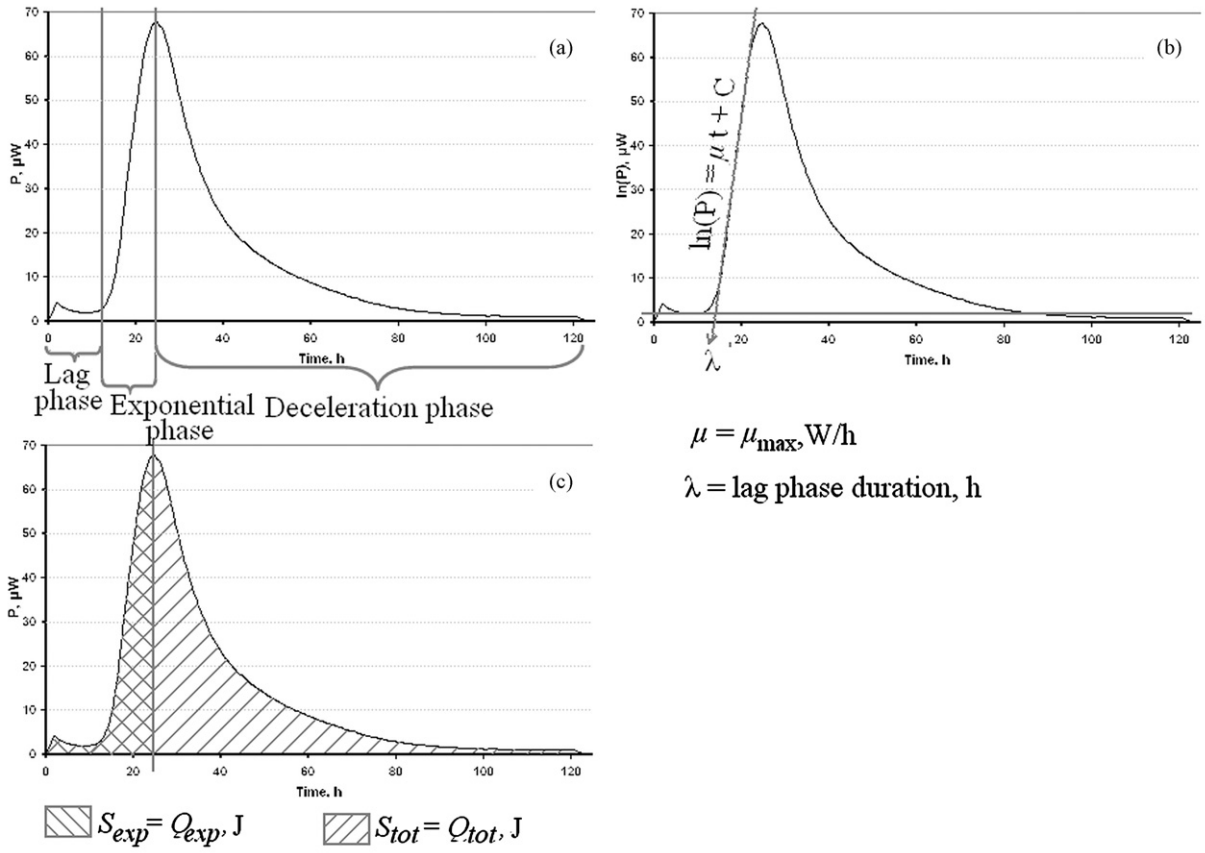

Fig. 1. The explanation of the processing of calorimetric power–time curves; (a) the division of calorimetric power–time curves into three phases–lag phase, exponential growth phase, and deceleration phase; (b) determination of the maximum growth rate (μ_{max}, h^{-1}) [3,16,17] and the lag phase duration (λ, h); (c) heat amounts determined—Q_{exp} is the heat evolved during the exponential phase and Q_{tot}—the total heat produced during the experiment.

baseline stability (over a period of 24 h) of ±0.2 μW characterize the instrument. The TAM III was operated in static ampoule mode (batch process) (see Wadsö [28]).

2.6. Analysis of power–time curves

3 mL ampoules with prepared cultures (see above) were placed into the TAM III. In all experiments heat flows (d*Q*/d*t*, μW) were measured. The curves that are the output of microcalorimeter are called power–time curves. Power–time curves obtained (see Fig. 1) were divided into three phases, essentially as ordinary growth curves of bacteria (Fig. 1(a)). Lag-phase which determines the adjustment period during which bacterial cells adapt to the new environment and start exponential growth [29] was defined in practice by the sensitivity of the microcalorimeter. In our case the growth was observed if there were about 10^5 cells in the ampoule (unpublished data). The second phase of the curve was exponential growth phase. In Fig. 1(a) it was assumed that the transition to the stationary phase (deceleration phase of growth) was started from the maximum value of the power–time curves.

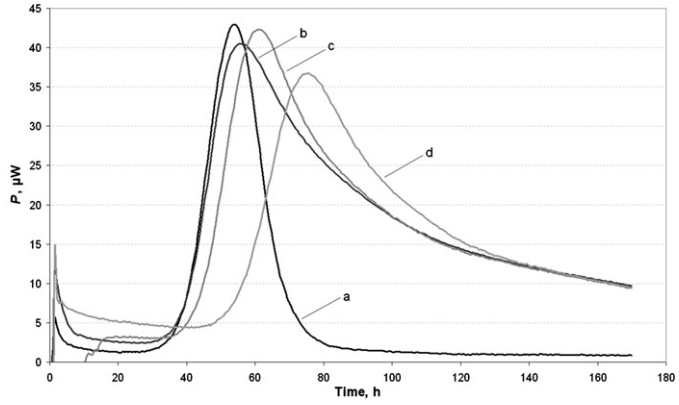

Fig. 2. Bacterial growth monitored by calorimetric power–time curves with differing initial substrate (glucose) concentration: a—2 g L^{-1}; b—20 g L^{-1}; c—50 g L^{-1}; d—100 g L^{-1}.

The total heat produced during the whole process of growth (area between the power–time curve and baseline, Q_{tot}, J) and the heat evolved during the exponential growth phase (area between the power–time curve, vertical line, that goes through the peak, and baseline, Q_{exp}, J) (Fig. 1(c)) as well as the average maximum growth rate (the slope of the tangent to the exponential phase, μ_{max}, W h^{-1}) and lag-phase duration (the crossing point of *X*-axis and the tangent, λ, h) (Fig. 1(b)) were determined using *TAM Assistant* program (v 0.9.1012.40, *SciTech Software AB*, *Thermometric AB*). All the multiple *Y*-axis graphs were drawn using *Microcal* (*TM*) *Origin* (v 6, *Microcal Software*, *Inc*) program (Fig. 3).

3. Results and discussion

3.1. Calorimetric growth curves in broth

The power–time curves describing growth of *L. lactis* IL1403 at different glucose concentrations (2, 20, 50, 100 g L^{-1}) and the same initial inoculation rate of 10^2 cfu mL^{-1} are presented in Fig. 2. Each curve presents average of three parallel power–time curves which were measured for the different environmental conditions studied.

Table 1
Parameters describing bacterial growth at different glucose concentration in CRM broth obtained from the processed power–time curves.

Glucose concentration, g L^{-1}	Maximum specific growth rate, μ_{max}, W h^{-1}	Evolved heat during the exponential growth phase Q_{exp}, J	Evolved total heat Q_{tot}, J	Lag-phase duration λ, h
2	0.2055[a]	1.4819	3.4544	29.71
2	0.2147[a]	1.5695	3.6604	29.42
20	0.1730[a]	1.7971	11.226	27.54
20	0.1732[a]	1.9582	11.302	27.80
50	0.1530[a]	2.1029	10.921	29.82
50	0.1533[a]	2.0755	10.825	29.29
100	0.1075[a]	2.3109	9.8581	34.86
100	0.1032[a]	1.9670	9.2376	33.93

[a] $R = 0.999$.

The standard deviation of the averaged curves was 0.355 μW which was quite low, confirming high reproducibility of the calorimetric method.

It is evident from Fig. 2 that calorimetric power–time curves measured were different in the case of different initial glucose concentrations. The power–time curve observed at low glucose concentration 2 g L^{-1} was almost like a Gaussian curve – symmetric – after rapid exponential growth phase similarly fast deceleration phase was taking place. With the increase of glucose concentration the power–time curves lost their symmetry, and growth deceleration phase became extended and prolonged. The power–time curves were processed as described in Section 2 and the numerical results obtained were presented in Table 1. The data obtained showed that the maximum specific growth rate decreased with the glucose concentration increasing which is in a good agreement with the observations made also earlier [30,31]. As seen from Table 1 the maximum specific growth rate (μ_{max}, W h^{-1}) decreased from >0.2 W h^{-1} to 0.1 W h^{-1} on the increase of glucose concentration from 2 g L^{-1} to 100 g L^{-1}.

The heat evolved during the exponential phase increased only slightly (from 1.5 J to 2 J) on the same change of glucose concentration. Assuming that growing bacterial cells produce equal amount of heat at different glucose concentrations it could be deduced that the number of bacteria produced during the exponential phase was almost the same in case of all substrate concentrations studied (see also Fig. 4).

The total evolved heat (Q_{tot}, J) was the smallest at 2 g L^{-1} glucose concentration (about 3.5 J) and it was approximately three times higher (about 10–11 J) in case of higher substrate concentrations studied (20, 50 and 100 g L^{-1}). Taking into account also the results of the additional experiments (see below) it could be assumed that at small glucose concentration the growth of the bacteria was limited by substrate (glucose) exhaustion, whereas at higher glucose concentrations physiological activities of the cells associated with the evolution of heat were continued even after the intensive growth of the biomass was stopped. At low glucose concentration the heat evolved during the exponential phase was 43% of the total, whereas at higher glucose concentrations the contribution of the exponential phase decreased to 17–21%. As noticed, the heat evolved during the exponential phase was practically the same in all growth conditions studied.

The duration of the lag-phase was the longest—34 h at the highest glucose concentration used. The shortest lag-phase was observed at glucose concentration 20 g L^{-1}, where it was equal to 27 h (Table 1).

In parallel experiments with the measurements of power–time curves growth of the bacteria was monitored also through the measurements of pH, lactic acid (*LA*, g L^{-1}) and glucose (*GLC*, g L^{-1}) concentrations changes in time in case of different initial glucose concentrations (Fig. 3) (see also Section 2). All the data measured in these parallel experiments were splined [32].

As seen from Fig. 3 the microbial growth curves could be divided into two groups also on the bases of the change of environmental parameters (pH, glucose and lactate concentrations): (a) in the case of low concentration of glucose (2 g L^{-1}) the substrate was exhausted in the end of the growth, and (b) the glucose was not exhausted in the end of growth in the case of all other concentrations studied.

In the case of low concentration of glucose (2 g L^{-1}) the active growth (see calorimetric power–time curve, black line) was observed starting from the 29th hour of cultivation, the maximum value of the power–time curve 22 μW mL^{-1} was observed at the 50th hour, and then the sharp decrease of heat production followed. The concentration of glucose decreased rapidly in parallel with the growth, and glucose (red line) was exhausted at the 60th hour. As expected, the production of lactic acid (blue line), and the change of pH (green line) were observed in parallel to the growth. The amount of lactic acid produced during the growth (~1.8 g L^{-1} or 0.02 mol L^{-1}) was almost the same as the amount of glucose consumed (~2 g L^{-1} or 0.01 mol L^{-1}) which was in a good agreement with the stoichiometry of lactic acid homofermentative metabolism according to which from 1 mole of glucose 2 moles of lactic acid are produced, i.e. the ratio of the masses of glucose consumed and lactate produced should be 1:1. When growth was fully stopped the pH was 4.3 (see Fig. 3).

As mentioned earlier in association with the analysis of power–time curves (see Fig. 2) the amount of heat produced during the exponential growth phase was practically the same in different growth conditions (about 2 J). As seen from the curves presented in Fig. 3 the maximum of the power–time curves was observed in all the cases, except the low concentration of glucose (2 g L^{-1}), at pH 4.3. The maximum of the power–time curve at low concentration of glucose was observed at pH 4.7 (according to Andersen et al. [30] the rate of glucolysis is considerably affected by acidity of the growth media starting with already pH 5). These facts obtained indicated that the exponential growth phase of the cultures of *L. lactis* most probably was terminated by pH (pH 4.3) at high initial concentrations of glucose in the culture media, and by exhaustion of glucose at the initial glucose concentration 2 g L^{-1}. The results obtained by us are in agreement with the results obtained earlier [33].

As seen from Fig. 3 the extended deceleration phases of heat production took place in parallel with the glucose consumption and lactic acid production at high initial concentrations of glucose. pH reached the minimum value pH 3.2 at around 100 h considerably earlier than glucose consumption or lactate production were stopped. These facts indicated that the final pH was determined by the buffering capacity of the growth media, rather than by the concentration of lactic acid in the media.

It must be noted that the consumption of glucose and production of lactic acid and dissipation of noticeable amount of heat was taking place during the post-exponential growth phase of cultivation which indicated that bacteria were slightly active even at pH 3.2 in the end of the experiments [21,23]. However, it should be noticed also that at higher concentrations of glucose, and especially during the post-exponential growth phase the ratio of lactic acid produced

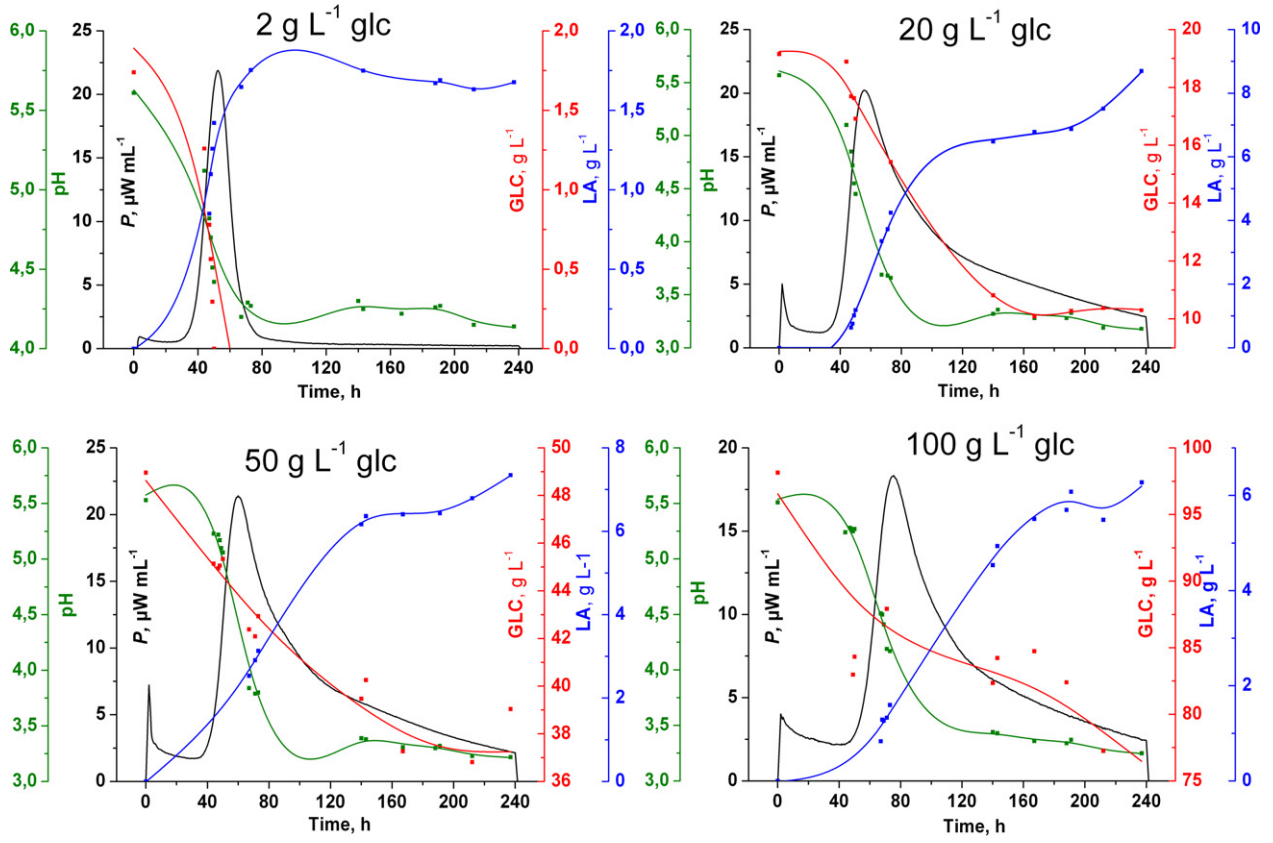

Fig. 3. Calorimetric power–time curves, pH change, lactic acid production and glucose consumption curves describing the growth of the bacteria in case of different initial glucose concentrations 2, 20, 50 and 100 g L^{-1}.

to glucose consumed was remarkably less than expected in the case of homolactic fermentation—lactic acid produced made up only 62% of the glucose consumed in case of 50 g L^{-1} GLC, and 22% in case of 100 g L^{-1} glucose.

All the data referred showed that the post-exponential growth phase (deceleration phase) is a complicated pattern of metabolic changes taking place after the exponential growth phase was stopped. Details of these patterns deserve certainly careful further studies using in addition to calorimetry also other methods.

Growth of the bacteria *L. lactis* was studied also using in addition to the measurements of power–time curves optical density and *plate-count* measurements. The data obtained were splined and shown in Fig. 4. During the growth in exponential phase the number of bacteria was proportionally increasing with the growth of OD. Exponential growth was ended at $1.5–2.5 \times 10^8$ cfu mL^{-1}; and OD = 0.40–0.68. During the post-exponential growth phase the OD values were staying practically constant, whereas the numbers of bacteria decreased about 10 times. The different behavior of growth characteristics in the post-exponential growth phase could be explained by assuming that OD showed not only living bacterial biomass which were able to multiply, but also partially autolysed or "dead" biomass. *Plate-count* method showed only viable cells.

Exponential phase curves measured by *outplating* or OD measurements were in a good agreement with the exponential phases of the power–time curves indicating that the latter could be used for the study of the bacterial growth during the exponential growth. A more complicated was the situation with the declining parts of the power–time curves. It is reasonable to assume that at least part of the heat evolved during the post-exponential growth phase was not reflecting the growth of biomass but some other physiological processes.

3.2. Conversion coefficients

If the stoichiometry of the 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 respectively. In this case Y_Q (J cfu^{-1}), the conversion, yield coefficient–amount of heat produced per cell synthesized, could be determined:

$$\frac{dX}{dt} = Y_Q \frac{dQ}{dt} \quad (1)$$

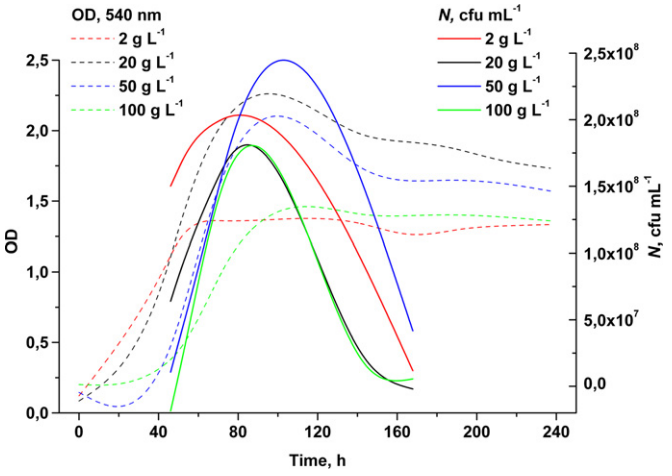

Fig. 4. The change of bacterial number (cfu mL^{-1}) and optical density (OD) in broth media with different substrate concentration (glucose 2, 20, 50, 100 g L^{-1}).

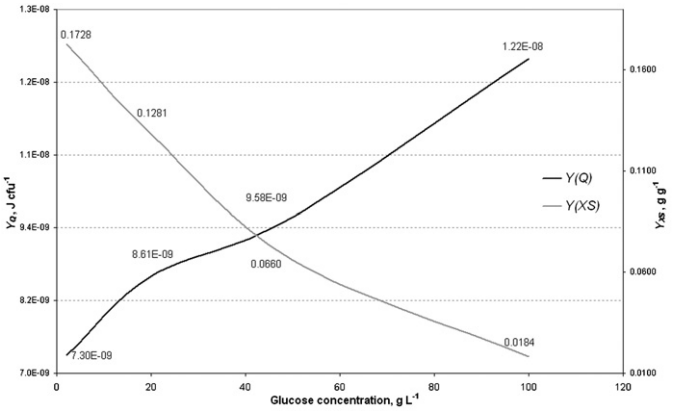

Fig. 5. Dependence of heat yield coefficient Y_Q ($J\,cfu^{-1}$) and Y_{XS} ($g\,g^{-1}$) determined for the exponential growth phase on different glucose concentrations 2, 20, 50, 100 $g\,L^{-1}$.

Knowing the value of Y_Q makes possible calculation of cell numbers and biomass from the power–time curves. For the calculation of Y_Q power–time curves and growth curves of biomass should be measured. Usually biomass is evaluated by optical density of the culture (OD), by number of cells in the culture broth determined by *outplating* (*N—bacterial number*, cfu mL^{-1}), and/or determined measuring dry biomass (*X*, g).

For the determination of constant k_1 which characterizes the relationship between optical density and bacterial cell number (cfu mL^{-1}) (Eq. (2)) the growth of *L. lactis* IL1403 was monitored in chemostat.

$$\Delta N = k_1 \times \Delta OD \tag{2}$$

monitored in chemostat. On the basis of the data collected the value of the constant was determined $k_1 = 1.63 \times 10^9 \pm 4.01 \times 10^8$ cfu. As known, bacteria are very actively growing in chemostat. The value of the same constant determined from the batch experiments presented in this paper was in exponential phase four times lower than in chemostat ($4.53 \times 10^8 \pm 7.7 \times 10^7$ cfu OD^{-1}).

The amount of biomass was estimated in chemostat experiments also through measurements of dry biomass. The value of the coefficient k_2 (Eq. (3)) was determined $k_2 = 0.32 \pm 3.05 \times 10^{-3}$ g OD^{-1}.

$$\Delta X = k_2 \times \Delta OD \tag{3}$$

On the basis of calorimetric *on-line* measurements of the power–time curves, *plate count* and OD (optical density) measurements the yield coefficients Y_Q, Y_{XS} were calculated. Both, Y_Q and Y_{XS} were calculated using the data of the exponential phase of the growth curves and corresponding intervals of the power–time curves in case of different glucose concentrations studied (Fig. 5).

As seen from Fig. 5, the heat yield was the lowest (7.30×10^{-9} J cfu^{-1}) at low glucose concentration (2 g L^{-1}). The heat yield value for the bacteria was growing practically linearly up to 1.22×10^{-8} J cfu^{-1} on increase of the glucose concentration. In full agreement with the observed behavior of the Y_Q the yield coefficient Y_{XS} which characterizes efficiency of biomass formation was decreasing on the increase of the glucose concentration—see Fig. 5. The value of Y_{XS} determined at the initial substrate concentration of 2 g L^{-1} was comparable to the values determined in the literature [31,34]. However, the values of Y_{XS} decreased remarkably on the increase of the initial glucose concentrations. It may indicate that homolactic growth was changed for some other scheme of fermentation of glucose (see de Vos and Hugenholtz [35]).

It should be emphasized that the calculation of Y_Q and Y_{XS} and their physical status is different here from the growth of bacteria on a single carbon source mineral media. It has been shown that bacteria *L. lactis* do not grow on mineral media. They need complex media for supporting their growth. However, there do not exist a medium where the consumption of the other than glucose components of the complex media could be measured. We have estimated that the consumption of other components (amino acids, vitamins, etc.) did not exceed 10% of the consumption of glucose in the chemostat experiments. Calculation of the values of parameters defined as yield coefficients Y_Q, Y_{XS}, etc. in these circumstances gives indicative values of the conversion coefficients, which, however, could be used in the interpretation of the experimental results obtained in the similar conditions.

4. Conclusion

The results obtained showed clearly that calorimetry is a reproducible and sensitive method for the continuous monitoring of anaerobic growth of *L. lactis*. Supplemented and combined with the results of parallel measurements of HPLC, *OD*, number of bacteria, etc. multichannel calorimetry is becoming a powerful method for the quantitative studies of bacterial growth.

References

[1] L. Yi, L. Xi, Q. Songsheng, S.J. Ping, J. Biochem. Biophys. Methods 45 (2000) 231–239.
[2] L. Gustafsson, Thermochim. Acta 193 (1991) 145–171.
[3] H. Vandenhove, Adv. Instrum., Data Interpret., Control Biotechnol. Process. (1998) 121–158.
[4] I. Lamprecht, Thermochim. Acta 405 (2003) 1–13.
[5] L. Wadsö, F.G. Galindo, Food Control 20 (2009) 956–961.
[6] D.A. Mitchell, O.F. von Meien, N. Krieger, F.D.H. Dalsenter, Biochem. Eng. J. 17 (2004) 15–26.
[7] M. Riva, D. Fessas, A. Schiraldi, Thermochim. Acta 370 (2001) 73–81.
[8] C. Alklint, L. Wadso, I. Sjoholm, J. Sci. Food Agric. 85 (2005) 281–285.
[9] U. von Stockar, L.A.M. van der Wieler, J. Biotechnol. 59 (1997) 25–37.
[10] M. Antwi, K. Barnaerts, J.F. Van Impe, A.H. Geeraerd, Int. J. Food Microbiol. 120 (2007) 71–84.
[11] P.D.G. Wilson, T.F. Brocklehurs, S. Arino, D. Thuault, M. Jakobsen, M. Lange, J. Farkas, J.W.T. Wimpenny, Van Impe J.F., Int. J. Food Microbiol. 73 (2002) 275–289.
[12] I. Boe, R. Lovrien, Biotech. Bioeng. 35 (1990) 1–7.
[13] A. Menert, M. Liiders, T. Kurissoo, R. Vilu, J. Therm. Anal. 64 (2001) 281–291.
[14] W.W. Forrest, D.J. Walker, Biochem. Biophys. Res. Commun. 13 (1963) 217–222.
[15] J.-S. Liu, I.W. Marison, U. von Stockar, J. Therm. Anal. 56 (1999) 1191–1195.
[16] A.E. Beezer, Thermochim. Acta 380 (2001) 205–208.
[17] X.M. Rong, Q.Y. Huang, D.H. Jiang, P. Cai, W. Liang, Pedosphere 17 (2007) 137–145.
[18] Z.-D. Nan, Y. Xiang, S.-Q. Cheng, X.-C. Zeng, H.-L. Zhang, J. Therm. Anal. Calorim. 63 (2001) 423–431.
[19] T. Maskow, W. Babel, J. Biotech. 101 (2003) 267–274.
[20] B. Schäffer, S. Szakály, D. Lörinczy, Thermochim. Acta 415 (2004) 123–126.
[21] J. Higuera-Guisset, J. Rodriguez-Viejo, M. Chacon, F.J. Muñoz, N. Vigues, J. Mas, Thermochim. Acta 427 (2005) 187–191.
[22] M. Peitzsch, B. Kiesel, H. Harms, T. Maskow, Chem. Eng. Process 47 (2008) 1000–1006.
[23] M. Winkelmann, R. Hüttl, G. Wolf, Thermochim. Acta 415 (2004) 75–82.
[24] J. Yao, L. Tian, Y. Wang, A. Djah, F. Wang, H. Chen, C. Su, R. Zhuang, Y. Zhou, M.M.F. Choi, E. Bramanti, Ecotoxicol. Environ. Saf. 69 (2008) 289–295.
[25] X. Wang, Y. Liu, B. Xie, X. Shi, J. Zhou, H. Zhang, Mol. Nutr. Food Res. 49 (2005) 350–354.
[26] Y. Chen, J. Yao, F. Wang, Y. Zhou, H. Chen, N. Gai, H. Chen, K. Chen, T. Maskow, B. Ceccanti, P. Trebse, G. Zaray, Curr. Opin. Microbiol. 57 (2008) 258–263.
[27] TA Instruments http://www.tainstruments.com/default.aspx?siteid=11.
[28] I. Wadsö, Thermochim. Acta 394 (2002) 305–311.
[29] I.A.M. Swinner, K. Bernaerts, E.J.J. Dens, A.H. Geeraerd, J.F. Van Impe, J. Food Microbiol. 94 (2004) 137–159.
[30] A.Z. Andersen, A.L. Carvalho, A.R. Neves, H. Santos, U. Kummer, L.F. Olsen, Comput. Biol. Chem. 33 (2009) 71–83.
[31] M. Papagianni, N. Avramidis, G. Filiousis, Microb. Cell Factories 6 (2007) 16.
[32] D.S.G. Pollock, Smoothing with cubic splines, in: Queen Mary and Westfield College, The University of London, England, 1993, http://webspace.qmul.ac.uk/dsgpollock/public_html/PAPERS/SPLINES.PDF.
[33] A. Razvi, Z. Zhang, C.Q. Lan, Biotechnol. Prog. 24 (2008) 852–858.
[34] S.J. Heijnen, Trends Biotechnol. 12 (2004) 483–492.
[35] W.M. de Vos, J. Hugenholtz, Trends Biotechnol. 22 (2004) 72–79.