2016 XIX ISBC Conference

International Society for Biological Calorimetry

June 22 – 24, **Basel**, Switzerland

Department of Biomedical Engineering

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ISBC 2016 Abstract book

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ISBC 2016 program

June 21	
18:00-20:00	Welcome reception. Meeting between summer school attendees and conference participants in a very informal setting. Early registration for congress participants **Location:** Center of Biomechanics & Biocalorimetry c/o Dept. Biomed.Engineering (DBE) Gewerbestr. 14 4123 Allschwil  Bus 48 Stop: im Brühl
June 22	
8:00-915	**Registration - Welcome coffee** **Location:** Schönenbuchstrasse 9, 4055 Basel, Switzerland. Oekolampad building  Tram 6 Stop: Allschwilerplatz
9:15-9:30	Welcome talk
9:30-12:00	**Session 1: Biomedical applications of calorimetry**
9:30-9:50 Muntean et al.	Analysis of microcalorimetric post antibiotic challenge response of bacteria-drug interaction: *Staphylococcus aureus* vs Cefoxitin
9:50-10:10 Gysin et al.	Microcalorimetric study of *Trypanosoma congolense* growth and growth inhibition by antitrypanosomal drugs
10:10-10:30 Solokhina et al.	Mycobacteria biofilm activity and susceptibility to antimycobacterial agents
10:30-11:00	**Coffee break - Poster viewing - discussion**

11:00-11:20 Lerchner et al.	A chip calorimetry based method for the real-time monitoring of red blood cell sickling
11:20-11:40 Astasov et al.	Calcium affinity to exopolysaccharides of cariogenic species
11:40-12:00	**To be announced**
12:00-13:30	**Lunch break**
13:30:16:40	**Session 2: New instrumentation and novel approaches**
13:30-13:50 Suurkuusk	The new generation TAM – TAM IV (sponsor presentation)
13:50-14:10 Leelaram et al.	Biocalorimetry a better monitoring tool in comparison with oxygen uptake (OUR) and carbon dioxide evolution (CER) rates in Yeast Inulinase production
14:10-14:30 Jansson	The calScreener: Four Years Down the Road- Application Diversity (sponsor presentation)
14:30-14:50 Mohan et al	Development of fermentation calorimeter with improved sensitivity for bioprocess monitoring and control applications
14:50-15:20	**Coffee break - Poster viewing - discussions**
15:20-15:40	C3 title to be announced (sponsor presentation)
15:40-16:00 Matthews et al	Applications of the NanoDSC (TA instruments) (sponsor presentation)
16:00-16:20	Calmetrix: title to be announced (sponsor presentation)
16:20-16:40	Syrris / Blacktrace: title to be announced (sponsor presentation)

June 23	
8:30-9:00	**Welcome coffee - registration** **Location:** Schönenbuchstrasse 9, 4055 Basel, Switzerland. Oekolampad building  Tram 6 Stop: Allschwilerplatz
9:00-12:20	**Session 3: Environmental calorimetry**
9:00-9:20 Rodenfels	Energetics of early vertebrate development
9:20-9:40 Kumar et al.	Role of monoculture in bacterial consortium in biotransformation of Azo dye: A Biocalorimetric investigation
9:40-10:00 Rohde et al.	Walk the line: Enhanced bioproduction using calorespirometry
10:00-10:20 Bölscher et al.	From soil calorimetry to climate change modelling: Temperature response of microbial resource-use efficiency
10:20-10:50	**Coffee break - Poster viewing - discussion**
10:50-11:10 Bravo et al.	A multi-method approach study on cadmium-immobilizing bacteria into Cocoa crops from northeastern Colombia
11:10-11:30 Maskow et al.	Never Ending Story: The development of photocalorimetry
11:30-11:50 Kula et al.	Changes in the chemical composition and metabolic activity in the green algal cells in response to silicon
11:50-12:10 Burnecki et al. Pres. by Saja	Assessment the toxicity of nickel using endosymbiotic *Paramecium bursaria*
12:10-12h30	Continuous monitoring by isothermal calorimetry of fungal colonization of wood materials

12:30-14:00	**Lunch break**
14:00-17:00	**Session 4: Food and related product calorimetry**
14:00-14:20 Wadsö	Studying grain germination by isothermal calorimetry
14:20-14:40 Yusof et al.	The Use of Isothermal Calorimetry to Measure the Metabolic Heat Production of Spinach Leaves as Consequences of Vacuum Impregnation Treatment
14:40-15:00 Torres et al.	Thermoanalytical investigations of honey produced by some species of stingless bees in Guainia, Colombia
15:00-15:30	**Coffee break**
15:30-15:50 Dodoo et al.	Evaluating the gastrointestinal fluid tolerance of personalized probiotics using calorimetry
15:50-16:10 Adamberg et al.	Metabolism of polysaccharides by fecal microbiota – a microcalorimetry study
16:10-16:55	**Lavoisier lecture by Prof. Nieves Barros**
19:00-22:30	**Congress diner**
June 24	
8:30-9:00	**Welcome coffee** **Location:** Schönenbuchstrasse 9, 4055 Basel, Switzerland. Oekolampad building  Tram 6 Stop: Allschwilerplatz

9:00-12:00	**Session 5: Biotechnology and calorimetry**
9:00-9:20 Brueckner et al.	Comparison of Tunable Diode Laser Absorption Spectroscopy and Isothermal Micro-calorimetry for Non-invasive Detection of Microbial Growth in Media Fills
9:20-9:40 Hagedoorn et al	Feel the heat of an enzyme - calorimetry of enzyme catalyzed reactions
9:40-10:00 Jacobs	Absolute, label-free measurement of enzyme activity by microcalorimetry
10:00-10:20 Linkuvienė et al	Thermodynamics of carbonic anhydrase – inhibitor binding for drug design
10:20-10:50	**Coffee break - Poster viewing - discussion**
10:50-11:10 Müller et al.	Probing Protein Adsorption on Surfactant-Coated Nanoparticles with Isothermal Titration Calorimetry
11:10-11:30 del Río et al.	Worth reconsidering the Wiseman Isotherm?
11:30-12:00	**Poster session**
12:00-13:30	**Lunch break**
13:30-15:00	**Closing session: open topics**
	- next ISBCs
	- education in calorimetry, feedback from this year summer school
	- integration of calorimetry, link with other societies
	- topic proposed by the audience

ISBC 2016 Poster session

Abenojar et al.	Polymerization kinetics of acrylic bone cement: effect of two different antibiotics.
Águas et al.	A Combined Calorimeter/Biosensor for the Study of Biomolecular Interactions
Lee et al.	Design of a micro calorimeter for biological samples
Kabanova et al.	Application of microcalorimetric method for the study of the growth of starter cultures in milk
Lerchner et al.	Continuous monitoring of drug effects on tissue samples by segmented flow chip calorimetry
Pinzon et al.	Arrhenius Parameters for the pyrolysis of stingless bees waxes in Norte de Santander, Colombia
Wadsö et al.	Studying glucose-amended soil samples with isothermal calorimetry

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Abstracts by alphabetical order

Polymerization kinetics of acrylic bone cement: effect of two different antibiotics.

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In this work the influence of addition of vancomycin and cefazolin antibiotics is evaluating in relation with the polymerization kinetic. Antibiotic-loaded bone cements are widely used for primary hip arthroplasties due to the intractability and high cost of infections. Cement with antibiotic has been shown as one of the most effective composite for therapeutic arms in both the prophylaxis [1] as in the treatment of periprosthetic infection [2,3]. Before new cements are clinically implemented, it is essential to investigate their thermal properties.
Thermal and chemical necrosis are the two major problems associated with the usage of bone cements. In this study, PALACOS®R+G, commercial bone cement, was evaluated using differential scanning calorimetry (DSC), dynamic methods from 0 to 200 ºC at 5 ºC/min for determining glass transition temperature. The second test was an isothermal one; a temperature range of 10, 20 and 30 °C and duration of 30 min were chosen; a second segment of dynamic scanning from the isothermal temperature to 180°C at a scan rate of 10°C/min was added, this assay was performed to calculate the free residual monomer percent and kinetic parameters. The influence of the addition of two antibiotics, Vancomycin and Cefazolin in various quantities, on the rate and heat of polymerization and glass transition temperature of resulting polymer was investigated, and the kinetic parameters were calculated by Kamal equation.
The glass transition temperatures, for the antibiotic-loaded bone cements and of the basic bone cement, were similar. Besides, under isothermal method, the addition of the two antibiotics had no discernable adverse effects on the polymerization kinetics. These conclusions imply the thermal safety of the new antibiotic-loaded bone cements, in terms of the bone cement polymerization.

Keywords: Bone cements; antibiotics; polymerization kinetic; thermal properties

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Metabolism of polysaccharides by fecal microbiota – a microcalorimetry study

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Dietary fibers promote the healthy gut microbiota and overall wellbeing of the host. The aim of this study was to compare the impact of different polysaccharides (resistant starch, levan, inulin, arabinogalactan, xylan, pectin) and a glycoprotein mucin on growth and metabolism of the fecal microbiota *in vitro* by using isothermal microcalorimetry (IMC). Fecal samples from healthy donors were incubated in phosphate-buffered defined medium with or without supplementation of a dietary fiber. The generation of heat, microbiota composition, and concentrations of metabolites during the growth were determined.

The multiauxic power-time curves generated during sequential degradation of oligo- and polysaccharides were sample and substrate-specific. All substrates except chitin were fermented with generation of 2-8 J/ml. Different metabolite patterns were in accordance with the microbial dynamics. General distribution of bacterial taxa shows some similarities (more *Bacteroides ovatus*, less *Bifidobacterium adolescentis*) for arabinogalactan, xylan and levan. In different from FOS and inulin, arabinogalactan, xylan and mucin enhanced formation of propionic acid, whilst mucin and pectin did not promote lactate production or it was converted to other acids. Mucin fermentation resulted in the highest amounts of butyrate. Associated growth of levan-degrading (e.g. *Bacteroides*) and butyric acid-producing (e.g. *Faecalibacterium*) taxa was observed in levan-supplemented media.

The study shows that the effect of different dietary fibers on colon microbiota varies in great extent. The data from *in vitro* experiments can be applied in development of high-fiber diets to modulate the microbiota by diet composition. IMC combined with analytical methods is an effective methods for screening the impact of dietary fibers on function of fecal microbiota.

Acknowledgements. *The project has received funding from the European Regional Development Fund (projects No. 3.2.0701.12-0041, managed by Archimedes Foundation and EU29994), & Institutional Research Funding (IUT 1927) of the Estonian Ministry of Education and Research.*

A Combined Calorimeter/Biosensor for the Study of Biomolecular Interactions

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The study of biological recognition is an area of considerable interest, important both in basic science as in applied research. For example, understanding the thermodynamics of binding is very useful to modern pharmacology, namely in the drug design field. Although a number of methods are available to measure binding, only calorimetry can afford its complete thermodynamic characterization. However, heat is not a specific property, often rendering the calorimetric experiments both very time consuming and of complex interpretation.

The recent technique of Quartz Crystal Microbalance (QCM) with Heat Conduction Calorimeter (HCC) [1], applied to liquid samples, allows overcoming those limitations. The biosensor provides the detection properties of their immobilized biological compounds to the calorimeter, allowing to assign the heat measured specifically to the reaction under study.

We have been developing a QCM/HCC system to screen the interactions between several examples of biomolecules and ligands. It consists on a non-conventional differential QCM coupled with a conventional differential HCC.

Current conventional HCCs are still lacking in sensitivity with regards to QCM biosensors. In this work we developed calibration techniques together with flow optimization in order to increase the HCC sensitivity.

Keywords Quartz Crystal Microbalance; Heat Conduction Calorimeter

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Calcium affinity to exopolysaccharides of cariogenic species

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In cariogenic biofilms, exopolysaccharides (EPS) provide an abundance of primary binding sites with different chemical functional groups such as carboxylic acids, phosphates and amines and form the core of the matrix-scaffold. Among these groups carboxylic acid and phosphates are known to bind calcium and other biologically important cations like magnesium, iron or zinc. During caries development calcium gets dissolved from enamel, however little is known whether this dissolved calcium is bound within the cariogenic biofilm. The aim of the study was to investigate and refine the role for the acid-base and calcium binding properties of the EPS of *Steptococcus mutans*, *Lactobacillus rhamnosus*, and *Candida albicans*. Acid-base titration of the EPS revealed that it contained mostly acidic proton binding sites. Some binding sites near neutral and alkaline pH were observed, however in much lower concentrations. Isothermal titration calorimetry (ITC) showed that EPS from bacterial strains had a binding affinity one order of magnitude higher to calcium than to lactic or citric acid. However, for EPS purified from *C. albicans*, titration data revealed relatively weak binding thereby not allowing estimation of an accurate binding affinity. In conclusion, the study reveals that within the biofilm matrix EPS can bind calcium with a higher affinity than organic acids thus, providing the cariogenic species with a possible mechanism to withstand higher concentrations of calcium ions which unbound could have toxic effects.

Keywords: caries, biofilm, exopolysaccharide matrix, calcium, isothermal titration calorimetry

Role of monoculture in bacterial consortium in biotransformation of Azo dye: A Biocalorimetric investigation

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Natural bacterial metabolisms and interactions are generally regarded as complex, the complexity of the bioprocess increases with the addition of more than one species in the reaction[1]. Bacteria isolated from the native habitat, can effectively perform an identical task in artificial laboratory condition mostly in the presence of a similar microbial community[2]. This phenomenon is due to the symbiotic relation between the microbial community and division of labour among the species. Biotransformation of azo dye by bacterial consortium has been taken as a model reaction and bioreaction calorimeter (Bio-RC1e) has been used to monitor the bacterial community.
Heat dissipation is the universal factor in the biological reactions[3]. The present study unsnarls the role of monoculture in the community, and the possible trade-off (metabolite) mechanism involved in the biotransformation of an azo dye between the species could be identified. By studying the role of individual species in a community, the reaction can be further engineered to do the desired task efficaciously. The results are valuable in identification of dominant – recessive species in the bacterial community and to fingerprint the unique metabolism of monoculture. The study will also signify the use of unique heat flow pattern in predicting the species responsible for active metabolism in the bacterial consortium.

Keywords: Natural bacterial community; microbial interaction; pathway predictions; bioenergetics.

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From soil calorimetry to climate change modelling: Temperature response of microbial resource-use efficiency

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Microbial soil respiration is a major flux in global greenhouse gas emissions. Its size is determined by the resource-use efficiency of the decomposer community and the availability of soil organic carbon for microbial decomposition. Experimental evidence suggests that the former is temperature sensitive[1] and varies among land-use management systems[2]. Most Earth system models, however, consider resource-use efficiency as a constant property. We tested the temperature sensitivity of resource-use efficiency across land-uses applying a calorimetric approach. The results were then incorporated into an Earth system model to assess the consequences for projected soil respiration and thus potential impacts on our future climate.
Soils were sampled from arable, grassland, ley-farming, and forest research sites exposed to a boreal climate (64°07´N, 19°27´E). The samples were amended with two carbon substrates and incubated at a set of temperatures ranging from 5 to 20 °C. Resource-use efficiencies were calculated from heat production and residual substrate[2] when 15 % of the added substrate was utilized. Land-use specific temperature sensitivity curves were fitted to the data and incorporated into the Q model[3] using Sweden as a case study.
Our results show that the temperature response of microbial resource-use efficiency is varying across land use management systems, a hitherto unknown phenomenon. Resource-use efficiencies in arable soils were not temperature sensitive and microbes residing in forest soils were most temperature sensitive. Comparing projections of soil respiration assuming (i) constant or (ii) temperature sensitive resource-use efficiency revealed differences in projected CO_2 emissions. Our findings emphasize the need to understand temperature responses of microbial resource-use efficiency. Further studies should establish reliable proxies for resource-use efficiency which can be exploited in model approaches.

Keywords: soil organic carbon, resource-use efficiency, temperature sensitivity, land use, isothermal calorimetry, soil carbon model

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A multi-method approach study on cadmium-immobilizing bacteria into Cocoa crops from northeastern Colombia

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Cadmium (Cd) is a non-nutritive metal occurring naturally on earth-crust and one of the biggest challenges in Cocoa (*Theobroma cacao* L.) seeds quality in South America. Cadmiun content in grains of cocoa is regulated due to its impact on public health but very few is known regarding soil microbiota related with cadmium dynamics at Cocoa subsurface soil. The aim of this research was to assess microbiological and geophysical aspects to understand the dynamics of total-Cd, available-Cd and Cd-immobilizing bacteria in Cocoa soils at northeastern Colombia. To achieve such goal, we performed an integration of i.) a 2D geoelectrical resistivity profiling, ii.) a culturing assay of soil microorganisms with inversed Petri dish plating method, iii.) a sequential fragmentation of Cd in soils and iv.) an isothermal microcalorimetry assay (IMC), to determine dynamics of cadmium and Cd-immobilizing microorganisms in Cocoa soils from three regions. The regions i. Arauca, ii. Boyacá and iii. Santander were selected because of their importance in Cocoa production over all the country and the farms due to the high cadmium content in seeds there. Crops from Santander region did showed major Cd-immobilizing microorganisms (37 bacterial and 7 fungal isolates) in comparison with other sampled sites (26 and 8 bacterial isolates, 2 and 0 fungal isolates in Boyacá and Arauca, respectively). The bacterial isolates CdDB30 and CdDB41 have shown major metabolic capacities to tolerate Cd (2.60 to 2.52 maximum heat produced for CdDB30 and 2.21 to 2.32 in CdDB41 at 6 and 24 $mg.L^{-1}$ $CdCl_2$, respectively – in addition theses isolates did not show a decrease in growth rate when exposed to Cd with higher reduction capacities of Cd^{+2} (0.240 and 0.220 h^{-1}, respectively). On the other hand, a farm in Boyacá did showed so far the higher level of available-Cd in soil (0.152 mg kg^{-1}) whereas farms in Arauca and Santander did showed lower levels (0.027 and 0.019 mg kg^{-1}, respectively). The correlation coefficient between geoelectrical profiles and total-Cd was high (R=0.84), as well as between available-Cd and Cd-immobilizing bacteria (R=0.82), which could be related to major presence of both chemical forms of cadmium at the top and bottom horizons (70% of all the soil profiles performed). The data have shown as well, an inverse correlation between soil pH and total-Cd at the top and bottom horizons at each profile. Further research will be focus on determine the impact of adding autochthonous Cd-immobilizing bacteria i.e. CdDB30 and CdDB41, in pot experiments with seedlings of *T. cacao* L. and cadmium.

Keywords Cadmium, 2D electrical resistivity, soil pH, Cd-immobilizing microorganisms

Comparison of Tunable Diode Laser Absorption Spectroscopy and Isothermal Micro-calorimetry for Non-invasive Detection of Microbial Growth in Media Fills

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Two methods were investigated for non-invasive microbial growth-detection in intact glass vials as possible techniques for automated inspection of media-filled units. Tunable diode laser absorption spectroscopy (TDLAS) was used to determine microbially induced changes in O2 and CO2 concentrations within the vial headspaces. Isothermal microcalorimetry (IMC) allowed the detection of metabolic heat production. *Bacillus subtilis* and *Streptococcus salivarius* were chosen as test organisms. Parameters as robustness, sensitivity, comparability and time to detection (TtD) were evaluated to assess method adequacy.

Both methods robustly detected growth of the tested microorganisms within less than 76 hours using an initial inoculum of <10CFU. TDLASO2 turned out to be less sensitive than TDLASCO2 and IMC, as some false negative results were observed. Compared to the visual media-fill examination of spiked samples, the investigated techniques were slightly slower regarding TtD.

Although IMC showed shorter TtD than TDLAS the latter is proposed for automating the media-fill inspection, as larger throughput can be achieved. For routine use either TDLASCO2 or a combination of TDLASCO2 and TDLASO2 should be considered. IMC may be helpful for replacing the sterility assessment of commercial drug products before release.

Keywords: microcalorimetry, tunable diode laser absorption spectroscopy, microbial growth, sterility testing, media fill.

Assessment the toxicity of Ni using endosymbiotic *Paramecium bursaria*

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Paramecium bursaria (Ehrenberg 1831) is an unicellular ciliate widely distributed in freshwater environments. Cells of the green *P. bursaria* contain endosymbiotic algae which release photosynthetic products to the host cell and in exchange the ciliate provides the endosymbionts with nitrogen components and CO_2. Algae bearing paramecia can grow better and are more resistant to environmental pollutants than algae free ciliates. Exposure of protist cells to heavy metals can induce the synthesis of low molecular weight, thiol rich proteins. *P. bursaria* can be used as bioindicator to evaluate the environmental pollution. The aim of this study was to determinate the sensitivity of *P. bursaria* cells and its endosymbionts to nickel chloride by microscopic observation of cells behavior, rate of cell divisions and by measuring the fluorescence emission spectra and metabolic activity

Paramecium bursaria cells were cultured on a lettuce medium (Sonneborn1970) inoculated with *Klebsiella pneumonia* (SMC) at temperature 18°C, under light/dark conditions (12L (12D). The ciliates were incubated in the solution with $10^{-3}g/dm^3$, $10^{-4}g/dm^3$,$10^{-5}g/dm^3$, $10^{-6}g/dm^3$ and $10^{-7}g/dm^3$ of $NiCl_2$.

Results showed that high concentration of nickel ions ($10^{-3}g/dm^3$, $10^{-4}g/dm^3$) have a lethal effect on *P. bursaria*. After incubation in the solution with $10^{-5}g/dm^3$ of $NiCl_2$ paramecia remained alive but immobilization by suppressing the co-ordination of the ciliature was observed. There was also registered the decrease in the rate of cell division. The analysis of photosynthesis of green endosymbionts showed a significant reduction of photosynthetic intensity (in the blue and red light spectrum) compared to the control group. The nickel chloride significantly affects the metabolic activity of *P. bursaria* cells. This effect depends on the concentration solution. In the $10^{-5}g/dm^3$ of $NiCl_2$ solution was observed the greatest decrease specific thermal power (endothermic processes). In control and the smallest concentration $NiCl_2$ solution ($10^{-7}g/dm^3$), were showed only exothermic processes, which indicates on better growth and vitality of cells. Results obtained has shown that the monitoring of metabolic activity of *P. bursaria* cells by using isothermal calorimetric methods allows rapid evaluation the level of tolerance and sensitivity this ciliate to the presence of nickel ions in the aquatic environment.

Keywords *Paramecium bursaria*, $NiCl_2$, immobilization, cell division, fluorescence metabolic activity

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Worth reconsidering the Wiseman Isotherm?

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The use of Isothermal Titration Calorimetry (ITC) begins in the second half of the 60's of the last century along two paths. One was the North American line; it was applied to chemical reactions using homemade equipments in different laboratories. The other was the Swedish line; it yielded commercial calorimeters. In the 80's the sensitivity of these instruments was increased and in the 90's the isothermal titration calorimetry becomes popular through the massive commercialization of these instruments and the economic interests related to applications in biology, biochemistry, biophysics, bioengineering and pharmacy. The success of these applications was such that this experimental technique was re-defined only in terms of free energies, enthalpies, entropies and binding constants. In fact, developments and applications in other areas have lost popularity and the interest of potential users.
The accessible price of these instruments, the velocity in producing results, and the success in terms of amount and suitability of these results made this technique to be something like a "product line" included in the "catalog of services" of many laboratories. Meanwhile, the result of this large scale utilization has been that the thermodynamic fundament of the measurements and their in-depth thermodynamic interpretation on a rational understanding have been left on the side.
One of the most used tools in isothermal titration calorimetry is the Wiseman Isotherm. It describes the binding of a ligand to a macromolecule with a 1:1 stoichiometry and it has been extended to more complex binding models. After its detailed analysis there are reasons to think that the Wiseman isotherm is an artifact. In this work we show an alternative approach based upon a rigorous thermodynamic treatment [1-3].

Keywords: Binding, Thermodynamics, Isothermal Titration Calorimetry

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Evaluating the gastrointestinal fluid tolerance of personalized probiotics using calorimetry

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In recent times there has been a heightened interest in probiotics and the probiotic market is projected to be worth US$ 96.0 billion globally by 2020. This interest also arises from the increasing number of health claims attributed them. Despite these numerous claims, the viability of probiotic species after delivery have been questioned, with some arguing that probiotics don't contain the number of cells indicated on the packs and others suggesting bacteria cannot survive exposure to the harsh pH of gastric acid. To address these issues, we are currently developing an on-demand method of probiotic formulation using ink-jet printing wherein bacteria are printed onto oral films. We evaluate the tolerance of these ink jetted formulations to gastric fluids using isothermal microcalorimetry. This technique has been used previously to study single microorganisms as well as mixed cultures [1]. The isothermal calorimeter used in this work was the 2277 Thermal Activity Monitor whilst on-demand production of probiotics was done with the aid of a modified thermal inkjet printer (HP 5940). The probiotic (*L. acidophilus*) formulation was prepared by ink jetting the bacterial suspension onto edible starch paper. Droplets dried quickly under ambient conditions. The films were then suspended in 3 mL growth medium in a sterile ampoule and growth characteristics were observed with calorimetry. The next set of experiments involved suspending the printed substrate in simulated gastrointestinal fluids (simulated gastric fluid, simulated intestinal fluid and pig intestinal fluid) for 2 hours at 37 °C. 30 μl of the resultant medium was then added to 2970 μl of pre-warmed (37 °C) growth medium in sterile ampoule. The ampoules were hermetically sealed by crimping in all instances and calorimetric readings taken for a period of 40 hours. Thermograms obtained were expressed in the form of power (mW) against time (hours). Integration of these thermograms resulted in the typical sigmoid shape characteristic of bacterial metabolism with distinctions observable in terms of onset of metabolism as well as the time taken to attain the stationary phase. The cumulative energy generated from metabolism was also take into consideration in analysis. The results obtained clearly showed that probiotics cannot not tolerate gastric fluids with a baseline signal being obtained.

Evaluations in simulated intestinal fluid and pig intestinal fluid however showed good tolerance observed in the form of relatively high intensity power signal. In conclusion, the calorimeter has been used here in the evaluation of an ink jetted probiotic formulation in developmental stages and from the results, an indication of targets for delivery and barriers to overcome during delivery have been obtained.

Keywords: probiotics, isothermal calorimetry, gastrointestinal fluid tolerance

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Isothermal calorimetric for enzymatic hydrolysis kinetics

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The objective of this study is to monitor kinetics, closed to the optimal operating conditions, when depolymerisation of straw occurs giving a maximum of fermentable sugar. In particular, the role of an enzyme recently identified, Cellobiose dehydrogenase (CDH), is investigated when this one acts together with identified cellulases.
The temperature affects the amount of heat generated. Indeed, the heat at 50 °C is greater than that released at 45 °C and 40 °C. In addition the increase of the temperature is concomitant with the height of the peak. It is also clear that the heat flow quickly drops in the case of reaction at 50 °C compared to 40 °C. This result can be explained by the fact that the reaction is faster at 50°C so the available substrate decreases rapidly. These results confirm the influence of the temperature on the enzymatic activity.
To verify if the registered heat can be correlated with the degree of hydrolysis (the amount of sugar produced / unit time), glucose produced was measured by a colorimetric method using glucose RTU kit. These tests were performed in triplicates.
Calorimetric signal was at least used to calculate kinetics. Validation on a simple substrate is performed.

Keywords hydrolysis, straw, kinetics

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Microcalorimetric study of *Trypanosoma congolense* growth and growth inhibition by antitrypanosomal drugs

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The tsetse-fly transmitted parasite *Trypanosoma congolense* is the most important causative agent of African animal trypanosomiasis, also called Nagana, in sub-Saharan Africa [1]. If left untreated, infected animals will succumb and eventually die. The most efficient way of control depends mainly on chemotherapy. However, due to extensive drug use and the administration of sub-therapeutic doses, drug resistance has developed and is now widely spread, rendering current drugs ineffective against infection [2].
Isothermal microcalorimetry is a highly sensitive and simple tool, which can be used to study the growth of the pathogenic protozoans *Plasmodium falciparum* and *Trypanosoma brucei* [3]. We present the first microcalorimetric experiments with *T. congolense.* This method enables to study small growth differences between different strains or different subpopulations. Unlike *T. brucei*, *T. congolense* grows *in vitro* in two distinct phases, one attached to the base of the culture vessel, whilst the other swims freely near the surface of the culture medium. We compared the phenotypes of the two phases via microcalorimetric real-time measurements.
This technology can additionally be utilised to monitor parasite inhibition in the presence of drugs, enabling the determination of parameters, such as onset of drug action and time to kill [3]. Exemplarily, we present *T. congolense* growth inhibition by a standard drug, used for the treatment of Nagana.

Keywords *Trypanosoma congolense*; Nagana; microcalorimetry; growth inhibition by drugs; parasite chemotherapy

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Feel the heat of an enzyme - calorimetry of enzyme catalyzed reactions

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In order to measure the performance of an enzyme, usually, changes in substrate or product concentrations are measured. This means that, in principle, for every enzyme and every substrate/product a different assay has to be developed. This is currently a bottleneck in high-throughput approaches to systematically engineer industrially useful biocatalysts. Furthermore, the activity of many enzymes has been reported towards artificial substrates, e.g. with a fluorogenic group attached. It is not trivial to measure the activity of enzymes towards their natural substrates. Calorimetry offers a way to bypass the need to measure substrate/product levels, but instead measure the heat that is being developed (or consumed) during a chemical reaction.
A challenge is, however, to determine accurate kinetic parameters (i.e. k_{cat}, K_M values) for reversible enzymes, enzymes that produce insoluble products or utilize insoluble substrates. Furthermore, the kinetics of the heat evolution in a calorimetric measurement does not directly correspond to a real time observation of the chemical kinetics. In order to circumvent this problem we have developed a new label-free approach for obtaining initial rate of enzyme activity from heat measurements, which we have named initial rate calorimetry (IrCal) [1]. This approach is based on our finding that the ITC data recorded directly after injection of the substrate, are correlated the initial rates. Using IrCal we were able to accurately determine the kinetic parameters of different enzymes with their natural substrates, e.g. proteases with protein substrates.
Because heat evolution or consumption is a label-free property of almost all chemical reactions, the IrCal approach holds promise in the fundamental study of enzymes and in the use of calorimetry for high throughput screening of enzyme activity.

Keywords Isothermal titration calorimetry, enzyme kinetics, Initial rate calorimetry IrCal

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Absolute, label-free measurement of enzyme activity by microcalorimetry

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Enzyme activity assays are performed by almost every scientist in the biosciences. However, this hardly ever results in an absolute activity measurement (the number of substrate turnovers/time) under in vivo-like conditions as labeled substrates have to be used. As a consequence, accurate specific activities of enzymes for in vivo or application conditions are simply not available. Present research activities in life sciences and synthetic biology need these data for further optimization of their systems. Microcalorimetry offers a generic read out for enzyme kinetic parameters by detecting the heat release during the enzyme catalyzed reaction. This technology enables the use of natural, label-free substrates. Isothermal Titration Calorimetry (ITC) has been used for many enzyme studies, however the technology is very time consuming and challenging with respect to data interpretation [1]. Recent developments in both instrumentation and data interpretation open up new possibilities for accurate and high throughput enzyme calorimetry. Progress will be presented on the accurate determination of enzyme kinetic parameters by measuring the initial rates of enzymatic reactions using ITC [2], on the automation of chip-based flow calorimetry [3] and on modular coupling of the flow calorimeter with analytical technology for enzyme purification and product identification. This unique combination of powerful analytical technologies will provide fundamental enzymology knowledge with industrial relevant applications.

Keywords enzyme; kinetics; chip calorimeter;

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Acknowledgement: This work was carried out within the BE-Basic R&D Program, which was granted a FES subsidy from the Dutch Ministry of Economic affairs, agriculture and innovation (EL&I)

The calScreener: Four Years Down the Road- Application Diversity

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The calScreener calorimeter concept was first presented to the ISBC audience four years ago at the Leipzig meeting as a working concept. Since then a number of different biological applications has been tested to evaluate both the performance of the equipment as well as finding the most suitable areas for this type of calorimeter.

The calScreener is a commercially available calorimeter and was envisioned as a tool for cell based assays based on calorimetry. The solution provides high sample throughput combined with small sample volumes and pre-sterilized consumables to facilitate the growth and analysis of mammalian cells, bacteria and other living matter.

The areas tested for applications development include a wide variety of fields. calScreener has been tested for monitoring of bacterial growth, both planktonic and biofilm-forming bacteria as well as for the detection of slow growing mycobacteria. It has also been employed for the testing of bacterial growth in fresh water production and distribution. Further testing has employed testing of fungal pathogens in agricultural development programs as well as anti-parasite drug development for tropical diseases. A large proportion of the application development has been focused around the monitoring of cellular activities in metabolic disease and adipose tissue differentiation. Also cell killing potency of mAb development has been tested using the system.

One of the main interests in testing a wide variety of possible applications has been to find the areas where calScreener can make the larges impact, to try to gain interest and momentum for the calorimetry based assays in a wider audience. The most favorable areas have been in bacterial detection especially for monitoring of biofilm formation and antibacterial compound screening as well as for the metabolic diseases area.

What we conclude from the testing and applications development is that given the right setting it is easy to convince a wider audience that calorimetry based cell assays can provide novel insights into research that is not possible with conventional technology and that calorimetry does not need to be confined to a smaller group of users, it is ready for a well-deserved large breakthrough in modern cell biological research.

Application of microcalorimetric method for the study of the growth of starter cultures in milk

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Growth of different starter cultures in milk, 2x concentrated milk and the mixture of these milks in case of different rennet concentrations (0, 1.2 and 2.4 mg/mL) was studied using thermal activity monitor TAM III in parallel to iCinac pH controlling system. Milk concentrated by reverse osmosis has a great developmental potential for innovative fermented dairy products. Utilization of concentrated milk allows to reduce production time, increase the product yields and minimize the amount of waste (for example whey).
The growth of two quark starter cultures containing two different *Lactococcus lactis* subspecies and three different *Lactococcus lactis* species + *Leuconostoc mesenteroides* were studied in different milks. Further, the effect of the temperature (30C and 42C) on the growth of the mixture of mesophilic and thermophilic starter culture bacteria was investigated. The results obtained showed that microcalorimetric curves provide remarkably more information regarding the growth and metabolic peculiarities of starter cultures in comparison to standard pH curves that are commonly used for the description of starter culture activity in dairies. The maximum specific growth rate and amount of heat produced during growth are higher in concentrated milk due to higher concentrations of lactose and free amino acids. The pH at the end of bacterial growth is higher in concentrated milk in comparison to ordinary milk due to higher buffering capacity. The addition of rennet has a minor negative effect on bacterial growth both in milk and in concentrated milk.
The results of this work are the basis for further research and understanding of the peculiarities of bacterial growth in concentrated milk for the development and production optimization of innovative dairy products.

Keywords: microcalorimetry; bacterial growth; starter culture; milk fermentation; concentrated milk

Changes in the chemical composition and metabolic activity in the green algal cells in response to silicon

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Silicon (Si) is a micronutrient necessary for the life of all organisms. In plants accumulates in the cells of the epidermis, mesophyll, xylem and sclerenchyma, mainly in cell walls [1, 2]. In algae the largest amount of silicon are collected by diatoms, which needed it mainly for the production of cell encasement. Deficient in this element results in inhibition of cell division, synthesis of protein, nucleic acids, fatty acids, and chlorophyll, and also makes dysfunction of photosynthesis [3]. Therefore, it is suggested that supplementation with silicon medium can have a positive impact on the greater vitality of others outside diatoms algae, their resistance to adverse environmental conditions and increase in biomass. Algal biomass has found application in various branches of industry. Their potential lies in the relatively small surface necessary for cultivation in comparison to the typical energetic plants.

The aim of the study was to evaluate the chemical composition and metabolic activity of cells of algae – *C. vulgaris* and *S. armatus*, grew on medium enriched silicon. Estimated metabolic activity, growth, changes in the morphology and chemical composition of algae cells. The strains of *C. vulgaris* and *S. armatus* have been cultured on the medium Kessler and BBM respectively, in which a solution of study sample was used silicon instead of distilled water.

The results show that a solution of silicon significantly speeds up the growth of algae biomass, while causing the significantly lower their metabolic activity. The analysis of the course of specific thermal power-time curves ($mW \cdot OD^{-1}$) indicates that algae cells of both species growing on the control medium show a significantly higher emission rate of thermal power (with *C. vulgaris* 6 times and *S. armatus* about 2 times) relative those growing on medium with silicon. Studies have confirmed that isothermal calorimetry is a quick way to evaluate the productivity of algae. In addition, it is shown that the amount of heat emitted by the cells, regardless of the strain of algae is inversely proportional to biomass production.

Keywords algae; silicon; isothermal calorimetry; the chemical composition; metabolic activity; morphology

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Design of a micro calorimeter for biological samples

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There are numerous biological processes which are related to heat production and the heat information can be used in various applications, for example the investigation of the bacteria metabolism [1]. So it is very useful to develop a device which can measure small amount of heat rate corresponding to nanowatt level. In this study, a design of microchip calorimeter is developed and the expected fluid and thermal results of the chip are presented which are obtained by using CFD software, COMSOL. The microchip is composed of a microchannel, in which biological samples are inserted, a heater made of platinum and temperature sensors made of several thin film thermopiles. The expected performances of the microcalorimeter such as heat rate resolution are also presented.

Keywords microcalorimeter, bacteria metabolism, thermopile

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Biocalorimetry a better monitoring tool in comparison with oxygen uptake (OUR) and carbon dioxide evolution (CER) rates in Yeast Inulinase production.

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The design of experiment and quality by design (QbD) in any industry should have advanced monitoring and control systems in place. In bioprocess industry, Paucity of suitable sensors and online monitoring tool affects the product yield and cost [1]. Real-time monitoring the metabolic state of the cell can improve the overall batch efficiency. High sensitive real-time heat flux measurement using biocalorimetry (BioRC1) can be efficiently employed for this purpose. In recent past high cell density (HCDS) cultures of *Kluyveromyces marxianus* [2] and recombinant *E coli* cells [3] was achieved on the basis of biocalorimetric heat signals, in which, heat based feed-forward control helped in maintaining specific growth rate.

In our study heat release pattern helped in monitoring the biomass growth and inulinase production better than oxygen uptake rate and carbon dioxide evolution rate. In batch inulinase production, after substrate depletion the OUR and CER values were minimal. This implies that OUR and CER are mainly due to sucrose metabolism with a minor contribution from enzyme expression. This observation emphasizes the usefulness of biocalorimetric monitoring. Mere OUR and CER monitoring will not throw light on enzyme production as both deplete as substrate depletes. When enzyme expression starts to peak the heat flow pattern is steady. This change is not reflected in CER and OUR but observed in heat release profiles.

Further our study reiterates feed forward control of specific growth rate in fed-batch cultures can be used for achieving higher inulinase activity in BioRC1. The heat based empirical model can be used for real-time prediction of inulinase production and culture's metabolic status.

Keywords Biocalorimetry; metabolic heat; *Kluyveromyces marxianus;* inulinase.

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A chip calorimetry based method for the real-time monitoring of red blood cell sickling

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Sickle-cell disease (SCD) is a hereditary blood disorder characterized by an abnormality in the oxygen-carrying hemoglobin molecule in red blood cells. Caused by a point mutation in the ß-globin gene, sickle-cell hemoglobin polymerizes into a 14-stranded polymer when in its deoxy state and depolymerizes when it is well oxygenated [1]. This leads to a propensity for the cells to assume an abnormal, rigid, sickle-like shape under certain circumstances. Sickle-cell disease is associated with a number of acute and chronic health problems, such as severe infections, attacks of severe pain, and stroke and there is an increased risk of death.

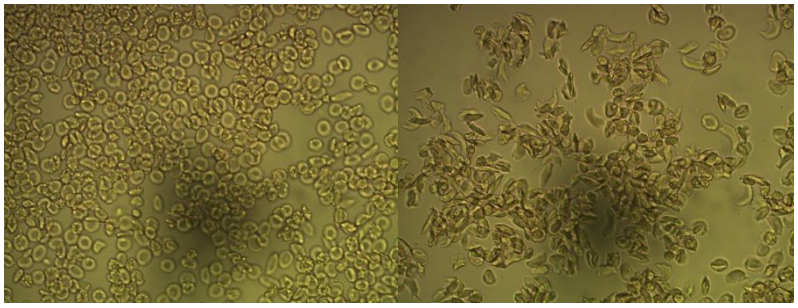

Fig. 1: Oxygenated (left) and de-oxygenated (right) red blood cells of a SCD patient.

Abnormalities in sickle-cell blood are also reflected by changes in the metabolic activity of erythrocytes as demonstrated by calorimetric measurements several years ago [2]. The comparison of the heat production rate of blood samples obtained from SCD patients with those from healthy control persons showed an increase by factor of two for SCD erythrocytes. However, it was not yet shown to which extent sickled red blood cells contribute to the higher heat production by itself.
In general, the fraction of sickle cells in oxygenated SCD patient's blood is low (see Fig. 1, left). In order to study effects of sickle cell formation in real-time a continuous monitoring of metabolic heat rates as response to the de-oxygenation of the sample is required. We will demonstrate new features of a segmented flow chip calorimeter which allow the defined oxygenation and de-oxygenation of blood cell samples during the measuring process. Using the new technique, sickling and de-sickling processes could be monitored in real-time.

Keywords: segmented-flow chip calorimetry; erythrocytes; sickle cell disease

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Continuous monitoring of drug effects on tissue samples by segmented flow chip calorimetry

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Already early on, the potential of calorimetry as diagnostic tool for the analysis of drug response was investigated. However, it is questionable to which extent calorimetry can compete with the numerous powerful and well established analysis methods which, in general, are useful for high-throughput screening and which quite often exhibit a considerably high information depth. Probably, calorimetry will never become a routinely used screening tool in bio-medical diagnostics. As a consequence, the search for exclusive applications of the method is reasonable. Calorimetry is indispensable if the enthalpy is a relevant parameter. The thermodynamic analysis of ligand binding and the formation of DNA structures is an important example. Furthermore, calorimetry can compete with state-of-the-art methods in case of complex systems. Most screening methods are based on optical detection techniques and, consequently, they fail when opaque, porous or multi-phase materials have to be studied. And last but not least, the time-dependency of drug effects can be analyzed more precisely than by endpoint methods, in particular in case of highly inhomogeneous samples.

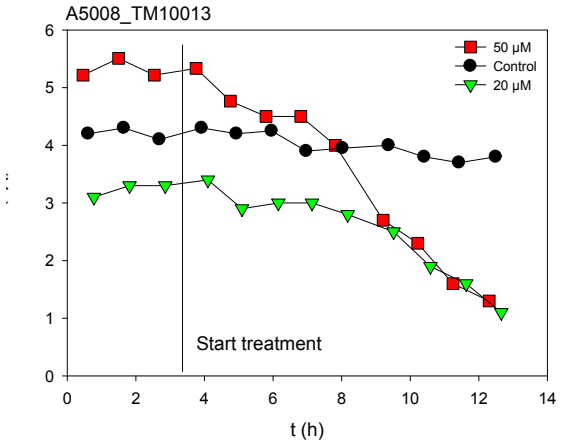

Fig. 1: Effect of the treatment of colon cancer tissue samples with

In the presented work, we demonstrate the capability of a segmented flow chip calorimeter [1] to analyze drug effects on cancer tissues in real-time. Segmented flow calorimeters comprise advantages of batch calorimeters as well as of conventional flow calorimeters. Spatially restricted samples enable enhanced throughput. The possibility to control the medium is a precondition for the study of drug treatment responses in real-time. As an example, we investigated the cytotoxic effect of staurosporine and 5-fluorouracil on colon cancer tissue (Fig. 1). The continuous measurement of the heat production of the tissue samples enabled the precise determination of the onset time of the drug effects.

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Thermodynamics of carbonic anhydrase – inhibitor binding for drug design

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Target-based drug design is usually based on protein-ligand interaction measurements when a binding hit compound is found after high-throughput screening. A hit is optimized by varying chemical groups of the compound and following not only the affinity (Gibbs energy (ΔG, K_d), one of the most important thermodynamic parameters that tells us about the strength of the interaction and stability of the complex), but also the change in enthalpy (ΔH) and entropy (ΔS) that provide additional information such as formation of hydrogen bonds [1].
Isothermal titration calorimetry (ITC) is a method of choice that can directly and model-independently determine the ΔH while the K_d is determined via minor dependence on the model. The entropy can be calculated from their difference often leading to a compensatory effect. ITC is also very helpful in dissecting the energies of all reactions involved in the complex formation. To determine the protein-ligand energetics, so called *intrinsic* binding parameters, the protonation/deprotonation reactions must be subtracted from the ITC-observed parameters. Only the intrinsic binding parameters should be used for structure – thermodynamics relationships in drug design.
Carbonic anhydrase (CA) enzyme, an ubiquitous protein found in all living kingdoms, is a good model system for the thermodynamics of protein-ligand binding. Furthermore, several CA isoforms are promising drug targets against such diseases as glaucoma, edema, cancer, etc. It is known that primary sulfonamides inhibit CA by binding to the zinc anion in the active site [2]. We have varied the functional groups and prepared over 700 compounds that yielded a lot of information about the thermodynamics of protein-ligand interaction leading towards inhibitors of extremely high affinity [3]. Based on the 50+ protein-compound crystal structures, interesting structure-thermodynamics correlations were drawn and will be discussed.

Keywords isothermal titration calorimetry; carbonic anhydrase; sulfonamide derivatives, drug design.

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Never Ending Story: The development of photocalorimetry

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Photocalorimetry is the unique direct measurement method to quantify the efficiency of photosynthesis, which is a process used by plants and other organisms to convert light energy into chemical energy that can be later used to fuel the organism's activities. Approximately 5.7 x 10^{24} J/year of solar energy are irradiated to the earth's surface. Plants and photosynthetic organisms utilize 0.05 % of this solar energy in fixing CO_2. The amount consumed by human beings 0.005 % [1] illustrates the high potential for energy development. Unfortunately, the effective energy concentration related to the area is small (< 1 kW/m^2) at most. Such low effective energy concentrations, limit the use of solar energy as a primary energy source, and elevate the costs associated with its accumulation and transmission. However, solar energy fixation by photosynthetic microorganisms does not incorporate the use of complex, technical systems, and indeed have relatively minimal investment and resource requirements. Additionally, these technologies are generating minimal amounts of waste.

Recognizing the importance of photosynthesis for ecology and technology first attempts to measure calorimetrically the photosynthetic efficiency were already done in the late 1930's [2]. Inspired by the reinvention of photocalorimetry by Wadsö [3] and others a new impetus came in the photo-calorimetric research at the begin of our century. Today, despite the great potential of this technique and the still existing lack of direct measurement methods, the photocalorimetric research seems to go to sleep. One reason for that surprising inactivity is the usually low photosynthetic efficiency of 3–6% [4]. Absorbed light that is unconverted is dissipated primarily as heat, with a small fraction (1–2%) re-emitted as chlorophyll fluorescence at longer (redder) wavelengths. This challenges extremely the accuracy of calorimetric measurements. It will be even harder, if changes in metabolism should be recognized. In the paper we will discuss the difficulties and solutions of photocalorimetric monitoring. The success of our method will be demonstrated at the example of light adaptation of *Chlamydomonas reinhardtii*.

Keywords Photosynthesis; efficiency, Photocalorimetry; *C. reinhardtii*

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Applications of the NanoDSC

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This talk with be an introduction to the myriad different applications of Differential Scanning Calorimetry for the examination of biological molecules. Several different examples will be shown for how this instrument can be used to elucidate various important functions of proteins and nucleic acids.
By monitoring the amount of energy required to increase the temperature of a biological solution through a range of temperatures, we can analyze the melting of a protein or nucleic acid molecule. This allows us to get a complete thermodynamic understanding of the unfolding of that molecule as well as its stability. We can not only analyze how this stability changes with different buffers and additives, but can even examine the different properties of various domains within the same molecule. Aside from looking at single molecules, this technique gives us access to interactions between molecules and can even help elucidate disease states from simple blood or other tissue samples.

Keywords: NanoDSC, Protein, Disease Diagnosis

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Development of fermentation calorimeter with improved sensitivity for bioprocess monitoring and control applications

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Determination of enthalphy of biochemical conversion in a bioprocesses is a vital parameter and could be related to various process variables like microbial growth rate [1], O_2 uptake rate [2], CO_2 evolution rate [3] etc. A conceptual design was fabricated on the principle of heat balance calorimetry and resolution of the system was improved in the following ways.

1)High sensitive RTDs were installed in both reactor and jacket side and a robust temperature control strategy elicits response even variation in temperature of the order of 1 mK.

2)Effect of ambient temperature fluctuations on the estimated calorimetric signals was reduced by a vacuum jacketed surface and the heat loss through head plate was minimized by thermostatting it to the reactor operational temperature

Design principle with 2 independant PID controls incorporated to control the heat input contributed by compensation heater and cryostat to the calorimeter. A precisely tuned PID controller of the heater was employed, which responds dynamically to a much smaller heating inputs applied to the system. Resolution of 35 – 40 mW/L and stability of 0.9 mW/L.h was achieved irrespective of variation of ambient temperature from 22 - 30°C. With this control strategy Hyaluronic acid fermentation by *Streptococcus zooepidemicus* was successfully monitored and the heat production rate (6.5 W/L during exponential phase) was in good agreement with CO_2 production rate.

Comparatively a better resolution was achieved by incorporating cascade control strategy involving 2 PID controls, in which electrical heater acts a primary control element and their output regulates a dynamic input to the cryostat set temperature and in turn acts as a secondary control element. This control circuit also maintains constant temperature difference of 0.2°C between jacket inlet and outlet temperature. External disturbance over jacket temperature fluctuation was significantly overcome by this technique.

Keywords: Heat compensation calorimetry, Resolution, Compensation heater, Cascade control, Hyaluronic acid, *Streptococcus zooepidemicus*

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Probing Protein Adsorption on Surfactant-Coated Nanoparticles with Isothermal Titration Calorimetry

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Nanomaterials in general are currently being investigated in biomedicine for various applications. Usually, this involves intravenous injection, where the nanomaterial comes into contact with the components of blood. Once a nanoparticle (NP) is incubated with human blood plasma, proteins immediately cover its surface, forming a so called ’protein corona‘ that creates a new biological ‘identity’[1]. Isothermal titration calorimetry (ITC) is a suitable technique to determine thermodynamic parameters characterizing the adsorption process of single proteins onto NPs. In the past years, the attachment of polyethylene glycol chains to the surface of a NP (PEGylation) has been the method of choice to reduce protein adsorption and obtain a ’stealth effect’, i.e. a non-uptake of the NP by macrophages [2], resulting in an increased blood circulation time. Besides PEGylation, a promising new approach to control the interaction between proteins and NPs is the stabilization of the latter with specifically tailored surfactants. Simple adsorption of surfactants would reduce the preparative effort compared to PEGylation.

As a model system, polystyrene NPs were coated with different poly(phosphoester)-based surfactants, since poly(phosphoester)s were also shown to reduce unspecific protein adsorption [3], and their binding affinity was determined with ITC.

Next, the interaction of the poly(phosphoester)-coated NPs with plasma and human serum albumin was quantified in comparison to uncoated NPs. Depending on the surfactant binding affinity, we were able to show that the protein adsorption is tunable and the desired effect of reduced unspecific adsorption can be controlled.

Keywords protein corona; stealth effect; nanoparticles; surfactants; poly(phosphoester)s; isothermal titration calorimetry

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Analysis of microcalorimetric post antibiotic challenge response of bacteria-drug interaction: *Staphylococcus aureus* vs Cefoxitin

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Microcalorimetry has been used to screen for bacterial contamination in clinical and industrial samples. Another application of microcalorimetry has been to rapidly detect growth of resistant bacterial and fungal species based on standard Clinical and Laboratory Standards Institute (CLSI) guidelines for drug susceptibility testing. An alternative way to evaluate antibiotic activity is represented by kill-time curves in which a bacterial suspension is challenged with a~~n~~ concentration of antibiotic.

The objective of this study is to evaluate a series of real-time Cefoxitin (FOX) challenges versus an exponentially growing culture of *Staphylococcus aureus* to evaluate bacteria-drug interaction.

Isolates of *Staphylococcus aureus* (ATCC 43300 – MRSA – and 29213 – MSSA) were first plated overnight on Trypticase Soy Agar. Discrete colonies were re-suspended in Muller Hinton (MH) broth and again left to grow overnight. The resulting liquid culture was pelleted by centrifugation, washed with and suspended in fresh MH broth to 0.5 McFarland units and pipetted into a standard batch microcalorimetric cell. Within 5 minutes of preparation, a varying concentration of FOX was added to the mixture and the cells were hermetically o-ring sealed and incubated at 37 °C in the Setaram microDSC 3 calorimeter.

In our study, the post-antibiotic microcalorimetric signature of *S. aureus* was significantly different for the MRSA and MSSA strain for the interaction with FOX. The differences are evident within 2 hours, suggesting that this technique may be used for fast MRSA-MSSA differentiation. The obtained thermograms are readily amenable to logistic-type growth modeling, with fitting parameters that scale with the antibiotic concentration and/or strain type. This suggests microcalorimetry can quickly pick out bacterial metabolic decline suggestive of a metabolic inhibitory/bactericidal response within the first hours of bacteria-drug interaction.

Keywords microcalorimetry; staphylococcus; cefoxitin

Arrhenius Parameters for the pyrolysis of stingless bees waxes in Norte de Santander, Colombia

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Bees offer variety of products with potential applications in different areas [1] (health, food, cosmetics, toiletries, etc.) [2]; within these products, wax is one that stands out for its diverse application and it has been very useful for mankind as found evidence of its use since 7000 B.C. [3]. Beeswax is solid at room temperature, complex in quantity and variety of components which leads to both its appearance and properties depend much of their origin [4]

Arrhenius Parameters for the pyrolysis of bee waxes were calculated using samples from three different parts of the nest (involucrum, brood and food storage post) of *Melipona compressipes*, *Melipona fuscipes*, *Melipona favosa favosa*, *Trigona* (*Tetragonisca*) *angustula*, *Trigona Tetragonisca Trigona (Frieseomelitta) nigra paupera*, *Bombus atratus*, *Scaptotrigona* sp., *Nanotrigona* sp. and Africanized honey bee. Thermal analyses were carried out using a simultaneous differential scanning calorimetry/thermogravimetric analysis (DSC/TGA) instrument (type SDT-Q600, T.A. Instruments, Delaware, USA). Samples (10 to 20 mg) were heated at 10 °C/min from room temperature to 800 ºC in inert atmosphere of dry nitrogen. The parameters were calculated by linearization and fitting data of the thermal behavior with the kinetic model KAS (Kissinger-Akahira-Sunose). The ANOVA analysis revealed significant differences between and within species, and the Hierarchical Cluster Analysis (HCA) showed that data were grouped according bee tribes. Pre-exponential factor decreases exponentially with the temperature interval and increases with the activation energy.

Key words Pyrolysis, Arrhenius, wax, bees

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Energetics of early vertebrate development

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The development of a multicellular organism poses an extraordinary challenge for metabolism, which must fuel cellular growth, division, motility and morphological changes. Proliferating cells such as cancer cells rewire their metabolism to produce the biomass necessary for growth and to increase the rate of cell proliferation. Thermodynamically, cell proliferation can be either fast and wasteful, or efficient and slow. However, which strategy embryonic cells use and how the energetic cost of cell proliferation relates to the rate of cell proliferation is far from understood. Here we use early zebrafish *Danio rerio* cleavage stage embryos as a model system to investigate the energetic economy of a fertilized oocyte undergoing ten synchronous minimal reductive cell cycles solely comprised of S and M phase in the absence of volumetric growth. We use isothermal calorimetry and micro-respirometry to directly measure the heat dissipation rate (R_Q) and oxygen consumption rate (R_{O2}) of embryos *in vivo.* Our preliminary data show that R_Q is composed of an increasing and an oscillatory component. Remarkably, R_Q oscillations correlate with the embryonic cell cycle and match its period. Thus, zebrafish embryonic cells metabolically cycle suggesting different energetic requirements of the cell cycle phases. Furthermore, early embryogenesis solely relies on aerobic metabolism and R_{O2} increases during cleavage stage, which coincides with the increasing trend of R_Q. Interestingly, the experimental oxycaloric equivalent per cell cycle deviates from the theoretical Thornton constant and becomes less negative, from -330 kJ/mol O_2 to -290 kJ/mol O_2, with increasing cell divisions. These results demonstrate the first step towards understanding the energetic economy of early vertebrate embryogenesis and how it is connected to the rate of embryonic cell divisions.

Keywords *Danio rerio,* embryogenesis, ITC, respirometry, cell cycle

Walk the line: Enhanced bioproduction using calorespirometry.

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The sustainable production of fuels and industrial bulk chemicals by microorganisms in biotechnological processes is promising but still facing various challenges. In particular, toxic substrates require an efficient process control strategy. Methanol, as an example, has the potential to become a major future feedstock due to its availability from fossil and renewable resources. C1 compounds such as methanol are discussed as third generation feedstock due to high synthesis capacities and its potentially sustainable production from natural gas, agricultural waste materials and biogas. C1 compounds do not competing with food or fodder production. However, besides being toxic, methanol is highly volatile. To optimize its dosage during microbial cultivations, an innovative, predictive process control strategy based on calorespirometry, i.e. simultaneous measurements of heat and CO_2 emission rates, was developed. The initial idea is based on two well-known facts: calorimetry delivers real-time information on growth parameters such as the specific growth rate and substrate consumption rate [1] and calorespirometry is proposed to detect shifts in growth stoichiometry [2]. For these reasons, we have hypothesized that calorespirometry enables an adapted feeding for different phases (i.e. growth and product formation). The calorespirometric control strategy is demonstrated exemplarily for growth and polyhydroxybutyrate formation of the methylotrophic bacterium *Methylobacterium extorquens* on methanol and compared to alternative control strategies [3]. Applying the new approach, the methanol concentration could be maintained far below a critical limit, while increased growth rates of *M. extorquens* and higher final contents of the biopolymer polyhydroxybutyrate were obtained [4]. We believe that our calorespirometric process control is also applicable to other feedstocks and other products and thus of general interest for biotechnology.

Keywords biocalorimetry; respirometry; process control; methylotrophy; *Methylobacterium extorquens*

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Mycobacteria biofilm susceptibility to antimycobacterial agents

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A lot of research on drug susceptibility of mycobacteria in planktonic cultures has been conducted with. However, only little to no data is available for mycobacterial biofilms, while being one of the biggest challenges in terms of antibiotic treatment [1]. Although it is known that biofilms tend to be more resistant to antibiotics and antimicrobial stressors compared to planktonic bacteria of the same species, there is limited number of standardized systems or protocols which enable to study this problem deeper. Within this study isothermal micro-calorimetry (IMC) was used to investigate the dose-dependent influence of different antibiotic drugs on mature biofilms, in order to determine potential treatment option (i.e., higher antibiotic concentrations). IMC allows to investigate the growth of biofilms before, during and after antibiotic treatment in real time and enables very sensitive (µW) and continuous measurements of metabolic activity [2]. The goal of the study was to measure the metabolic biofilm activity of slow-growing *Mycobacterium smegmatis* and *Mycobacterium phlei* in presence and absence of antibiotics. Therefore, biofilms were grown on nylon filters and subsequently transferred on solid 7H9 medium first without antibiotic and second with antibiotic by using the same undisturbed model biofilm each time. This approach sheds light on metabolic growth of mycobacterial biofilms being under antibiotic treatment and demonstrated the very high resistance of such biofilms toward isoniazid. This in turn provides new data about mycobacterial antibiotic resistance that will be helpful in a clinical setting.

Keywords: mycobacterial biofilm, drug susceptibility

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The new generation TAM – TAM IV

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About 35 years ago the first multichannel isothermal microcalorimeter system was described [1]. It was initially called BAM – Bio Activity Monitor but later changed to TAM – Thermal Activity Monitor. TAM has been in continuous development since it was introduced and is a truly modular and flexible microcalorimeter system that can be configured in many different ways depending on application and technique of measurement. Microcalorimetry has the advantage of being general, nondestructive and very sensitive, thus enabling metabolic activities to be monitored, in real time and under a wide variety of conditions, on samples such as intact tissue biopsies or cell cultures and small animals, insects or plants.
Recently the fourth generation TAM, TAM IV, was released and offers new possibilities for biological microcalorimetry:

- An extended temperature range down to 4 °C is now allowing biological and ecological samples or systems to be studied under sub-ambient and in some cases more realistic conditions.

- A new accessory which can collect voltage signals from miniature probes that can be inserted into reaction vessels in the calorimeter. This probe can be a pH or an oxygen probe adding more information to a microcalorimetric measurement.

- Availability of ampoules and micro reaction systems with additional inlets were probes or light sources can be guided into the reaction vessels to be able to initiate a reaction inside the calorimeter.

This presentation will review the new features and possibilities with the new TAM in the field of biological microcalorimetry

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Thermoanalytical investigations of honey produced by some species of stingless bees in Guainia, Colombia

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Bees are fundamental for the survival of humans through their role as pollinators due to these insects are probably best adapted to floral visits. Stingless bees (Tribe: Meliponini) are eusocial insects by 22 genus with over 500 species [1] that living in every tropical and southern subtropical areas throughout the world [2]. They are known as ‘‘stingless bees’’ because the sting and associate structures of the female bee are greatly reduced. These insects produce honey called ‘‘pot-honey’’ because the container in which they store the honey has the shape of a pot. The honey produced by stingless bees is greatly appreciated, not only as food, but as medicine also.

In this project supported by Ricola Foundation (Switzerland), thermal properties of Colombian honey samples were determined. The honeys were collected in the indian Reservation, La Ceiba, located in the Amazon Region of Colombia of the species *M. eburnea*, *M. marginata*, *M. compressipes*, *T. angustula*, *Cephalotrigona* sp., *M. crinita* and *Scaptotrigona* sp. Thermal analyses were carried out with dry nitrogen (100 mL/min) using simultaneous differential scanning calorimetry/thermogravimetric analysis (DSC/TGA - TA Instruments SDT-Q600, Delaware, USA). The samples weighed ~10 mg and the temperature scans began at room temperature (~20 °C) and ended at 260°C with a heating ramp at a rate of 5ºC min^{-1}. The equipment was previously calibrated using high purity zinc as a temperature standard and with sapphire for the heat flow.

Thermal analysis showed the presence of four thermal transitions trs_1, trs_2, trs_3, trs_{4a} and trs_{4b}. These results were used to perform a multivariate chemometric treatment, finding that between species there were significant differences. These results show that the differential scanning calorimetry (DSC) can be used to distinguish honey from each species (fingerprint), and to determine whether it has been adulterated.

Key words Honey, stingless bees, differential scanning calorimetry, thermogravimetric analysis

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Studying glucose-amended soil samples with isothermal calorimetry

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In soil science, respiratory measurements are a common practice when evaluating microbial activity, and often glucose is added to (i) assess maintenance energy requirements, (ii) determine the amount of microbial biomass or (iii) examine microbial carbon use efficiencies. Such studies can for example be made in an isothermal calorimeter (Bölscher et al., 2016). However, some care is needed to ensure robust measurements. This presentation will discuss advantages and pitfalls of using isothermal calorimetry within soil sciences.

Soil respiration requires sufficient oxygen throughout the measurement. If this is not guaranteed than samples need to be aerated during the measurement. If this is not made, the lowered oxygen concentration and the correspondingly increased carbon dioxide concentration will influence the soil activity negatively.

The aim of an experiment with a glucose-amended soil sample can be to evaluate an efficiency of the soil organism community. It should be noted that there are several different ways to define efficiency (see Bölscher et al. 2016), and to evaluate the carbon conversion efficiency a calorespirometric measurement is needed (Wadsö and Hansen 2015).

Soil experiments can be made in all types of isothermal (heat conduction) calorimeters, with volumes from more than 100 mL to a fraction of one milliliter. Small vials can ensure high sample throughput which will allow the use of high replication numbers for each treatment. However, larger vials may be preferable for several reasons: (i) larger samples will produce results with a higher signal-to-noise ratio, (ii) a smaller sample in a relatively large reaction vial reduces changes in gas composition, (iii) large samples are more representative for in-situ conditions due to the inhomogeneous nature of soil, (iv) large vials will enable us to construct microcosms, and isothermal calorimetry can then be combined with other methods such as computer-aided tomography (Nunan et al., 2006) to analyze the heterogeneous soil structure.

Keywords isothermal calorimetry, respiration, soil, glucose amended

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Studying grain germination by isothermal calorimetry

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Seed germination is an important process in agriculture that can be studied by isothermal calorimetry, see for example the study on soybean germination by Schabes and Sigstad (2006). It is also of interest to study seed germination in some industrial settings, for example in the malting industry where barley grain is germinated as part of the malting process.

The imbibition and germination of barley and other grains takes a few days and do in most cases consume the oxygen in a closed calorimetric vial. The vials do therefore need to be equipped with means of aeration, and this can be arranged either with continuous or discontinuous flow of humidified air to minimize the disturbances from evaporation.

The results from barley grain germination studies by isothermal calorimetry contain many features – for example peaks – that can be used to quantify the complex germination process. The extraction and use of indices derived from calorimetric results is interesting, for example if we want to correlate the result of isothermal calorimetry with the result of other methods.

This presentation will discuss the use of isothermal calorimetry to study grain germination, both from a method perspective (supply of water, supply of oxygen, removal of carbon dioxide) and from a results perspective (indices, correlations with traditional methods).

Keywords isothermal calorimetry, seed germination, grain germination, barley malting

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Continuous monitoring by isothermal calorimetry of fungal colonization of wood materials

L. Wadsö[1], S. Johansson[1] and S. Bardage[2]

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[2] SP Sustainable Built Environment, Stockholm, Sweden

Wood degrading fungi (decay fungi) are the main decomposers of plant material in nature, and also cause damage to man-made wood structures. We have developed a calorimetric method to study the colonization of wood-materials by decay fungi; a method that can be of use both to increase understanding of the colonization process, and as a method to test the durability of wood-materials. As isothermal calorimetry gives a time resolved result during the test period, it contains much more information than, e.g., standard mass loss measurements at the end of a test.

We make samples in which non-degraded wood materials are placed in contact with wood that is heavily infested with a decay fungi. We than place these combined samples at controlled moisture content and temperature in an isothermal calorimeter and study the development of the colonization during, typically, five weeks. The samples are aerated during the experiment.

The results of our measurements during fungal colonization and degradation of wood are surprisingly complex (an example is shown in Fig. 1), showing several stages, and in some cases long term oscillating behavior of the heat production rate (as for heat treated wood samples in Fig. 1).

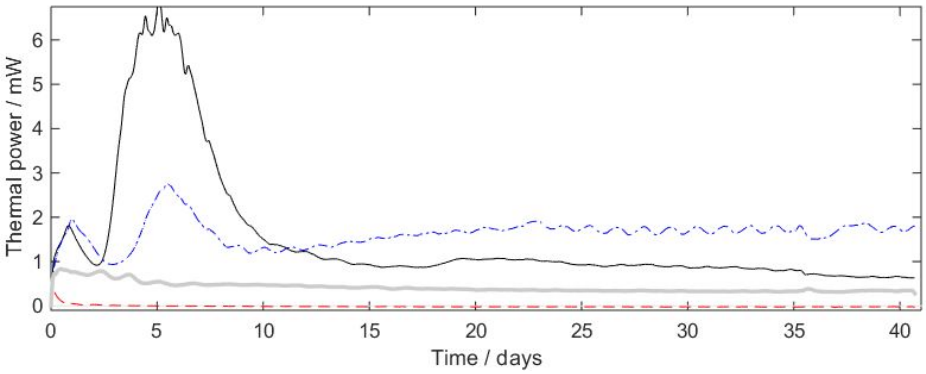

Figure 1. *Example of results from experiments in which wood was colonized and degraded by the brown rot decay fungus Postia placenta. Materials: spruce wood (solid black line), heat treated wood (blue dash-dotted line), impregnated wood (red dashed line), and a reference (thick gray line).*

Keywords isothermal calorimetry, decay fungi, *Postia placenta*, wood, wood products

The Use of Isothermal Calorimetry to Measure the Metabolic Heat Production of Spinach Leaves as Consequences of Vacuum Impregnation Treatment

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[3] Department of Building Materials, Lund University, P.O Box 118, SE-221 00, Lund, Sweden.

Fruits and vegetables undergo aerobic respiration to produce about 455 kJ of heat per mol of oxygen consumed. The respiration of plant tissue is affected by several factors e.g. storage temperature, humidity, post-harvest handling, substances used during processing, and many others. In principal, the metabolism and respiration in the plant tissue produce heat that can be measured by isothermal calorimetry. In this case, the main purpose of the study was to explore the use of isothermal calorimetry as a means of quantifying the metabolic activity of baby spinach leaves after impregnating with several substances that are commonly used in the food industry. Calorimetric measurements were carried out when the spinach leaves were harvested and placed into the calorimeter at 20 °C and the thermal power was recorded for 3 h. The same leaves were later treated by either immersion or vacuum impregnation (VI) for 1 h with solutions of ascorbic acid and citric acid (both pH 3.2 and 4.5), calcium lactate (pH 7.1) and sucrose solution (pH 5.7). Once the treatment finished, the leaves were placed again into the calorimeter and the thermal power was recorded. The leaves, either immersed or VI treated with different substances, increased their metabolic heat production as compared to the non-treated leaves (average thermal power i.e. 660 μW/g). Whilst the highest metabolic heat production was produced by the leaves that were impregnated with sucrose solution (average thermal power i.e. 1486 μW/g), the heat production was also pH dependent (higher metabolic heat production was observed when treated with more acidic solution). These results indicate that impregnation of metabolizable substance with higher concentration may trigger the metabolic activity of the plant tissue, providing substrate for the tissue to modulate the cell's activity, leading to higher thermal power. Complementary experiments were done to further asses the metabolic activity of the impregnated leaves at 9 °C, to mimic the real processing line of vegetables in food industry as well as to determine the implications on intrinsic

sugar metabolism. As the respiration of fruits and vegetables was reduced when handled at low temperature, lower metabolic heat production was recorded by the calorimetry for the spinach leaves when treated with those aforementioned solutions. The present study makes a notable contribution towards understanding the correlation of different substances affecting the metabolic heat production in spinach leaves, providing insights on the metabolic consequences of VI in plant tissue.

Keywords; Isothermal Calorimetry, Spinach, Metabolic Activity, Sucrose, Ascorbic Acid, Citric Acid

Pandora's box: Chemicals induce epidemics in microbial communities.

Juan Xu[1], Feng-lei Jiang[1], Yi Liu[1], Bärbel Kiesel[2], Thomas Maskow[2]

[1] *State key laboratory of Virology & Key laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education), College of Chemistry and Molecule Sciences, Wuhan University, Wuhan 430072, P.R. China*

[2] *Department of Environmental Microbiology, Helmholtz Centre for Environmental Research – UFZ, 04318 Leipzig, Germany*

Bacteriophages are viruses infecting bacteria. They can be virulent and lyse bacteria directly after infection or they can be temperate and integrate their DNA into the bacterial chromosome (so called prophage) and survive. These prophages undergo a symbiotic strong relationship. They are passed to the next generations of cells but can presumably be activated to cause phage propagation and cell burst by several chemicals and UV light in lab cultures but also in the environment. Therefore, prophages could be called molecular time bombs [1] on one hand side. On the other hand side, evidences are piling up, that's the coevolution of prophages and their hosts have led to positive effects for the host, e.g. by horizontal phage mediated gene transfer. Real time monitoring devices are needed to clarify the role of prophages in pure cultures and microbial communities.

Calorimetry was proven to provide real-time insights into the activation of prophage propagation [2]. The applied fermentation calorimetry that allows a combination of mass and energy balances provides a coherent picture of the infection process. Unfortunately, fermentation calorimetry is too laborious and too complicate to allow a fast screening of different chemicals as potential detonators of the molecular time bomb. Chip calorimetry is well suited for monitoring metabolic reactions on phage infections however it does not allow combining mass and energy balances [3]. For these reasons, we try here for the first time to develop isothermal microcalorimetry and isothermal titration calorimetry as monitoring tools for activation of silent viral infections. We want to explore, how much information is at least necessary to characterize the calorimetric curve in terms of the infection process. For that purpose, the infection processes were thermokinetically modelled.

Keywords: Chemical test; Bacteriophage; Prophage; Calorimetry; *E.coli*

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3. Mariana Morais, F., Buchholz, F., Hartmann, T.et al., Chip-calorimetric monitoring of biofilm eradication with bacteriophages reveals an unexpected infection-related heat profile. J.Thermal Analysis & Calorimetry, 2014. 115 2203-2210.

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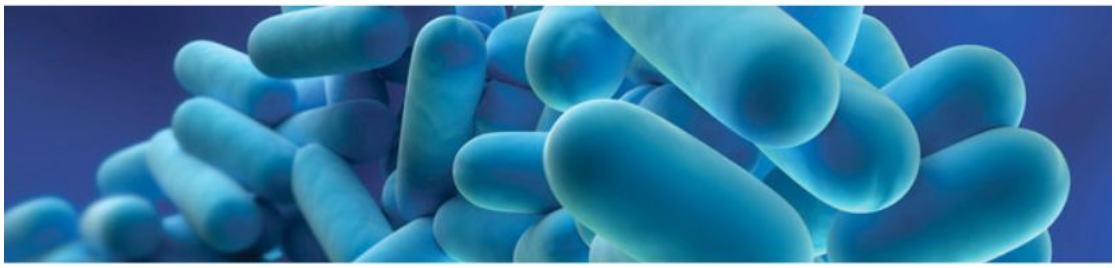

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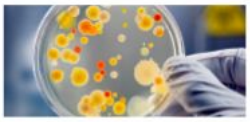
Spoilage and shelf life
Metabolic response
Preservation techniques
Yeast studies
Fermentation



Microbiology

Bacterial growth studies
Microbial detection
Infectious diseases
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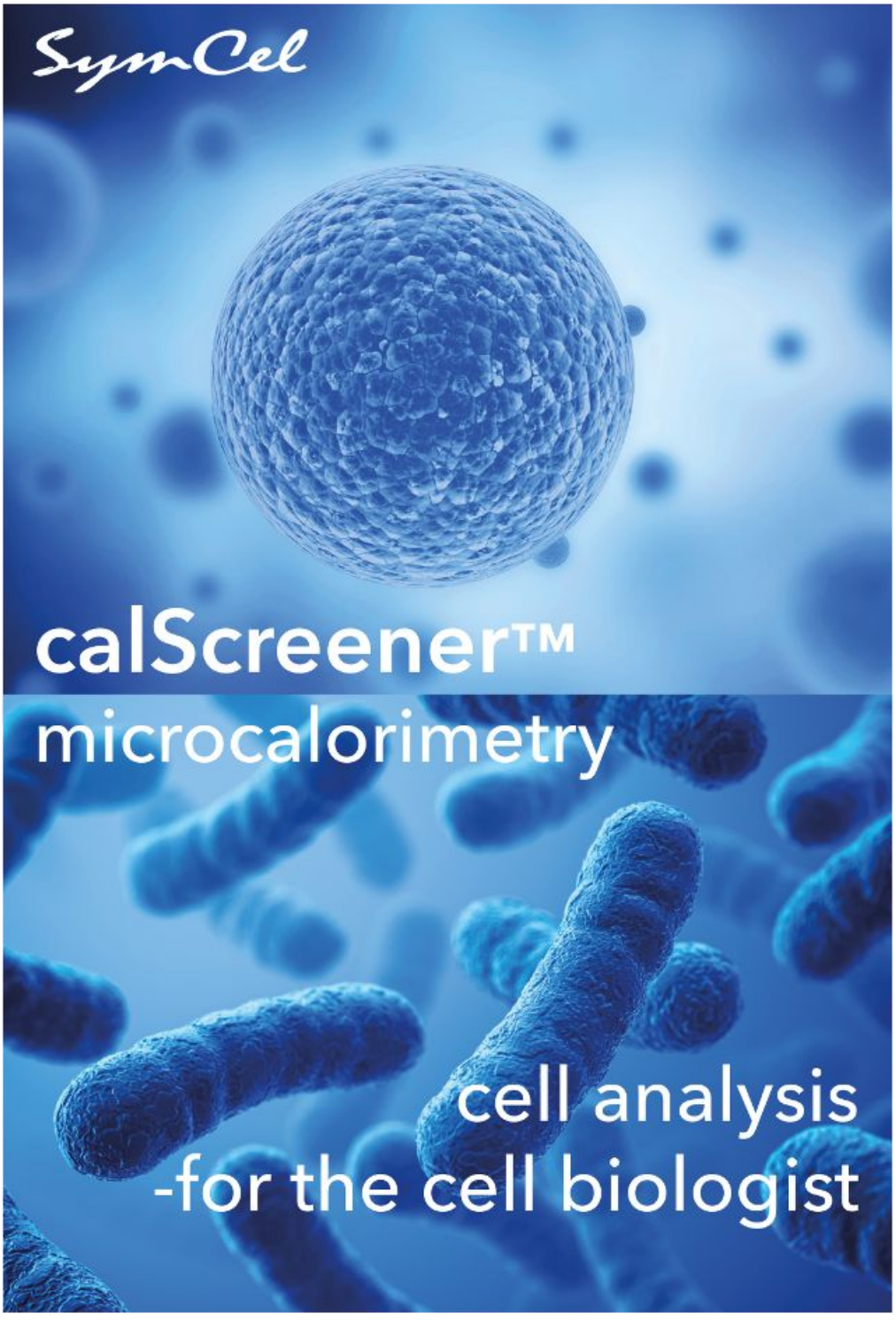
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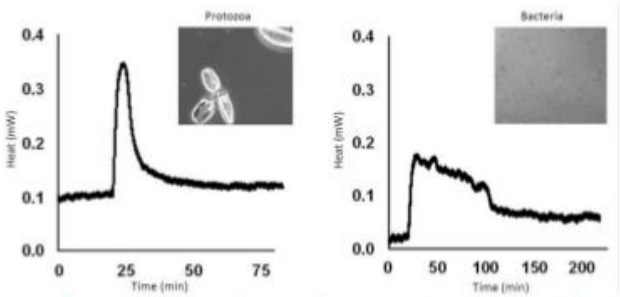
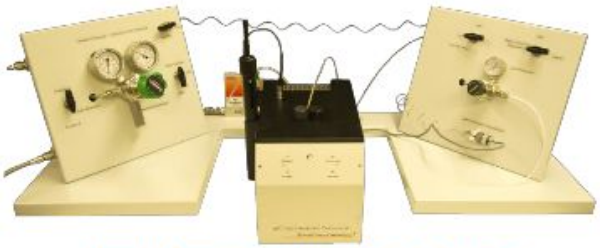


Rate of heat production of rumen microbes at 39°C (Data courtesy of Timothy J. Hackmann, The Ohio State University. USA)

Areas of application:

- Food spoilage
- Microbial growth
- Protein denaturation
- Thermal stability
- Heat capacity (Cp) determination
- Reaction kinetics
- Excipient compatibility
- End point determination



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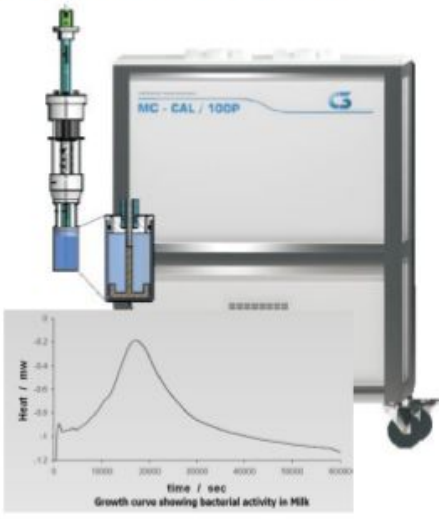

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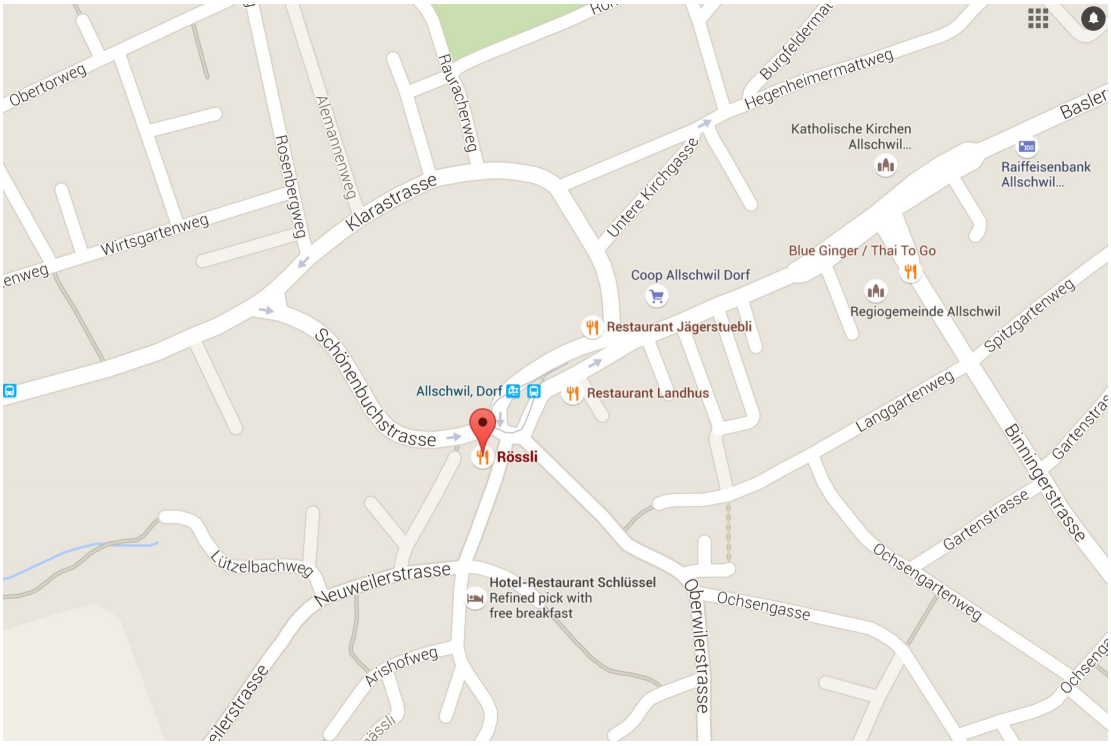

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