XVIII ISBC

International Society for Biological Calorimetry

Conference

1 - 4 June 2014, Lund, Sweden

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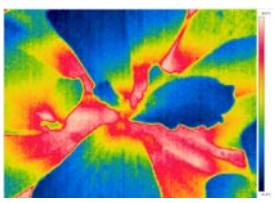

Cover picture story

The cover picture is the thermal image of a pistia grown in water shown in the picture to the right. The pictures were taken in the green house of the Botanical Garden at Lund University by Yujing Li.

Preface

Dear Colleagues,

Welcome to Lund and Lund University for the XVIII ISBC meeting. It is now 41 years since 1973, when the first meeting in this series was held at Chelsea College in London. At that time I had not even considered doing calorimetry (I was only 12). We now have the 18th meeting and are a rather small, but stable and lively scientific community that meets every second year in different places, mainly in Europe.

From the first years of ISBC, the conferences have broadened their scope from isothermal measurements on living cells and organisms to a wider range of instrumentation (isothermal, scanning, titration…) and a broader range of study objects. The latter is exemplified by the theme of the 1997 meeting: "Biothermodynamics: from Human Beings to Molecules". This ISBC meeting – which was my first such meeting – was held in Ascona in Switzerland and was hosted by this year's Lavoisier medalist Urs von Stockar. I remember from that meeting that there were several presentations on irreversible thermodynamics – a subject that seems to be much less popular now. On the other hand I believe that industrial applications have come more in focus.

This year's conference is called "Biocalorimetry of the future" and we have received many interesting presentations that point in the direction of future uses of biocalorimetry. Concerning the subjects covered at XVIII ISBC the range is very wide; we could have called our conference "From Elephants to small molecules" or "From ecology to small molecules", even further broadening the range of subjects being presented.

The ISBC meeting on the last day of the conference (8.30-9.00) is open to all ISBC members (the only way to become member of ISBC is to attend a conference). What I write above gives rise to some questions that we can discuss during the conference and at the ISBC meeting:

- Is the present scope of ISBC good?
- Are we happy with the size or our meetings (about 75 participants) or do we want to grow and attract more participants to our meetings?
- Should we attempt to increase the use of calorimetry in the bio-field?
- Should we make a new try outside Europe 2020 (we have proposals for the next two ISBC venues; these will be presented at the meeting)?

Lars Wadsö
Chairman ISBC XVIII
22 May 2014, Lund Sweden

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TA Instruments got the broadest product line in the industry, with a tiered focused product strategy within the key areas: Microcalorimetry (with the models TAM AIR and TAM III), Thermal Analysis and Rheology.

TAM AIR - Isothermal Calorimetry

Monitoring the thermal activity or heat flow of chemical, physical and biological processes provides information which cannot be generated with other techniques. Isothermal calorimetry is a powerful technique for studying heat production or consumption and is non-destructive and non-invasive to the sample. The TAM Air offers unmatched sensitivity and long-term temperature stability with flexible sample requirements.

The TAM Air is the ideal tool for large scale calorimetric experiments, capable of measuring several samples simultaneously under isothermal conditions. This system is especially well-suited to processes that evolve or consume heat over the course of days and weeks such as cement and concrete hydration, food spoilage, microbial activity and more.

TAM Air: Reliable, Robust, Versatile

The 8-Channel Standard Volume TAM Air is ideal for more homogenous samples while the 3-Channel Large Volume calorimeter can accommodate the more heterogeneous samples as well. The shape of the heat flow curve will reflect the process under study.

TAM Air: Reliable, Robust and Versatile

TAM III - Isothermal Microcalorimetry

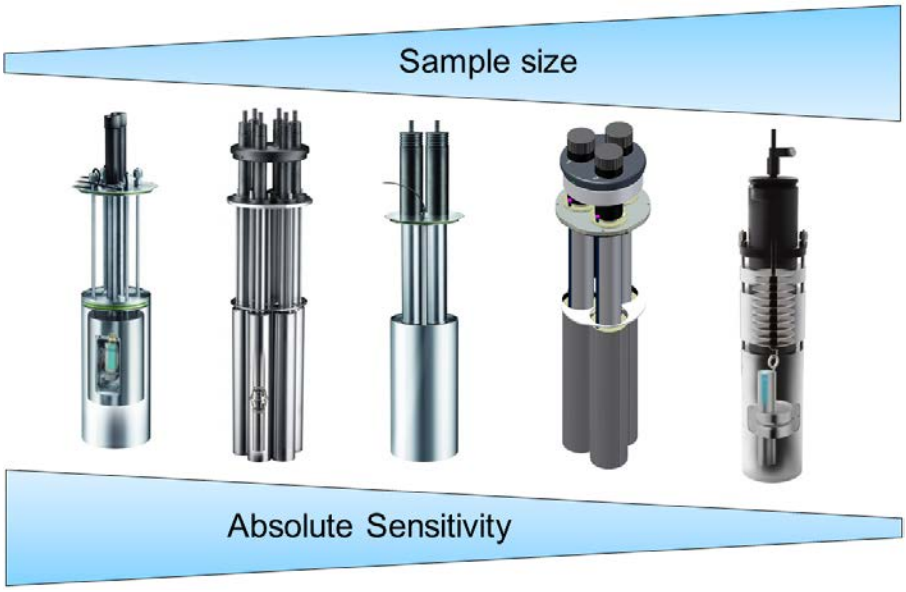
TAM III represents an ultra-sensitive heat flow measurement which is complementary to TA Instruments differential scanning calorimeters. Based on the pioneering Thermometric technology, TAM III offers maximum sensitivity, flexibility, and productivity. It can be used with the most sensitive microcalorimeters and a wide variety of accessories to control the experimental conditions.

TAM III offers maximum sensitivity, flexibility, and performance. It can be used with the most sensitive microcalorimeters and a wide variety of accessories to precisely control the experimental conditions. Up to four independent calorimeters can be used simultaneously with TAM III, to perform repetitive or different types of experiments. TAM III is totally modular and enables multiple calorimeters to be added to increase sample capacity or functionality. With the addition of a multicalorimeter holding six independent minicalorimeters, the sample throughput is substantially increased. TAM III employs patented thermostat technology to precisely control the liquid bath temperature to within 0.0001 °C, and can be operated in isothermal, step-isothermal or temperature-scanning mode.

TAM III Technology

All chemical, physical and biological processes result in either heat production or heat consumption. Microcalorimetry is a versatile technique for studying this thermal activity in terms of heat, heat flow and heat capacity. TAM III offers unmatched sensitivity, long-term stability and high measuring capacity. The modular design, coupled with a wide range of accessories and auxiliary equipment, offers unrivaled flexibility.

Microcalorimetry can be completely nondestructive and non-invasive to the sample. It seldom requires any prior sample treatment nor does it limit analysis to a physical state of the sample. Solids, liquids and gases can all be investigated. Microcalorimetry does not require that a sample has a particular characteristic to enable measurement like FTIR, UV-Vis, NMR etc. Microcalorimetry is a direct and continuous measurement of the process under study. Unlike other analytical techniques that give “snapshots” of data, microcalorimetry gives real-time data continuously as the process proceeds.



Unmatched TAM III flexibility: with a variety of different possibilities

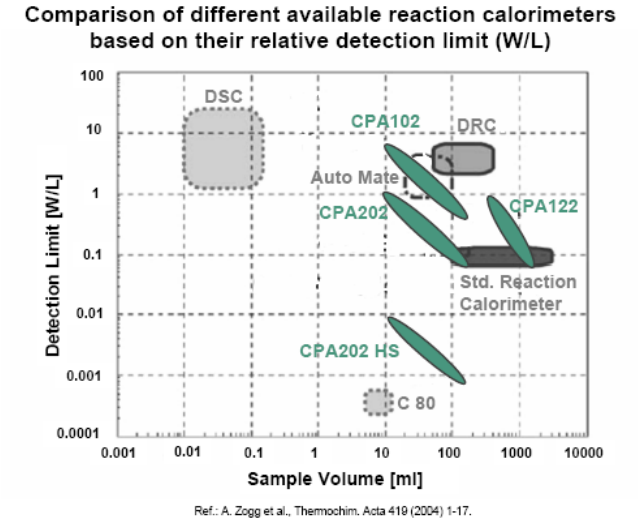
Contact your local TA representative for more details concerning the available solutions.

We don't believe in "one size fits all".

You might agree with us after having checked the new ChemiSens **High**Sens **reactor**

The new **High**Sens reactor from ChemiSens enables you to expand the frontiers of conventional reaction calorimetry beyond present imagination.

The diagram below, originally presented by A. Zogg et. al. shows the detection limit vs sample volume for different available reaction calorimeters



The CPA202HS clearly demonstrates a dramatic paradigm shift!

With detection limits down to 0.5 mW/l and a resolution of 0.1 mW, you will be able to study chemical as well as physical processes of vital importance in crystallisation, dissolution, micellisation etc.

The working principal of the HighSens reactor has much in common with the CPA202 True Heat Flow reactor. Both employ the same active, thermally isolating shield in the form of a precision liquid thermostat.
Further, the ingenious use of "Peltier- element" technology, to drive heat flows in the desired direction at precise power levels.

During operation, the reactor is well protected inside the liquid-filled thermostat. This helps reduce external disturbances to a minimum.

ChemiSens
Reaction Calorimeter Systems

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Applications

Ideal applications for the ChemiSens **High**Sens Reactor;

- Adsorption kinetics
- Crystallisation
- Desorption studies
- Dissolution of tablets
- Heat of adsorption
- Mass transfer investigations
- Micellisation studies
- Studies of polymorphism
- Wetting of solids
- Biological processes

Specification

Temperature range	-40ºC to 150ºC
Pressure range	Vacuum to 10 bar
Resolution	0.1 mW
Volume, gross	200 ml
Volume, practical	10 to 150 ml, continuously variable
Time constant	20 –30 s
Max power	1 W
Materials	Glass, SS316, HC276 and Tantalum
Thermal modes	Isoperibolic, scanning, set new temperature
Probes	IR, Raman, UV, pH, pressure, oxygen etc

Feature summary

- True Heat Flow sensor; factory calibrated once and for all
- Time efficient and ease-of-use operation
- Wide range of accessories, stirrers, dosing devices for powders and pastes, pumps and gas control valves
- Power signal on-line in real-time for use together with ProFind™ enabling automated experiment and process optimization

Shortcomings in stirring, dosing, sampling, installation of probes and pressurising have been general and serious disadvantages in high sensitivity calorimeters up until now.

Let High*Sens *with the proven CPA202 open new frontiers in your reaction calorimetry

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ONE CALORIMETER ...

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ACCELERATE DEVELOPMENT OF PRESERVATIVES FOR LONGER SHELF LIFE

UNDERSTAND THE KINETICS OF MICROBIAL ACTIVITY

STUDY RESPIRATION RATE AND SPOILAGE OF FRESH-CUT PRODUCE

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USE LARGE SAMPLE VIALS THAT ARE ADAPTED FOR MANY FOOD TYPES

ASSESS THE EFFICIENCY OF FERMENTATION PROCESSES

STUDY THE RELATIONSHIP BETWEEN SALINITY AND SEED GERMINATION

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Table of Contents

Conference program

Sunday 1 June 2014

18:00 - 20:00 Registration and Reception, Lund Museum of Public Art

Monday 2 June 2014

08:00	**Registration**	
09:00	**Welcome**	
09:05	**Lavoisier lecture by Urs von Stockar**	
	Energy dissipation and performance in microbes, animals, and distillation columns	
10:00	**Break**	
10:30	**Session 1: Animals**	
10:35	Y. Montanholi	Bovine feed efficiency evaluated through indirect calorimetry and infrared imaging
11:00	A. Torres	FTIR and thermal analysis of nest paper in nine species of Polistinae wasps from Colombia
11:15	H.K. Cammenga	The gonadal fat deposits of the African Elephant - A latent heat storage?
11:30	M. Jakubowska	The heat production of blue mussel *Mytilus edulis trossulus* - is there a synergistic effect of water acidification and oxygen deficiency?
11:45	D. Singer	Microcalorimetry and Microrespirometry of Human Placental Samples: A Feasibility Study
12:00	**Lunch**	
13:30	**Session 2: Animals (continued)**	
13:35	J. Röttgers	Heat Flow Measurement in Human Preterm Neonates: Towards a "Calorimetric" Incubator
13:50	P. Junghans	Indirect calorimetry and N balance measurements may be affected by endogenously produced nitrogen
14:05	L. D. Hansen	Effects of temperature and modified atmospheres on diapausing 5th instar codling moth metabolism
14:20	**Presentations of posters for poster session A: Animals and Food**	
14:30	**Break and Poster session A: Animals and Food**	
15:30	**Session 3: Food**	
15:35	R. Vilu	Isothermal microcalorimetry and development of systems biology of bacteria growing in solid state and opaque liquid media
16:00	R. Vilu	Metabolism of prebiotic fructans by colon microbiota studied by isothermal microcalorimetry
16:15	P. Rocculi	Effect of vacuum impregnation with calcium salts on quality and metabolic aspects of fresh-cut melon
16:30	F. Gómez Galindo	Modulating metabolic activity by controlled vacuum impregnation of spinach leaves
16:45	S. Tappi	Effect of cold plasma on fresh-cut fruit tissues metabolism
17:00	**End of day 1**	

Tuesday 3 June 2014

08:30 Session 4: Instrument and method

08:35	L. Wadsö	The ice calorimeter of Lavoisier and Laplace
08:50	J. Lerchner	Chip calorimetry of aggregated biological samples in segmented flow
09:05	Wonhee Lee	Parylene microfluidic calorimeter integrated with vanadium oxide thermistor
09:20	S. Paufler	Difficulties in interpretation of high resolution anaerobic biocalorimetric data due to non-perfect reference analysis
09:35	I. Wadsö	Will isothermal microcalorimetry ever become a routine method for estimation of 'bio-activity'?

09:50 Break

10:30 Session 5: Instrument and Method (continued)

10:35	J-H. Ferrasse	Preliminary modeling of a continuous calorimetric set-up for aerobic measurement
10:50	U. Hess	High Sensitive Reaction Calorimetry for Anaerobic Biotechnology
11:05	C. Ortmann	Investigation of contaminated soils
11:20	M. Suurkuusk	New and modernized isothermal calorimeters open new possibilities

11:35 Presentations of posters for poster session B: Method and Instrumentation, and Microbiology

12:00 Lunch

13:30 Session 6: Microbiology

13:35	T. Maskow	How reliable is isothermal microcalorimetry of growing cells in the wake of oxygen limiting conditions?
14:00	M.-T. Weichler	Calorimetric Process Control for Microbial Product Formation Using Toxic Feedstocks
14:15	M. Fredua-Agyeman	A real-time study of defined mixed cultures of bacteria
14:30	T. Hartmann	Antibacterial activity of commercial nAg and nTiO2 against beads-grown *P. putida* biofilms – a chip-calorimetric study
14:45	O. Braissant	Determining the antimicrobial properties of coatings and porous biomaterials by taking advantage of isothermal microcalorimetry

15:00 Break and Poster session B: Method and Instrumentation, and Microbiology

16:00 – 17:00 Social event

19:00 – 22:00 Conference dinner, "Pelarsalen", Lund University Building

Wednesday 4 June 2014

08:30 ISBC Meeting

09:00 Session 7: Ecology, Soil and plants

09:05	L. D. Hansen	Completing the temperature function in metabolic theories of ecology
09:30	T. Bölscher	Microbial energetics of soils exposed to different temperatures and land uses
09:45	Anke M. Herrmann	Revisiting the terrestrial carbon cycle: New insights into microbial metabolism

10:00 Break

10:30 Session 7: Molecular

10:35	V. Kocherbitov	Application of sorption calorimetry for studies of hydration of biomolecules
11:00	E. Sparr	How small polar molecules protect membrane systems against osmotic stress
11:15	S. Winzen	Protein Adsorption on Nanocapsules: Demonstrating the Differences between the Hard and Soft Corona
11:30	K. Honarmand Ebrahimi	Isothermal titration calorimetry for biocatalysis: Measuring metal ion binding to proteins and catalytic activity of enzymes

11:45 Presentations of posters for poster session C: Soil, Plants and Molecular

12:00 Lunch and Poster Session C: Soil, Plants and Molecular

13:30 Session 8: Molecular (continued)

13:35	D. Matulis	Thermodynamics of anticancer fluorinated lead binding to target carbonic anhydrases by isothermal titration calorimetry and thermal shift assay
14:00	J. M. del Río	Application of the Gibbs-Duhem equation to the study of the relation between forward and reverse titration in ITC
14:15	K. Bhadra	Isoquinoline alkaloids and their binding with DNA: Calorimetry and thermal analysis applications.

14:30 – 15:00 Closing session

Posters

A1	Y. Cardona	Thermoanalytical analysis of wood used for nest sites of the carpenter bee *Xylocopa lachnea*
A2	A. Torres	Combustion calorimetric investigations of nest paper in wasps from Colombia
A3	A. Torres	Host Preference and Performance of Lichenivorous *Astylus* sp. adults (Coleoptera: Meliridae) in Relation to Lichen energy contents and heat production rates
A4	M. Normant	Energy value of Norway lobster *Nephrops norvegicus* (Linnaeus, 1758) from the North Sea
A5	M. Normant	Oxygen-related behaviour and heat output in the Baltic clam *Macoma balthica*

A6	K. Dymek	Enhanced metabolic activity of spinach baby leaves as a consequence of pulsed electric field treatment (PEF) and vacuum impregnation (VI)
A7	N. Kabanova	Application of microcalorimetric method for the study of diffusion processes in gelatin matrices
A8	L. Wadsö	Lactic acid fermentation studied by isothermal calorimetry
A9	S. Tappi	Influence of ripening stage on metabolic heat production of fresh-cut kiwifruit and melon
A10	K. Fransson	Calorimetry, a useful method for determination of unfrozen water in food below freezing temperature
B1	L. Wadsö	A new calorespirometric vessel for isothermal calorimeters
B2	T. Hartmann	Segmented-flow technology enhances chip calorimetry
B3	G. Lewis	The application of chipCAL, a flow microcalorimeter (FMC) for high throughput analysis of enzyme activity
B4	O. Braissant	Combination of isothermal microcalorimetry and mass-spectrometry for rapid drug susceptibility testing in suspected urosepsis
B5	C. Paul	Isothermal microcalorimetry with reporter bacteria for assessment of nutrient content in source and processed drinking water
B6	O. Braissant	Autophagy: a possible survival strategy for *Candida spp.*
B7	M. Kula	The influence of spectral composition of light on the metabolic activity of Chlorella emersonii and Botryococcus braunii in the dark
B8	J. Lerchner	Chip calorimetry on spheroids: Study of proliferation and viability
C1	C. Albèr	Hydration of Sodium Hyaluronate Studied by Sorption Calorimetry and Differential Scanning Calorimetry
C2	E. Dejmková	Studies of mutant lectin binding behaviour by microcalorimetry
C3	Ö. Topel	Thermodynamics of interaction between L-serine and TiO2 nanoparticles
C4	Q. D. Pham	The effect of terpene on phospholipid membranes
C5	V. Morkūnaitė	Recombinant production and calorimetric characterization of human carbonic anhydrase IX
C6	I. Rakipov	Calorimetric study of intermolecular interactions of model biomolecules in aqueous-organic solvents: the ratio "structure-property"
C7	H. Trębacz	Thermal denaturation of fibrilar collagen in tissues - effects of glycation
C8	A. Skoczowski	Does storage protein composition influence metabolic activity of wheat seedlings?
C9	J. Lerchner	Sensitivity of chip calorimetry to study the degradation of the soil organic matter
C10	N. Barros	Factors influencing the calculation and evolution of calorespirometric ratios in soils determined by calorimetry
C11	Y. Salmanca	Simultaneous measurements of microbial energetics, methane and CO_2 in soils with varying water contents
C12	J. Dziejowski	The use of glucose, cellobiose and cellulose for calorimetric studies of soil microbial processes
C13	N. Barros	Calculation of the Activation Energy to describe sensitivity of soil organic matter degradation to temperature: Application of TAM III

List of participants

	Lastname	Firstname	Affiliation	Country
1	Albèr	Cathrine	Malmö University	Sweden
2	Barros	Nieves	University of Santiago de Compostela	Spain
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67	Wadsö	Ingemar	Lund University	Sweden
68	Wadsö	Lars	Lund University	Sweden
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71	Weichler	Maria-Teresa	Helmholtz-Centre for Environmental Research	Germany
72	Vilu	Raivo	Tallinn University of Technology	Estonia
73	Winzen	Svenja	Max Planck Institute for Polymer Research	Germany
74	von Stockar	Denise	private	Switzerland
75	von Stockar	Urs	Swiss Federal Institute of Technology Lausanne	Switzerland
76	Zacharias	Marc	Calmetrix	USA

Collections of Abstracts

Lavoisier lecture

Energy dissipation and performance in microbes, animals, and distillation columns

U. v. Stockar

Institut des sciences et de l'ingénierie chimiques (ISIC) Station 6, Swiss Federal Institute of Technology Lausanne (EPFL), Switzerland

A new point of view in analyzing Gibbs energy dissipation in growing microorganisms is proposed. Gibbs energy dissipation has a direct link to the biomass yield $Y_{X/S}$: the more energy substrate is catabolized instead of being used in anabolism for building up new biomass, the smaller the yield will be. This usually also leads to a larger heat generation, which may be measured in calorimeters. Therefore, minimization of the energy dissipation would clearly present a biological advantage for growing microbes. On the other hand, the amount of Gibbs energy dissipated is linked to the driving force of growth. Reducing it would slow growth down. As a result, growing microorganisms dissipate in reality considerable amounts of Gibbs energy.

This lecture will propose maintenance metabolism as an explanation why evolution has not favored strains growing with less dissipation but higher biomass yields: such high efficiency strains would grow so slowly that maintenance would dominate carbon and energy substrate consumption, because it has to repair the constant thermal deactivation and decay of biomolecules, which occur at a fixed rate. The biomass yield as a function of Gibbs energy dissipation must therefore go through a maximum.

It is proposed to use the existence of a maximum as a method for predicting biomass yields of microbial strains of interest. Based on an experimental database for a very large variety of microbes, this method will be tested and compared to older, more empirical correlations for predicting yields.

It will also be shown that the optimization problem between fast operation resulting in high costs for energy dissipation on the one hand, and slow operation generating high maintenance costs on the other is very general, occurring even in technical systems such as distillation column design. Finally, it appears that increasing the scale of operation both in technical and biological systems, such as animals, may reduce maintenance requirements. This raises the intriguing question whether the reduction of maintenance costs was the very reason for the evolution of larger organisms such as animals on our planet.

Keywords Gibbs energy dissipation, maintenance costs, non-equilibrium thermodynamics.

References

von Stockar U., Optimal energy dissipation in growing microorganisms and rectification columns. J. Non-Equib. Thermodyn. 2014, in press

Session 1-2: Animals

Bovine feed efficiency evaluated through indirect calorimetry and infrared imaging

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The beef industry has been emphasizing the improvement of feed efficiency. However, the direct selection for improved feed efficiency is cost prohibitive, making the indirect selection through biomarkers a promising alternative. Calorimetric studies are fundamental for identifying and validating potential biomarkers. This study aimed to characterize the circadian calorimetric profile of cattle with known feed efficiency, as well as, verify associations between whole animal calorimetry with *ex vivo* liver oxygen consumption and infrared images from different body locations. Pregnant *Bos taurus* crossbred beef heifers (n=36; age: 704±23 days; body weight: 659±42 kg; days in gestation: 244±4) with known feed efficiency, calculated similarly as detailed by Montanholi et al. (2013), were sampled using a four-head-chamber-open-indirect-calorimeter, comparable to the system described by Odongo et al. (2008). The calorimeter was set to perform 6 min of sampling in each chamber plus the reference channel for a full cycle every ½ h over 24 h of breath gases sampling (CO_2, O_2 and CH_4). Heifers were concomitantly infrared imaged on an hourly basis, with images taken from eye, snout, feet, hind area and flanks. Water and *ad libitum* feed were offered and heifers were allowed to have their postural choices, which were assessed using an accelerometer. Room temperature and humidity were also recorded. At the conclusion of the whole animal calorimetry assessment, heifers were immediately liver biopsied through an intercostal trocarization. Liver samples were stored in a modified Krebs-Henseleit buffer (37 °C, pH 7.4) and 0.5149±0.1198 g of liver tissue were incubated (8.6±2.4 min after collection) for 5 min in a one-chamber-closed-indirect calorimeter similar to the system described by Ninness et al. (2006), these authors also describe the equation used to calculate the O_2 consumption by the liver sample. Means of the 12 highest and 12 lowest feed efficient heifers were compared. Results indicate that more feed efficient cattle have greater oxygen consumption by the whole body (819 vs. 743 W, P=0.01) and by the liver tissue (2.54 vs. 1.57 μmol/min/g DM, P=0.03); more efficient cattle also produce more CH_4 (153 vs. 124 ml/min, P=0.02). Infrared images indicated lower near-infrared radiation emissions in more feed efficient cattle at the eye-globe, caudal view of the feet (i.e. 31.8 vs. 32.9 ^{o}C, P=0.03) and both flank regions. No differences were observed between feed efficiency groups for the remaining body locations infrared imaged. These results provide evidence to support that improved feed efficiency in the bovine is associated with increased metabolic rate and diminished radiant heat loss.

K**eywords:** beef cattle; circadian heat production; methane; thermography; tissue calorimetry.

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FTIR and thermal analysis of nest paper in nine species of Polistinae wasps from Colombia

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Paper wasps exhibit typical nest site selection (inside or out of doors) were they construct paper covers for their nests using different architectural features and paper qualities that are required to improve the thermoregulatory behavior [1]. The material used to build the nests varies by species and includes long fibers and woody pieces slightly rotten and intensively chewed by the wasps as the basic material and a chitin-like secretion of their saliva glands as adhesive [2].

We examined nest paper samples from brood cells and the involucrum of different wasps' species native to South America: *Apoica* sp., *Polybia* sp., *Sinoeca* sp. and *Polistes* sp. The infrared spectra of the samples were taken with an IR Prestige-21 FTIR (Shimadzu, Japan) spectrometer, while thermal analyses were carried out with a TA Instruments SDT-Q600 simultaneous DSC/TGA. Dry nitrogen (100 mL/min) served as purge gas for the calorimetric cells. Individual samples (~15 mg) were placed in alumina pans. Temperature scans began at 30 °C in all experiments, followed by a heating ramp at a rate of 10 K min^{-1} up to a temperature of 600°C. FTIR spectra revealed the presence of different organic functional groups (O-H, C=O, C=C, C-H, CO and C-CO-C), comparison of the absorption frequencies showed that no large differences in the position of bands were present in the samples.

DSC analysis exhibited an endothermic peak (~90^{o}C) due to the loss of absorbed water, a transition from endothermic to exothermic effects around 210^{o}C and three exothermic peaks; the first one at 348^{o}C is connected with the thermal degradation of cellulose, the second one at 435^{o}C with that of lignin, while the last one at 510^{o}C with components formed during pyrolysis. TGA analysis showed that chemically not-bound water appears between 60 and 100 ^{o}C and amounts from 3.4 to 10.2% of the total mass. The percentage weight loss of the samples was less than 21%. The FTIR data were analyzed qualitatively, using the software OriginPro 8.6 test version. TGA/DSC data were treated with a TA Universal Analysis 2000 software and the statistical analysis software OriginPro 8.6 was used to perform the statistical analyses of variance (ANOVA), followed by Bonferroni *post-hoc* tests (P<0.05) to determine whether there are significant differences between and within species.

Keywords: Wasps; paper nests; TGA/DSC; FTIR; thermoregulation.

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The gonadal fat deposits of the African Elephant - A latent heat storage?

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Almost all male mammals carry their gonads outside their body. This prevents overheating of the sperm cells, for their vitality and fertility, higher temperatures are more detrimental. The family of elephants also includes manatees and hyraxes. Male elephants carry their gonads inside their body.

Elephants form two important groups: African (*Loxodonta africana*) and Asian (*Elephas maximus*) elephants. The mean body temperature of elephants is similar to that of humans, namely 36 – 37 °C. Elephants can not sweat, so their body temperature does not decrease by evaporation of water because their skin has no sweat glands. The African Elephant which mainly populates savannas, regulates its body temperature by balancing heat by means of its oversized ears and by spraying its body with water if it is available, and, of course, if possible, by staying in the shade. The Indian Elephant in contrast inhabits rather damp, forested areas and its ears are correspondingly much smaller than those of its African relative.

Especially the male sexually mature African elephant carries around his gonads enormous fat deposits that can each be up to 60 kg in mass. The fat stores are in Africa, for example, used for hair and leather care but also for food. The biological significance of the fat pads is controversial to this day. *Inter alia*, they were considered as an energy reservoir for periods short in nutrition. W. PITRA introduced some years ago therefore the hypothesis that the male sexually mature elephant needs his fat deposits as a protection against overheating of his gonads. According to this hypothesis, the fat pads provide hence a latent heat buffer to avoid its gonads from becoming overheated, in the case the elephant for an extended time is exposed to intensive sunlight.

We have therefore carried out a large number of calorimetric measurements on samples from the fat pads of both male and female African and Indian elephants. We have also compared fat samples of juvenile with such of mature male elephants and also examined the fat of the related manatee. The measurements have shown that the fat of the male, sexually mature African elephant exhibits a different melting behaviour than that of females, the Indian elephant and the manatee. The major part of the latent heat is absorbed by melting in the region between the regular body temperature and 42 °C, which is precisely in the range of critical overheating.

Cyclic measurements with repeated periods of heating and cooling the fat samples in the calorimeter have shown that melting and re-solidification of the elephant fat is completely reversible, provided that a temperature of 42 °C is not exceeded. Temperatures of 42 °C and more are (similar to humans) for the elephant on a permanent fatal. All findings confirm that the fat pads of male, adult African elephants protect the gonads of the animal against overtemperature – at least for an extended period of time.

The heat production of blue mussel *Mytilus edulis trossulus* - is there a synergistic effect of water acidification and oxygen deficiency?

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The ocean acidification is considered as one of the main threats to marine biodiversity. In the Baltic Sea the effect of atmospheric CO_2 on water pH is additionally reinforced by the eutrophication process. High biological production and decomposition of organic matter, especially near the bottom layers, also results in oxygen deficiency – the second anthropogenic problem which seriously threatens marine organisms. Therefore, we investigated the effect of decreased water pH and oxygen saturation on the total metabolic rate of blue mussel *Mytilus edulis trossulus* – one of the the key components of Baltic benthic communities.

Heat dissipation measurements were carried out in a self-made isothermal twin calorimeter of the Calvet type described by Normant et al. (2007), equipped with flow-through system described by Jakubowska et al. (2013). Bivalves were placed singly in the calorimetric vessel and exposed to gradually changing pH (8.1 – control, 7.5 and 7.0) over a period of 36 hours. The experiment was performed at two values of oxygen saturation (~100% and ~20%).

The resting metabolic rate was not significantly ($p > 0.05$) affected neither by pH nor the oxygen saturation. There was also no significant ($p > 0.05$) effect of the interacion of both factors on this process. At high oxygen saturation most bivalves exhibited their natural, diverse behavior, regardless of pH. At low oxygenation most individuals were inactive. The lowered oxygen saturation significantly ($p < 0.05$) affected the contribution of time spend for active metabolic rate to the whole measurement time. However there was no effect ($p > 0.05$) of lowered pH on this parameter, which indicates that oxygen deficiency may affect the Baltic population of *M. edulis trossulus* more seriously than acidification.

Keywords *Mytilus edulis trossulus*; Baltic Sea; ocean acidification; oxygen deficiency; total metabolic rate

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Microcalorimetry and Microrespirometry of Human Placental Samples: A Feasibility Study

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Background and Aim: Mammalian placental tissue exhibits an unusually high hypoxia tolerance. However, the underlying mechanisms are still poorly understood. Data from perfusion experiments suggest that the placental O_2 consumption decreases with decreasing O_2 supply. This kind of "hypoxic hypometabolism" is known from lower hypoxia-tolerant animals and has been observed in mammalian neonates, as well. Since in animal tissues, microcalorimetry and microrespirometry had proven to be useful tools to study hypoxia tolerance, a small pilot study was done to test their applicability to human placental samples.

Materials and Methods: Specimens from the fetal side of human placentae were taken after delivery and transferred to the lab. A total of 12 specimens were subdivided in 4 samples of comparable size and studied by both microcalorimetry (2277 Thermal Activity Monitor, ThermoMetric, Sweden) and microrespirometry (Oxygraph 2K, Oroboros, Austria) with and without addition of glucose to the storage medium (PBS). Another three series of tissue samples of varying size were studied by microrespirometry alone, again with (n=11) or without addition of glucose to the storage medium (Ringer's solution), the latter either directly (n=8) or following a 1,5 hours interval after delivery (n=8).

Results and Discussion: With both methods, reproducible data were obtained. Using placental samples of varying size, a typical "crowding effect" was observed. In the micro-*respirometric* measurements, a low specific O_2 consumption rate was found which remained virtually unchanged over hours and was slightly higher with than without glucose addition. In the micro*calorimetric* experiments, surprisingly high and slowly declining heat output rates were recorded, comparable to earlier findings in preserved mammalian muscle tissue. The long-lasting use of minimal amounts of ambient O_2 (and glucose) may reflect a "hypoxic hypometabolism", possibly related to the placenta's capacity of downregulating its synthetic and transport processes in favor of O_2 transport to the fetus. The sustained high levels of heat production may be indicative of an elevated glycolytic capacity which would fit the concept of metabolic analogies between placental and tumorous tissue ("Warburg effect").

Conclusion: Microcalorimetry and microrespirometry are suitable tools to study placental metabolism. Preliminary data are in accordance with the high hypoxia tolerance reported earlier. Both a "hypoxic hypometabolism" favoring O_2 diffusion to the (aerobic) shell and a high glycolytic capacity in the (anaerobic) core of tissue samples might be causative. In future studies, a more subtle preparation of samples and a completion of respirocalorimetric by histological and/or biochemical methods will be crucial to obtain meaningful results.

Keywords: Human Placenta, Hypoxia Tolerance, Microcalorimetry, Microrespirometry

Heat Flow Measurement in Human Preterm Neonates: Towards a "Calorimetric" Incubator

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Background and Aim: Contemporary intensive care incubators provide a "thermoneutral" environment to preterm neonates, allowing them to keep their body temperature constant in spite of immature thermoregulation. However, due to endo- and exogenic factors, the babies' own basal metabolic rate increases after birth, thus interfering with heat balance. Earlier attempts to record neonatal metabolic rates by either direct calorimetry (in accordingly rebuilt incubators) or O_2 consumption measurement (using head hoods or face masks) have not found their way to clinical routine. In this study, the usability of a miniaturized heat flow sensor to assess the postnatal metabolic increase in human preterm neonates was tested.

Materials and Methods: 29 measurements were done in 20 preterm neonates (mean gestational age 29 5/7 ± 1 1/7 weeks, mean birth weight 1385 ± 298 g,), ranging from day 2 to day 27 after birth. Miniaturized heat flow sensors, consisting of two thermistors separated by an insulating layer of known thermal conductivity, were attached to the patient's forehead, abdominal wall (liver area), and lower leg. "Thermoneutrality" was assumed when the gradient between central (rectal) and peripheral (lower leg) temperature was < 2.0, 1.0, or 0.5 °C, respectively. Heat flow rates were calculated from the difference of inner and outer sensor temperatures, and used to figure out the basal metabolic rate by three alternative algorithms.

Results and Discussion: The hypothesis that under "thermoneutral" conditions, the heat flow from the body surface should reflect the baby's basal metabolic rate, was confirmed: Heat flow rates paralleled the postnatal metabolic increase as is known from the literature. One single measuring point and the simplest calculation algorithm did not lead to worse results than a multi-site, multi-parametric approach, at least as far as the time course of metabolic increase is concerned. Interestingly, however, a clear increase in heat flow rates was observed with increasing axial (central-peripheral) temperature gradients, suggesting that even under contemporary incubator care, "thermoneutrality" may not always be achieved.

Conclusion: In our study of human preterm neonates undergoing incubator care, a novel miniaturized heat flow detector and a simple calculation algorithm provided metabolic data that paralleled the postnatal metabolic increase known from the literature. The strong dependence of heat flow rates on axial temperature gradients puts into question the current incubator settings with respect to "thermoneutrality". Beyond its importance for appropriate thermal care, continuous metabolic monitoring ("calorimetry") in intensive care incubators would help adjust the food and oxygen supply to the babies' individual metabolic demand.

Keywords: Preterm neonates, metabolic rate, incubator care, heat flow

Indirect calorimetry and N balance measurements may be affected by endogenously produced nitrogen

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After oral administration of [$^{15}N_2$]urea (1.5 mmol, 95 atom% ^{15}N), we found that breath N_2 was significantly ^{15}N-labelled [1]. The result suggests that molecular nitrogen in breath must be produced endogenously. Based on a metabolic model, the endogenous N_2 production was estimated to be 0.40 ± 0.25 mmol kg^{-1} d^{-1} or 2.9 ± 1.8 % of the total (urinary and faecal) N excretion in fasted healthy humans (*n* = 4). In patients infected with *Helicobacter pylori* (n = 5), the endogenous N_2 production was increased to 1.24 ± 0.59 mmol kg^{-1} d^{-1} or 9.0 ± 4.3 % of the total N excretion compared to the healthy controls ($p < 0.05$). [2].

We conclude that indirect calorimetry [3] and N balance measurements [4] may be affected by endogenously produced nitrogen, especially in metabolic situations with elevated nitrosation, for instance in oxidative and nitrosative stress-related diseases such as *H. pylori* infections.
It remains to be investigated in humans and animals if other factors such as the quantity or quality of dietary protein, physical exercise, lactation, etc. may also increase the endogenous N_2 production.

Keywords Stable isotope ^{15}N breath test; Endogenous molecular nitrogen; Indirect calorimetry; Helicobacter pylori; Humans; Nitrosative stress

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Effects of temperature and modified atmospheres on diapausing 5th instar codling moth metabolism

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The oxygen and capacity limitation of thermal tolerance (OCLTT) has been established in aquatic insect larvae, but OCLTT has not been shown to generally apply to terrestrial insects. Previous research indicates that heat treatments in combination with high concentrations of carbon dioxide and low concentrations of oxygen may be effective for controlling diapausing codling moth, a quarantine pest in walnuts, but treatment requires long times and the killing mechanism is unknown. In this study, the effects of temperature and modified atmospheres on metabolism in diapausing 5th instar codling moth (*Cydia pomonella*) was investigated with multi-channel differential scanning calorimeters, one equipped with an oxygen sensor. O_2 consumption and metabolic heat rates in air were measured simultaneously at isothermal temperatures from 5 to 50°C at 5°C intervals. Both rates increased with increasing temperatures from 5 to 40°C. The ratio of metabolic heat rate to O_2 consumption rate at temperatures ≤40°C shows that a portion of the metabolic heat is from normal anabolic reactions of metabolism. At 45 and 50°C in air, O_2 consumption and metabolic heat rates dropped to near zero. These results indicate that treatment of walnuts in air at >45°C for a short period of time (minutes) is effective in killing diapausing 5th instar codling moth larvae. Continuous heating scans at 0.4°C/min were used to measure metabolic heat rates from 10 to 50°C with air and modified atmospheres with lowered oxygen and high carbon dioxide. A rapid increase was observed in heat rates above 40°C in scans with $O_2 \geq 11\%$. Taken together with the isothermal results showing no metabolic heat production or oxygen uptake at 45 and 50ºC, these results demonstrate that thermal damage to cell membranes and loss of control of oxidation reactions is the lethal mechanism at high temperature when $O_2 \geq 11\%$. The data from scans with $O_2 \leq 2\%$ and high CO_2 show the effects of oxygen limitation as postulated by the OCLTT. However, CO_2 anesthesia appears to protect larvae from oxygen limitation at high temperature. These results show that treatment of walnuts in air at temperatures >45ºC will rapidly kill diapausing 5th instar codling moths.

Thermoanalytical analysis of wood used for nest sites of the carpenter bee *Xylocopa lachnea*

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Female carpenter bees of the genus *Xylocopa* make large (~13 mm diameter) tunnels into unpainted and weathered softwoods for nests; just large enough for the bee to enter. Usually these bees reuse nesting sites for many years unless they are disturbed. Little is known about nesting-site selection usually, and knowledge is anecdotal rather than experimental. Selection of nesting material may be elaborate [1].

The same species may choose either soft, easily penetrable material which gives a less durable nesting site, or a harder material that offers better protection. Shallow excavations at the nesting sites can be regarded either as examination of the substrate prior to drilling or as unsuccessful attempts to start nests [2].

To attempt to understand the mechanisms used by these bees for the selection of places to build their nests, we analyzed wood from fences with and without bee nests, and sawdust from the entrance of the nests. Thermal analyses were carried out with dry nitrogen (100 mL/min) using simultaneous DSC/TGA (TA Instruments SDT-Q600). The samples weighed ~10 mg and the temperature scans began at 20 °C and ended at 700°C with a heating ramp at a rate of 5 K min^{-1}.

TGA analysis showed that the total amount of water varied from 7.2 to 10.1% of the total mass. DSC analysis revealed a transition from endothermic to exothermic effects around 210^{o}C and two exothermic peaks; the first one was connected with the thermal degradation of cellulose at 289-300^{o}C and the second one of lignin at 369-387^{o}C. The quantity of lignin from sawdust corresponded approximately to twice that found in the solid wood samples.

Keywords: wood; TGA; DSC; carpenter bee *Xylocopa*; lignin; cellulose.

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Combustion calorimetric investigations of nest paper in wasps from Colombia

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Some insect species - among them mainly paper wasps - construct paper envelops for their nests to improve the thermoregulatory behaviour. They use slightly rotten, intensively chewed wood as the basic material and the chitin-containing spittle secretion of their saliva glands as cement. The multi-layered envelope contains the combs which are constructed from nearly the same material [1].

Energetic investigations of nest paper samples used by wasps for building different parts of their nests like brood cells and involucrum were performed by means of combustion calorimetry. We examined samples from brood cells and involucrum of different species of wasps like *Apoica* sp., *Polybia* sp., *Sinoeca* sp. and *Polistes* sp.

A customer built modification of the Phillipson microbomb calorimeter was used for the energy content determination. Combustion experiments were run at room temperature and at a pressure of 2.5 MPa. Ignition was obtained by an Ohmic discharge of a 5000 µF capacitor and a nickelin fuse wire of 0.1 mm diameter.

Samples for combustion calorimetry were burnt in gelatine capsules that were kept till use above silica gel in an exsiccator. The thermal signal of the calorimeter was registered with a datalogger UNIDAN^{PLUS} (ESYS, Berlin, Germany) and graphically analysed for the heat of combustion. The combustion heats of the samples vary between 18.1± 2.1 and 22.8±1.2 J/mg.

Keywords: Wasps; paper nests; combustion calorimetry.

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Host Preference and Performance of Lichenivorous *Astylus* sp. adults (Coleoptera: Meliridae) in Relation to Lichen energy contents and heat production rates

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Host use by herbivores is closely related to properties such as nutrient content and chemical defense. Lichen species are known for the low quality of their nutritional intake and level of chemical defense. Therefore, feeding on lichens is assumed to be more critical than for species feeding on higher plants [1].

Lichen species *Usnea bogotensis, Usnea rocellina, Rimelia cetrata, Rimelia reticulata, Flavopunctelia flaventior, Rimelia revoluta, Hypotrachyna reticulata* were reported in previous studies as host lichen of *Astylus* adults and larvae. Using combustion calorimetry techniques and analysis of consumption and mass gain of individuals, we estimated the consumption index (CI) and the conversion efficiency of food (ECI). A customer built modification of the Phillipson microbomb calorimeter was used for the energy content determination. Combustion experiments were run at room temperature and at a pressure of 2.5 MPa. Ignition was obtained by an Ohmic discharge of a 5000 µF capacitor and a nickel in fuse wire of 0.1 mm diameter.

Consumption and utilization measurements revealed that *U. rocellina* was of the highest quality, although the relative consumption rate was highest on *R. cetrata*. Our results indicate that different secondary chemicals have different effect against lichenivores or that adults are either well adapted to certain chemicals or that these chemicals may have other roles than antiherbivore function for lichens [2]. There were no significant differences in CI and ECI, showing that each species of lichen biomass provides beetles regardless of differences in consumption. U. *rocellina and U. bogotensis* were consumed in greater quantities, which are probably related to their availability in the habitat of *Astylus* sp.

Keywords: *Astylus*, Lichen, Andean forest, trophic relationship, nutritional intake and energy.

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Energy value of Norway lobster *Nephrops norvegicus* (Linnaeus, 1758) from the North Sea

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The Norway lobster *Nephrops norvegicus* is a commercially important species being caught in Europe in amounts exceeding ca. 65,000 tones annually. It plays an important role in the food web. On one hand the muscular tail of *N. norvegicus* known as 'scampi' is willingly consumed by humans, but on the other this crustacean is a prey for commercially harvested fish, like Atlantic cod *Gadus morhua*, Thornback ray *Raja clavata* or small-spotted catshark *Scyliorhinus canicula*. To find out what is the energy content of the whole specimen as well as of its muscular tail the studies on *N. norvegicus* (total length 95.60–153.80 mm) collected in 2007 from the North Sea were performed with the means of modified Phillipson KMB-2 type microbomb calorimeter type MI 100 [1]. Additionally, organic matter content in the studied material was analyzed by ashing in a muffle furnace at a temperature of 450°C [2]. Three replicates of each measurement were performed for each sample.

The mean energy value of *N. norvegicus* from the North Sea (n = 12) amounted to 10.79 ± 1.84 J mg^{-1} dry wt and was significantly ($p < 0.05$) correlated with specimen total length as well as with organic matter content which averaged to 59.56 ± 4.38 % dry wt. The abdominal muscle (n = 29) was characterised by the energy value of 17.48 ± 1.22 J mg^{-1} dry wt and organic matter content of 87.62 ± 1.98 % dry wt.

Keywords *Nephrops norvegicus*; combustion calorimetry; energy value; organic matter content

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Oxygen-related behaviour and heat output in the the Baltic clam *Macoma balthica*

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Behavior and rate of heat output in the Baltic clam *Macoma balthica* (n=10, shell length 14.00 ± 1.19 mm, total dry mass 0.191 ± 0.063 g) were studied during gradual exposure to reduced oxygen saturation from 100% through 54% down to 8% (T = 10^{o}C, S = 7‰) in a Calvet calorimeter [1]. This species lives buried in the sediment in both tidal regions or deeper waters, where it oft suffers environmental hypoxia or even anoxia. Its behaviour is manifested in shell opening and closing and in stretching out inhalant and exhalant siphons to maintain contact with the overlying water.

The high inter-individual variability in behaviour of the studied bivalves was observed. Some of them were inactive or exhibited temporal activity, whereas in other clams peaks of activity appeared more or less rhythmically. Moreover, differences in behavior were also observed in the same specimen at different oxygen saturations making the comparison of results hard. Despite these difficulties, results demonstrate shortening of activity time with the reduction of water oxygen saturation.

At control conditions (100% O_2) the resting and active heat dissipation rates in *M. balthica* averaged to 0.107 ± 0.031 and 0.231 ± 0.081 mW g^{-1} dry wt, respectively. The resting heat dissipation rate was significantly ($P < 0.05$) related to the specimen's total dry weight. Reduction in oxygen saturation affected only the active heat dissipation rate in *M. balthica* causing significant ($P < 0.05$) lowering of the ratio between active to resting rates. It seems that the drop in water oxygen saturation induces changes in behavior and reduces activity of *M. balthica* what in turn leads to reduction in the total heat output. Behavioural changes as well as the ability of rapid shift in rate or type of the standard metabolism are common responses of infaunal invertebrates to adverse environmental conditions.

Keywords *Macoma balthica*; direct calorimetry; heat dissipation; behaviour; deoxygenation

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Session 3: Food

Isothermal microcalorimetry and development of systems biology of bacteria growing in solid state and opaque liquid media

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With the introduction of multichannel isothermal microcalorimetry TAM III a new era in studies of growth of microorganisms in solid state and opaque media began. Development of the method of serial dilutions and use of advanced molecular biology methods including omics methods in parallel with the microcalorimetric experiments made possible application of systems biology approaches to the growth processes of microorganisms in unconventional solid state and liquid (opaque) media.

Growth of *Lactococcus lactis* IL1403 in solid agarose and gelatin gels at different glucose concentrations and different inoculation rates was studied. The results obtained allowed to develop quantitative models of growth of average bacterial colonies in the media.

Growth of *Streptococcus termophilus* ST12 was monitored in milk reconstituted from 10 kGy gamma-irradiated and not-irradiated low heat skim milk powder (LHSMP), both with and without rennet added. In parallel to calorimetric measurements the changes of concentrations of amino acid, sugar and lactic acid amounts were determined and pH measurements of culture media were carried out. Maximal specific growth rates μ_{max} (W h^{-1}), heat produced during different growth stages Q_{TOT} (J mL^{-1}), Q_{EXP} (J mL^{-1}) and lag–phases λ (h) duration were obtained by processing calorimetric curves. Typical dual-peak power-time curves of diauxic growth of *St.termophilus* were registered in reconstituted milk prepared from LHSMP at 35˚C and 40˚C. Power-time curves were remarkably different in irradiated LHSMP (iLHSMP). No diauxia was observed in iLHSMP at 40˚C. Moreover, lag-phase duration was prolonged approximately by two hours by irradiation. The specific growth rate decreased in the rennet curd starting from a certain number of the bacteria in the samples.

A detailed analysis of intracellular metabolic fluxes of the bacteria studied was carried out on the basis of all the data obtained using Single Cell Models.

Keywords microcalorimetry; lactic acid bacteria; colonial growth; solid-state fermentations; metabolic modeling; systems biology

Metabolism of prebiotic fructans by colon microbiota studied by isothermal microcalorimetry

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Fermentation of inulin HP (DP_{ave}~23), fructooligosaccharides (FOS) from inulin - DP<10 (both from Orafti, Belgium); levan (DPave~40000) and levan FOS DP<10 (University of Tartu, Estonia) by colon microbiota was studied in isothermal microcalorimeter TAM III. Consumption of carbon sources and amino acids, as well as production of organic acids, gases, and amino acids during growth in a defined phosphate buffered medium (initial pH 7.1-7.2) was analyzed and a metabolic model of growth of the consortia was developed.

Sequential degradation of oligo- and polysaccharides resulted in multiauxic growth reflected in power-time curves. It was shown that levan and levan- or inulin-type FOS were metabolized with shorter lag phase and with higher growth rates compared to inulin. Hydrolysis of longer oligosaccharides continued slowly over 100 h with the slower specific growth rates while part of the longer oligosaccharides were not degraded. In the medium without any carbon source the growth stopped after exhaustion of energetic amino acids.

The results obtained showed that isothermal microcalorimetry in combination with other analytical methods can be applied for the continuous monitoring of growth of complex consortia of bacteria on oligosaccharide substrates and for quantitative metabolic studies of prebiotics.

Keywords microcalorimetry; growth of colon bacterial consortia; metabolic modeling

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Effect of vacuum impregnation with calcium salts on quality and metabolism of fresh-cut melon

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Vacuum impregnation (VI) is a promising technology for solutes introduction into the internal structure of porous food, exploiting a mass transfer known as hydrodynamic mechanism [1]. Compared to the more traditional dipping treatments, VI gives a deeper and more homogeneous distribution of solutes (e.g. preservatives, bioactive compounds) into the tissues. Several studies have been conducted on the use of calcium salts in dipping treatments to retain flesh firmness and structure of melon (being softening one of the main factors limiting its shelf-life) [2], but calcium introduction using VI has not been extensively studied yet. In this research isothermal calorimetry coupled to respiration rate assessment was used as a tool to investigate the effect of VI with calcium salts on tissue metabolic heat production response of fresh-cut melon. Fresh-cut melon (*Cucumis melo* L. var *Reticulatus*) has been subjected to VI treatment using a vacuum controller, in a isotonic solution with a ratio product:solution 1:4 (w:v). Process parameters (rate of vacuum application and release) were selected on the basis of preliminary tests. The effects of minimum vacuum pressure (200 or 600 mbar) and calcium lactate concentration (0 or 5%) were evaluated on weight gain (%), textural parameters, metabolic heat production by TAM Air isothermal calorimeter and respiration rate (O_2 consumption and CO_2 production) through a static method. Results showed that texture was improved by high calcium concentrations and low vacuum levels, although at this condition the weight gain was the lowest. It was also observed that samples subjected to the lowest vacuum level (200 mbar) showed a significant reduction of metabolic heat production compared to control sample, while the effect of inhibition attributed to vacuum pressure seemed to be contrasted by the presence of calcium in the solution. Actually, after height hours, metabolic heat production was higher in sample impregnated with calcium salts compared to control. This effect may be due to the activation of metabolic reactions within the cell membranes in the presence of calcium salts. Respiration rate results showed that in all the samples a lower O_2 consumption was detected, while no significant differences were found in the rate of CO_2 production. Generally, it seems that the variation of metabolic heat production was not simply related to a change in the cell aerobic respiration rates, but to more complex physiological phenomena.

Keywords Vacuum Impregnation; calcium; fresh-cut melon; metabolic heat production

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Modulating metabolic activity by controlled vacuum impregnation of spinach leaves

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Vacuum impregnation (VI) is a unit operation in which porous products are immersed in solutions of various compositions and/or concentrations and subjected to a two-step pressure change. The first step (application of vacuum) consists on the reduction of the pressure in the solid-liquid system. During this step, the gas in the product pores is expanded and flows out until mechanical equilibrium is achieved. When the atmospheric pressure (second step) is restored, the residual gas in the pores is compressed and the external liquid flows into the pores (Tylewicz et al., 2013). In this study calorimetric measurements provided evidence of a drastic increase of spinach leaf gross metabolism as a consequence of vacuum impregnation (VI) at a minimum pressure of 150 mbar with isotonic solutions of trehalose and sucrose. With the application of VI, extracellular air is replaced by the impregnation solution, potentially limiting tissue respiration to any remaining volume of air in the tissue. However the observation that the impregnated leaves showed photosynthetic activity strongly suggest that not all air was exhausted during VI. Hence impregnation of spinach tissue appears to reach a maximum with remaining gas filled compartments. Metabolic inhibitors were impregnated together with the sugars, showing that the short-term metabolic response responsible for the drastic increase in gross metabolism upon VI depends on the mitochondrial oxygen consuming pathways. Interestingly, the metabolic effect following the impregnation with mannitol was less pronounced and comparable with water impregnation, suggesting that the strong metabolic effect reported here is only seen for molecules that can be metabolized and provide energy to the cells (*e.g.* sucrose and trehalose).

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Effect of cold plasma on fresh-cut fruit tissues metabolism

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Cold atmospheric gas plasma is an ionised gas characterized by active particles such as electrons, ions, free radicals and atoms that is produced by applying energy to a gas mixture. When atmospheric air is used as working gas to generate non-equilibrium plasma discharges, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are formed.

Recently, new applications of cold gas plasma were proposed for food processing. Its oxidative power was tested in order to preserve the qualitative characteristics of fresh-cut fruit and vegetables, with promising results in terms of enzymatic activity reduction [1,2]. However, its effect on tissue metabolism is still largely unknown.

Isothermal calorimetry is a general measurement technique that can provide useful indication of the general metabolic activity of fruit and vegetables, as well as metabolic responses to stress due to different processing operations [3,4,5].

In this research the isothermal calorimetry was used as a tool to investigate the effect of cold plasma on tissue metabolic response in three different fresh-cut fruit (apples, kiwifruit and melon). For each fruit, different treatment times were chosen on the basis of preliminary tests. In particular treatment times of 5+5, 10+10 and 15+15 min per side for apples, 10+10 and 20+20 min for kiwifruit and 15+15 and 30+30 min for melon were chosen. During 24 h at 10°C, metabolic heat production was measured by TAM-Air isothermal calorimeter and O_2 consumption and CO_2 production were monitored through a static method. Moreover enzymatic activity (polyphenoloxidase for apples, peroxidase and pectinesterase for kiwifruit and melon) was assessed before and after the treatment.

Results showed that cold plasma treatment was able to slow down the endogenous heat production in all three fruit tissues considered, proportionally to treatment time. Investigated tissues showed a partial conversion of the tissue respiratory metabolism from aerobic to anaerobic, and a slight inactivation of enzymatic activity was observed after treatment.

Our results confirm that isothermal calorimetry provides a versatile and high sensitive tool for conducting fundamental metabolic studies on the effect of innovative processing operations on fresh-cut fruit and vegetable tissues.

Keywords cold plasma; fresh-cut fruit; tissue metabolism; isothermal calorimetry

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Enhanced metabolic activity of spinach baby leaves as a consequence of pulsed electric field treatment (PEF) and vacuum impregnation (VI)

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To improve the freezing tolerance of spinach leaves a pretreatment in teo steps is applied: vacuum impregnation (VI), which is used to infuse the cryoprotectant in the extracellular spaces of plant tissue and pulsed electric field (PEF) treatment, which is used to introduce the cryoprotectant into the intracellular space of the leaf cells via pores created in the cell membrane. This pretreatment leads to the presence of the cryoprotectant in the outer and inner side of the cell membrane and allows the effective protection of the cells against freezing injury. Calorimetric measurements are used in this study to analyze the influence of the pretreatments (VI and PEF) on the short term metabolic response of spinach leaves.
Spinach baby leaves were placed in a stainless steel ampoule equipped with the pipe to apply vacuum and with electrodes to apply PEF treatment. Cut piece of the spinach leaf was placed in the ampoule and the rate of metabolic heat (thermal power) was recorded for the leaf without treatment (control), after VI treatment and after PEF treatment. The signal was recorded for the combination of VI and PEF treatments and for the PEF treatment only.
The preliminary results show that the thermal power increased after VI treatment by approximately 85 %. Further application of PEF on the previously VI sample resulted in an increase of the thermal power by approximately 87 %. Application of PEF treatment on the nontreated leaf shows an increase of the thermal power by approximately 88 %.

Keywords Vacuum Impregnation; Pulsed Electric Field treatment; Spinach; Metabolic activity

Application of microcalorimetric method for the study of diffusion processes in gelatin matrices

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Growth of *Lactococcus lactis* IL1403 in solid gelatin gels at different glucose concentrations of 2, 10 and 20 g L^{-1} and different inoculation rates was studied using thermal activity monitor TAM III. Inoculation rates from 10^0 to 10^6 cfu mL^{-1} with the increment of 10 were investigated at each glucose concentration in order to study the growth of the colonies of different sizes. The main focus of the study was to investigate the influence of gelatin concentration on the growth of bacteria and diffusion processes. Gelatin concentrations of 10, 15, 20, 25, 30 and 35 % (wt wt^{-1}) were examined. In order to obtain additional information for the interpretation of calorimetric power-time curves the changes of glucose and lactic acid concentrations and pH of culture media were measured in parallel to calorimetric measurements. The sizes of colonies were also determined using microscope during the bacterial growth at different time moments of the calorimetric growth curves. The patterns of pH changes around the colonies made visible using bromocresol purple dye indicator allowed characterize diffusion processes of lactate during the growth. The results obtained showed that the increase of gelatin concentration starting from 15% (wt wt^{-1}) negatively influenced the growth rate of bacteria but the heat production was increased. Calculated diffusion coefficient of lactate decreased with the gelatin concentration increase. All the parameters measured were used in combination for the elucidation of the details of growth of the individual average colonies and diffusion processes of lactate.

Keywords: microcalorimetry; bacterial growth; lactic acid bacteria; colonial growth; solid-state fermentations; diffusion processes

Lactic acid fermentation studied by isothermal calorimetry

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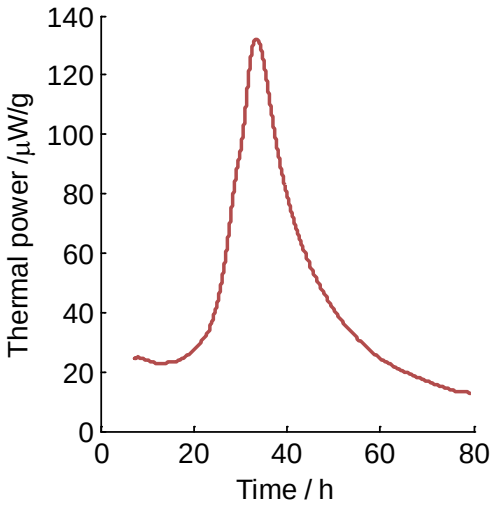
Lactic acid fermentation is used to preserve vegetables, for example in Korea ('kimchi') and Germany, Poland and other Eastern European countries ('sauerkraut'). To make these products, cut vegetables are mixed with salt and left in containers to which air does not have access. Helped by the saline environment, lactic acid bacteria present on the vegetables will grow, produce carbon dioxide, and lower the pH to levels that other microorganisms cannot tolerate. They also consume the oxygen and inhibits the growth of aerobic organisms (mold).

In this study, whole carrots were grated, mixed with 1% sodium chloride and mixed by hand. About 100 g samples were then charged into 125 mL ampoules equipped with thin 10 cm stainless steel tubes to release the produced carbon dioxide, but prevent oxygen from entering the ampoule. The grated carrots were held down in the liquid by a weight. The thermal power was measured at 20 °C with a BioCal isothermal calorimeter (Calmetrix Inc. USA).

All specimens showed a strong peak in thermal power at 30-40 h (see example curve to the right), and all samples had a pH of about 4 after the measurements, indicating that the lactic acid fermentation was successful. Note the rather high initial thermal powers from the shredded vegetables.



Isothermal calorimetry is an interesting technique for studying biological processes in food science [1], for example are fermentation processes easy to monitor; see for example published papers on rye bread [2], cheese [3], and milk [4] fermentation.

Keywords isothermal calorimetry, carrots, lactic acid fermentation

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Influence of ripening stage on metabolic heat production of fresh-cut kiwifruit and melon

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Fresh-cut fruit are metabolically active tissue that show physiological reactions to mechanical stress suffered from minimal processing operations, such as peeling and cutting. The loss of cellular compartmentalisation promotes the contact between enzymes and substrates and an overall increase of metabolic activity [1]. As a consequence of wounding, the plant starts a number of protective processes that lead to an increase of the produced metabolic heat [2], so that the energy released by the cell can be considered the sum of the ‘‘normal’’ metabolic activity and that originating from wounding stress produced by the cells near the cut surface [3]. Many studies have shown that the more advanced the stage of ripeness is, the more susceptible the fruit is to wounds, hence to minimal processing [4] thus emphasizing the fact that maturity influences stress tolerance. Although several research projects have been carried out regarding fresh-cut fruit quality, some basic aspects are still unknown, mainly because only a few studies focused on fresh-cut fruit metabolic response to processing stress. The aim of this work was to evaluate the influence of ripening stage on fresh-cut kiwi fruit and melon tissues in terms of quality and general metabolic parameters. For this purpose three different ripening stages for each fruit were selected. For each ripening stage the fruits were peeled and cut and stored in controlled conditions (climatic chambers at 10 °C, 90% RH) for 3-4 days. During storage, quality indices as soluble solid contents, titratable acidity, colour and texture were monitored. Metabolic assessment was carried out evaluating the endogenous metabolic heat using isothermal calorimetry, and monitoring O_2 consumption and CO_2 production simultaneously. Differences were found in the evolution of the quality indexes during storage; in particular fruits at the lowest ripening stage showed the slowest quality degradation, both in terms of softening and visual quality modifications. Metabolic heat production results were different at the different ripening levels investigated, giving evidence for a strict connection between the physiological state of the tissue and the character of its wounding response.

Keywords Fresh-cut fruit; ripening stage; tissue metabolism; isothermal calorimetry

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Calorimetry: a useful method for determination of unfrozen water in food below freezing point

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Freezing is a common method for the preservation of foods. However, the quality of frozen foods may vary. This can, for example, be in the form of block formation of frozen vegetables (i.e. vegetable stick together in a frozen solid block), which occurs along the entire distribution chain [1]. Block formation is caused by the existence of unfrozen water, a frequent phenomenon in frozen food. Knowledge about the amount of unfrozen water at different temperatures will enable the determination of a critical temperature for the storage of food items. Despite this, the available scientific data for unfrozen water in food products and its determination is rather limited [2]. The aim of the present work was too investigate the amount of unfrozen water at relevant production and thawing temperatures of green peas to better understand when any why block formation occurs in the product.

The instrument used for the experiments was a CPA202 True Heat Flow (THF) reaction calorimeter from ChemiSens AB, Lund, Sweden. The amount of unfrozen water in green peas was calculated based on the required energy for thawing. The required energy was determined by small stepwise temperature increases within the temperature range of interest. The energy required for a certain temperature increase was determined by integrating the THF over time.

The results show that the energy required to thaw green peas with high sugar content is higher compared to green peas with high starch content. This factor has to be known and compensated for in order to design an efficient freezing process. Since the result depends on the chemical composition of the specific food, the required energy has to be determined for each food item in order to design and optimize the freezing processes.

The amount of unfrozen water increases gradually over the studied temperature range. However, at a temperature above -6°C the increase of the amount of unfrozen water is significantly higher. Based on empirical knowledge, this is most likely a critical temperature during the freezing process and handling of green peas to avoid block formation and therefore also quality issues.

Keywords Unfrozen water, frozen food, quality, CPA202 Reaction Calorimeter

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Session 4-5: Instrument and method

The ice calorimeter of Lavoisier and Laplace

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The most frequently shown image in presentations at the International Society of Biological Calorimetry (ISBC) is probably the guinea pig in the ice calorimeter of Lavoisier and Laplace. This iconic image could be the emblem of ISBC and we are proud to represent a line of instruments – calorimeters – that have a history of more than 200 years. In this presentation I will shortly tell the story of this ice calorimeter.

The ice calorimeter was made public in 1783. It was then simply called a *machine* (it was not until 1789 that Lavoisier coined the term *calorimeter*), and was purposely described as the perfect device to study all different types of processes involving heat: heat capacity, combustion, respiration etc. The instrument was about 1 m high and contained a cage for the sample surrounded by two metal cylinders. Ice was placed between the cage and the inner cylinder, and the volume between the two cylinders was also filled with ice. When a sample produced heat in the calorimeter, this would melt the ice in the inner cylinder, which was then at 0 °C. The water could then be collected through a tap in the bottom of the instrument and weighed as a measure of the produced heat. As the ice in the outer cylinder would also melt (as it was in thermal contact with the ambient) this would also be at 0 °C, and there was thus no heat exchange between the two ice compartments; the outer cylinder served as an adiabatic shield.

The ice calorimeter is an odd instrument as it has become an iconic object in chemistry – and possibly even more so in ISBC – despite the fact that no one ever published any scientific results with it, except Lavoisier and Laplace [1]. The truth is that the ice calorimeter of Lavoisier and Laplace was very difficult to work with, something that contemporary scientists pointed out. One main problem was that it was not certain that all liquid water would flow out of the calorimeter as some of it could be retained between the melting ice particles. It was not until Bunsen 1870 invented a better functioning ice calorimeter (in which the volume change of the melted ice was used as a measure of heat) that ice calorimetry was again used in science. Another problem was that the instrument could only be used in the winter, and then only at certain temperatures.

Although the ice calorimeter of Lavoisier and Laplace did not work very well, it fulfilled its purpose as a pedagogical tool [2]. The ice calorimeter was used in the argumentation of the new ideas. If one understood the functioning of the ice calorimeter, one would also, for example, understand that not all thermal events could be discussed only in terms of heat capacity, as was a common idea. When the guinea pig respired inside the calorimeter, it melted ice as a sign of its metabolic heat production (or combustion as Lavoisier called it) and this was one of the most important events – maybe the single most important event - in the history of biological calorimetry.

Keywords Lavoisier, Laplace, ice calorimeter

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Chip calorimetry of aggregated biological samples in segmented flow

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In contrast to batch calorimeters, devices operated in flow-through mode allow the long-term maintenance of well-defined cultivation conditions (e. g. pH, oxygen tension, carbon source) to biological materials. Therefore, they are well suited for the study of transient effects caused by drug treatment [1, 2]. However, usual flow-through calorimeters require the fixation of aggregated samples in the measuring chamber which drastically reduces the sample throughput. Applying the segmented-flow technology [3], we present for the first time a calorimeter for the study of solid and aggregated biological materials in flow-through.

In the newly developed segmented-flow calorimeter, solid particles or biological aggregates up to 1.5 mm are immersed in segments of aqueous solutions. The segments are transported to the heat flow detector of the calorimeter by a carrier liquid. The interfacial tension between the aqueous segments and the water immiscible carrier liquid ensures plug flow and prevents sample dispersion. At a typical segment volume of 11 µL, a volume specific calorimetric resolution of 2 mW L^{-1} was achieved.

The proper functioning of the new calorimeter was proved by measuring the metabolic heat production of biofilms cultivated on glass beads and sulfidic ore particles. For the first time, the heat production of spheroids and human hair follicles could be analyzed in flow-through. The measurement of the basal microbial activity of small soils samples demonstrates the capability of the device to measure solid samples with quite coarse texture. With the successful implementation of the segmented-flow technology, the application range of chip calorimetry is considerably extended.

Keywords chip calorimetry, segmented flow, biofilms, soils, spheroids, hair follicles

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Parylene microfluidic calorimeter integrated with vanadium oxide thermistor

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Microfluidic chip calorimeters can provide high-throughput measurements with minimal sample volume, which will greatly reduce the time and cost for broad range of applications (e.g., drug discovery). However, it has been challenging for chip calorimeters to provide both high sensitivity and precise sample-manipulation capabilities. One of the breakthroughs was made by vacuum-insulation of thin-film microfluidic system. In the previous research vacuum-insulated parylene microfluidics enabled measurements of the heat of reaction from pL scale samples with a few nW resolution [1]. The parylene microfluidic system provides excellent thermal properties and physical strength to build a thin-film microfluidic channels, however conventional fabrication technique has problems of long fabrication time and contamination from residual photoresist [2]. We overcame these problems by molding and bonding parylene layers with initiated chemical vapor deposition (iCVD) with polymer bonding layers[3]. We improved temperature resolution using a vanadium oxide (VOx) based thin-film thermistor, which has a high temperature coefficient of resistance (TCR). The improved microfluidic chip calorimeter can be especially useful for monitoring cell growth and metabolic rate changes from controlled stimuli.

Keywords Chip calorimeter, Parylene microfluidics, iCVD, Vanadium oxide thermistor

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Difficulties in interpretation of high resolution anaerobic biocalorimetric data due to non-perfect reference analysis

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Microbial driven biological conversions are used to synthesize industrial relevant products from readily available feeding materials. Especially, anaerobic processes can be used to yield products with a high energy content compared to aerobic bioconversions due to a lower degree of oxidation. This makes anaerobic bacteria for example interesting for the production of gaseous and liquid biofuels. Real time monitoring tools are essential to effectively control such industrial applications to increase productivity, product quality, and process stability. Given that heat flux measurement reflects changes in stoichiometry and kinetic of bioconversion processes immediately, calorimetry has the potential to be a great tool for monitoring such processes. Forming the enthalpy balance of a reactor allows the determination of the metabolic heat production rate in an easy way [1]. Yet the higher energy content, lower level of oxidation, of the products of anaerobic bioconversions also means that less energy is dissipated throughout the conversion process. These tiny heat production rates are challenging the calorimetric devices and difficulties may arise to apply enthalpy balances for data interpretation. Particularly to develop thermodynamic models, bringing measured heat signals into well agreement with stoichiometric conversion rates gained from reactor experiments might proof to be intricate.

For exploring the information content of calorimetric signals of anaerobic bioprocesses the heat production rate of *Clostridium acetobutylicum* was monitored using a high resolution reaction calorimeter. The heat production rate was analyzed and related to conventionally derived growth kinetics and product formation during the different metabolic phases of the acetone-butanol-ethanol (ABE) fermentation. Since *Clostridium acetobutylicum* is a well-studied bacterium additional information on kinetics, stoichiometry, genetics, and proteomics are available from literature.

Changes between different metabolic phases can easily be retraced by the calorimetric signal. However, the quantitative data interpretation based on advanced kinetic models combined with enthalpy balances proofed to be very challenging. Small errors in chemical analysis of intermediates and products lead to huge differences in predicted enthalpies easily changing the whole conclusion of a model. This means that the calorimetric measurement provides much more accurate process information than the chemical reference analysis (on- and off-line). The challenge is now to combine the high resolution on-line signal of heat production with other on-line (e.g. gas production and analysis, pH) and off-line signals (concentration of biomass, substrate, products etc.) to yield more detailed and more accurate information regarding the ABE fermentation process. Advantages and problems of a calorimetric approach analyzing anaerobic bioconversions processes will be discussed and progress in developing monitoring tools will be presented in detail.

Keywords: anaerobic bioprocesses, process control, renewable energies, *Clostridium acetobutylicum*

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Will isothermal microcalorimetry ever become a routine method for estimation of 'bio-activity'?

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All living organisms produce heat and isothermal microcalorimetry (here called IMC), can thus, in principle, always be used to monitor their activity, continuously and over long periods of time (days, at the µW level), without interfering with the samples. The sensitivity of IMC is higher than most other techniques used to measure a functional property of organisms and results are expressed in real time. However, calorimetry is a non-specific technique, which often limits its usefulness in practical work. Further, the low sample throughput for conventional IMCs is a serious disadvantage, especially for work in the applied areas. Despite these shortcomings, there have been many enthusiastic reports from studies where IMCs were used as 'bio-activity monitors', especially in areas related to environmental problems and the medical field. However, after 40 years of exploratory work, IMC has still not become widely used. Will there ever be a breakthrough?

Chip calorimeters have very low time constants and use extremely small sample volumes, typically in the order of 10 s and 10 nl, respectively. For conventional IMCs corresponding values are in the order of minutes and milliliters, respectively. Chip calorimeters have therefore a much higher sample throughput than the IMCs and have in this respect a great advantage over IMC. However, chip calorimeters are less sensitive than IMCs, which will limit their use, *e.g.* in work with dilute cell suspensions, and they are not suitable for measurements of heterogeneous materials like microorganisms in soil or of relatively large samples, such as most tissue preparations and small animals. I believe that further developments of multi-channel IMCs, if possible in combination with specific analytical sensors, are the most realistic approach to reduce, but not overcome, the problems with IMC.

I will argue that the properties of IMCs and chip calorimeters are complementary and that both instrument groups within a few years' time can develop to the level of routine instruments, dedicated for use as bio-activity monitors in some fields of applied biology. I will also express some views on current exploratory studies, development work, experimental procedures, detectability and accuracy, especially with regard to IMCs.

Keywords isothermal microcalorimeter; chip calorimeter; biological activity; environmental; medical.

Preliminary modeling of a continuous calorimetric set-up for aerobic measurement

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Calorimetric measurements are now considered as a routine tool to determine enthalpy values for microorganisms. Even if it remains some unsolved comprehension on growth mechanism [1], assess of the measured value once integrated is not questioned. The use of power curve has also been studied and proved to be useful for comparison [2].

The measurement of growth rate is more linked to the power curve. In the case of anaerobic measure there is no need to use an open system, and more likely the CO_2 produced can be trapped and a useful technique of respirometry has been developed based on this. The use of a closed and tight system with slight change in pressure corresponds to an ideal case for calorimetry.

When using dilute aqueous suspension, it is convenient to increase the quantity of matter in order to increase the signal/noise ratio and to get significant power curve. Practically, in aerobic case, this means that quite rapidly the oxygen depletion is happening. A mean to overcome is to supply continuously oxygen in the calorimetric cell. Doing this and to be sure that intrinsic kinetic is measured without limitations, it is necessary to ensure bubbling condition to dissolve sufficient oxygen. The oxygen flow in turn tends to evaporate the water of the solution: the concentration is affected and the latent heat of vaporization interferes with the heat of reaction associated to the kinetics.

Based on a continuous set-up that will be presented, the aim of this model is to determine how far the direct reading of the power curve could be interpreted as a kinetic curve.

Keywords oxygen transfer; evaporation; kinetics

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High Sensitive Reaction Calorimetry for Anaerobic Biotechnology

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In recent years anaerobic bioprocesses have been drawn into focus for bio based synthesis of organic compounds like solvents (acetone, 2,3 butanediol) or as sources for liquid (bioethanol and biobutanol) or gaseous fuels (hydrogen or biomethan). Anaerobic bioprocesses also have potential to be used for waste degradation like the Anaerobic Ammonium Oxidation (Anammox). However, to efficiently employ those on an industrial level real time process, control strategies are needed. For aerobic bioprocesses, yielding heat signals of some W/l, reaction calorimetry has already proven to be a valuable tool for process analysis. However, anaerobic processes usually deliver maximum heat signals of a few hundred mW/l. Calorimeters with a very high sensitivity (5mW/l and better) are therefore needed. Microcalorimeters can access this signal range but real process conditions are not reflected by this technology and they are usually too limited to incorporate additional analysis methods (on- and offline) needed to comprehensively understand the process. Reaction calorimeters fulfil these requirements but are usually not sensitive enough.

The CPA202 reaction calorimeter from ChemiSens AB has been equipped with a specially developed high sensitivity reactor, HighSens, for biotechnological applications. The sensor has a sensitivity of 1mW/l and is therefore unique for reactor types with volumes of 100 to 200ml. The reactor can be equipped with various automated dosing and sampling lines for liquids and gases as well with additional sensor such as pH, pressure or more sophisticated ones, like IR or particle size sensors. The measuring principle of the ChemiSens calorimeter offers a number of significant advantages over conventional calorimeters:

(1) Due to its design, it does not require any additional calibration after an initial one that is already performed at the factory.
(2) Consequently, changes in experimental conditions such as changes in pressure, liquid level, stirring rate or viscosity do not require any calibration procedures.
(3) Even the high sensitivity reactor has an extremely stable baseline within +/-1mW/l. This allows a reliable quantitative evaluation of the heat production curves.
(4) The measured heat data do not require any post experimental correction and can potentially be used as process control parameter.

The presentation will show several cases where the CPA HighSens reactor has been used for (e.g. Hydrolysis of Methyl Paraben as example for a chemical calibration reaction but also, anaerobic digestion/ product formation)

Investigation of contaminated soils

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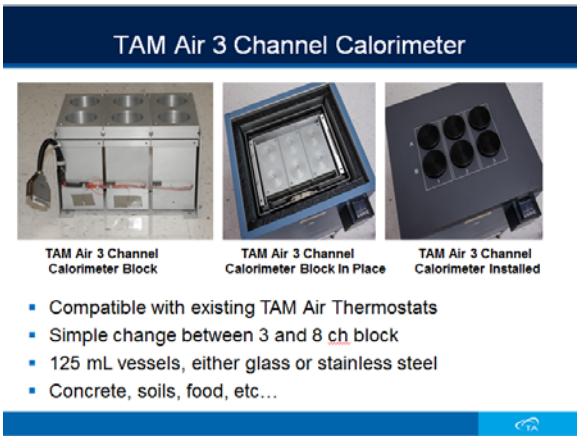
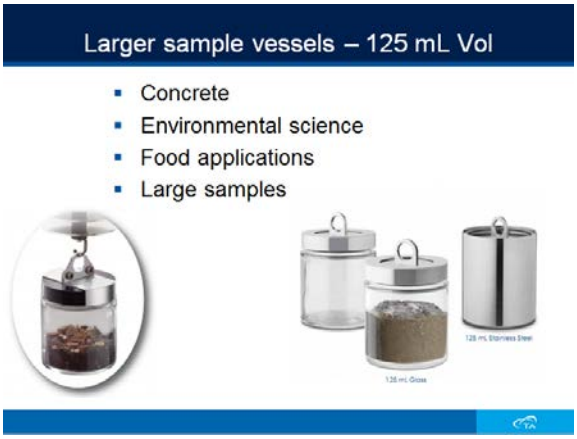
TA Instruments is known for a long time as producer of high quality calorimeters. Beside the high sensitive top-bench instruments dedicated to the Life Sciences, Nano ITC and Nano DSC, the TAM is well established for a long time as a very versatile and powerful instrument for all kinds of calorimetric applications. The rather simple TAM Air on the other hand was implemented as a robust tool in the field of construction chemistry and has fulfilled its duty especially in the cement industry for decades.

However, in case of larger or more inhomogeneous samples TA could provide nothing above the 20ml volume vessels. Because of this the reproducibility, e.g. in investigations of concrete, was rather poor. The 3 Channel block of TAM Air which has recently been introduced by TA Instruments now fills this gap. It consists of three twin channels which house vessels of 125ml volume, either glass or stainless steel. This 3-Ch-block can easily replace a common 8-Ch-block of present TAM Air thermostats. Beside the requirements within construction chemistry mentioned above it also enables investigations of larger samples of food or larger amounts of soils in ecological research or whenever inhomogeneous nature of samples requires higher volumes.





In this talk we will provide first data on heat flow measurements of soils which are contaminated at different levels compared to natural control sediments. We expect that the metabolic heat of the microbial community in such soils considerably differ with the level of contamination.

New and modernized isothermal calorimeters open new possibilities

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A little over 30 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.

Due to high sensitivity and in many cases low availability of sample, calorimeters have been developed for smaller sample volumes >1, 4 and 20 ml. However, there is also need for sensitive calorimeters for larger samples; it may be different heterogenic systems as soil, sediment or food, a sample that needs a certain atmosphere or a larger sample that will not fit into the smaller reaction vessels. Recently two different types of Macrocalorimeters were introduced. These calorimeters have a sample volume of 125 ml and open new possibilities for this type of samples/systems to be studied.

Another microcalorimetric technique is flow calorimetry, where a solution from an external reacting system can continuously be pumped through the calorimeter. This could be a fermentor for the study of the fermentation process or it could be a cell suspension where the effect of different additives can be tested. A new type of flow calorimeter is being developed and is described in this presentation.

Keywords flow calorimeter, macrocalorimeter

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A new calorespirometric vessel for isothermal calorimeters

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Calorespirometry - the combination of isothermal calorimetry and respirometry (the measurement of oxygen consumption and/or carbon dioxide production) – has been used in a number of recent studies, see for example references [1; 2]. The most common method of calorespiromenty is the one presented by Hansen and coworkers. In this method calorimetric measurements are made both with and without a carbon dioxide trap (a small vial with sodium hydroxide solution) in the ampoule [3]; sometimes pressure change rate is also measured [4]. From the measurements of thermal power and pressure change rate, with and without the carbon dioxide trap, thermal power, oxygen consumption rate, and carbon dioxide production rate can be calculated. These three parameters can then be used in further evaluation of the performance of the studied biological system [5].

It is a draw-back with the above method that one has to take up and open the vial to insert and remove the carbon dioxide trap, as isothermal calorimeters take time to recover from the thermal disturbances when samples are being charged. I have therefore built a calorespirometric vessel containing a carbon dioxide trap that can be opened and closed when the vessel is down in the calorimeter. The vessel fits into the TAM Air (TA Instruments, USA) isothermal calorimeter and also contains a pressure sensor. The principles of this instrument and the results of some measurements will be presented.

Keywords: isothermal calorimetry, respirometry, calorespirometry

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Segmented-flow technology enhances chip calorimetry

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The adaptation of the segmented-flow technology (SFT) to chip calorimetry considerably extends its application range, in particular for the study of (micro-) biological materials. Primarily, the SFT was developed to handle samples of picoliters or nanoliters in micro-fluidic systems [1]. Samples dissolved or suspended in aqueous droplets are forced through the fluidic channels by a water-immiscible carrier liquid. Due to the interface tension, plug flow characteristic is achieved which is the precondition for an increased throughput. Moreover, the formation of spatially limited plugs enables the defined transport of solid or aggregated samples through the measuring device. As an effect of the viscous entrainment of the carrier liquid and the capillary pressure inside the droplets, a thin lubricant film is present between the droplets and the walls. The thin film protects the walls against contamination by the sample (e. g. biofilm formation) and prevents cross-talking.

The design of calorimeter components like measuring chambers, heat-exchangers, injection ports, and transport channels is a particular challenge if they should meet the requirements of the SFT. Thus, all components of the fluidics must be highly hydrophobic to prevent attachment of the aqueous sample to the channel walls. Further, only moderate changes in the cross-sections of the fluid pathway can be tolerated to avoid fragmentation of the droplets. Therefore, the adaptation of the SFT to calorimetry is reasonable only for miniaturized, chip-based calorimeters.

In the presented work, we describe a new chip calorimeter for liquid samples which fits the requirements of SFT. Based on numerical simulations, the calorimeter components were optimized with respect to calorimetric sensitivity and heat exchange efficiency. The dynamics of the signal generation under segmented-flow conditions was analyzed to find optimal settings of the operation parameters for maximum throughput and requested signal-to-noise ratio. Furthermore, it was proved that thin films of carrier liquid suppress undesirable biofilm formation in the measuring chamber. The proper work of the segmented-flow chip calorimeter is demonstrated by monitoring the growth kinetics of bacterial cultures and by the measurement of the metabolic heat production of mammalian cells.

Keywords chip calorimetry; segmented flow;

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The application of chipCAL, a flow microcalorimeter (FMC) for high throughput analysis of enzyme activity

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Calorimetry has been successfully used for the measurement of heat exchange in biomolecular interactions in the drug discovery, fermentation, food, textile, and detergent industries, however, for numerous applications classical calorimeters are not suitable because of their high reagent consumption and length of time taken for analysis. Automation of these systems has also been very difficult thereby limiting throughput. These problems can be addressed by automating miniaturised calorimeters containing microfabricated thermopile chips.

TTP Labtech's flow microcalorimeter (FMC), chipCAL is a low cost, low volume flow calorimeter which is ideally suited for label free, high throughput screening of a variety of biological processes such as enzyme activity or microbial metabolism. Enzyme and substrate are passed through a thermodynamic cell simultaneously and thermodynamic changes are detected. With miniaturised flow channels, sample volumes as low as 15 µL can be applied without reducing the heat flow detection limits of about 30nW.

In this poster we present data that illustrates two applications of chipCAL. In one example chipCAL was used to detect an exothermic reaction associated with the amide hydrolysis of asparagine initiated by the enzyme, asparaginase. This reaction reduces the levels of acrylamide, a carcinogen found in carbohydrate foods when cooked at high temperatures. 15 µL of substrate and 15 µL of enzyme were injected simultaneously into the flow cell and mixed. The measurement of thermal change was determined using the microvolt response and calculating the enthalpy of the reaction. An estimation of the Km and Vmax can subsequently be calculated. The entire reaction can be completed in less than 10 mins.

In a separate example chipCAL was used by Barros et al, Spain to measure soil microbial degradation reactions. The sensitivity of this technique was determined for small bulk mineral soil samples and after fractionation of samples with different procedures. Close correlation between heat rates was found between this method and the more traditional isothermal calorimetry method with larger samples.

Keywords flow calorimeter, enzyme activity, asparaginase, acrylamide, soil microbial degradation, automation

Session 6: Microbiology

How reliable is isothermal microcalorimetry of growing cells in the wake of oxygen limiting conditions?

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Metabolic heat production is directly related to the number of cells, the kinetics and stoichiometry of their bioconversions. Approximately 100 000 aerobically growing bacteria or 100 myocardial cells generally produce enough heat to be measurable in conventional isothermal microcalorimeter in real time. The easiest and most economical way to measure such heat signals are experiments in closed, unstirred ampoules in multichannel instruments. The convincing advantages of these simple method lead to many applications in, but not limited to, medicine, pharmacy, environmental sciences, and for the control of food and tap water. The most serious concern however, in regards to the accuracy of such experiments, arises from oxygen depletion inside closed calorimetric ampoules and the influence of oxygen availability on the heat signal. We show that oxygen bioavailability shapes the heat signal to large extent. Therefore, here the balance between oxygen sink (cellular activities) and oxygen source (diffusion) in dependency on the spatial biomass distribution is quantitatively evaluated for the first time. Thereof, rules and suggestions which lower the influence of oxygen bioavailability on the outcome of calorimetric experiments are derived.

Keywords Isothermal Microcalorimetry, Biothermodynamics, Oxygen balance, Cells

Calorimetric Process Control for Microbial Product Formation Using Toxic Feedstocks

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When intending to use microorganisms for product formation, process control is one of the most delicate aspects to be considered. Only minute changes in fermentation conditions may considerably affect the aspired synthesis rates. Accordingly, every step of the fermentation has to be optimized to achieve a maximal formation rate and yield of the desired product.

Currently, by using traditional feedstocks such as simple sugars and fatty acids, industrial biotechnology directly competes with food production for agricultural resources. In search for alternative substrates, methanol is discussed due to high synthesis capacities and its potentially sustainable production from natural gas, agricultural waste materials and biogas. However, in contrast to other carbon sources, methanol is highly volatile and acts toxic at high concentrations. Its application as a substrate within fermentation processes therefore calls for an efficient and innovative real-time control strategy.

In this study, we decided on biocalorimetry as a basis for an optimal control of substrate feed. Every metabolic flux is via law of Hess quantitatively related to the heat production rate. Thus, calorimetry provides real-time stoichiometric and kinetic information of the fermentation process. Furthermore, compared to other monitoring tools currently used for bioprocess control, for example the oxygen uptake rate, biocalorimetry has a higher sensitivity and a much finer resolution. This allows reacting faster and more efficiently to metabolic changes.

For testing the newly developed control strategy, cultivation experiments were performed with *Methylobacterium extorquens*. This methylotrophic bacterium has been shown to utilize methanol fast and efficiently while accumulating polyhydroxybutyrate (PHB) within its cell. The biopolymer hence served as the product to be optimized.

In first fermentation experiments, we were able to show an exact correlation between the heat produced and other growth patterns. This allowed us to develop algorithms for calorimetric control of the optimal substrate feed. By applying this method, we achieved better growth rates on methanol as well as a higher final PHB concentration. Additionally, we were able to extend this control strategy to other substrates, e.g. formic acid. Even for this higher toxic and thus more challenging substrate, biocalorimetry proved as a very efficient tool for process control.

Keywords biocalorimetry; real-time process control; methanol, polyhydroxybutyrate

A real-time study of defined mixed cultures of bacteria

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Bacteria exist as mixed cultures of different species in their natural environments. Most laboratory experiments are however done on pure species of bacteria. Interactions that may occur and their consequent effects on the metabolisms of the individual species are missed by this approach. The potential of isothermal microcalorimery to identify and discriminate between species of bacteria has been established [1]. Such discrimination or characterization can be useful to study mixed cultures. In this work, we explored isothermal microcalorimetry to study defined mixed cultures of two species. The mixed cultures studied were *P. aeruginosa* and *S. aureus*, *P. aeruginosa* and *E. coli*, *S. aureus* and *E. coli*, *P. aeruginosa* and *B. lactis*, *S. aureus* and *B. lactis*, *E. coli* and *B. lactis*, *L. acidophilus* and *C. difficile* and *B. lactis* and *C. difficile*.

Each species was firstly inoculated to a cell density of 10^6 cfu/mL in broth and studied as pure culture. In mixed culture studies, the two species were introduced into broth to give an equivalent inoculum of 10^6 cfu/mL each. Measurements were taken with a TAM 2277 (TA Instruments Ltd., UK) set at 37 ± 0.1^{o}C and at amplifier setting of 1000 μW

Power-time curves were characteristic for the species in the different media studied. A mixed culture of *P. aeruginosa* and *S. aureus* at equal densities exhibited metabolism synonymous to *P. aeruginosa* alone, which correlated with other studies [2]. When the density of *P. aeruginosa* was exponentially decreased, *S. aureus* gradually recovered showing power-time curves that demonstrated this. Mixed cultures of *P. aeruginosa* and *E. coli* at equal densities showed power-time curves representative of both species demonstrating the co-dominance of the organisms. *S. aureus* and *E. coli* mixed culture behaved metabolically like *E. coli* alone. Mixed cultures of *B. lactis* with *P. aeruginosa*, *S. aureus* and *E. coli* showed power-time curves representative of all species. Plate counts at the end of the TAM experiments however showed complete inhibition of *P. aeruginosa*, *S. aureus* and *E. coli*. The mixed cultures of *L. acidophilus* and *C. difficile* and *B. lactis* and *C. difficile* lacked the characteristic curve of *C. difficile* and were superimposed on either the sole culture of *L. acidophilus* or *B. lactis*.

The use of isothermal microcalorimetry has been able to demonstrate competition between species.

Keywords defined mixed culture; two species; isothermal microcalorimetry

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Antibacterial activity of commercial nAg and $nTiO_2$ against beads-grown *P. putida* biofilms – a chip-calorimetric study

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Nanomaterials are expected to improve our lives in many ways. Photoactive metal oxides and metallic nanomaterials have attracted great attention due to their broad antibacterial capabilities and have now penetrated the consumer and medical products market. This, in turn, has led to the growing concern over the potential impact of these nanomaterials on the natural environment since production, use and disposal will inevitably lead to discharges to air, soils and aquatic systems [1].

It is well known that photoactive metal oxides like TiO_2 form reactive oxygen species (ROS) when activated by UV or solar light. ROS generated by nanotitania ($nTiO_2$) can cause oxidative damage to cell membranes and inactive microorganisms. Although the bactericidal mechanism of metallic nanomaterials such as nanosilver (nAg) is still under discussion, ROS generation and release of silver ions which penetrate the cell membranes seem to be important [2]. However, a profound understanding of the effects of nanomaterials in complex systems is still lacking. In this respect, biofilm communities are a more pertinent model system for consideration of nanomaterial toxicity in environmental systems than planktonic cells [3].

While nanomaterial effects on planktonic bacteria can be easily quantified by conventional methods (e. g. optical density, cfu), biofilm analysis however represents a complex issue requiring more sophisticated approaches due to the inhomogeneous nature of the samples. In this context, it becomes clear that calorimetry may represent an interesting alternative.

In the presented work, we studied the concentration-dependent effect of commercial nAg and $nTiO_2$ on growth and inactivation of *P. putida* mt-2 biofilms cultivated on glass beads ($d \leq 106$ µm). Using a newly developed segmented-flow chip calorimeter [4], it will be demonstrated that calorimetry is ideally suited to overcome the inhomogeneity that is typically associated to biofilm samples and is a major challenge for all other test methods.

Keywords chip calorimetry; nanomaterial; *Pseudomonas putida*; biofilm

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Determining the antimicrobial properties of coatings and porous biomaterials by taking advantage of isothermal microcalorimetry.

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Monitoring bacterial growth and activity at the surface or inside of porous solid substrates is often a complicated task and usually requires destructive assays. Therefore assessing antimicrobial properties of porous implant material or coatings is still challenging and relies mostly on microscopy or on conventional culture methods. Both of these approaches only provide endpoint measurements and bear several drawbacks as the cells have to be stained and / or detached from the original substrate.

In this study we investigate the use of isothermal microcalorimetry to monitor bacterial growth at the surface of two antimicrobial coatings. Since hydroxyapatite is known for its good biocompatibility, the first coating tested was silver doped hydroxyapatite. Similarly we tested calcium hydroxide coatings because calcium hydroxide as been widely used for its antimicrobial properties in dentistry. To assess the antimicrobial efficacy of each coating, the coatings were applied on titanium coupons (ca. 15mm in diameter) using vacuum plasma spraying for hydroxyapatite and electrodeposition for calcium hydroxide. Then 10^5 bacteria (*Stapylococcus epidermidis*) were inoculated at the surface of the coating and the coupon was placed in a microcalorimetric vial containing solid Luria agar with the inoculated hydroxyapatite or calcium hydroxide side facing the medium. This setup ensures that the inoculum will form a biofilm at the interface between the medium and the coating.

The metabolic heat production was measured over time using a TAM air calorimeter and the antibacterial activity of each coating was calculated using the growth parameters (i.e., the growth rate and the lag phase duration) derived from the calorimetric data. Using this approach we were able to show that more than 99.99% of the original inoculum deposited on the two different coatings was killed.

Keywords: isothermal microcalorimetry; antimicrobial coating; hydroxyapatite; Calcium hydroxide.

Combination of isothermal microcalorimetry and mass-spectrometry for rapid drug susceptibility testing in suspected urosepsis

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Urosepsis is defined as sepsis originating from the urinary tract. It might progress toward severe sepsis and septic shock both associated with high mortality. Thus rapid drug susceptibility testing is of major importance to improve early empiric antimicrobial therapy. In this context, pathogen identification could be achieved within a few minutes using MALDI-TOF MS. Once the pathogen has been identified, it is possible to select a set of antimicrobials and determine the susceptibility of the pathogen by using isothermal microcalorimetry. Here we present results obtained using 15 bacterial strains isolated from different patient with suspected urosepsis. We compared the results obtained with the isothermal microcalorimeter to those obtained with the most commonly used automated drug susceptibility testing system (i.e., Vitek2).

With respect to the speed of the technique, isothermal microcalorimetry was able to deliver results within 7 hours as the Vitek systems needs 16 to 48 hours. The seven hours include 30 minutes for sample handling, 6 hours of measurements and 30 minutes for data processing and calculation of an inhibition index. Such fast processing is extremely important because in septic shock every hours after the first hour increase the risk of mortality by approximately 8%. In addition, the accuracy isothermal microcalorimetry was ca. 93% with a sensitivity of 95% and a specificity of 91%. Due to their slow action the beta-lactam class of antimicrobials was the most difficult class to assess. However, still resistant strains were all identified correctly.

Keywords: isothermal microcalorimetry; urosepsis; drug susceptibility testing.

Isothermal microcalorimetry with reporter bacteria for assessment of nutrient content in source and processed drinking water

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During the transformation of raw source water to drinking water, nutrients are removed by bacteria growing as biofilms within sand filters. These biofilms are uncharacterized, but during metabolism of the components of the source water will produce heat which could be detected using isothermal microcalorimetry (IMC). Traditional measurement of biologically available carbon has used a biofilm grown from source water to metabolize the organic carbon. As IMC can assess metabolism of bacteria growing as biofilms (1) measuring the nutrient content of drinking water could also use a biofilm growing within the vials of the IMC and exposed to drinking water of varying nutrient content. IMC has been used to detect bacterial contamination of drinking water with high sensitivity and could also identify the contaminant, since different bacterial species have specific metabolic and kinetic profiles (2). In addition, IMC is desirable for routine monitoring of drinking water as it does not require additional reagents, making it cost effective; and it produces fast, real-time measurements.

In order to determine if IMC can be used to monitor changes in biologically available organic carbon when raw water is processed, the metabolic activity generated by different types of bacterial growth media, and drinking water samples representing 4 stages of the drinking water treatment process was assessed. Raw water, filtrate after rapid and slow sand filters and distributed drinking water were combined with defined bacterial strains (*E. coli* JM109; *Novosphingobium aromaticivorans* strain DSMZ 12444) after determining appropriate cell densities and media controls for each strain. Metabolic activity was observed as heat and related to the total organic carbon of the water samples, and stage of drinking water processing.

Keywords isothermal microcalorimetry; reporter bacteria; organic carbon; drinking water

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Autophagy: a possible survival strategy for *Candida spp*.

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Urinary tract infections caused by *Candida albicans* represent a significant healthcare problem. Although antifungal therapy is often efficient in the short term, relapses are still common.

Understanding the dynamic and the mechanisms allowing the survival of *Candida albicans* in urine especially in the presence of inhibitory concentrations of antifungals is of certain importance to avoid such relapses and associated morbidities and mortalities. Here we used isothermal microcalorimetry (IMC) to monitor the metabolic activity of *C. albicans* inoculated in artificial urine added with fluconazole, fluorocytosine and amphotericin B. The results were compared with conventional cell counts and with the glucose consumption in artificial urine. For all antifungals, peak in the metabolic activity measured using IMC were very closely related to the peaks in cell number. As expected only amphotericin B acted as a fungicidal agent killing a very large proportion of the original inoculum. This resulted in a metabolic activity that was not detectable for the first 4 days. On the contrary with fluconazole and flucytosine no decrease of the cell count was observed, and a weak metabolic activity was recorded for up to 31 days. Noteworthy metabolic activity and growth were maintained for up to 15 days after the resources (glucose and proteins) in the medium were exhausted. In this context, we hypothesize that autophagy might be the mechanism providing the necessary resources for maintaining the metabolic activity and allowing the formation of candidal chlamydospores. Although autophagy needs to be further confirmed by cytological observation, this emphasizes the role of autophagy in the resistance to starvation. Indeed, it is not uncommon to observe an increase in cell counts in starved *Candida* cultures in which autophagy is confirmed. In addition since efflux mechanisms (i.e., efflux pumps) require energy to pump the antifungal out of the fungal cells, it also underlines that autophagy might be an important process allowing resistance to antifungal treatment as well.

Keywords: isothermal microcalorimetry; urinary tract infections (UTI); *Candida*; fluconazole, autophagy.

The influence of spectral composition of light on the metabolic activity of *Chlorella emersonii* and *Botryococcus braunii* in the dark

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Strains of *Chlorella emersonii* and *Botryococcus braunii* are listed among the species of algae containing highest amounts of lipids in the cells. The spectral composition of light is one of the key parameters in the culture of algae in photobioreactors. *Chlorella emersonii* (SAG 2334) and *Botryococcus braunii* (SAG 807-1) have been cultured in photobioreactors. The light sources applied were the LED matrices emitting blue-red light (BRL), blue-red-far red light (BRFRL) or fluorescent lamps emitting white light (WL).

The aim of the study was to determine the effect of spectral composition of light used during the day on the metabolic activity of *C. emersonii* and *B. braunii* strains in the dark (night).

The analysis of the course of specific thermal power-time curves indicated that the cells of *C. emersonii* cultured under BRFRL showed a higher emission rate of thermal power than those cultured under the BRL or WL in particular. Specific thermal power-time curves for the *B. braunii* culture were significantly different from those observed in *C. emersonii*. The lowest emission values of thermal power had cells growing under BRFRL, while similar thermal power values were observed for the cells cultured under BRL and WL. Interestingly, after approximately 6 hours of measurement, the appearance of peaks was recorded on the thermal power-time curves of *B. braunii* culture, regardless of the spectral composition of light used, while in *C. emersonii* this phenomenon was detected only for BRL. The time of occurrence of these peaks preceded the moment of turning on the lights in photobioreactor, by about 1 hour (end of the night). Therefore, we can assume that this effect is related to the day/night rhythm of algae. This phenomenon also shows an increase in the intensity of metabolism of algae at the end of the night ("waking up of the algae") and a preparation of cells to light.

Specific thermal energy produced by the cells of *C. emersonii* was several times higher than the energy of *B. braunii* cells. In addition, *C. emersonii* cells growing under BRFRL had significantly higher thermal energy compared to those cultured under WL. In contrast, no statistically significant differences in thermal production have been found for the *B. braunii* cells that would correlate with the spectral composition of light applied.

The data shows that the spectral composition of light plays a key role in the photobioreactor culture of *C. emersonii* and *B. braunii* by regulating the metabolic activity of cells. However, this influence is strongly dependent on the strain of algae.

Keywords: algae, *Chlorella emersonii*, *Botryococcus braunii*, isothermal calorimetry

Chip calorimetry on spheroids: Study of proliferation and viability

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Multicellular spheroids, formed by self-assembly of single cells, are commonly used as a three-dimensional cell culture model in drug delivery, drug screening, tissue engineering, and stem cell biology [1, 2]. A 3D cell structure mimics the cell-cell and cell-extracellular matrix interactions like tissues *in vivo* regarding the spatial, temporal, physical, and chemical properties determining hereby the cellular phenotype outcome. Cells in 2D monolayers are characterized by much contact of the cells to the solid surface and the medium, but no gradients of nutrients, growth factors or waste products affect the cells. Therefore, some cells lose their normal phenotype once taken out of the tissue and cultured as 2D cell culture. Nowadays different 3D culturing methodologies are used in order to simply generate cost effective spheroids in controlled-environment in high-throughput to provide standardized 3D cell models for the particular biomedical research.

2D cell-based analytical protocols are well-established, whereas 3D methods pose new analytical challenges. When turning to 3D analysis, many assays are rendered useless. This is particularly valid for high-throughput screening methods where predominantly fluorescent and chemiluminescent signals are analyzed. Because of its real-time capabilities, chip calorimetry offers new possibilities for the quantification of proliferation and viability of spheroids. For the first time, we measured the metabolic heat rate of small numbers of spheroids using a new segmented-flow based chip calorimeter.

In our experiments, we used spheroids of murine fibroblasts L-929 cells which were cultivated by the hanging drop method [1], a simple method where cells in suspension spontaneously aggregate in a drop to a spheroid. In each experiment, one to five spheroids were placed in a sample segment containing 6 µL of nutrient solution. The sample segments were transported by a carrier liquid into the measuring chamber of the chip calorimeter. The measured heat production rate was strictly linearly dependent on the number of spheroids placed in a sample segment. For a single spheroid, cultivated for 6 days starting with one thousand cells, a heat production rate of about 0.5 µW was determined. The heat production rates of the spheroids which were cultivated for the same period but with different starting cell numbers correlated with their sizes.

Keywords chip calorimetry; segmented flow, spheroids

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Session 7: Ecology, Soil and Plants

Completing the temperature function in metabolic theories of ecology

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Because the temperature function is incomplete, the metabolic (MTE) and the dynamic energy budget (DEB) theories of ecology cannot fully explain many of the documented correlations between the success or failure of organisms, the properties of ecosystems, and the climate. As currently formulated, MTE cannot describe performance of poikilotherms over the entire range of relevant temperatures and lacks explanatory power because: 1) the molecular basis for MTE has not been explicated, and 2) MTE does not contain any principles for natural selection. Adding ”congruency" (see below) as a principle for natural selection and including a molecular mechanism in the theory based on well-established properties of proteins neatly fixes these shortcomings. The molecular basis of the theory is needed because enzymes catalyze and control nearly all activities of living organisms. Therefore, it is not surprising that the rate/temperature relationships of these activities (i.e., performance curves), are congruent with those of enzyme activity versus temperature. The rates of the activities that organisms do in order to survive and reproduce are thus described by the same equation as the microscopic processes catalyzed by enzymes. Simply put, the form of the equation that describes enzyme kinetics *in vivo* also describes the temperature dependence of performance curves.

Microbial energetics of soils exposed to different temperatures and land uses

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Soil organic matter is the largest carbon pool in terrestrial ecosystems. It is two to three times the amount of carbon in the atmosphere[(1,2)]. The fate of soil organic carbon in an ecosystem is determined during its decomposition by microbial metabolic activity. Temperature changes may alter both microbial metabolism as well as community composition of active microorganisms. Energy is required to maintain metabolism, and to understand soil microbial energetics and its temperature dependence may improve our knowledge of the global carbon cycle and help in refining climate change modelling[(3,4)].

We investigated microbial energetic patterns of soils exposed to temperatures at the range of 5 °C to 20 °C under different land uses by isothermal calorimetry. Soil samples were taken from forest, arable, grassland and ley farming long-term research sites situated in a boreal climate (sites are closely located to Umeå in Northern Sweden). We determined the heat signals for those samples after soil amendments with different substrates representing simple to complex organic matter. In addition, respirometric measurements were performed parallel on separate soil samples.

We will present our findings illustrating microbial energetics and respiration in soil ecosystems exposed to different land uses and temperatures. Our findings will be linked to the microbial community composition determined via phospholipid fatty acid (PLFA) analysis. Results will be discussed in relation to their potentials in how far they can be incorporated into soil organic matter modelling.

Keywords: Isothermal calorimetry; soil microbial energetics; soil temperature, land use; microbial community composition; substrate complexity

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Revisiting the terrestrial carbon cycle: New insights into microbial metabolism

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Energy is continuously transformed in environmental systems through the metabolic activities of living organisms. In terrestrial ecosystems, there is a general consensus that the diversity of microbial metabolic processes is poorly related to overall ecosystem function because of the inherent functional redundancy that exists within many microbial communities. Here, we propose a conceptual ecological model of microbial energetics in various terrestrial ecosystems (e.g. Scandinavian arable systems or temporarily flooded systems in South East Asia). Using isothermal calorimetry, we show that direct measures of energetics provide a functional link between energy flow and the composition of belowground microbial communities at a high taxonomic level. In contrast, this link is not apparent when carbon dioxide (CO_2) was used as an aggregate measure of microbial metabolism. Our results support the notion that systems with higher relative abundances of fungi have more efficient microbial metabolism. Furthermore, we suggest that the microbial energetics approach combined with spectroscopic and aqueous chemical measurements is a viable approach to determine the effect of energy release from organic matter on metal(loid) mobility in soils and sediments under anaerobic conditions. We advocate that the microbial energetics approach provides complementary information to soil respiration for investigating the involvement of microbial communities in belowground carbon dynamics. Our results indicate that microbial metabolic processes are an essential constituent in governing the terrestrial carbon balance and that microbial diversity should not be neglected in ecosystem modeling. Quantification of microbial energetics incorporates thermodynamic principles and our conceptual model provides empirical data that can feed into carbon-climate based ecosystem feedback modeling. Together they disentangle the intrinsically complex yet essential carbon dynamics of soils to address important issues such as climate change.

Keywords Soil carbon; microbial energetics; microbial community; use efficiency; conceptual model

Does storage protein composition influence metabolic activity of wheat seedlings?

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Plant storage proteins are one of the chemical components of the grain that play an important role as respiration substrates for germinating seeds. Species and cultivars of common wheat (*Triticum aestivum* L.) have a great diversity of chemical composition of storage proteins (gliadins and glutenins). It is highly probable that the distinct structure of gliadins and glutenins affects the efficacy of metabolic activity during germination. To verify this hypothesis, we analyzed the changes in heat production of germinated seedlings of two closely related wheat lines differing in the composition of ω-gliadins as evidenced by electrophoresis (A-PAGE).

Thermal power-time curves for five growing seedlings of each wheat line were recorded by isothermal calorimetry (TAM III). Statistically significant differences were observed in heat production between the two wheat genotypes. Genotype lacking most of the ω-gliadins showed significantly higher values of heat production, expressed as J/g_{DW}, as compared with the second genotype containing a complete set of proteins. The lack of ω-gliadins caused a greater than expected effect of increased metabolic activity in the early stages of seedlings growth.

Keywords wheat, storage proteins, germination, calorimetry, metabolic activity

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Sensitivity of chip calorimetry to study the degradation of the soil organic matter

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Soil organic matter (SOM) is a complex macromolecule constituted by different fractions that differ in their red-ox state. To yield a model of SOM structure that can explain how the macromolecule is degraded constitutes an important goal in soil science [1]. But the only way to achieve that is by associating different SOM fractions taking part of the SOM macromolecule with their respective degradation rates. Most of those measurements use the rate of CO2 released by the biochemical reaction as the indicator of SOM biodegradation. However, this method may fail to express the biological activity correct in case of prevalent humidification, mineralization of humidified substances, or in case of changing environmental conditions, or if the content of oxygen, carbon, hydrogen and other elements distinguishes in the fresh added to soils organic matter. The detection of heat as indicator for energy transformation resulting from biological degradation processes may help to solve these challenges.

There are several methods to obtain the different fractions constituting SOM. Some of them involve soil treatments that yield very low quantities of SOM mass. Therefore, it could be interesting to develop and to design technical devices allowing the measurement of degradation rates of very low SOM quantities. Chip calorimetry can be an option but it was never tested before to study soil microbial degradation reactions.

We studied the sensitivity of this technique for detection of microbial degradation processes in small bulk mineral soil samples (mean carbon content 4 %) and after fractionation of samples with different procedures. The calorimetrically determined heat rate in samples of 7 mg was compared with results obtained by isothermal Calorimetry with much bigger samples of 0.3 g through well-established methods for soil.

The found results confirm close correlation between heat rates determined by both methods. The chip calorimetry technique was found even sensitive enough for reliable detection of the dynamics of heat flow rates with high resolution in time.

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Factors influencing the calculation and evolution of calorespirometric ratios in soils determined by calorimetry.

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The study of mechanisms of soil organic matter (SOM) stabilization is a topic of increasing interest due to its direct involvement in the carbon (C) sequestration capacity linked to a certain soil management. It comprises knowledge about SOM properties from different perspectives and their connection with the SOM biodegradability. The highly complex structure of SOM made necessary the search for new methods that can report the SOM chemical, physic-chemistry, thermal, and biological properties in order to evaluate the C sequestration capacity in base on the concept of SOM quality and its resistance to be degraded.

Calorimetry can play an important role in the assessment of the biological stabilization of SOM since it can directly measure the CO_2 and heat rates of SOM decomposition to yield calorespirometric ratios of the SOM biodegradation processes. Calorespirometric ratios are linked to the nature of the substrate being degraded and can inform about changes in SOM nature after a certain management. Previous work showed they can vary a lot in soils.

This work focuses on establishing the main factors influencing calorespirometric ratios of SOM biodegradation in order to improve the interpretation of their variability and the adequate protocols for their calculation by calorimetry. The main factors studied were soil sieving, humidity of samples, SOM chemical and thermal properties, soil management, and temperature.

Results showed that the main factor affecting the evolution of calorespirometric ratios were the spatial distribution of SOM and its interaction with the soil mineral matrix. Humidity did not affect them if working with percentages under 60 % of the soil water holding capacity. SOM chemical properties can yield calorespirometric ratios higher than those given for carbohydrates in some soils but the general evolution observed support the mechanistic models of SOM structure.

Keywords calorimetry, soil, biodegradation, soil organic matter.

Simultaneous measurements of microbial energetics, methane and CO_2 in soils with varying water contents

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Soils are structurally heterogeneous and external environmental conditions do not have a uniform effect throughout the soils, resulting in a large diversity of micro-habitats. In such complex environments, several metabolic processes occur simultaneously and thus deconvolution of heat profiles into various soil processes (e.g. aerobic and anaerobic soils processes) are required. However, a robust method is currently not available to deconvolute soil processes and method development is required in such heterogeneous systems such as soils.

Soils were taken from three different soil systems varying in soil management (arable soil; grassland soil and forest soil situated in Uppsala, Sweden) and exposed to various water contents yet constant 25°C. A range of different carbon substrates were added to the soils in order to stimulate microbial metabolism. Based on previous studies [1; 2], we further developed an experimental setup which allows simultaneous measurement of energy transformations, methane and CO_2 production in soil systems.

We will present preliminary data using this experimental set-up. Particularly, practical considerations in the use of this method with respect to experimental design, sample preparation and interpretation of data will be emphasized.

Keywords Soil; microbial energetics; water content; deconvolution

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The use of glucose, cellobiose and cellulose for calorimetric studies of soil microbial processes

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Cellulose, hemicellulose, lignin and other substances are the most important organic substrate groups for formation of soil organic matter [1]. Glucose and cellobiose are present in soil as a result of enzymatic hydrolysis of organic substrate. Glucose is very often used in soil microcalorimetric studies [2, 3]. Less attention has been paid to research on biodegradation of other saccharides, for example saccharose, lactose [4], cellulose [5].

Calorimetric determination of thermograms of glucose, cellobiose and cellulose biodegradation in soil was the aim of our studies. Additionally we investigated the influence of phosphogypsum on the progress of those processes.

Heat-conduction isothermal microcalorimeters were used for measurements. Each type of calorimeter contained four measuring cells and one as the reference with thermally inactive soil. The calorimeters were connected to a very stable thermostating system. Studies were conducted using the soil samples collected at the Agricultural Station of the University of Warmia and Mazury in Olsztyn. Biodegradation of glucose, cellobiose and cellulose in the amount of 1 mg $\cdot$ g^{-1} soil dry mass at the presence of ammonium sulphate (1 mg $\cdot$ g^{-1} soil dry mass) and water content corresponding to 60% of the water holding capacity has been examined. Biodegradation processes of cellulose in presence of glucose or cellobiose were also investigated. Microcalorimetric measurements were carried out at the temperature of 298.15 K.

Our results confirmed the hypothesis that microcalorimetric studies on biodegradation of glucose, cellobiose, cellulose and mixture these sugars can be a useful tool for better understanding of organic matter turnover in soil.

Keywords microcalorimetry; soil; saccharides; biodegradation; phosphogypsum

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Calculation of the Activation Energy to describe sensitivity of soil organic matter decomposition to temperature: Application of TAM III

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The sensitivity to temperature of soil organic matter (SOM) is receiving an increasing interest due to its importance in the global carbon (C) cycle and potential feedbacks to climate change. It constitutes a highly controversial topic in soil science due to different constrains involving the models employed, together with methodological limitations. Recent papers emphasize that decomposition of SOM should be described by a common set of principles of kinetic theory and environmental constrains, making the Arrhenius model an attractive option. It has been applied to specific enzymatic reactions taken place in soils but the enzymes for decomposition may be physically or chemically excluded from many of the organic-C substrates in the heterogeneous soil environment, limiting the response to temperature. When the model is applied using the CO2 rate of SOM decomposition, the observed sensitivity is mainly attributed to the labile SOM compounds limiting the information about the SOM recalcitrant fractions. Therefore, the introduction of new methods and indicators that can assess the sensitivity of the SOM macromolecule continuum to temperature on a more global basis are welcome. Calorimetry can be an attractive alternative if the SOM degradation is studied as a function of temperature. The design of TAM III permits such measurements in real time through a temperature scan mode. This method was never applied before to test the Arrhenius model to SOM degradation and for this reason it is necessary to develop protocols for those measurements.

We have applied and designed a preliminary protocol with TAM III to calculate the activation energies of soil samples collected through a depth gradient involving changes in the SOM composition. The calculation was run on short-term basis by continuous measure of the heat rate of SOM biodegradation through a temperature gradient from 18 to 35 ºC during one week. Results showed fast adaptation of microbial biomass to increasing temperature and enough sensitivity of the method to detect changes in the heat rate linked to the changing temperature. When the Arrhenius equation was applied the linear fits obtained allowed the quantification of the activation energies, which increased with depth from -30 to -48 kJ/mol, suggesting a sensitivity to the SOM composition. Values given by literature for easily degradable compounds are -30 kJ/mol while those for tannins are about – 70 k /mol.

Keywords Calorimetry, Activation Energy, Soil.

Session 8-9: Molecular

Application of sorption calorimetry for studies of hydration of biomolecules

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The method of sorption calorimetry [1] is designed for studies of hydration of various materials, including those of biological origin. A sorption calorimetric cell consists of two chambers connected by a tube. In one chamber a studied sample is placed while in the other chamber pure water is injected. During experiment, water evaporates, diffuses through the tube and is absorbed by the sample. The sorption cell is inserted into a double-twin calorimeter where thermal powers released in the two chambers are measured continuously and independently of each other. Processing these data one can calculate the sorption isotherm and the enthalpy of hydration of the studied material. Thus, in a sorption calorimetric experiment one obtains a full thermodynamic description of hydration: the Gibbs energy, the enthalpy and the entropy of hydration as functions of water content. This made possible to thermodynamically characterize phase transitions, glass transitions, adsorption, capillary condensation and other phenomena that take place during hydration.

The method was applied for studies of biopolymers, such as of microcrystalline cellulose, pig gastric mucin, xanthan gum, lysozyme, starch, hyaluronic acid. Interestingly, for a broad range of biopolymers, the enthalpy of hydration at zero water content was very close -18 kJ/mol [2]. These results indicate that the nature of biopolymer – water interactions in the glassy state is similar in all studied amorphous polymers. The difference in hydrophilicity arises from different numbers of functional groups that are able to interact with water.

Sorption calorimetric studies of lipids allowed distinguishing between enthalpy and entropy driven isothermal phase transitions [3]. Also, driving forces of hydration of liquid crystalline phases of lipids can be resolved.

Keywords: Hydration; Sorption calorimetry; Biopolymers; Lipids; Phase transitions; Glass transitions

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How small polar molecules protect membrane systems against osmotic stress

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Most organisms in nature are in one way or another affected by osmotic stress. Marine life is exposed to high salinity in ocean water, while terrestrial life has to deal with desiccating conditions in the form of low relative humidity (RH) and cold climate. For humans, the outermost layer of skin (called the stratum corneum, SC) is particularly exposed to osmotic stress from dry and cold climate of the external environment. One generally applied strategy of protection against an osmotic stress is to introduce a small water-soluble component (osmolytes) with low vapor pressure. In SC, The solute acts to reduce the chemical potential of the water, although it should preferably behave in a neutral way with respect to the functional components of the system. In the skin, the osmolytes are referred to as the natural moisturizing factor (NMF) and comprise a mixture of free amino acids, amino acid derivatives lactic acid, urea and glycerol.

In this study we investigate the thermodynamics of lipid hydration in lipid systems in the presence of osmolytes (urea, glycerol, TMAO and glucose). We aim at the mechanism of how these polar molecules with low vapor pressure protect the membrane system against osmotic stress/dehydration. We have selected ternary systems composed of dimyristoylphosphatidylcholine (DMPC)-osmolyte-water, as models to investigate the molecular mechanisms behind this protective effect with focus on factors that control the solid to liquid phase transition in the phopsholipid bilayers. We utilize a number of complementary experimental techniques, including sorption microcalorimetry, sorption microbalance, DSC, solid-state NMR and small and wide angle X-ray scattering (SAXS & WAXS). It was discovered that the osmotlytes stabilize the liquid crystalline bilayers at low relative humidities, whereas for the pure binary DMPC-water system, a solid gel phase is induced at 93% RH. From the sorption experiments, we conclude that the swelling pressure is sensitive to the phase state of the lipids, and that the addition of a second polar solute to the phospholipid-water system has a remarkably small effect on the swelling behavior when analyzed with respect to solvent volume. Finally, we compare the findings on polar molecules in lipid model membranes on to the effects of the same molecules in intact skin in dehydrated conditions.

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Protein Adsorption on Nanocapsules: Demonstrating the Differences between the Hard and Soft Corona

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When nanocapsules or particles come into contact with biological fluids like blood, adsorption of plasma proteins on the particle surface leads to a formation of the so called 'protein corona'.[1] This biomolecular interface can be divided into so deemed 'hard' and 'soft' protein coronas which depend on the binding affinities as well as the exchange rates between proteins and the nanomaterial.[2] As the protein corona can lead to physical changes and influences the biological response [3], a defined analysis is critical.

In this study we investigate the interaction of differently functionalized hydroxyethyl-starch (HES) nanocapsules with plasma as well as with single proteins (human serum albumin (HSA), apolipoprotein A1 (ApoA1)) by applying analysis techniques that provide information about both the hard and soft corona. To compare the different methods, first the hard corona formation of plasma proteins was examined with SDS-PAGE and a protein quantitation assay, giving a very similar pattern of adsorbed proteins for all types of capsules. We further applied isothermal titration calorimetry (ITC) and dynamic light scattering (DLS), both methods which require no extraction from the biological medium prior to analysis and with that allow the analysis of the soft protein corona. ITC revealed that a large number of plasma proteins (on the order of $1 \cdot 10^5$) are loosely associated with the capsules after adsorption and cause an increase of the hydrodynamic radius of around 80 nm, which was determined with DLS. The investigated processes are all slightly exothermic and do not indicate very strong interactions and with that represent the soft protein corona. Further, ITC reveals that the adsorption of HSA onto all kinds of investigated nanocapsules behaves very similar to that of plasma, making it a soft corona protein. In contrast, ApoA1 shows a much higher binding affinity to the capsules in the first place. However, only very few ApoA1 molecules are adsorbed to the surface, which is also confirmed by DLS measurements. This indicates that ApoA1 is only involved in the hard corona formation of the capsules. Additionally it was observed, that the capsules functionalized with amino groups show a very strong endothermic reaction with ApoA1. From this we conclude, that ApoA1 is being unfolded completely upon adsorption.

These experiments clearly demonstrate that not only the hard protein corona should be examined to determine biological impacts. ITC and DLS were proven to be suitable methods to distinguish between hard and soft corona proteins and to detect different adsorption behaviors of single plasma proteins.

Keywords nanocapsules; protein corona; isothermal titration calorimetry; light scattering

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Isothermal titration calorimetry for biocatalysis: Measuring metal ion binding to proteins and catalytic activity of enzymes

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Calorimetry is a powerful method to characterize binding of a ligand to its biomolecular scaffold, and to determine thermodynamic parameters, i.e. enthalpy and entropy, of this event. Moreover, calorimetry can be used to measure the heat that is developed during an enzymatic activity as a method to determine enzyme kinetic parameters. We discuss the potentials and challenges of using isothermal titration calorimetry (ITC) for studying the binding events and enzyme catalysis. The results for anaerobic Fe(II) binding to the ubiquitous iron-storage protein of life ferritin [1,2], and enzymatic hydration of CC-double bonds on different substrates will be presented. Our data for ferritin shows that the mechanism of Fe(III) storage is common among all three Kingdoms of life [2]. This suggests unity in biochemistry as proposed by A.J Kluyver [3] “From elephant to butyric acid bacterium – it is all the same”.

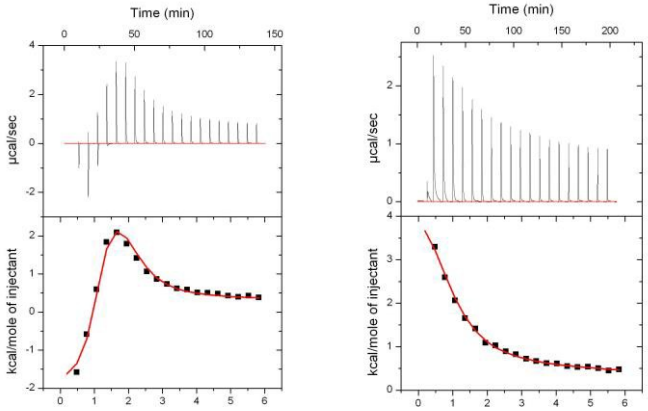

Figure 1. Difference in the thermodynamic parameters of Fe(II) binding to apo-ferritin and ferritin that has Fe(III) defines a common mechanism of Fe(III) storage.

Keywords Isothermal titration calorimetry, metal binding, enzyme catalysis, ferritin, hydration,

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Thermodynamics of anticancer fluorinated lead binding to target carbonic anhydrases by isothermal titration calorimetry and thermal shift assay

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The lead discovery in pharmaceutical design is often based on selecting the highest affinity compound that binds and inhibits the target protein function. However, the affinity alone provides limited information on the forces driving the binding event. Compounds with similar affinities often have very different enthalpic and entropic contributions due to the enthalpy-entropy compensation phenomenon. Such compounds have different reasons for binding. In the stages of optimizing the lead compound, it is important to take into consideration as many energetic contributions to the binding reaction as possible. The enthalpic term is a direct measure of the net change in the number and strength of the non-covalent interactions upon binding. However, it is important to carry out a number of controls and dissect the intrinsic enthalpy from other contributions occurring in solution due to linked reactions.

Isothermal titration calorimetry (ITC) is a method of choice in the pharmaceutical industry. However, the thermal shift assay (TSA, ThermoFluor) is a method that avoids the narrow window of ITC Kd measurements. In this work we present the determination of the intrinsic binding parameters of arylsulfonamide binding to carbonic anhydrase (CA) by ITC and TSA. Many human CA isozymes are established therapeutic targets and sulfonamide inhibitors are used for treatment of a wide range of disorders. Sulfonamide inhibitor binding reaction to CA is linked to at least two reactions, the protonation of zinc-bound hydroxide anion in the active site of CA, and deprotonation of inhibitor sulfonamide group. Detailed study of the interaction between CA and arylsulfonamides enabled us to dissect the protonation contributions to the binding reaction and allowed to obtain the intrinsic binding parameters. Combination of the intrinsic enthalpies, entropies, and the Gibbs free energies together with the crystal structures of compounds bound to target protein isoforms provide the direction of optimization of the compound binding affinity and selectivity towards the desired enzyme isoform. Compounds are mapped in the direction of increasing functional groups to correlate with the increments in the intrinsic thermodynamic parameters. The structure-thermodynamics correlations are used for the design of drug-like lead compounds with desired binding properties.

Keywords: isothermal titration calorimetry, thermal shift assay, ThermoFluor, intrinsic thermodynamic parameters.

Application of the Gibbs-Duhem equation to the study of the relation between forward and reverse titration in ITC

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By isothermal titration calorimetry [1] it is possible to measure the partial enthalpy of interaction of a ligand with a macromolecule. In this work we will see that, integrating the Gibbs-Duhem equation, it is possible to calculate the corresponding enthalpy of interaction of the macromolecule. This calculation is based upon rigorous thermodynamic arguments where the unique additional hypothesis is that solutions must be diluted. Forward titration can be characterized by the partial enthalpy of interaction of ligand and the partial enthalpy of interaction of macromolecule. In the same way reverse titration can be characterized by both partial enthalpies of interaction. In this work we propose an experimental criterion to identify when forward process and reverse process are equal or different. If both properties are equal for forward and reverse titrations as in Figure 1, processes are equal. Figure 2 shows an example when forward and reverse processes are different. Solid curves represent values calculated from the Gibbs-Duhem equation. Data were taken from literature [2,3].

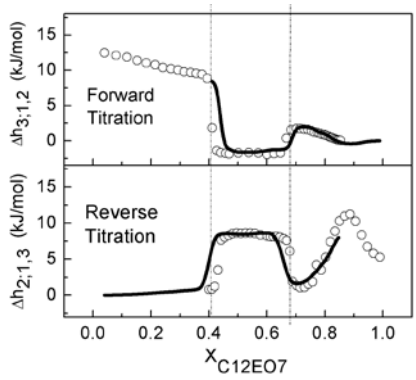

Figure 1: Interaction between a lipid membrane and a surfactant.

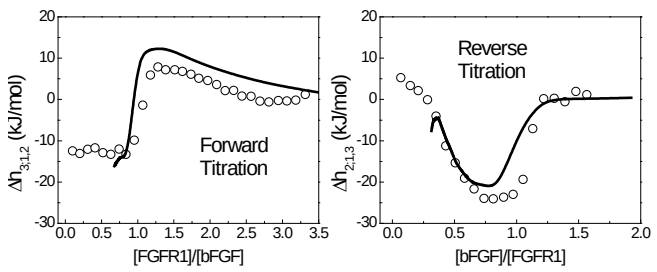

Figure 1: Interaction between the basic fibroblast growth factor (bFGF) and a receptor (FGFR1)

Keywords Thermodynamics; Isothermal Titration Calorimetry; Forward Titration; Reverse Titration

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Isoquinoline alkaloids and their binding with DNA: Calorimetry and thermal analysis applications.

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Alkaloids are a group of natural products with unmatched chemical diversity and biological relevance forming potential quality pools in drug screening. The molecular aspects of their interaction with macromolecules like DNA, RNA, proteins are being currently investigated in order to evolve the structure activity relationship. Isoquinoline constitute an important group of natural alkaloid. They have extensive applications in cancer therapy and a large volume of data is now emerging in the literature on their mode, mechanism and specificity of binding to DNA. Thermodynamic characterization of binding of these alkaloids to DNA may offer key insights into the molecular aspects that drive complex formation and these data can provide valuable information about the balance of driving forces. Various thermal techniques have been conveniently used for this purpose and modern calorimetric instrumentation provides direct and quick estimation of thermodynamic parameters. Thermal melting studies and calorimetric techniques like isothermal titration calorimetry and differential scanning calorimetry have further advanced the field by providing authentic, reliable and sensitive data on various aspects of temperature dependent structural analysis of the interaction. We present the application of different thermal techniques *viz.* isothermal titration calorimetry, differential scanning calorimetry and optical melting studies in characterization of drug-DNA interactions with particular emphasis on isoquinoline alkaloid-DNA interaction.

Hydration of Sodium Hyaluronate Studied by Sorption Calorimetry and Differential Scanning Calorimetry

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Sodium hyaluronate is a high molecular weight polysaccharide which is found in the biomatrix of the connective tissues in vertebrates[1]. It was first isolated in 1934 by Meyer and Palmer who named it *hyaluronic acid* after hyaloid (=glassy) and uronic acid[2]. However, *in vivo* it occurs exceptionally in the form of Na^+ salt and thus the name sodium hyaluronate is preferred. Owing to its biocompatibility and viscoelastic properties, sodium hyaluronate is used in variety of medical and cosmetic field such as in ophthalmic surgery, cutaneous wound healing and injectable dermal fillers[3].

In this work we study hydration of sodium hyaluronate (17kDa) by using the methods of isothermal sorption calorimetry[4] and differential scanning calorimetry (DSC). The method of sorption calorimetry was used to simultaneously monitor the water activity, a_w, and the partial molar enthalpy of mixing of water, H_w^m as a function of sodium hyaluronate-water composition. At low water content the enthalpy of hydration of sodium hyaluronate is exothermic ($H_w^m < 0$), which implies that it is in a glassy state. Upon further hydration, the value of H_w^m increases towards 0 kJ/mol H_2O. At water content of approximately 32% a step in water activity is observed as well as a sharp endothermic peak, which may correspond to a phase transition. Furthermore, data obtained by DSC was used to determine glass transitions and phase transitions in this system. By combining data from sorption calorimetry and DSC a phase diagram of the sodium hyaluronate – water system was constructed.

Keywords hyaluronate; hyaluronic acid; sorption calorimetry; differential scanning calorimetry; DSC; hydration; water activity

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Studies of mutant lectin binding behaviour by microcalorimetry

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Lectin-carbohydrate interactions are involved in bacterial recognition and adhesion to host cell glycoconjugates. Bacterial lectins help pathogens in adhesion to sugar moieties presented on the host cell surface. Therefore, lectins from pathogenic bacteria and their adhesion modes are the subject of intense study. On the other hand, understanding of the basic principles of the lectin-carbohydrate binding process is still important task and can be helpful for further lectin engineering by targeted mutagenesis methods. Lectins with tuned affinity can offer interesting perspectives in biomedical or biotechnology.

Our work is focused on the engineering of previously well studied lectins PA-IIL [1], CV-IIL [2], RS-IIL [3] and BC2L-A [4] from the PA-IIL lectin family. These lectins show a unique binding mode among bacterial lectins. They bind sugars trough two calcium cations with very high affinity (K_d in micromolar range). Lectins belonging to the PA-IIL lectin family originally come from different pathogenic organisms. They share sequence and structure similarities, but interestingly they differ in the binding specificity for the saccharides.

Site directed mutagenesis method was used for the engineering of lectins from PA-IIL lectin family. In our work, we focused on the mutagenesis of two different positions in the lectin sequences - position 22 in the "specificity binding loop 22-23-24" (29 resp.) and 98 position (97, 112 resp.), where the role of the water molecule in the lectin-sugar interaction has been also investigated. Effects of these mutations on the individual lectin binding behaviour were studied by isothermal titration microcalorimetry method (ITC). Crystallography served for study of molecular basis of binding modes of all prepared mutants.

Keywords: lectins, pathogens, mutagenesis, calorimetry

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Thermodynamics of interaction between L-serine and TiO_2 nanoparticle

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In recent years, it is of a growing interest in nanometer-sized particles and their biological application. However, it is urgently necessary to investigate nanoscale systems from energetic point of view since there is a lack of knowledge in this area. Isothermal titration calorimetry (ITC) is a powerful technique to study thermodynamic and kinetic properties in a system. It is highly sensitive to determine thermodynamic parameters of the systems with low energy change. Therefore, ITC is a potential technique to determine thermodynamic parameters in nanoscale systems such as nanoparticle-biomolecule interactions. In this respect, this study presents an application of ITC on thermodynamics of a nanoscale system consisting of nanoparticle and amino acid. The interaction between a neutral polar amino acid, L-serine with titanium dioxide (TiO_2) nanoparticles has been investigated by isothermal titration calorimetry (ITC) at 25^{o}C at pH=5.58.

Keywords TiO_2 nanoparticles; amino acid; L-serine; isothermal titration calorimetry (ITC)

The effect of terpene on phospholipid membranes

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Terpenes are the most abundant compounds in the essential oil extracted from plants in which they are responsible for scent and flavor. Essential oils have been used for a long time as fragrances or in preservation of food and medicinal applications due to their antioxidant and antimicrobial properties which reside in parts of the terpene constitutents. In the recent past many more interesting properties and activities that they possess have been discovered and applied widely in pharmaceutical, sanitary, cosmetic and food industries. They have been shown to have preventive and antitumorigenic activity as well as influences on the apotosis and differentiation. Moreover, in topical drug delivery they are potential penetration enhancers which can help to increase the percutaneous permeation. From a wide range of application, it can be seen that they exist in very diversed formulations from very low water content to excess water. The use of terpene, a natural product, is becoming more widespread as alternatives to synthetic chemical compounds. However, the mechanism of these activities have not yet been clarified. In this study, we want to investigate the effect of terpene on the DMPC (1,2-dimyristoyl-sn-glycero-3-phosphocholine) lipid bilayer phase behaviour and phase transition, structure and dynamics at different scales and hydration levels by combining complementray techniques including differential scanning calorimetry (DSC), solid-state NMR and X-ray Scattering. Employing Thymol and Geraniol which are two isomeric monoterpenes with linear and cyclic structures enables us to reveal the terpene structural effect.

Keywords terpene; lipid; Thymol; Geraniol; NMR

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Recombinant production and calorimetric characterization of human carbonic anhydrase IX

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Carbonic anhydrase (CA) IX is a tumor associated enzyme. It is responsible for pH regulation in the hypoxic environment in cancer cells. Expression of this protein in normal tissues is very low but it becomes highly overexpressed in various cancerous tissues under hypoxic environment [1]. Thus CA IX is pivotal target for anticancer therapy. There are 12 catalytically active CA isoforms in human body and they have only small differences in their active sites [2]. For this reason it is difficult to develop an inhibitor that would be selective exclusively for CA IX and not bind other CAs.

Our aim is to design CA IX – selective inhibitors and thus it is necessary to calculate the intrinsic parameters of inhibitor binding to CA IX. When CA IX was titrated by isothermal titration calorimetry with tightly – binding ligands, the observed binding parameters differed from the intrinsic parameters. In order to determine the intrinsic binding parameters, the pK_a and enthalpy value of zinc-bound water molecule protonation must be determined. For this reason, ethoxzolamide (EZA) was titrated in the pH range from 6 to 9 in phosphate and tris buffers. Observed thermodynamic binding parameters, the pK_a and enthalpy values, and the intrinsic parameters are compared with CA I.

Keywords isothermal titration calorimetry, carbonic anhydrase, intrinsic binding parameters, protein-ligand binding.

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Calorimetric study of intermolecular interactions of model biomolecules in aqueous-organic solvents: the ratio "structure-property"

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Intermolecular interactions has a significant influence on properties of many supramolecular and biological systems. They are specially important in conformational stability of proteins and nucleic acids. Quantification and analysis of hydrogen bonds in complex biological molecules is a very complicated task, so for scientific studies simple model compounds are used. Peptides and amides of carboxylic acid are most appropriate for this purposes.

In the present work specific interactions of alanine anhydride (AA), glycine anhydride (GA) and sarcosine anhydride (SA), 2-pyrrolidone (Py), formamide (FA), N-methylformamide (NMF), N-methylacetamide (NMA) and N,N-dimethylformamide (DMFA) and N,N-dimethylacetamide (DMAA) with different organic molecules were investigated. These compounds simulate structural fragments of different biological molecules.

Firstly, solution enthalpies of peptides, amides in proton donors, proton acceptors and amphiphilic molecules were measured, after enthalpies of solvation were determined. Based on these data enthalpies of specific interactions were estimated. It was shown that enthalpies of specific interactions of investigated peptides and amides with chloroform are different. This suggests different proton acceptors ability of all studied compounds. Peptides and amides are forming strong hydrogen bonds with proton acceptors and aliphatic alcohols. In the present work solution enthalpies of ethers, ketones, esters, nitriles, amines, alcohols, chloroform and water in 2-pyrrolidone, formamide, N-methylformamide, N-methylacetamide, N,N-dimethylformamide, N,N-dimethylacetamide were also determined. Influence of quantities and properties of proton-donor, proton-acceptor centers in molecules of peptides and amides on thermodynamic functions of solvation was analyzed. Secondly, absorbance of free and its complex molecules of peptides and amides in C=O stretching vibration frequencies, with proton acceptors and proton donors were investigated. It was shown that obtained C=O frequencies of these substances in inert and basic solvents linearly depend on solvent parameter S_{VW} [1], responsible for Van der Waals interactions. Sensitivity of the studied carbonyl groups to solvent is decreased in the order GA>AA>SA>NMF>NMA>DMFA. The C=O frequencies of peptide and amides with complexes of aliphatic alcohols and water (C=O…H-O) were investigated. Calorimetric and IR spectroscopy data may allow us to understand the process of many biological systems.

Keywords: peptides, amides, calorimeter solution, IR-spectroscopy, hydrogen bond.

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Thermal denaturation of fibrilar collagen in tissues - effects of glycation

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Formation and maturation of collagen fibrils, based on conversion of lysine and hydroxylysine residues to stable crosslinks, results in material with an optimal balance of strength and flexibility. However, during aging, products of nonenzymatic attachments of sugars - advanced glycation end products (AGEs), make the collagen less elastic and more susceptible to pathologic changes and impaired turnover.

The main purpose of the present study was to compare effects of *in vitro* induced AGEs on fibrilar collagen from different tissues obtained from adolescent and adult animals.

Samples of tendon, articular cartilage, meniscus, cornea, artery, cortical bone and demineralized cortical bone were used in the study. Stability of collagen molecules was investigated by differential scanning calorimetry. Glycation was induced by incubation in ribose (0.67 M). Level of glycation products was estimated by fluorescence spectroscopy (420–440 nm/370 nm and 390–400 nm/335 nm).

In all samples, except for bone, a denaturation endotherm was found between 58^{o}C and 72^{o}C. In bone thermal processes were observed up to 160^{o}C. Parameters of collagen denaturation were different in different tissues. Effects of ribose were also dependent on the type of tissue. The biggest changes were found in the cornea of adults, the smallest – in the artery of the younger animals.

The results were analyzed in the terms of the type of collagen, other components of the tissue and enzymatic and non-enzymatic crosslinks in the tissue.

Keywords ageing, collagen, connective tissue, DSC, glycation, spectrofluorymetry