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Program and Abstracts

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Plenary lecture

HOT PLANTS: THE PHYSIOLOGY AND BEHAVIOUR OF THERMOGENIC FLOWERS

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The flowers, inflorescences and cones of several groups of early seed plants produce heat during blooming. Some species are able to regulate heat production and maintain relatively constant floral temperatures in widely variable environmental temperatures. This presentation explores temperature regulation in plants from its initial discovery to current experiments. We have examined the phenomenon at different levels of organization, from the molecular control of heating to its ecological significance. Respiration rates can be extraordinarily high, far above those of even maximally active vertebrate animals, and floral temperature can reach 35 ºC above the air. Oxygen is supplied at a high rate by diffusion, and the morphology of the diffusive pathway is matched to demand.

The thermoregulatory control mechanism is still unknown, but investigations on gene expression, selective inhibitors and isotope fractionation reveal involvement of the alternative oxidase (AOX) and uncoupling protein (UCP), depending on the respiratory substrate (carbohydrate—AOX; lipid—UCP). Studies of the time course of responses to experimental changes in ambient temperature reveal a quick van ‘t Hoff response in the direction of the change, and then a slow regulatory adjustment in the opposite direction. The time-course of these responses differs greatly between species, and is associated with the precision of temperature regulation.

Thermogenesis is associated with scent production in most species, but temperature regulation continues past the attractive phase and is associated with insects (chiefly endothermic beetles) that reside for a day in a floral chamber, where they eat, digest and mate. Measurements of the energy costs of insect activity show that as little as 4 ºC elevation in floral chamber temperature in *Philodendron* can reduce the costs of insect activity 5-fold. Thus thermogenic flowers can provide a direct energy reward to insect visitors, and floral temperature regulation may make the reward independent of environmental temperature.

Symposium 1

S1O1

Real time bioprocess analysis from the synergetic utilization of calorimetry and impedance spectroscopy

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The dissipation of Gibbs energy (often in form as heat) is a general feature of biocatalysis with whole cells. The heat is tightly correlated with the bioconversions via the law of Hess. Thus, each essential change in the metabolic fluxes will be reflected in real time by the heat production rate. New developments ranging from the *Megacalorimetry to Chip- or Nanocalorimetry* in reaction vessels from the µL to m^3 scale will allow high throughput measurements even under "dirty" technical conditions. Following this argumentation line, the heat production rate is an "ideal" indicator for the bioprocesses. An essential counter argument is the missing specificity of the signal. But, combining calorimetry with additional monitoring tools and kinetic metabolic models will provide a deeper understanding of the bioprocesses. The most important additional parameter is the concentration of catalyst (i.e. the number of active/intact cells).

The impedance spectroscopy measures the interaction between cells and an alternating electrical field. The capacitance difference at two frequencies is assumed to be proportional to the number of intact cells [1] and the monitoring technology works even under technical conditions. We found that deviations from the assumed linear correlation can be interpreted in terms of intracellular product formations [2] and that this intracellular product formations can be quantified using the information content of the whole impedance spectrum (capacity vs. frequency) [3]. Combining the analytical potential of calorimetry (activity of the cells) and impedance spectroscopy (number of cells and product formation rates) provides the basics for new bioprocess analysis and control strategies. This potential will be discussed and evaluated.

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S1O2

Calorimetry and Pressure:

A review of technical solutions and applications

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In drug development, food or catalysis processes, pressure could be required to perfectly control a global formulation. Then, to understand compound behavior, unsuccessful reactions or transformations phases it appears interesting to mimic these processes monitored by the pressure. Setaram with Sensys and C80 apparatus is able to provide different solutions in function of the way of working:

- Analysis with no control of the pressure
- Analysis under static pressure
- Analysis under dynamic mode

Indeed, these calorimeters equipped with the 3D sensor based on the Calvet principle could work with different crucible types in specific pressure range.

Then, it has been possible in process safety to follow the decomposition of Chromophore with our C80 calorimeter in 100 bar batch cell allowing the pressure monitoring.

For this same C80 apparatus, a specific pressure cell has been developed to be able to measure the pressure variation till 200 bar during reaction between two compounds.

With our HP microDSCVII and a 1000 bar gas panel, we have followed the dissociation of methane hydrates. The curve is characterized by a peak associated to the ice melting and a peak due to the hydrate melting.

In a same way and with adapted equipment we could study for example the influence of pressure in crystallization or glass transition determination or determine the adsorption and desorption hydrogen on Mg alloy.

S1O3

NANO-CALORIMETRY OF BIOLOGICAL SAMPLES

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Recent applications of silicon chip based calorimeters have shown that the application of miniaturized calorimeters in biocalorimetry overcome some disadvantages of conventional calorimeters such as high cost and sample volume, low dynamics and poor flexibility [1]. On the other hand the construction of miniaturized calorimeters which are useful for biological applications is a great challenge because heat flow measurement in the lower nano-watt range is required. The reason is that the signal-to-noise ratio decreases dramatically with the proceeding miniaturization of the sample size if a slowly changing metabolic heat production has to be measured [2].

In the presented lecture

- the requirements for the measurement of the metabolic heat production of bacteria and single multi-cellular organisms will be discussed,
- our new solutions for chip-based nano-calorimetry with a detection limit of less than 20 nW will be presented (see also poster),
- an overview about relevant applications of the presented nano-calorimeters will be given, as for example the monitoring of the bacterial growth in extremely diluted suspensions, the on-line measurement of high-density bacterial cultures in bioreactors, the analysis of biofilm activities as well as the determination of the metabolic heat production of single fish embryos.

Details of the mentioned projects will be explained in separate lectures.

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S1O4

INTRODUCING THE NEW iTC$_{200}$™. LESS IS MORE

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Isothermal Titration Calorimetry (ITC) is traditionally used to find out what the driving forces of your protein-ligand complexes are. ITC determines both the strength and the mechanism of binding of two molecules, by monitoring the change in heat during complex formation. The thermodynamic parameters measured allow unique insights into the mechanism of binding in a single experiment.

With the introduction of the iTC$_{200}$,for the first time one can achieve gold standard binding affinities, both label free and without the need for immobilization, using as little as 5 to 10 µg of protein. Moreover, in addition to reduced sample usage, instrument operation is both simplified and faster, such that a binding affinity with mechanistic information can be achieved within half an hour.

A description of the use of ITC in biomolecular systems will be given with examples and there will also be an introduction to the new iTC$_{200}$.

S1P1

MINIATURIZED SENSOR-CALORIMETER

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The application of calorimetric methods for the investigation of the metabolism of cell cultures using different devices is well known. In earlier presentations [1] we have shown that for the interpretation of calorimetrically detected metabolic processes the simultaneous registration of additional culture parameters is necessary. However the possibilities to integrate additional sensors in conventional calorimetric systems, especially using small reactor volumes, are limited.

Figure 1: Miniaturized calorimetric reactor system with integrated chemical sensors

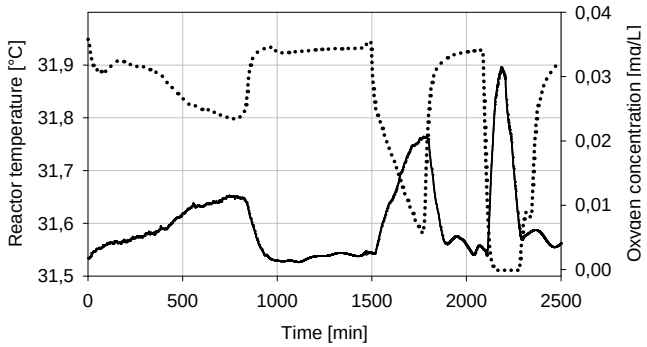

Figure 2: Response of the calorimetric (solid line) and oxygen sensors (dotted line) during the cultivation of *P. pantotrophus* to a threefold addition of 100 μmol glucose

This contribution presents a miniaturized reactor system based on a small-scale calorimeter (25 mL vessel) equipped with different chemical sensors (see Figure 1). In our system the universal thermal power, characterizing the physiological state of the microorganisms is combined with special electrochemical sensors. In comparison to established calorimeter, this newly designed combination as a whole significantly extends the range of application. The miniaturized sensors can be chosen from a pool of potentiometric and amperometric sensors, measuring pH-value, redox potential, dissolved carbon dioxide and glucose content, according to the fermentation process or to the analytical problem. This system is applicable for batch and continuous cultivations Fields of application are biotechnological process development, the production of cost-intensive products and the investigation of metabolic activity under different growth conditions (see Figure 2).

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S1P2

CHIP BASED NANO-CALORIMETER FOR BIO-THERMODYNAMIC APPLICATIONS

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It is a great challenge to design chip based micro-calorimeters for the study of processes with slowly changing heat power evolution like the metabolic heat production in micro-organisms or enzyme catalyzed reactions. The reason is that the signal-to-noise ratio dramatically decreases with the reduction of the sample size [1]. On the other hand the advantage of the application of miniaturized calorimeters in biochemical and bio-thermodynamic research is evident if high throughput and flexible use are taken into account. Further, chip calorimeters open new possibilities for the investigation of dynamic effects in micro-organisms.

In our presentation details a new designed chip calorimeter which satisfies the criteria for the investigation of biological micro-samples will be demonstrated. The presented calorimeter is an essentially improved version of a device which was recently published [2]. Due to a well-elaborated design of the thermostat a stability of the temperature of the thermopile heat power transducer of 20-30 µK could be achieved. This leads to a heat power resolution of less than 20 nW which corresponds to a volume specific resolution of about 3 mW l^{-1} assuming a 6 µl sample.

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S1P3

Mega- and Chip- Calorimetry for Bioprocess Analysis

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The metabolic activity correlates with heat dissipation of an organism. If the heat flow is detected calorimetrically, the quantity and dynamic of the heat signal will inform about stoichiometry and kinetic of metabolic conversations. The heat signal as an online and real time signal allows monitoring complex biological processes.

In reaction calorimetry the heat signal is obtained with a robust measurement technique and small time delay. In spite of these advantages the tests of calorimetric measurement in biotechnological processes are complicated, because calorimeters have not been optimized for biotechnological aspects and are relatively costly. The integration of calorimetric measurement technique in existing bioreactors (mega-calorimetry) or the development of miniaturised heat sensors (chip-calorimetry) could be a resource to overcome the disadvantages of conventional calorimeter and to point out the potential of calorimetry for biotechnological applications. The state of art will be presented on example of the bioprocess monitoring and control the biosynthesis of the compatible solute ectoine by *Halomonas elongata*.

The importance of such real time bioprocess analysis is caused by the sporadic variance of physiologic parameters (substrate affinity, growth rate and yield coefficients) and an inaccurate prediction of the process state of the cell population. Because of the indefinite behaviour of the cell population, batch or fed batch processes are forced in industrial applications. By using new monitoring algorithm it is possible to control continuous bioprocesses optimally. First examples should demonstrate the suitability of mega- and chip- calorimetry for bioprocess control.

S2O1

CALORIMETRIC STUDY OF MYOGLOBIN EMBEDDED IN TREHALOSE AND IN SUCROSE-WATER MATRIX

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As it is well known proteins embedded in saccharide-water matrixes of low content of residual water are preserved against adverse environmental condition as e.g. high temperature and extreme drought. Among saccharides trehalose (R-D-glucopyranosyl-R-D-glucopyranoside) has been found to exhibit the best protective effect [1]. To rationalize this peculiarity of trehalose it has recently been proposed that in *"dry"* protein-saccharide-water systems, a water-mediated hydrogen bond network, whose strength increases by lowering the residual water content, anchors the protein surface to the surrounding water saccharide solid matrix, thus coupling the internal degrees of freedom of the protein to those of the surrounding glassy matrix [2,3]. In particular a comparative study of MbCO embedded in sucrose, maltose and raffinose matrixes suggested such coupling to be the tightest in trehalose [4].

To investigate the above hypothesis, we performed a calorimetric study on myoglobin embedded trehalose-water and sucrose-water systems of low water content, in the temperature interval ~100K to ~400K. Glasses were obtained by drying sugar/myoglobin aqueous solutions to a water/sugar molar ratio lower than 10. Calorimetric upscans were measured with a rate of 10 $Kmin^{-1}$, after quickly cooling (500 $Kmin^{-1}$) the sample to liquid nitrogen temperature; as usual, the glass transition temperature of the samples was identified from the stepwise increase of the specific heat; an irreversible endothermic peak (at $T > 330$ K) indicated the protein denaturation.

In agreement with the above hypothesis, we observed that in samples of very low water content the protein denaturation temperature is slightly higher in trehalose than in sucrose. Measurements are on the way to detect the detailed dependence of such effect on the water/sugar ratio of the sample.

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S2O2

NOVEL CALORIMETRIC INVESTIGATION OF DIFFERENT HUMAN JOINT HYALINE CARTILAGE

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During recent years, knowledge of the nature and pathogenesis of degenerative joint deseases has increased. A limited number of papers have been published before on the subject of thermal analysis of human hyaline cartilage. Thermoanalytical techniques measure the change in physical or chemical properties of the sample as a function of temperature. There are many possible applications of thermoanalytical techniques: characterizations of active and inactive ingredients, routine analysis, and qualitative control. The main purpose of this study was to compare the altered metabolism in osteoarthritis, avascular necrosis, rheumatoid arthritis, degenerative spine diseases and shoulder arthropathy to normal hyaline cartilage by differential scanning calorimetry. Since, water content has not been measured, further aim of this investigation was to elucidate the importance of water content, and to establish the kinetic character of water loss effect of heating by thermogravimetric analysis. All samples that were extracted for this study were obtained during live surgeries. A new protocol had to be established before the detailed investigation could be performed. The use of DSC as part of thermal analysis was a reliable method for differentiating normal hyaline cartilage from rheumatoid samples. All samples showed a clear denaturation peak on the calorimetric curve, therefore volume of the curve was easily calculated giving the enthalpy change of the sample. These changes correlated with the water content of the samples. The calorimeter that was available for use proved to be adequate for these measurements.

APPLICATION OF ISOTHERM CALORIMETRY IN THE DEVELOPMENT OF FOODS CONTAINING PROBIOTIC LIVE FLORA AND ENRICHED WITH BIOAVAILABLE CA^{2+}

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The development of functional foods of probiotic effect based on the slime-producing strains isolated in the 1980s, and that of enriched with Ca on the utilization of the high Ca-containing whey of the quarg production in the Carpathian basin using fermentation. The probiotic properties of the slime-producing microbe strains isolated have been proved by in vitro and in vivo examinations.We have developed an isotherm DSC method to identify the probiotic microbes. The percentuel ratio of probiotic and other microbes was determined in the product by this technique. By utilization of quarg whey a special additive food for Ca-enrichment has been developed which is suitable to complete or enrich different foods (dairy, meat and bakery products).

The products developed are

- probiotic kefir (Synbiofir)
- probiotic sour cream
- probiotic butter cream
- poultry meat products completed with Ca^{2+}
- bakery products completed with Ca^{2+}.

Key words: isotherm calorimetry
probiotic microbes
additive-food for Ca^{2+}-enrichment
probiotic dairy products
foods completed with Ca^{2+}

S2P1

Isothermal Titration Calorimetry Studies of the Interaction of Surfactin Analogues with a Model Membrane

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Surfactin is a class of lipopeptide compounds that receive wide-spread attention in scientific and industrial areas. This arises from its hybrid amphiphilic structure (i.e. combining small surfactant and protein features) and numerous excellent properties it exhibits. Among these are the surfactant power, foaming and emulsifying properties, antiviral, antimycoplasma, and antibacterial activities. As a result, surfactin finds potential applications in agricultural, environmental, biotechnological, cosmetic and pharmaceutical fields. Its general structure consists of a heptapeptide linked to a ß-hydroxy fatty acid. This includes those from natural and chemical synthesis sources. Natural surfactin, produced mainly by *Bacillus subtilis*, is cyclic whereas synthetic one may be cyclic or linear. The lipid chain length varies from 12 to 17 carbon atoms that constitute homologous series while the peptide primary structure may differ in amino acid composition giving rise to isoform compounds. In previous studies, we showed that little changes in lipid chain length or amino-acid composition of the heptapeptide ring induced considerable differences in surface-active, foaming, and haemolytic properties [1-3].

In the present investigation, we evaluated the potential use of the isothermal titration calorimetry (ITC) for determining the surfactin structural effects on its interaction with liposome as model membrane. The binding of a natural cyclic and three synthetic linear surfactins varying in total ionic charge (2 or 3 carboxylic residues) and lipid chain length (C14 or C18) to large unilamellar vesicles (LUVs) of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine (POPC) was investigated by lipid titration into surfactin solutions in TRIS buffer pH 8,5 with 150 mM NaCl at 25°C. By fitting the cumulative heat curves as a function of lipid concentration, thermodynamic parameters (binding constant K, enthalpy, free energy and entropy) were calculated. All surfactins interact spontaneously with POPC vesicles ($\Delta G_D^{w\rightarrow b} < 0$, -7.6 to -9.2 Kcal/mol). The reactions of transfer were endothermics ($\Delta H_D^{w\rightarrow b} > 0$, 1.3 to 2.7 Kcal/mol) accompanied by positive variation of the system entropy ($\Delta S_D^{w\rightarrow b} > 0$). Thus, the binding of surfactins to the lipid membrane was driven by entropy, which was in agreement with the classical hydrophobic effect. Nevertheless, significant difference among surfactin structures emerged from the binding constant values (K) ranging from 6.6 mM^{-1} to 96.3 mM^{-1}. Based on this parameter, it was deduced that a cyclic structure and a more hydrophobic lipid chain were favourable on the surfactin binding affinity to POPC vesicles whereas an increase of the peptide ionic charge from -2 to -3 decreased it drastically. From these results, we can also conclude that both hydrophobic and electrostatic strengths were involved in such interactions.

In summary, ITC technique appears as a sensitive tool for evaluating the structural change effect on the binding affinity of surfactin to neutral phospholipid liposomes as model membrane. The results provide not only fundamental information such as the nature and degree of interactions but also enable us to select or design the most efficient molecule for many applications in biomedical fields. The binding step constitutes undoubtedly an important factor in the membrane-active properties of bioactive molecules.

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S2P2

DSC investigation of early pregnant uterus of the rat

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Spontaneous and elcited contractility is one of the most important physiological parameter of the pregnant uterus. The aim of the present work is the thermodynamic characterization of the myometrium of the early pregnant rat (days 3, 4, 5 and 6). Uterine motor activity was additionally recorded in these days for an interpretation of the relationship between biophysical and physiological parameters of the pregnant myometrium.
Dissected uterine rings were vertically mounted in a tissue bath containing 10 ml de Jongh buffer. Organ baths were kept at 37 C and oxigenized. The integrated tensions of the rings were measured with a strain gauge transducer and recorded with Isosys Data Acquisition System (Experimetria, Budapest). The initial tension of the rings was set to 1.5 g and samples were equilibrated for 90 min before the experiment. After this period a 7-min period of spontaneous activity was recored then contractions were elicited by 25 mM KCl. Finally, maximal tensions were determined by addition of 70 mM KCl which were used for normalization of the tensions.
It was found that uterine rings from 5^{th} day of pregnancy exhibits significantly higher motor activity, both spontaneous and K-stimulated.
During the calorimetric experiments the pieces of different samples have been prepared and measured within 6 hours of removal. The thermal denaturation was monitored by a SETARAM Micro DSC-II calorimeter. All the experiments were performed between 0 and 100 ^{0}C. The heating rate was 0.3 K/min. Conventional Hastelloy batch vessels were used during the denaturation experiments. The sample and reference vessels were equilibrated with a precision of ± 0.1 mg and there was no need to do any correction from the point of view of heat capacity between the sample and reference vessels. The data treatment after ASCII conversion was done by Origin 6.0. The calorimetric enthalpy change was determined with the aid of SETARAM two points fitting integration software.
The higher contractility was reflected in the calorimetric data too. In case of non-pregnant uterus the main transition temperature (T_m) was 61 ºC with 0.61 J/g calorimetric enthalpy (ΔH) change. During the development of pregnancy these parameters altered to 61.25 ºC and 1.18 J/g (3^{rd} day); 60.7 ºC and 0.83 J/g (4^{th} day); 60.6 ºC and 2.05 J/g (5^{th} day); 60.4 ºC and 1.21 J/g (6^{th} day).
With our investigations we could demonstrate that DSC is a useful and well applicable method for the investigation of myometrial function during pregnancy.

S2P3

Determination of certain technological parameters of margarine and mixed fat spread

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Nowadays most of the margarine and mixed fat spread producers select their elementary fats in such a way that nutritionally it can not be criticised (i.e. the fat based trans-fatty acid content is less than 2%) or it should be advertise able (i. e. favourable rate of ω-6: ω-3 fatty acids).

In our experiments the elementary fat models were the butter or the palm oil (containing both liquid and solid fat at 10 °C temperature) and sunflower oil (containing only liquid fat above -20 °C temperature). As a technological model crystallization process suitable for producing margarines or mixed fat spreads with water-in-oil emulsion system and reemulsification followed by fermentation process (EP A 1 289 3761) suitable for producing margarines and spreads with fat-in-water emulsion system were used. Products with 40 °C fat content were produced.

From our experiments the following ascertainment can be done:
1. The cooling rate of fat is 7 °C/min. in our crystallizer device.
2. From DSC curves recorded in temperature range of -20 °C and +60 °C at 0.3 K/min heating rate we have found that the liquid/solid fat rate is 22.5/77.5% in palm oil and 25/75% in butter at 10 °C temperature. During our technological experiments we have found that product with suitable firmness (penetration value is <200) from both palm oil and butter
-At minimum 20% solid fat rate with crystallization process and
-At minimum 10% solid fat rate with reemulsification process can be produced.
3. A formerly established selection (25.8% coconut + 74.2% sunflower oil, and 26.7% butterfat +73.3% sunflower oil, respectively) containing 20% solid fat were made up and crystallization DSC curves were recorded in temperature range of +60 °C – -20 °C at 0.3 K/min cooling rates, respectively. We found that most part of fat were crystallized till +20 °C temperature, so the outlet temperature was set at 19 °C for the production of products with water-in-fat emulsion system.

In summary it can be stated that the rate of solid fat in the product and the outlet (crystallization) temperature in case of products with water-in-fat emulsion system can be well determined with DSC-method.

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S2P4

Effect of AMP.PNP as a nucleotide analogue on F-actin filaments

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The actin cytoskeleton has a complex structure that provides the background to the wide range of intracellular functions. Actin filaments play essential roles in the dynamic and mechanical properties of eukaryotic cells. F-actin is a double-stranded, helical polymer built up from G-actin molecules. During a polymerization process the G-actin bound ATP is hydrolysed to ADP and P_i, through an ADP·P_i intermediate state.

We studied the effect of a non-hydrolysing ATP analogue AMP-PNP on the structure of actin filaments prepared from rabbit skeletal muscle in the presence and absence of phalloidin. Experiments were carried out with Differential Scanning Calorimetry (DSC), which is a powerful method to analyse changes of the protein structure induced by the binding of different nucleotides or toxins.

ATP can be hydrolysed rapidly to ADP through a transient ADP.Pi state. This intermediate state can be mimicked by the non hydrolysable AMP.PNP molecule which remains bound to the actin protomers for a long time and can preserve a stable, long-lived structure of actin filaments. AMP.PNP can be bound to the nucleotide binding cleft to replace the bound nucleotide.

In our DSC measurements we found that the structure of actin filaments became more stable when AMP.PNP and phalloidin (82°C) was added separately to the sample. In the presence of AMP.PNP the melting temperature (T_m) was 74°C while the addition of toxin shifted the T_m value to 82°C. When the AMP.PNP and the phalloidin were added to the sample in the same time the melting temperature was shifted to an even higher value (84.5°C). This result may indicate that there are different molecular changes behind the accumulated stabilising effect of the two compounds.

S3O1

Thermal transitions in actin

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Actin is one of the main components in the eukaryote cells which plays significant role in many cellular processes, like force-generation, maintenance of the shape of cells, cell-division cycle and transport processes.

The filamentous form of actin (F-actin) is the principal component of the contractile and motil systems. Different studies (biochemical and physical) indicate that actin filaments are flexible, and the dynamics of the filaments may have significant role in the contractile process.

In this study the thermal transitions of F-actin were studied to get information about the changes induced by binding of myosin to actin using DSC and EPR techniques. The main transition of F-actin was at 67.5 ˚C by EPR (the relative viscosity change was around 62 ˚C), while the DSC denaturation T_ms were at 60.3 ˚C for G-actin and at 70.5 ˚C for F-actin.

Applying the Lumry-Eyring model for the determination of activation energy, a “break” was observed both for G as well as for F-actin in the rate constant-1/T function. This indicates that different structural domains are present. The addition of myosin or heavy meromyosin (HMM) in different molar ratio of myosin to actin has changed significantly the EPR spectrum of spin-labeled F-actin, indicating the presence of a supramolecular complex.

In the actomyosin complex after the denaturation the different structural domains of myosin and actin were quite clear separable.

S3O2

S3P1

Complexation of niflumic acid with native and hydroxypropylated α- and β-cyclodextrins in aqueous solution

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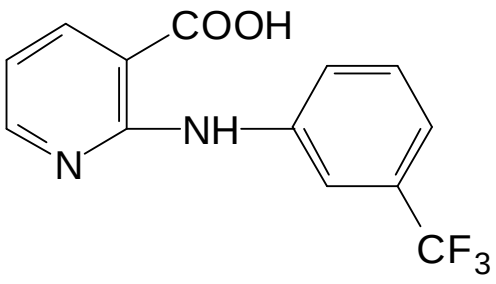
Niflumic acid 2-[3-(trifluoromethyl)anilino]nicotinic acid, NA, belongs to a class of nonsteroidal anti-inflammatory drugs and acts by inhibiting isoforms of cyclo-axygenase. It is also a noncompetitive inhibitor of chloride exchange. Niflumic acid has an activity to treat inflammatory rheumatoid diseases and relieve acute pains. It is used during the period of pains and after surgery and fever. It is obvious, that gastrointestinal absorption and therapeutic action of drugs depend on its solubility. Niflumic acid is a poorly water-soluble, therefore, its low aqueous solubility can reduce activity and restrict practical applications.

In presented work to increase the aqueous solubility and bioavailability of niflumic acid as well as to eliminate its unwanted side effects, the encapsulation by cyclodextrins (CDs) was studied. The results of the interactions between niflumic acid and native and hydroxypropylated α- and β-cyclodextrins investigated by calorimetry, H NMR, UV-vis spectroscopy and densimetry at pH = 7.4 (phosphate buffer) and T = 298.15 K were reported. It was stated that native and hydroxypropylated α- and β-CDs are able to form with niflumic acid the 1:1 inclusion complexes. Insertion of both phenyl and pyridine residues of niflumic acid molecule in the macrocyclic cavity of the all considered CDs takes place. However, the inclusion of the former residue is more preferential. The β-CD was found as more suitable complexating agents for niflumic acid since form with this acid more stable inclusion complexes.



Niflumic acid

S3P2

S3P3

S4O1

METABOLIC AND THERMAL PROPERTIES OF SOILS FROM THE ATACAMA DESERT

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The Atacama desert in Chile is one of the driest and most lifeless environments on Earth. It rains maybe once a decade. NASA examined these soils as a model for the Martian environment by comparing their degradation activity with Martian soil and to look for “the dry limit of life”. The existence of heterotrophic bacteria in Atacama soil was demonstrated by DNA extraction and by isolation of microorganisms, but they gave a very low numbers of colony forming units [1,2]. No data exist on the metabolic activity of microorganisms in these soils due to limitations of existing methodologies when applied to desert soils. Calorimetry was applied to obtain information on the metabolic and thermal properties of 11 soil samples collected at different sites in the Atacama desert. DSC was used to determine the pyrolysis properties of the carbon-containning matter and isothermal calorimetry was used to measure microbial metabolism and to quantify the biomass by the Sparling´s method. Total C and N were determined with an automated analyzer. Measurable organic matter exits in 9 of the 11 samples. Biomass was not detected in 5 of the 11 samples and no basal metabolism was recorded since all samples showed endothermal activity, probably from inorganic reactions with water. Six samples showed microbial activation after addition of glucose and water with the typical shape of a microbial growth reaction. Carbon content and nitrogen content and microbial activity after glucose amendment were correlated with the altitude of the sampling sites. The metabolism becomes more dissipative with increasing of altitude.

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S4O2

New insights into λ page infection program using calorimetry

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Bacteriophages are virus-like agents that attack bacteria in biotechnological processes as well as in ecosystems. For instance, it is estimated that up to 70% of marine bacteria are infected by phages [1]. A lot of phages are host specific to bacteria, and thus are more accurate and potent than antibiotics. This was already in 1920's considered as a basis for a phage therapy and will be potentially developed as a future answer to emerging multi-antibiotic resistances (e.g. MRSA). The specificity of phages is also advantageously used to identify pathogenic strains. Furthermore, phages are an important vehicle for foreign genes transport. Recognizing the huge importance of bacteriophages their classification, structure, replication and the mechanism of interaction with host organisms was investigated deeply. However, the shift from the host to phage governed metabolism (e.g. synthesis of toxins, viral genetic material, hull proteins, and helper proteins) is not yet well described.

Calorimetry as a real time non-invasive monitoring tool should put light into the complex mechanism of phage infections. Indeed, pioneering investigations applying microcalorimeter [2] show clearly the influence of T4 phages on *E. coli* heat production (lytic cycle). The next challenge is a quantitative description of the infection combining calorimetric data with metabolic material fluxes. For that purpose an improved reaction calorimeter [3] equipped with on-line gas analysis and providing enough sample material was applied. We tested the analytical potential of calorimetry with the respective biothermodynamic modeling at the example of a lysogenic λ phage infection. Simple λ phage infection models can not explain the seemingly contradicting evolutions of heat production, substrate consumption, growth rate and phage numbers (PFU). This means that calorimetry provides additional insights into the process and is thus indispensable for a holistic picture of the infection. We developed as a consequence a more complex model about the phage infection program which now explains consistently the seemingly contradicting results.

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S4O3

THERMOCHEMICAL STUDIES OF THE PHYSIOLOGICAL BURDEN OF OVER-EXPRESSING FOREIGN PROTEINS ON MAMMALIAN HYBRIDOMA CELLS GROWN IN BATCH CULTURE

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It is known that the over-expression of foreign proteins in recombinant host cells utilizes a significant amount of their metabolic resources and places a physiological (metabolic) burden on the host cells in terms of their growth rate [1,2]. This is because the expression of native and foreign proteins competes for the biosynthetic machinery and the available ATP. The objective of the current research is to examine the extent to which the cell can cope with these demands. The murine hybridoma type chosen for this study is the TB/C3 pEF cell containing the recombinant neomycin *neo*^{r} gene. The gene was amplified in stepwise fashion using a range from zero to 2.8 mM G418 aminoglycoside antibiotic to block translation and thus impart a selective pressure on the cells by the constitutive production of phosphotransferase to inactivate G418 [3]. The effect on the biosynthetic machinery was measured by (i) changes in cell number, and (ii) the production of the foreign protein, human immunoglobulin monoclonal antibody (IgG mAb). In terms of energetics, the activity of the catabolic half-cycle coupled to ATP production was registered principally by batch microcalorimetry but assisted by assays for major catabolic intermediates and measurements of the rate of oxygen consumption to assess the intensity of anaerobic glycolysis [4].

The results showed that G418 imposed a concentration-dependent metabolic burden on the TB/C3 pEF cells in terms of decreasing both the specific growth rate from the control at 0.042 to 0.024 h^{-1} at 2.8 mM G418 and the synthesis of IgG mAb from 6.9 to 4.1 × 10^{-7} ng s^{-1} per cell for the same concentration range. The microcalorimetric data gave an increased heat flux from 19 pW per cell in the control to 44 pW per cell at 2.1 mM and showed that the cells responded correspondingly to G418 concentration (to 2.1 mM) in terms of the demand for ATP, though at some sacrifice to cell growth and foreign protein production. This means that the mitochondria had considerable capacity to produce the required ATP rate and was only partially the limiting factor for biosynthesis. It was only at 2.8 mM G418 that anaerobic glycolysis became a marked factor with the calorespirometric ratio being at –569 kJ mol^{-1} O_2. The increased catabolic rate caused essentially by the phosphotransferase synthesis rapidly depleted substrates in the medium and triggered the early onset of apoptosis, a feature of these hybridoma cells.

Besides the increasing restriction in ATP rate at the highest G318 concentrations, the metabolic burden could be associated with: (i) the inhibition of the translation; and (ii) the cytoplasmic accumulation of the foreign protein and neomycin system in the cell cytoplasm.

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S4O4

MINIATURIZED CALORIMETRY – A NEW TECHNOLOGY TO FOLLOW BIOFILM ACTIVITIES

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In nature bacteria predominantly occur as sessile communities called biofilms. These adhered growing cells build complex structures and exhibit morphological and physiological differences compared to suspended growing cells. Even though calorimetry was applied successfully for the investigation of planctonic microorganisms, only very few studies concerning the investigation of surface-associated cells are known. Biofilm examinations usually require a flow-mode-system, but due to high costs and low throughput capacities conventional flow-through-calorimetry is rarely used. However, biofilm poisoning [1,2] or detachment [3] could be detected calorimetrically within a few minutes. The presented chip-calorimetric method provides a new approach for the investigation of surface associated cells. This technique offers a flow-through mode as well as high-throughput capacities due to exchangeable flow-cells, which allow separation of cultivation and measurement.

In the experiments different biofilms of *Pseudomonas putida* PAW340 cells were cultivated and calorimetrically investigated. Comparative analyses were performed with confocal laser scanning microscopy as well as oxygen measurements. The results indicate that biofilm activity can be described accurately with this new technique [4].

Furthermore we examined the potential of chip-calorimetry to monitor and investigate antibiotic action against microorganisms growing in a biofilm. Now surface adhered *Pseudomonas putida* PAW340 cells were exposed to different antibiotic treatments. For example tetracycline was used to study the influence of a bacteriostatic antibiotic, kanamycin as a bactericidal antibiotic. The realtime observation of the processes qualifies this technique as a monitoring tool, but additionally physiological information can be derived. For this purpose similar experiments were also performed with planctonic cells, the comparison of the results may indicate some phenotypic specialties of the biofilm mode of growth.

Based on these results further possibilities for the application of the new chip-calorimetric method will be evaluated.

S4O5

Energetic considerations of fast haloadaptation

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Bacteria are often imposed on fluctuations in salt concentrations varying in amplitude and frequency. The survival and fitness of the bacteria depends highly on the strategies to cope with these hostile environments and the respective energetic costs. One of the adaptation strategies includes the synthesis and intracellular accumulation of small organic molecules serving as osmotic counterweights. Because these compounds don't interfere with the metabolism they are called compatible solutes. It was found that compatible solutes stabilize proteins, enzymes or whole cells against freezing, heating, drying and different denaturing agents [1]. This property has attracted commercial attention. Thus, the conversion efficiency of the carbon source into the compatible solute is also highly important for its biotechnological production.

It is speculated that cells have to spend a lot of ATP units to survive in high saline environments for the production of compatible solutes [2]. Own experiments and maximum metabolic flux estimations contradict the speculation and advise that one mole of glucose can be converted into one mole of ectoine (an abundant compatible solute) requiring maximal one mole ATP [3]. Furthermore, it was found that the synthesis of compatible solutes is the main adaptation process from the energetic point of view. The reaction enthalpy of ectoine synthesis is calculated to be endothermic or slightly exothermic dependent on the estimation of the energy content of ectoine.

If the pure haloadaption is really endothermic and the growth is exothermic then calorimetry allows distinguishing clearly between both metabolisms. The entropic driving force for the reaction should be small due to the slight structure changes and reaction stoichiometry additionally to the expected small or counteracting enthalpic driving force. The question arises how fast bacteria can adapt to rapidly increasing salinity if the driving forces are so small.

To verify these theses we confronted exponentially growing halophilic bacteria (strain *Halomonas elongata*) with suddenly increased salt concentrations. Bacteria adapt to the salt shock within approx. 3 hours by synthesizing ectoine with a highest ever observed formation rate. Online exhaust-gas analysis (CER or OUR) as well as calorimetric measurements indicates considerable changes in adaptation mechanism. To separate the influence of the solution enthalpy of salt on the result we used two calorimetric techniques, i.e. complete heat balanced bioreactors BioRC1 (Mettler Toledo, Switzerland) and a microcalorimeter TAM II (Thermometric, Sweden) used in a measuring loop. We found a dramatically reduced heat production rate per mole of glucose consumed during the adaptation phase in comparison to the undisturbed growth phase, according our thesis. Real endothermic adaptation processes can not be excluded because growth processes was not completely suppressed during the adaptation process. Thus, our next attempts target to separate growth and haloadaptation processes to verify our thesis.

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S4O6

Application of microcalorimetric method on interaction between heavy metals and envrionmental microorganisms

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Abstract

The functions of envrionmental microorganisms are essential to the environment because of their roles in cycling mineral compounds, decomposing organic matters, promoting or suppressing plant growth and other biophysical processes (Critter et al., 2004). Thus, microorganisms and their controlled processes are essentail for the long-term sustainability of ecological and agricultural systems. Addition of inorganic and organic matter promotes changes in chemical and physical properties of the envrionment and the biodiversity of the microbial communities can be influcenced by the added chemicals. However, many studies of ecological effects on microbes in environmental medium are short-term. On the other hand, microorganisms also play an important role in degrading many agrochemicals and accumulating heavy metals. This action can promote a decrease in the toxicity of many inorganic and organic compounds and influcence the health of the nature.

The acute toxicity text for toxic chemicals is very important, because an acute toxicity study can establish relationship between the dose of toxicant and its effect on the tested organism. Using growth metabolism of microbe as the environmental risk assessment process is attracting more interest. Bioenergetic investigations should be the most important in ecotoxicology for assessment of harmful properties of substances. These are closely related to the applicability of calorimetry in biology and environmental science.

The aims of the studies are to investigate the dose- response relationships between heavy metals (Cr, As) and soil microorganism community or pure microorganism, and to prove the availability of microcalorimetric methods to assess the influence of toxic agents on the environment and human health with the power-time curves generating a lot of kinetic information, By analysis of the thermogenic curves, kinetic parameters, such as rate constant for microbial growth, total yielding metabolic heat and peak power for microbial acticity can be evaluated in laboratory conditions, conbining other routine microbiological methods.

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S4P1

Energy and material fluxes during growth and haloadaptation of the non-conventional yeast *Debaryomyces hansenii* revealed by calorimetry.

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Non-conventional yeasts have attracted scientific and commercial attention due to high pH- and halotolerance, versatile substrate spectra and simple cultivation [1]. Unfortunately, they are characterized by highly complex growth and adaptation patterns. The exploitation of such strains for the decontamination of the environment, as cell factory or as hosts for biosensor developments requires easy, non-invasive real-time monitoring tools to analyze the complex metabolic regulation patterns. Calorimetry appears particularly suitable for that task, because it fulfills all of these requirements and delivers additionally insights into the main metabolic fluxes using the law of Hess. Recent calorimetric developments promise applicability under technical "dirty" conditions and efficiency for high-throughput measurements.

We assayed the analytical potential of biocalorimetry at the example of the haloadaptation of the non-conventional yeast *Debaryomyces hansenii*. By means of calorimetric monitoring, thermokinetic analyses and additional off-line analytics we were able to show that information about the metabolic network, growth and product syntheses under certain conditions can be easily gained. The complex heat production pattern does not only indicate the formation/consumption of intracellular pools, moreover, it was even possible to explain the heat production rate quantitatively for a few examples by using variances in the main metabolic fluxes [2]. We examined the haloadaptation of *D. hansenii* under different growth conditions (i.e. batch and continuous cultivation). Surprisingly, we found different sequences of stress regulations (e.g. synthesis of arabitol and glycerol) depending on the nature of the growth limitation. Different "bioenergetic costs" for synthesizing arabitol and glycerol are possible explanations for the sequence of stress regulations. The strong correlation between thermokinetically modeled heat production and experimental data emphasize the analytical potential of calorimetry.

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S5O1

Adaptation of Growth and Respiration of Three Varieties of *Caragana* to Environmental Temperature

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Growth and respiratory characteristics of *Caragana korshinskii* from Wushen and two different seed sources of *C. davazamcii* from Helinger and Yijinhuole, all grown at the same conditions, were determined by measuring metabolic heat and CO_2 production rates by isothermal calorimetry at 5°C intervals from 10 to 40°C. Substrate carbon conversion efficiencies and growth (anabolic) rates were calculated from the measured data. Differences in substrate carbon conversion efficiency, respiratory rates, and temperature responses of respiratory rates among the three accessions all contribute to produce differing temperature responses of growth rate. Planting seeds from these seed sources outside their native ranges will probably not be successful because of the differences in temperature adaptation.

S5O2

COMPARISON OF RESPIRATORY AND GROWTH CHARACTERISTICS OF TWO CO-OCCURING SHRUBS FROM A COLD DESERT, *Coleogyne ramosissima* (blackbrush) and *Atriplex confertifolia* (shadscale)

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Coleogyne ramosissima Torr. (blackbrush) and *Atriplex confertifolia* [Torr. & Frem.] Wats. (shadscale) are cold desert shrubs from different families. Despite very different life histories they often grow in close geographic proximity in the Great Basin and the Colorado Plateau between 800 and 2000 m elevation. Blackbrush and shadscale are soil specialists; blackbrush is almost completely confined to shallow, coarse-textured soils, while shadscale is a specialist on deep, fine textured soils. Shadscale is a salt-collector and more salt tolerant and occupies more saline soils. The purpose of this study is to compare the ecophysiology of slow growing and reproducing blackbrush with the ecophysiology of faster growing and reproducing shadscale. Metabolic heat and carbon dioxide production rates were measured by calorespirometry on tissue from wild plants and on lab-grown seedlings at temperatures from 10 to 35°C at 5°C intervals. Substrate carbon conversion efficiencies and anabolic (or growth) rates were calculated from the respiration data. Heats of combustion, ash content, and carbon and nitrogen contents were also measured. The growth rate of blackbrush was found to be approximately half that of shadscale because of both lower respiration rate and efficiency. Blackbrush and shadscale were found to have similar temperature responses at lower temperatures, but blackbrush begins growing earlier in the spring and can grow at higher temperatures when water is available. Blackbrush was observed to reproduce heavily when summer rain is abundant.

S5O3

TEMPERATURE DEPENDENCE OF CYANIDE-RESISTANT RESPIRATION BY TERRESTRIAL PLANTS

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Quantifying the temperature response of carbon use efficiency in plant respiration is necessary for predicting the effects of global warming on the global carbon budget. The ratio of the activities of the alternative and cytochrome oxidase paths in plant tissues can be determined by measuring 18O/16O discrimination by total respiration and by the alternative oxidase. Total carbon use efficiency can be calculated from the ratio of measured respiratory heat and CO2 rates. Oxygen isotope discrimination and heat and CO2 rates were measured as functions of temperature from 15 to 35°C. This paper tests a hypothesis that relative activity of the less efficient alternative oxidase path changes with temperature as a means of regulating intracellular phosphorylation potential during diurnal temperature changes, and thus changes carbon use efficiency. Oxygen isotope discrimination (22.9±0.4‰) and %AOX activity (30±5%) were found to be invariant with temperature, and invariant among the three species measured (C. pepo, N. sativa, and V. faba), thus falsifying the hypothesis. Carbon use efficiency was found to increase with increasing temperature in all three species. The variable efficiency must be caused by temperature variable activity of uncoupling proteins, NADH dehydrogenases, ATP phosphateses, or other inefficient pathways that allow variable coupling between ATP synthesis and use in anabolism.

S5O4

THE INFLUENCE OF EXTRACTS FROM SUNFLOWER AND MUSTARD LEAVES ON THE GERMINATION OF MUSTARD SEEDS

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The aim of this investigation was to perform studies on allelopathy using FT-Raman spectroscopy and isothermal calorimetry.

The aqueous extracts from sunflower or mustard leaves (5% (w/v)) were separated on the SPE Columns (solid phase extraction) on two fractions: water and "methanol". The last one was evaporated to dryness and dissolve in water. The seeds of mustard were pre-germinated on water for 24 hours and then germinated on the crude extracts from sunflower or mustard leaves and on the particular fractions after SPE. The heat production rate during germination of the seeds was measured by isothermal calorimetry at 21°C (metabolic activity of the seeds). The chemical changes in the cotyledons of seeds, caused by various extracts, were measured by FT–Raman spectroscopy. The significances in the spectra of seed's cotyledons chemical composition were analyzed by the Cluster Analysis (on the basis of Raman spectra).

The heat production rate during seeds germination depends on the applied extracts. Crude sunflowers and mustard extracts strongly inhibited seeds germination. The "methanol" fractions from mustard and sunflower extracts have a similar influence on the pattern of heat efflux from mustard seeds. The similar situation is observed with water fractions, however this extracts influence on seeds germination in a less degree.

The results obtained from FT–Raman spectroscopy shows, that extracts caused the chemical changes in cotyledons of mustard seeds. The most changes between Raman spectra performed from mustard seeds germinated on various extracts are visible in the range of saturated and unsaturated fatty acids, carotenoids and flavonoids.

The application of the Cluster Analysis was useful to analyse the differences in chemical composition of seeds. The analysis of the Raman spectra in the range from 100 to 4000 cm^{-1} indicates the presence of two groups of samples. In the first group are control seeds and they are completely different under chemical composition than the samples in the second group. To this group belong the seeds, which germinated on the extracts from mustard leaves. The same situation is observed for the sunflower extracts.

The results indicate that the isothermal calorimetry and the Raman spectroscopy are very useful tools for the investigation of the influence of allelopathic compounds on the metabolic activity of plants.

S5O5

DESIGN AND FIRST EXPERIENCE OF USING A LIGHT-EMITTING DIODE (LED) AS THE INBUILT LIGHT SOURCE FOR A CUSTOMISED DIFFERENTIAL PHOTOCALORIMETER

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Johansson and Wadsö [1] described a photocalorimetric module for the commercial TAM heat conduction batch microcalorimeter (Thermometric AB, SE-17561 Järfälla, Sweden) and applied it to measure the rate of photosynthesis in spinach leaves. Mukhanov and Kemp [2] modified it for use on cultures of the halotolerant chlorophytic microalga, *Dunaliella maritima* and interpreted the results in terms of nonphotochemical quenching. In both these applications, the incident light from an external xenon lamp was divided by a beam splitter and directed to the two vessels of the differential system by light guides. In seeking further technological improvements, however, the following advantages of light-emitting diodes (LEDs) are potentially attractive for photocalorimetric measurements: (i) they are more efficient in terms of producing more light per watt than other alternatives; (ii) they can be tuned to a narrow spectrum to emit light of an intended colour without the use of colour filters, (iii) their output as light intensity as a function of current is very close to linear; (iv) they do not change their color tint as the current passing through them is lowered; (v) they light up very quickly, achieving full brightness in microseconds; (vi) they have a relatively long useful life; and, finally, (vii) they are small in size. Thus, the xenon lamp-based system was replaced by a pair of white LEDs embedded directly in the test and control calorimetric vessels. The LEDs had independent electrical circuits to achieve manually the balance between the vessels in terms of the heat outputs resulting from the incident light. The test vessel was additionally equipped with PTFE tubing for changing the liquid phase in it using a vacuum pump and a syringe. This procedure improved the reproducibility of the results because it avoided extracting the vessel from the instrument and opening it to change the liquid phase.

The photocalorimeter was applied to measure the instantaneous heat flow of *D. maritima* grown at 50 μmol photon m^{-2} s^{-1} in an incubator at 25 °C with a 24-white LED lamp. In experiments combining both photocalorimetric and respirometric methods, the culture was exposed to normal and higher light intensities (up to 150 μmol photon m^{-2} s^{-1}) to observe the energetic consequences of acute light stress. At the first stage of every experiment, light calibrations were performed with the Bold's culture medium in the vessels. Then, the vessels were elevated to the middle, thermostatic position and the medium in the test vessel was exchanged to the algal culture.

The results appeared encouraging and are discussed in this paper from the standpoint of the accuracy and reproducibility of the measurements. The new approach may provide a promising technological basis to more precisely balancing the light inputs to the photocalorimetric vessels. In particular, the prospects are considered for developing an automatic device with a digital feedback control of the current in the LED circuits.

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S5P1

THE INFLUENCE OF VARIOUS HERBAL EXTRACTS ON THE GERMINATION OF MUSTARD SEEDS

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The aim of the studies was the investigation of the impact of herbal extracts on the germination of mustard seeds (the allelopathy phenomenon).

The seeds of mustard were pre-germinated on water for 24 hours and then germinated on selected aqueous herbal extracts (angelica, arnica, ribwort, camomile, hypericum, fennel, milfoil, sage and thyme). The concentration of extracts was 5% (w/v). Impact of herbal extracts on the metabolic activity of seeds was measured by isothermal calorimetry at 21°C (the heat production rate). The chemical changes in the plant tissue, caused by herbal extracts, were measured by FT–Raman spectroscopy. The significances of differences in chemical composition of seed's cotyledons were analyzed by Cluster Analysis (on the basis of Raman spectra).

The heat production rate during seeds germination depends on the extracts. The extracts from fennel and milfoil, sage and thyme, arnica and angelica, camomile and ribwort have a similar influence on the pattern of heat efflux from mustard seeds. During seeds germination on extracts from arnica, milfoil, sage and thyme the same endothermic reaction was observed. It was found, that this effect was not associated directly with seed's metabolism. It was the effect of the chemical reactions between unidentificated compounds in exudates from seeds and in herbal extracts.

On the basis of FT–Raman spectroscopy ascertains, that herbal extracts caused the various chemical changes in cotyledons of mustard seeds. The most changes are visible in the range of fatty acids (saturated and unsaturated fatty acids), carotenoids and flavonoids.

The Cluster Analysis occurred very useful for the classification of the differences in chemical composition of seeds. The analysis of the Raman spectra in the range from 100 to 4000 cm^{-1} indicates that, two separate groups of extracts can be named based on the effects which they caused to chemical changes in cotyledons of mustard seeds. To the first group belong the extracts from sage, hypericum, camomile and thyme and to the second one the extracts from angelica, arnica, ribwort, fennel and milfoil. Nevertheless, analysis in the range of 1236 – 1371 cm^{-1} (fatty acids marker bands) indicates on the five groups of extracts, which affected seeds metabolism in similar way. There are: 1) sage and thyme, 2) arnica and angelica, 3) milfoil and fennel, 4) ribwort and camomile, 5) hypericum. On the other hand, the same pairs of extracts caused similar changes in the pattern of heat efflux from mustard seeds. Thus, influence on the metabolism of fatty acids, carotenoids and flavonoids seems to be correlated with heat production rate during germination seeds on these extracts.

The obtained results indicate, that the isothermal calorimetry and FT–Raman spectroscopy are very useful tools to investigate the allelopathy and the influence of chemical compounds on the metabolism of seeds.

S6O1

ENDOTHERMY OF SCARAB BEETLES IN THERMOGENIC FLOWERS

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Over a thousand species of heat-producing flowers in the Neotropical forests are pollinated by large dynastine scarab beetles. We investigated the patterns of activity of beetles (*Cyclocephala colasi*) in the inflorescences of a large arum lily (*Philodendron solimoescence*) in French Guiana. We observed beetles inside inflorescences in the field and related activity to respirometry and body temperatures measured by needle thermocouples and an IR camera in the laboratory. Beetles were active during the night inside the floral chambers, where they mated and fed, and they rested the following day. The 24-hour cycle of respiration mirrored this, with bouts of endothermy at night (particularly in the early evening), and low rates during the day. Flying beetles had thoracic temperatures up to 9 ºC above ambient, but the temperature excess was less than 2 ºC inside the floral chamber. Floral chamber temperature during the night averaged 26.6 ºC while ambient air was 22.8 ºC. Beetles artificially exposed to temperatures less than 27 ºC showed bouts of intense endothermy during which respiration could increase over 80-fold. The energy cost of activity increased at lower ambient temperatures, because active beetles maintained thoracic temperature generally between 30 – 36 ºC at ambient temperatures down to 16 ºC. Respiration rate was correlated with elevation in thoracic surface temperature, leading to the prospect of using surface temperature as an indirect measure of metabolic heat production.

S6O2

Thermal investigations of a nest of the stingless bee *Trigona (Frieseomelitta) nigra paupera* Provancher in Colombia

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Abstract

Thermal investigations on a colony of the Colombian stingless bee *Trigona (Frieseomelitta) nigra paupera* were performed by means of thermometry and direct calorimetry.

These stingless bees are of slender and delicate appearance and display brood cells without contact between them in a disorganized cluster, unlike the brood combs of other species. Ten *Frieseomelitta* species are recognized in the world distributed from Mexico to Brazil, two of them are in Colombia [1,2].

A nest with approximate 2000 individuals was transferred from a "rational" hive to a twin calorimeter, where the bees continued their normal life and development. Two camping boxes ("Poor Man's Calorimeter, PMC") with Peltier elements as cooling systems were used as a differential system. Each box had a volume of 8 L and a sensitivity of 19.2 mV W^{-1}. The PMCs were modified to give forager bees a free access to the environment. The colony was monitored calorimetrically and thermometrically. 10 kΩ NTC resistors were distributed at special points of the brood and storage pots. The thermometric and calorimetrical signals were recorded continuously by a four channel data logger.

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S6O3

Calorimetric investigations of discontinuous ventilation in insects

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A number of insects show the interesting behaviour of discontinuous ventilation, which helps to survive difficult environmental conditions like hibernation and droughts. The carbon dioxide produced in the metabolism is released in short pulses after longer intervals of closed tracheae thus decreasing the water loss from the body (danger of desiccation!) and the energy consumption for opening the stigma. Rhythmic movements of the abdomen often play a similar role, but occurring more frequently than the bouts of CO_2 release.

Such structures were observed by the authors by direct and indirect calorimetry for hibernating hornets, for bumble bees, mealworms and cockroaches. Data from the literature concerning discontinuous ventilation are discussed also.

S7O1

Do shell deformities influence the metabolic rate of blue mussel *Mytilus edulis*?

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The blue mussel *Mytilus edulis* is a widely distributed species inhabiting temperate and polar waters where it forms dense beds in intertidal or subtidal zones. This bivalve is of great interest for fisheries and therefore it is harvested from wild or commercially cultivated in many regions, except of the brackish seas, where specimens of *M. edulis* are significantly reduced in size. It was observed that in field populations of blue mussel shell morphology exhibits considerable variations which are attributed to factors like age, density or locality. Shell deformities might be also of mechanical origin or can result from exposure to toxicants (e.g. TBT), parasitic infections and diseases. Nevertheless, shell abnormalities may affect shell length/soft tissue ratio regarded as one of the condition indices in bivalve molluscs. The highly mineralized shell protects mussels against the external environment, whereas all physiological functions take place in organic soft tissue. Moreover, due to uneven edges some mussels are not able to tightly close their shells forcing them for permanent exposure to external conditions. Bivalves are known to be able for rapid shift in type or rate of the standard metabolism which is possible due to the gaping behaviour (shell opening and closing).

To verify the hypothesis that mussels with shell deformities may have different metabolic rates compared to those with regular shells both, morphological analyses and heat dissipation measurements were conducted on *M. edulis* collected in the southern Baltic Sea in the winter 2008. Mussels with shell deformities were separated and classified into different groups: (1) elongated, with lateral indent, (2) with scarred shells, (3) egg-shaped, (4) with uneven edges/ not tight and (5) with tumour. Next the length, width and height of shell as well as wet and dry mass (shell and soft tissue, separately) of each specimen were measured. Water content and condition indices were calculated. To find whether shell deformities may influence the metabolic rate, *M. edulis* with regular shells and with abnormal shells (of equal length) were studied in an isothermal calorimeter of the Calvet type. Animals were placed separately in the vessel containing 15 ml of sterile filtered and aerated water of 10 °C temperature and 7‰ salinity. For better interpretation of power-time curves in regard to activity some measurements were performed with mini cameras incorporated into the calorimeter.

S7O2

Impact of Ionic Liquids on the Metabolic Rate of *Gammarus tigrinus*

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Ionic liquids represent a highly solvating, non-coordinating medium in which a variety of organic and inorganic solutes are able to dissolve. Due to the unique features these chemicals became more and more popular in chemistry as well as in different industry branches. Despite extensive application of different ionic liquids their effect on living organisms is poor known. It especially concerns aquatic organisms which are particularly exposed to these chemical substances due to their physicochemical properties.

The aim of this study was to examine the impact of the ionic liquids 1-butyl-3-methylimidazolium chloride [bmim]Cl on the metabolic rate of the amphipod *Gammarus tigrinus* – a non-indigenous species in European waters. Animals (adult males of length > 10 mm) were collected in the coastal waters of the Baltic Sea in February 2008. Metabolic rate of *G. tigrinus* was determined based on the heat dissipation measurements conducted in the isoperibol differential twin calorimeter. The sensitivity of the instrument calorimeter was 101 $\mu V\ mW^{-1}$. Measuring vessel was equipped in bullet camera which allowed for observations of organism's behavior and for better interpretation of power-time curves. Single animal was placed in the vessel filled by 15ml of aerated water of temperature 15°C and salinity 7‰. The metabolic level was recorded over 4-6 h and then 2 ml of ionic liquids was added to the vessel. Shift in metabolic level was registered over the next 12 h.

Results showed a marked decrease of the metabolic rate following exposure to [bmim]Cl. The effect of [bmim]Cl on metabolic rate was concentration-depended. The metabolic rate was depressed by 18% at the concentration of 0.25mM and by 43% at 2mM. It can be concluded that [bmim]Cl has inhibitory effect of on metabolic processes in *G. tigrinus*.

S7O3

COMPARATIVE STUDY OF DSC PATTERN, COLOUR, TEXTURE AND WATER-BINDING CAPACITY OF SHRIMPS DURING HEATING

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Shrimp continues to be the most important commodity traded in value terms, accounting for 16.5 percent of the total value of internationally traded fishery products in 2004. In 2004, more than 41 percent (or 2.5 million tonnes) of total shrimp production was of farmed origin. The per capita availability of crustaceans between 1961 and 2003 increased more than threefold, from 0.4 kg to 1.5 kg. In Europe, shrimp imports increased in 2005. The share on shellfish in fish consumption in Germany increased from 12% to 14% in 2005. Import of shellfish into Germany in same year amounted 85, 000 mt.

Despite this importance of shrimp, literature is almost lacking on reports dealing with changes in functional properties and quality caused by heating shrimps while influence of freezing has been investigated more in depth. This is particularly surprising because both brown shrimp (*Crangon crangon*) and northern shrimp (*Pandalus borealis*) are mainly cooked directly after catching.

The objective of the study was therefore cooking shrimp to different core temperatures in the range 30-80 °C and to monitor changes in quality by measuring texture, colour and water- binding capacity. To explain changes observed DSC curves and gel electrophoresis pattern taken on the differently cooked shrimp were discussed. On this way the following commercially important shrimps were compared: deepwater rose shrimp (*Parapenaeus longirostris*), brown shrimp (*Crangon crangon*) and northern shrimp (*Pandalus borealis*). Behaviour of shrimp after cooking regarding colour, texture and water-binding capacity was found to be species-specific. DSC curves taken on differently heated shrimp differed markedly. With increasing temperature the enthalpy of denaturation decreased significantly.

Farmed shrimps imported mainly from South-East Asia into Germany include Black Tiger Shrimp (*Penaeus monodon*) and White Shrimp (*P. vannamei*). DSC curves taken on both species were compared with each other and regarding the influence of origin as well as use of additives.

S7P1

Bioenergetics of the Chinese Mitten Crab *Eriocheir sinensis*

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Human mediated introductions of alien species are regarded as the global problem due to ecological and economical consequences they can cause. Our current knowledge on species introductions is largely based on abundance data, whereas the information on adaptive capacity and growth of non-indigenous species as well as on their functional role in new environments are rather sparse. One of the 100 world's worst alien invasive species is the Chinese mitten crab *Eriocheir sinensis*, which due to the large size (carapace width up to 10 cm) as well as occurrence in high abundances might be the serious threat to local biodiversity. Although this species has been introduced to European waters almost 100 years ago still little is known on its physiology. Beside better understanding of *E. sinensis* functioning in European waters such an information could help to estimate its the impact on the native ecosystems.

The basic physiological rates (consumption, excretion, respiration) were studied in adult *E. sinenis* specimens (carapace width 55–68 mm) collected in the autumn of 2006 and 2007, in the coastal waters of the Baltic Sea (salinity 7‰, temperature 11ºC). Crabs were fed with soft tissue of blue mussel *Mytilus edulis trossulus*. Oxygen consumption measurements of starved and fed animals were conducted over 24 hours in the flow respirometer with a chamber of 1200 ml volume. Two needle electrodes OX-N (Unisense, Denmark) connected to pA-meter PA2000 (Unisense, Denmark) measured oxygen partial pressure at the entrance and exit of the measuring chamber. Signal was registered every 30s by data logger (LD-20; CIE, Taiwan). The ammonia excretion rate of starved and fed crabs was determined by the phenol-hypochlorite method. Physiological rates (e.g. consumption, excretion and respiration) were converted to energetic equivalents to determine energy balance of an single organism as well as to calculate energy available for growth.

S8O1

Differential scanning calorimetric examination of the degenerated human palmar aponeurosis in Dupuytren disease

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The Dupuytren contracture - degenerative shortening of the palmar aponeurosis - is a common disease of the hand in Europe. The aetiology of the degenerative changes in the collagen structures is still not clear. To describe the clinical manifestation of the disease we use an international classification according to Iselin. The treatment of the Dupuytren contracture is basically surgical, the exact time of surgery is depend on structural and functional changes in the aponeurosis. Differential scanning calorimetry (DSC) is a well established method for the demonstration of thermal consequences of local and global conformational changes in biological systems. It has never been applied for the investigation of human palmar aponeurosis. According to the present study the thermograms may prove and follow the changes in the structure of aponeurosis collagen in different stages of Dupuytren disease.

Our hypothesis was that in Dupuytren disease there is a clear pathological abnormality in the tissue elements building up the palmar aponeurosis, which is responsible for the disease. Besides examining healthy structures of the aponeurosis with differential scanning calorimetry (DSC) we planned to carry out investigations of collagen destruction caused by the disease. A calorimetric examination of this type has not yet been carried out on international level.

Our aim was to prove with the examinations that there is a definitive difference in the structure of the healthy (4 samples from healthy cadaver) and pathological (2-2 samples from all of four stadium according to Iselin) aponeurosis, which can be reproduced.

The pieces of different samples have been prepared and measured within 6 hours of removal. The thermal denaturation of different parts of human samples was monitored by a SETARAM Micro DSC-II calorimeter. All the experiments were performed between 0 and 100 ^{0}C. The heating rate was 0.3 K/min. Conventional Hastelloy batch vessels were used during the denaturation experiments. The sample and reference vessels were equilibrated with a precision of ± 0.1 mg and there was no need to do any correction from the point of view of heat capacity between the sample and reference vessels. The data treatment after ASCII conversion was done by Origin 6.0.

DSC scans clearly demonstrated significant differences between the different types and conditions of samples (control: T_m = 63 ^{0}C and ΔH_{cal} = 4.1 J/g, stage I.: T_m = 64 ^{0}C and ΔH_{cal} = 5.2 J/g, stage III.: T_m = 60.2 ^{0}C and ΔH_{cal} = 5.3 J/g). The heat capacity change between native and denatured states of aponeurosis samples decreased with the degree of structural alterations indicating significant water loosing. These observations could be explained with the structural alterations caused by the biochemical processes.

With our investigations we could demonstrate that DSC is a useful and well applicable method for the investigation of collagen tissue of the human aponeurosis. Our results may be of clinical relevance in the future i.e. in the choice of the optimal time of surgical therapy of different clinical level Dupuytren contractures.

S8O2

Differential scanning calorimetric examination of transverse ligament of the wrist in carpal-tunnel disease

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The carpal-tunnel syndrome – compression of the median nerve by the transverse liga-ment of the wrist - is a serious disease of the human hand. The incidence of this disease is increased year by year in the European countries, because of the monotonous motion of hand during work. The electrophysiological changes in the median nerve can be easily followed by electroneurography (ENG). The degenerative changes in the collagen structures of the carpal ligament cause shrinking of the ligament and compression of the nerve. The aetiology of these degenerative changes is still not clear. Differential scanning calorimetry (DSC) is a well established method for the demonstration of thermal consequences of local and global conformational changes in biological systems. It has never been applied for the investigation of transverse ligament of the wrist. According to the present study the thermograms may prove and follow the changes in the structure of ligaments collagen in different stages of carpal-tunnel disease.

Our hypothesis was that in carpal-tunnel disease there is a clear pathological abnormality in the tissue elements building up the transverse ligament of the wrist, which is responsible for the disease. With differential scanning calorimetry (DSC) we planned to carry out investigations of collagen destruction caused by the disease.

Our aim was to prove with the examinations that there is a definitive difference in the structure of the healthy (4 samples from healthy cadaver) and pathological (10 samples from different electrophysiological stadium) ligaments, which can be reproduced. We demonstrate that there are relationships between the electrophysiological changes in the nerve and the structural changes in the transverse ligament.

The pieces of different samples have been prepared and measured within 6 hours of removal. The thermal denaturation of different parts of human samples was monitored by a SETARAM Micro DSC-II calorimeter. All the experiments were performed between 0 and 100 ^{0}C. The heating rate was 0.3 K/min. Conventional Hastelloy batch vessels were used during the denaturation experiments. The sample and reference vessels were equilibrated with a precision of ± 0.1 mg and there was no need to do any correction from the point of view of heat capacity between the sample and reference vessels. The data treatment after ASCII conversion was done by Origin 6.0.

With our investigations we could demonstrate that DSC is a useful and well applicable method for the investigation of collagen tissue of the human carpal transverse ligament. DSC scans clearly demonstrated significant differences between the different types and conditions of samples (control: T_m = 61.3 ^{0}C and ΔH_{cal} = 4.04 J/g, stage I.: T_m = 62 ^{0}C and ΔH_{cal} = 4.3 J/g, stage II.: T_m = 61.5 ^{0}C and ΔH_{cal} = 5.17 J/g, stage III.: T_m = 61.85 ^{0}C and ΔH_{cal} = 8.44 J/g). After these investigations we can choose the optimal time of surgical therapy of different clinical level Carpal tunnel syndrome too.

S8O3

Calorimetric examination of the human meniscus

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Introduction:

The aim of our experiment was to examine the thermal consequences of the degenerative changes in the human meniscus with thermodynamic methods.

Material and methods:

We used 3 healthy and 21 degenerated meniscus samples for the examination. The healthy samples were taken from cadavers, these showed no macroscopic abnormalities. The pathological samples were removed in the course of knee operations from patients suffering from osteoarthritis and traumatic meniscus rupture. These samples showed signs of macroscopic and microscopic degenerations. The measurements of these samples have been made after a standard preparation with the SETARAM Micro DSC-II calorimeter. The data processing was made by the program Origin 6.0.

Results:

We found significant differences in the calorimetric enthalpy and heat capacity not only between the healthy and degenerated samples, but between males and females, too (Men: young-3.46; middle ages-4.93; old-3.63 J/g. Females: middle ages-6.03-; old-6.34 J/g).

Conclusion:

Based on this examination we can see that the thermodynamic method is suitable for the verification of the degenerative changes in the meniscus. The results verified the decreased thermal stability of the degenerated meniscus.

S8O4

DSC analysis of human fat tissue in alcohol-induced avascular necrosis of the femoral head– a preliminary study.

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Backgound: The osteonecrosis or avascular necrosis of the antero-superior part of the human femoral head (ANFH) often causes incongruity in the hip joint and leads to severe pain and disability in middle aged patients. This abnormality often requires surgical intervention, mainly total hip arthroplasty (THA). The possible pathomechanisms of ANFH are fat embolism, arterial occlusion, fatty necrosis of ostecytes and intraosseous hypertension. Several etiological factors have been proposed in the literature, that can lead to this condition, alcohol abuse, steroid therapy, metabolic changes, dyslipidaemia are the most common causes. In some case we can not verify any factor, these are the so called idiopathic ANFH cases.

We observed macroscopic variation in color and consistency of the subcutaneous fat tissue in patients with ANFH compared to osteoarthitis or hip fracture during THA procedures.

Aim: We proposed that the changes due to the altered fat metabolism that contributed to the ANFH can be observed in the subcutaneous fat tissue in the molecular level.

Material and methods: We obtained subcutaneous fat tissue during THA from a patient with ANFH due to alcohol abuse and compared with an otherwise healthy patient who underwent surgery due to traumatic hip fracture. For histological analysis HE, and oil red staining were utilized. From frozen tissue gas chromatography looking at the variation of medium and long chain fatty acids (C12-C22) and DSC analysis were performed.

Results: No histological changes were notified in the size, shape of adipocytes and in the cells of the septae of the connective tissue. Gas chromatography showed, that the patient with alcoholic ANFH had less long chain fatty acids.

DSC revealed, that in case of non-necrotic sample as a reference, during heating between 0-100ºC: two separable transitions with T_m=5.7 and 9.9ºC, total ΔH= -20.8J/g; and heating between -20-100ºC: T_m=-10.9 and 4.95ºC, total ΔH= -75.8J/g endotherms could be detected. In case of alcohol-induced avascular necrosis we have found endotherms between 0-100ºC with: T_m=7.3ºC, total ΔH= -26.9J/g, and heating between -20-100ºC: T_m=-0.25ºC, total ΔH= -103.3 J/g thermal parameters.

Conclusion: The alteration in the fatty acid profile did not cause histological changes, but we were able to detect it with analytical methods e.g. DSC and gas chromatography.

S8O5

Differential scanning calorimetric examination of the esophagus after implantation of special stents, designed for the management of acute esophagus variceal bleeding. An experimental study.

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Massive bleeding from esophagus varices presents a life threatening complication of portal hypertension. No effective method of treatment is available until now, that would guarantee high grade of patient wellness during the conditioning and investigation phase until the definitive treatment could be introduced. The aim of this study was to evaluate the tissue response to esophagus stents - designed for manage acute variceal bleeding - in animal experiment.

Self-expandable nitinol stents (stent-1) were introduced into the esophagus of six porcines. Another twelve porcines were undergone the same procedure, using the special biodegradable stents (stent-2) made of PDO (polidyoxanon). Histological investigations of the stented esophagus segments were observed after 2 and 4 weeks at the first 12 animals. To monitor the time of stent degradation, histology were performed 7 and 12 weeks after the implantation in the biodegradable group (3-3 animals). Differential scanning calorimetric examination (DSC), which is a well-established method for the demonstration of thermal consequences of local and global conformational changes in biological systems, was performed in all esophagus sample.

Focal erosion of the esophagus segments was more explicit in the group stent-1 at the histology. On the 7th week all of the biodegradable stent were in the stomach and on the 12th week these were completely solved. DSC examination showed, that using the stent-2 the final results are closer to the healthy control, but its all sample exhibit more stable thermal data (greater calorimetric enthalpy and higher melting temperature) as the control or stent-1.

This experiment showed that the new self-expandable stents are safety and suitable procedure without deterioration of the esophageal wall. According to our DSC results the thermal denaturation of intact esophagus, its mucosa and muscle fragments revealed significant differences compared to healthy sample in favour the new biodegradable stent. Safety and efficiency in the experimental model had encouraged us to apply this method successfully patients with bleeding esophagus varices. The long term goal is to show that stent placement could be an effective way of decreasing or stabilising the acute bleeding from ruptured esophagus varices in cirrhotic patients.

S8P1

Characterization of human cartilage in degenerated spine disease with differential scanning calorimetry

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Every level of the spine is composed of a disc in the front and paired facet joints in the back. The disc acts as a shock absorber in between the vertebrae, whereas the paired facet joints restrain motion. As the facet joints age, they can become incompetent and allow too much flexion, allowing one vertebral body to slip forward on the other. This slippage is known as a degenerative spondylolisthesis

The change of energy in thermal processes can be measured by Differential scanning calorimetry (DSC). A limited number of papers have been published before on the subject of thermal analysis of normal and degenerative human hyaline cartilage. One research group has concluded that structural manifestation of osteoarthritis appears as a remarkable change of thermal stability of hyaline cartilage samples. The data on the calorimetric enthalpy changes proved to be inconsistent.

The purpose of this study was to further characterize the altered metabolism in human degenerated cartilage that promotes disease progression. A new protocol had to be established before the investigation. Degenerative human cartilage (intervertebral disc, facet joint and vertebral end-plate) was obtained during 11 posterior lumbar spine interbody fusion procedures performed at the University of Szeged.

The thermal properties of samples were determined by differential scanning calorimetry (Mettler-Toledo DSC 821e apparatus). From the DSC curves the decomposition temperature, the transition temperature range and the total calorymetric enthalpy change were calculated.

With the rise of temperature an endothermic reaction was observed in all of the cases. The enthalpy change of the process initiated by the temperature change showed marked difference between the normal and pathological groups. Greatest change in the enthalpy was observed in the intervertebral disc samples: -1600.78 J/g (SD:141.25). Consequently these samples required the largest amount of energy for decomposition. Denaturation caused by heating in the normal human hyaline cartilage needed -1492.86 J/g (SD:202.42) energy. The average enthalpy change during the calorimetric measurements in the vertebral end-plate samples was -1196,91 J/g (SD: 361,10) and -969,29 J/g (SD: 287,39) in th facet joint. Statistical tests proved these calculations to be significantly different ($p<0.05$). All samples showed a clear denaturation peak on the calorimetric curve.

This study clarifies the previously reported thermoanalytical results, with acquiring normal cartilage from live surgery, thus providing similar sample environment. The investigation was performed in a relatively short period of time compared to the earlier reports. The use of thermal analysis could be a simple and effective method for controlling the relationship between biomarkers and disease progression. Characterization of the altered metabolism in cartilage that promotes disease progression should lead to future fundamental treatment options that can prevent structural damage.

S8P2

Novel calorimetric properties of human cartilage samples in rheumatoid arthritis

K. Tóth, G. Sohár, Z. Aigner, F. Greksa and P. Szabó-Révész

During recent years, knowledge of the nature and pathogenesis of rheumatoid arthritis has increased, and management of the disease has improved. Pathogenic mechanisms have showed that irreversible loss of articular cartilage begins relatively early. A limited number of papers have been published before on the subject of thermal analysis of degenerative human hyaline cartilage but RA has not been studied previously. A new protocol had to be established before the investigation.

The purpose of this study was to further characterize the altered metabolism in human RA cartilage that promotes disease progression.

The thermal properties of samples were determined by differential scanning calorimetry (Mettler-Toledo DSC 821e apparatus) and thermogravimetry (MOM Derivatograph). From the calorimetric curves the decomposition temperature, the transition temperature range and the total calorimetric enthalpy change were calculated. Cartilage was obtained during 26 hip and knee arthroplasty procedures performed at the University of Szeged. Preoperatively the diagnosis of RA was established. All tissues were yielded in accordance to legal regulation. Samples were stored in physiological buffer before the measurement at a temperature of 4 °C for not longer then 6 h.

With the rise of temperature an endothermic reaction was observed in all of the cases. The enthalpy change (denaturation caused by heating) of the process initiated by the temperature change was observed in RA at: - 1366,53 J/g (SD: 154,18). All samples showed a clear denaturation peak on the calorimetric curve. Denaturation peak in normal cartilage was at 47,09°C (SD = 6.99).

Previously, these methods have not been used for this purpose. Water molecules' binding mode may have an important consequence in pharmacokinetics. The use of thermal analysis could be a simple and effective method for controlling the relationship between biomarkers and disease progression.

S8P3

Calorimetric properties of degenerative human shoulder joint hyaline cartilage

János Csotye, Zoltán Aigner, Gellért Sohár, Piroska Szabó-Révész and Kálmán Tóth

Thermoanalytical techniques measure the change in physical or chemical properties of the sample as a function of temperature. There are many possible applications: characterizations of active and inactive ingredients, routine analysis, and qualitative control. Differential scanning calorimetry (DSC) involves the heating or cooling of a sample and reference and the measurement of the differential heat flow between the with respect to temperature. The change of energy in thermal processes can be measured, so it can be used in the same way for qualitative and quantitative studies.

The specific causes of osteoarthritis are unknown, but are believed to be a result of both mechanical and molecular events in the affected joint. The new paradigm of OA considers it as a heterogeneous disease with numerous factors leading to its pathologic hallmark of cartilage loss

During different shoulder operative procedures performed at the Orthopedic Department, University of Szeged, degenerative human hyaline cartilage was obtained from 10 patients and normal cartilage from 11 knee. All tissues were yielded in accordance to legal regulation, international ethical concerns, and patients' consent. After the operation, a disc (5mm in diameter) was removed under sterile conditions, and excess bone was removed. The disc was stored in 20 ml saline for transportation at room temperature. Mean storage time was 6 hours (min: 1 hour, max: 26 hour). Preoperatively the diagnosis of the patient was established on basis of the patient history, clinical signs and radiological findings.

The thermal properties of samples were determined by differential scanning calorimetry (Mettler-Toledo DSC 821e apparatus, Mettler-Toledo GmbH, Switzerland). Samples were heated from 0 to 80 °C. The heating rate was 0.3 °C/min. Conventional Hastelloy batch vessels were used with 40 µl sample volume. All the DSC measurements were proceeded in Ar atmosphere and the flow rate was 100 mL/min. Samples were stored in physiological buffer before the measurement at a temperature of 4 °C for not longer then 12 h.

From the DSC curves the decomposition temperature, the transition temperature range and the total calorymetric enthalpy change were calculated.

Greatest change in the enthalpy was observed in normal cartilage: -1492,86 J/g (SD = 202,42), in the degenerative samples -1250,96 J/g (SD = 199,49). Therefore, denaturation caused by heating was larger in the normal human hyaline cartilage. Consequently these samples required the largest amount of energy. Statistical tests proved these calculations to be significant. Denaturation peak in normal cartilage was at 49.77 °C (SD = 5.09), in the shoulder samples it was similar at 46.3 °C (SD = 9.22).

Characterization of the altered metabolism in cartilage that promote disease progression should lead to future treatment options that can prevent structural damage. Since damaged articular cartilage has a very limited potential for healing, prevention is fundamental in treatment.

S8P4

DSC analysis of human fat tissue in steroid induced ostheonecrosis – a preliminary study.

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Nontraumatic ostheonecrosis of the femoral head is a rather common disorder causing disability of the hip joint. It develops in the high load-bearing region of the femoral head. The necrotic subchondral region of the bone looses of its load-bearing capacity, the chondral surface collapse which results the incongruity of the joint.

Aseptic necrosis of the femoral head appears to have a number of aetiological factors. The disease frequently occurs in disorders of lipometabolism, after steroid medication, in chronic alcoholism and sickle-cell anaemia and there is also a group with idiopatic aetiology. In case of steroid induced type, the intramedullar adipogenesis is thought to be a capital step causing the ischemic necrosis of the bone. The final surgical therapy of the joint disorder is to replace the destructed joint by a total endoprothesis. The intraoperative observation of the authors is, that the fat tissues of the patients with ostheonecrosis macroscopically deviates from other patients fat tissues. The relationship between the steroid therapy and the occurrence of nontraumatic ostheonecrosis of the femoral head is well known for a long time, but the pathobiological mechanism underlying the induction of adipogenesis by steroids has not been fully elucidated.

The main purpose of this study was to examine the intraoperative obtained gluteal fat tissue with DSC. 5 tissue samples were taken intraoperatively from steroid treated patients with femoral head ostheonecrosis, and 5 samples were taken from non-necrotic patients to build a control group. The calorimetric measurements were drawn a parallel with histological sections and with results of a gas chromatographic analysis.

The histological sections showed no significant differences compared with the control group. Our preliminary finding suggests that DSC could be a sensitive and specific method in the structural analysis of steroid induced human fat tissue abnormalities. The DSC curves correlate with chromatographic analysis of the tissue fatty acids (Steroid treated, heating between 0-100ºC: T_m=5.7ºC, ΔH=-15.8J/g; heating between -20-100ºC: T_m=-9.96 and 5.85ºC, ΔH= -59.17 and -16.2J/g. Non-necrotic, heating between 0-100ºC: two separable transition with T_m=5.7 and 9.9ºC, total ΔH= -20.8J/g; heating between -20-100ºC: T_m=-10.9 and 4.95ºC, total ΔH= -75.8J/g.) Further investigations are needed with higher sample rate and under other anamnestic circumstances too. According to the authors expectations DSC could help by the understanding the pathobiological mechanism leading to the ostheonecrosis as well as to better identify the aetiological factors.

S8P5

DSC analysis of human fat tissue in idiopathic avascular necrosis of the femoral head– a preliminary study.

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Backgound: The osteonecrosis or avascular necrosis of the antero-superior part of the human femoral head (ANFH) often causes incongruity in the hip joint and leads to severe pain and disability in middle aged patients. This abnormality often requires surgical intervention, mainly total hip arthroplasty (THA). The possible pathomechanisms of ANFH are fat embolism, arterial occlusion, fatty necrosis of ostecytes and intraosseous hypertension. Several etiological factors have been proposed in the literature, that can lead to this condition, alcohol abuse, steroid therapy, metabolic changes, dyslipidaemia are the most common causes. In some case we can not verify any factor, these are the so called idiopathic ANFH cases.

We observed macroscopic variation in color and consistency of the subcutaneous fat tissue in patients with ANFH compared to osteoarthitis or hip fracture during THA procedures.

Aim: We proposed that the changes due to the altered fat metabolism that contributed to the ANFH can be observed in the subcutaneous fat tissue in the molecular level.

Material and methods: We obtained subcutaneous fat tissue during THA from a patient with idiopathic ANFH and compared with an otherwise healthy patient who underwent surgery due to traumatic hip fracture. For histological analysis HE, and oil red staining were utilized. From frozen tissue gas chromatography- looking at the variation of medium and long chain fatty acids (C12-C22)- and DSC analysis were performed.

Results: No histological changes were notified in the size, shape of adipocytes and in the cells of the septate of the connective tissue. Gas chromatography showed, that the patient with idiopathic ANFH had less long chain fatty acids than the normal control, however these changes were not as marked as with alcoholic or steroid induced ANFH.

DSC revealed similar changes in the thermodynamic parameters: non-necrotic sample during heating between 0-100ºC exhibited: two separable transition with T_m=5.7 and 9.9ºC, total ΔH= -20.8J/g; heating between -20-100ºC: T_m=-10.9 and 4.95ºC, total ΔH= -75.8J/g, while in idiopathic avascular necrosis the results in same circumstances were - heating between 0-100ºC: T_m= 8.0ºC, total ΔH= -14.25J/g; heating between -20-100ºC: T_m=-0.1ºC, total ΔH = -100.9 J/g.

Conclusion: The alteration in the fatty acid profile did not cause histological changes, but we were able to detect it with analytical methods e.g. DSC and gas chromatography.

S8P6

DSC EXAMINATION OF INTESTINAL TISSUE FOLLOWING WARM ISCHEMIA AND REPERFUSION

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Mucosal lesions are characteristic features of the small bowel during intestinal warm ischemia and reperfusion (I/R). The most commonly employed method used for estimating intestinal injury is conventional histology determined by grading system. We aimed to compare the conventional histology and Differential Scanning Calorimetry (DSC) method by measuring structural changes in small bowel tissue following warm I/R in animal model.

Warm ischemia/reperfusion groups were designed with occlusion of superior mesenteric artery in Wistar rats (n=20). In Group I 1 hour ischemia followed by 3 hours reperfusion, and in Group II 3 hours ischemia followed by 1 hour reperfusion. Small bowel biopsies were collected after laparotomy (control) at the end of the ischemia as well as reperfusion periods. We evaluated tissue damage on hematoxylin/eosin-stained sections by light microscopy and they were quantitatively analyzed using the software Scion Image. Separating the small bowel tissue, thermal consequences of structural changes of mucosa, muscular layer and total intestinal wall were detected by DSC.

In Group I histological findings were corresponded to an injury grade 1, showing minor clefting with the villus epithelium. In Group II the injury was grade 3 after ischemia, characterized by severe destruction in mucosal thickness, denudation of villi and lesion in crypts, which was further deteriorated by the end of the reperfusion. These changes were significant by quantitative analysis ($p<0.05$). DSC data have supported these observations: after 1 hour ischemia the transition temperature (T_m) was the same as in case of control for mucosa, but the calorimetric enthalpy decreased by about 30%. In case of 3 hours warm ischemia the T_m changed from 55 to 46 ºC and the calorimetric enthalpy was only half of the control one.

Present work demonstrated complex structural analyses of intestinal injury following I/R. The thermal parameters indicate the thermodynamic consequences of structural destruction, which provides basis for further investigation in different intestinal stress models. (Supported by OTKA F046593, Bolyai Scholarship of the Hungarian Academic of Science)

S9O1

OSMOTIC AND ACTIVITY COEFFICIENTS OF SOME QUATERNARY AMMONIUM SALTS IN AQUEOUS SOLUTION AT 298.15 K, 293.15 K AND 298.15 K.

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ABSTRACT

The osmotic coefficients of aqueous solutions of trimethyloctylammonium, decyltrimethylammonium, dodecyltrimethylammonium and tetradecyltrimethylammonium bromides were measured using the isopiestic method. The reference aqueous solutions had as solute NaCl and were checked against solutions of KCl and sucrose. The temperatures were 298.15 K, 293.15 K and 288.15 K. From the osmotic coefficients the activities of solvent were calculated and from these, the activity coefficients of the solutes were obtained. The effect of the length of the longer hydrocarbon chain is analyzed.
The necessary equipment was built following the design of Blanco and Amado [1] which has been used successfully in several others works from this laboratory [2].

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S9O2

PROPERTIES AND FUNCTION OF SELECTED (BIO-)SURFACTANTS FOR BIODEGRADATION OF CONTAMINANTS

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Crude oil and its constituents such as long-chain hydrocarbons or their derivatives are widespread environmental pollutants. Particularly the low bioavailability of the lipophilic substances causes their persistence and poses a crucial limitation to the speed and success of the microbial bioremediation process. However, a large number of terrestrial and aquatic microorganisms are capable of degrading such contaminants. Among the microbial strategies to increase the bioavailability of hydrocarbons are the hydrophobisation of the cell surface and the excretion of emulsifiers. Such biosurfactants are usually formed during growth on long chain alkanes, for instance by the genera Rhodococcus, Nocardia and Corynebacterium [1]. Due to their biological origin, these surfactants are associated with a lower toxicity, a higher environmental compatibility and a higher biodegradability in comparison to their synthetic counterparts [2, 3]. By contrast, the antimicrobial properties of some of them are already well investigated [4, 5].

Our investigations are concerned with the evidence of an ecological, effective and calculable application of biosurfactants to enhance the bioavailability of pollutants. Therefore both thermodynamic fundamentals of the surfactant interaction and microbial and toxicological questions have to be answered. In the contribution first results of the calorimetric investigations relating to the effect of surfactant enhanced bioavailability of hydrophobic pollutants are presented. Mainly the isothermal titration calorimetry (ITC) is used for the investigation of the appropriate interactions. Thus the first step is the determination of the critical micelle concentration (cmc) of the applied surfactants, i.e. of the commercially available biosurfactant rhamnolipid (Jeneil Biosurfactant Company (USA)), a trehalose tetraester, from the Institute of Biosciences on our university in Freiberg and a synthetic, well-investigated surfactant, sodium dodecyl sulphate (SDS) as reference substance. Furthermore the interactions between the selected pollutants, namely *n*-hexadecane and pristane, and the (bio-)surfactants are determined. Further experiments referring to the interactions between the cell surface of the bacteria and the pollutant and the surfactant respectively will be carried out afterwards. The calorimetric investigations provide a complete thermodynamic characterisation of the interactions. Hence, the results will enable us to distinguish between the two main cases [6] of the effect of surfactants concerning enhanced biodegradation.

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S9O3

THERMODYNAMIC STUDY OF THE INFLUENCE OF POLYOLS AND GLUCOSE ON THE THERMAL STABILITY OF HOLO-BOVINE α- LACTALBUMIN

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ABSTRACT

Thermodynamic studies about protein behaviour in different solvents give valuable information about solute-solvent interactions and solvent effect on protein stability [1,2]. Protein thermal stability in buffer and dilute aqueous solutions of erythritol, xylitol, sorbitol, inositol and glucose was evaluated by fluorescence spectroscopy and circular dichroism. Results show that at the conditions of the study, the transition is reversible and occurs as a change between two states [3,4]. At low concentrations these cosolutes do not have stabilization effect, and even, some of them destabilize the protein. However, at higher cosolute concentrations, all of them stabilize the native protein conformation.

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S9P1

CRYSTALLISATION AND MELTING BEHAVIOUR OF FISH OIL MEASURED BY DSC

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Fish oil which is characterised by important amounts of poly-unsaturated ω-3 fatty acids attach increasing importance within functional foods. Numerous studies reveal positive effects (antiarrhythmic, antithrombotic, anti-inflammatory) in prevention and/or treatment of a considerable number of diseases. In this context discussed effects such as the lowering of blood pressure and triglyceride concentration, the inhibition of artherosclerosis, the enhancement of tensibility of arteries and the development of brain capacity and visual function in babies underpin their importance.

Recently attention is directed not only on the chemical composition of edible oils, particularly regarding fatty acids, but also on physical methods that allow fast and relatively easy the identification and discrimination of oils. To this end Differential Scanning Calorimetry (DSC) be deployed. DSC measurements yield in information on thermal effects, characterised by changes in enthalpy and their temperature range such as melting and crystallisation. To our knowledge this application of DSC be mainly confined to vegetable oils and in present times particularly on olive oils. However, information on fish oil is rather rare.

The aim of the investigation presented here was to take DSC curves in the temperature range +20 to – 40 °C on several fish oils and fish oil capsules to visualise the crystallisation and melting behaviour and to compare transition temperatures and enthalpies. The measurements were performed using a PerkinElmer DSC 7 device equipped with a PerkinElmer Intra cooler
II and Pyris software. Fish oil samples of about 7 mg were weighed accurately in aluminium pans and sealed. The influence of pre-treatment of fish oils at 50 °C was also investigated. DSC curves obtained display partly significant differences and it is tried to discuss their causes.

S10O1

ISOTHERMAL MICROCALORIMETRY AND CHARACTERIZATION OF 'BIOLOGICAL ACTIVITY' IN SOIL

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Soil is a complex matter and its physical, chemical and biological properties vary greatly between sites and may change significantly with time, for example due to agricultural activities, deforestation or pollution effects. The properties of soil are of prime global significance with respect to ecology, climate, economy and human health. Clearly, it is essential to have a range of experimental methods available, by which soil qualities can be characterised on a routine level.

Isothermal microcalorimetry is a well established technique for the characterization of living matters in soil, but very few scientific groups are active in this field and the method is not used in any routine measurements.

For a modern instrument the thermal power detection limit, reproducibility and baseline stability are adequate for a wide range of investigations on soil. However, further development is much needed with respect to sample throughput and interpretation of the calorimetric signals, as well as in the control of samples before and during the experiments.

In some cases results from the measurements are discussed in terms of thermodynamic properties. However, usually the instruments are primarily employed as general analytical tools, i.e. as 'monitors' for the biological processes. The lecture will include a brief discussion of the different directions of isothermal microcalorimetric work on soil, with a focus on methodological problems.

S10O2

DETERMINATION OF CALORESPIROMETRIC RATIOS IN SOILS BY CALORIMETRY

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Calorespirometric ratios constitute a well known parameter of living systems that can be calculated by calorimetry. They have been widely used in the study of metabolism of plants, cells and insects. These ratios can provide interesting and sometimes surprising information about complex biological systems through different thermodynamic modeling of the efficiency of the metabolic reactions and the oxidation state of the substrates. Calorespirometric ratios have not previously been determined for the metabolic reactions in soils. Understanding soil respiration has become an important goal since the Kyoto protocol considers soil exploitation as one of the sources involved in global warming. Thus, it is necessary to develop methodologies that can inform about soil microbial metabolism .

A calorimetric procedure to determine the calorespirometric ratios of microbial reactions in soils was used to study the basal metabolism and C mineralization in nine soil samples collected from different sites in Asturias (Spain). Calorimetric determinations were done with a Calorimetry Sciences Corporation model 4400 IMC with three samples channels and one reference channel. Ten gram soil samples in 30 mL, sealed serum bottles were continuously monitored for 24-48 hours. C mineralization was studied with 1 g of soil amended with 0.2 ml of a nutrient solution containing 1.5 mg of glucose and 1.5 mg of ammonium sulphate in 2 ml stainless steel ampoules in a prototype 16-channel calorimeter. All measurements were done at 25 ºC. CO_2 rate was determined from the increased heat rate from introducing a vial of 0.4 M NaOH into the ampoule with the soil sample.

Calorespirometric heat per CO_2 ratios for basal metabolism vary among the soil samples and in some cases are far from the 200-460 kJ /mol typical of heterotrophic metabolism of carbohydrates. The CO_2 rate was ~ 2 nanomol g^{-1} s^{-1} in most of the soil samples. Two soils had CO_2 rates an order of magnitude lower. The difference appears to be related to the amount of organic matter in the soil. As expected, addition of glucose caused an exponential increase in heat and CO_2 rates, and altered the calorespirometric ratio.

S10O3

CALORESPIROMETRY: A NEW APPROACH FOR ASSESSING MICROBIAL EFFICIENCY IN SOILS

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Soil C content, predicted by soil organic matter models, is sensitive to changes in the microbial efficiency parameter. Available data on microbial efficiency come largely from the aquatic ecology literature. There is very limited soils data, due primarily to methodological constraints of current methods. Calorespirometry provides a new approach for assessing microbial efficiency in soils. Calorespirometry provides more information than CO2 evolution alone, including estimates of metabolic efficiency and the type of substrate being utilized (in terms of its oxidation state). Calorespirometry couples the simultaneous measurement of CO_2 (R_{CO2}) and heat (R_q) production rates by isothermal calorimetry to thermodynamic models of the carbon use efficiency. The ratio of heat to CO_2 production rates is related to metabolic efficiency by
$R_q / R_{CO2} = 455(1-\gamma_S / 4) – 114(\gamma_S - \gamma_B) [\varepsilon / (1- \varepsilon)]$
where ε = substrate carbon conversion efficiency, γ_S = oxidation state of substrate C, and γ_B = oxidation state of microbial biomass (-0.3). Results to date suggest that efficiency increases with decreasing temperature in unamended soil and that substrate use changes with temperature, with more reduced substrates being utilized at lower temperature.

S10O4

Calorimetric Applications and the Oceans Biodiversity

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Oceans waters cover almost 71% of the earth's surface and because of their great depth they form more than 99% of the available living space on the planet. The amazing collection of habitats populated by a bewildering variety of organisms causes that the ocean life is extremely diverse and unevenly distributed, both in space – horizontally and vertically and in time. The time scale might be short-term (e. g. daily cyclicity or seasonality) or long-term (eutrophication or climate modification). According to the environment (tropical or cold, full marine or brackish, shallow or and deep, estuary or open ocean) aquatic organisms have to cope with biotic and abiotic factors. This is possible by the development of a diversity of morphological, behavioural, physiological and biochemical mechanisms which allow for acclimatization or adaptation to occupied habitats.

Calorimetric determinations are an important element of the studies concerning adaptation and performance of aquatic organisms. One of the examples is conduction calorimetry used for metabolic rate determinations. The great advantage of this method is the fact that it includes all chemical reactions and the energy turnover (aerobic and anaerobic) taking place in cells of living organisms. Moreover, it allows for continuous energetic registration especially important when studying aquatic organisms exposed to stress caused by environmental factors. Some animals are able to shift not only the rate, but also the type of their standard metabolism utilizing anaerobic metabolic pathways. In such a case indirect respirometric methods do not provide reliable information.

Another tool applied in studies of aquatic organisms is combustion calorimetry. The calorific value of an organism depends on its biochemical composition which is the expression of an animal's adaptation to its environment. Every species is characterised by an own range of energy content, however, it may vary also according to different intrinsic and extrinsic factors. The knowledge of the energy content of a studied species provide information on its suitability as a food source and - together with the data on abundance and biomass - might be used to estimate energy resources in the region of research.

S10O5

Insect Energetics and the Kyoto Protocol

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The Kyoto Protocol is mainly concerned with reduction of green house gas emissions. Its importance has been dramatically increased with growing scientific evidence that climatic changes, rise of global temperature, change in precipitation patterns, lengthening of growth periods and alterations of plant and animal distributions are already under way.

Here we present three examples for possible impacts of climate change on insect energetics: (1) A honeybee hive is a highly ordered, complex system being strongly dependent on its immediate environment. Temperature increases of several degrees and an earlier starting, up to 2 weeks prolonged vegetation period allow for an advanced start of breeding and thus larger hives and changed energy balances. (2) Hornet and wasp colonies die in the autumn leaving only the inseminated younger queens for hibernation. The energy storages they collected in late summer are crucial for surviving the winter. A strongly reduced metabolism at low temperatures and discontinuous ventilation allow for an economical consumption of the stores and guarantee success. Elevated temperatures endanger the outcome. (3) Some arctic and alpine plants show a sophisticated interplay with their pollinators that sunbath in their bowl-shaped and solar tracking blossoms. Increasing temperatures would introduce more common and frequently occurring insects to do the pollinating job and thus to destroy this fragile ecosystem.

S10O6

THE APPLICATION OF CALORIMETRY TO A WIDE RANGE OF PROBLEMS FROM ENERGY FLOWS IN AQUATIC MICROBIAL COMMUNITIES TO THE OCEANIC BIOLOGICAL PUMPS AND THE EFFECTS OF GLOBAL WARMING

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Key biogenic factors controlling the capacity of the oceans for taking up natural and anthropogenic CO_2 are the biological organic carbon pump and the calcium carbonate pump. These sometimes called "biological pumps", are components of the global carbon cycle and, thereby, influence Earth's climate. The organic carbon pump is the flux of biologically produced organic matter from the euphotic zone of the ocean to the deep waters below it. The carbon thus removed from the upper layer is replaced by carbon dioxide from the atmosphere. The phenomenon of the carbonate pump is a result of the activity of calcifying organisms (e.g. corals but mostly unicellular coccolithophores and foraminifera) and involves reactions of $CaCO_3$ production, precipitation and dissolution. Particulate inorganic carbon (calcium carbonate mineral) produced by the calcifying biota in the surface layer sinks to the deep ocean and is stored in the geological archive. At the same time, the process of biogenic calcification is a source of CO_2 to seawater so, depending on the ratios between the rates of calcification, photosynthesis and respiration in the water column, the ecosystem can be a net sink or a source of atmospheric CO_2. A further direct link between the global climate and the marine biota is dimethylsulphide (DMS), a volatile sulphur compound produced by planktonic protists and released to the atmosphere. It is responsible for the formation of cloud condensation nuclei to give cloudiness which can affect the backscattering of solar radiation and, hence, have an influence on climate. In the water column, the formation of DMS from its precursor, b-dimethylsulphoniopropionate (DMSP), is accomplished by a number of biological processes, such as phytoplankton senescence, cell lysis, bacterial degradation, or grazing by zooplankton. From the above, it can be seen that both the pumps and the DMS production are localized essentially in the upper water column and are controlled predominantly by the microbial component of the aquatic ecosystem. Consequently, the structure of the pelagic microbial food web and its functional activity determine the complexity of the pathways of carbon and DMSP-DMS transformations in the ocean, feedback controls of the processes and, finally, their efficiencies.

The major driving force behind the "machinery" of the pumps and, at least partly, of DMS production is the energy flow through the microbial community, manifested as its respiration and heat dissipation. Hence, quantifying and interpreting it correctly is crucial to understanding the mechanisms which control the pumps and the DMS production in the ocean,, thereby to affect the global climate. It is surprising, however, that the bioenergetic aspect of the problem generally is disregarded and, as a result, there is a huge discrepancy in the amount of data on material and energy flows in the aquatic ecosystems. It is still a common and, undoubtedly, a bad practice in aquatic microbial ecology to approximate the energy expenditures of a community from its biomass production, assuming there is relatively high and constant growth efficiency. Indirect methods of measuring the energy flow, for example from the oxygen uptake rate, still prevail and sufficient progress has not been made over the last few decades to involve innovative methodology. This methodological void is ideally occupied by calorimetry – a powerful tool for measuring the energy (heat) flow directly – not instead of, but rather in tandem with, a respirometric approach, i.e. calorespirometry involving measurements of the ratios of heat flux to CO_2 and O_2 to furnish information about catabolic and anabolic pathways and growth efficiencies as well as energy flows. In addition, the photocalorimetric method looks highly promising for quantifying both the photosynthesis and respiration of marine phytoplankton and probing the phenomena like photoinhibition and non-photochemical quenching on the global scale. Nevertheless, for the potential to be realized, nano-scale rather than microcalorimetry has to be adopted using chip technology which has the advantages of making the instruments: (i) more compact for use on-board ship in field studies; and (ii) relatively inexpensive. This will usher in a new era. The study was supported by EC INTAS grants 03-51-6196 and -6541. [1]Present address: *Institute of Biology of the Southern Seas, NASU, Nakhimov ave., Sevastopol 99011, Crimea, Ukraine.*

S10P1

INFLUENCE OF THE AMOUNT OF RETARDANT ON THE EFFECTS OF SOIL HEATING

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Galicia, (NW Spain) is one of the most affected regions by forest fires in Europe, where thousands of forest hectares have been devastated in the last years, and even several human lives have been lost. Likewise the combustion of organic matter, that takes place during the fires, throws out to the atmosphere a great quantity of carbon dioxide, which is one of the causative gases of the greenhouse effect, being necessary an important reduction of these emissions in the next years, according to the criteria of the Kyoto Protocol. In that way, several types of chemical agents, flame retardants, gain acceptance as effective tools for the fire fighting [1-3].

In this work, the effect of the addition on the soil of different amounts of a polimer subtance, Firesorb, used as a flame retardant was studied. Samples of two soils treated with three different doses of this retardant were heated in an oven at 230ºC and 350ºC to simulate medium and high intensity fires, respectively. Unheated soil samples with and without Firesorb were used as control.

From the combustion enthalpy of the organic matter and the ignition temperature, calculated with a Differential Scanning Calorimeter (DSC), the effect of the heating on the organic matter of these soils samples was estimated, and hence the effect of the retardant addition in relation to the dose added. The method was exposed and used in previous works of our research group [4, 5] .

Firstly, an increase of the ignition temperature was observed in unheated samples treated with retardant versus non treated, implying a delay in the thermal degradation of soil and therefore an increase in its resistance to the fire.

The effect of the retardant was clear after the heating at 230ºC, especially in the most organic soil, characterised by a minor organic matter loss and minor ignition temperature in treated samples, particularly in those with the highest content of retardant.

Despite of significative differences in the combustion enthalpy of the organic matter in samples treated and heated at 350ºC against non treated have been found, these values corresponded to losses of organic matter between 80% and 90%, this is, it was practically destroyed and recovery will be practically impossible. Although of the soil treated needed more time and more heat supply to raise the temperatures corresponding to high intensity fires, when this temperature was reached, the effectiveness of the retardant was almost null.

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S10P2

A CALORIMETRIC PROCEDURE TO ASSES THE EFFECT OF SOLID-LIQUID EFFLUENTS FROM ANAEROBIC DIGESTERS ON SOIL MICROBIAL ACTIVITY

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The transition to a sustainable energy economy is one of the most important issues facing us in the 21st century. It involves the introduction of new energy sources for economic reasons dealing with the arising prices of a barrel of oil and for ecological reasons dealing with the sustainable use of our energy resources to minimize the impact of CO_2 in the atmosphere. One of the alternatives for synthesizing renewed energies is the conversion of biomass to biogas via anaerobic digestion. Biomass is a renewable energy resource which includes a wide variety of organic supplies. If slaughtering houses wastes are used as biomass source, the anaerobic digestion yields also a solid-liquid effluent that could be used as soil fertilizer. A calorimetric procedure was developed to study the properties of that effluent and how affects to the soil microbial metabolism. DSC was used to study the pyrolysis properties of the effluent and that of the soil. ITC calorimetry was applied to study the microbial activity in the effluent and to asses on its effect on the microbial activity of the soil where the industrial digester will be situated. The calorimetric data were studied together with the chemical and biological properties of that residue. Results showed that effluent is constituted by low levels of carbon and high levels of nitrogen. The power-time curves of the effluent have the typical shape of microbial growth yielding microbial growth rate constants between 0.37 and 0.53 h^{-1} for about 4 and 11 hours. This activity was strongly influenced by the fat content of the effluent that inhibits the growth reaction. The analysis of the thermograms obtained by DSC of the soil sample mixed with the effluent, showed a decrease of the heat of combustion of the mixture with time, suggesting activation of the soil organic matter degradation. The ITC results of the soil-effluent mix revealed a clear increase of the basal metabolism of that soil, given by a value of the soil mass heat rate, $J_{Q/S}$, from 0.75 J $g^{-1}d^{-1}$ to 2.0 J $g^{-1}d^{-1}$. These features suggest the stimulation of heterotrophic metabolism by the effluent which permits to evaluate the variation of the oxidation state of the soil organic matter caused by the effluent. This calculation was done through the Thorton´s rule equation [1, 2] and showed that the oxidation state of the organic matter increases with time in the soil-effluent mix. These results fit well with the shift in the basal metabolism observed in the power-time curves. The nitrogen content of the effluent stimulates the microbial activity that uses the soil organic matter as carbon source. This is a very typical effect reported for nitrogen fertilizers [3].

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S10P3

Seasonal Study of Different Properties of a Pasture Soil Using Calorimetric Techniques

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Two calorimetric techniques, Isothermal Calorimetry and Scanning Differential Calorimetry (DSC) were applied to study the microbial activity and the thermal properties of a soil collected in a pasture located in Viveiro (Galicia, NW Spain). The study was performed over 1 year. Samples were seasonally collected (spring, summer, autumn and winter) in order to check the influence of environmental climatic conditions on the microbial activity [1], on the combustion enthalpy of organic matter and on the thermal degradation temperature [2]

The calorimetric study was complemented through a deep analysis of the most important soil physical (temperature, moisture, texture, density, porosity, plasticity index, hydraulic conductivity constant, structure and field capacity), chemical (C-to-N ratio and pH) and biological properties (organic matter content and most probable number of microorganisms or MPN) together with some environmental data (for example mean temperature, rainfall and moisture)

The physical-chemistry properties have a strong influence on the thermal properties [1] and on the microbial activity in soil given by calorimetric indexes. Now it is intended to study the sensitivity of these indicators to the seasonal environmental conditions.

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S10P4

Calorimetric Seasonal Characterization of a Corn and Bean Culture Soil

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The soil exploitation has been considered by the Kyoto protocol to be under control due to its significant contribution to the global warming. For this reason it is important to provide new methodologies that can contribute to the sustainable development of the soil system. Differential Scanning Calorimetry (DSC) and Isothermal Calorimetry are applied to provide the thermal properties and information about the microbial activity in a Corn and Bean arable land. Soil samples were collected during one year at the different seasons (summer, autumn, spring and winter) to give the influence of the climatic data on the soil. The Thermal Activity Monitor (TAM, Thermometric) was used to study the soil microbial activity registered as power-time curves [1]. The direct analysis of the curves gives the microbial growth rate constant, the length of the lag phase and the duration of the exponential growth phase (Peak time). From the DSC curves, the combustion enthalpy of the organic matter and its thermal degradation temperature could be obtained [2]. Both, organic matter and microbial activity are considered two important indexes of soil quality. The proposed parameters have been demonstrated to be sensitive to well known physical-chemistry soil properties. Now it will be tested how they are affected by environmental data to introduce the role of the climatic conditions of the sampling areas on the soil thermal properties and on the microbial activity characterized by calorimetric indexes.

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S11O1

The Potential and the Challenges in Integrating Thermodynamics into Systems Biology

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Systems biology is experiencing fast development, as a result of the development of high throughput experimental techniques as well as computational power. The goal is to integrate as much as possible biological data in the mathematical models that would eventually allow the creation of a completely quantitative in silico model of a live cell. However, in spite of the enormous amount of information that goes into a genome-wide quantitative analysis of a functional cell, systems biology results in mathematically underdetermined models, and instead of an exact solution one often has to deal with a whole solution space (Edwards and Palsson, 2000).

In an attempt to reduce this solution space, it was recently proposed to incorporate thermodynamics into system biology analyses (Beard et al, 2002 and 2004; Qian et al, 2004; Henry et al, 2006 and 2007; Kümmel et al, 2006). Indeed, thermodynamic feasibility analyses (TFA) can point out those solutions that satisfy all mass balances but are not allowed from a thermodynamic point of view. The authors were however confronted with such an overwhelming mass of unknown physical-chemical parameters that they had to introduce dramatic simplifying assumptions. Thus, the standard Gibbs energies of reaction were often estimated based on simple group contribution methods, the metabolite concentrations were either assumed to be 1 M throughout or they were adjusted within a very wide, conservative span of values, the solution were sometimes assumed to be ideal, the influence of ionic strength and of pH was either allowed for based on data estimated from group contribution methods or neglected altogether (Maskow and von Stockar, 2005).

In order to check to what extent these simplifications are justified, and if a TFA based on them is still able to provide meaningful and useful information, we have performed a thorough analysis of the thermodynamics of the well known pathway of glycolysis. The rationale behind this is that a) the result of TFA is known since glycolysis is undoubtedly a feasible pathway, b) most of the thermodynamic data as well as cytosolic concentrations for the glycolytic intermediates are known, c) glycolysis is an entirely cytosolic pathway, which simplifies the problem by avoiding the necessity to account for the compartmentalization of the cell.

Based on the mathematical procedure described by Alberty (2003) the corrections to the published standard Gibbs energies caused by changes in acid dissociation, magnesium complex formation, and ionic strength may be calculated for each reaction of the glycolytic pathway and for different pH, magnesium concentrations and ionic strengths. These transformed standard Gibbs energies were used in the subsequent TFA in order to check whether the pathway appears thermodynamically possible with and without different simplifying assumptions.

It turned out that the result of the TFA is extremely sensitive to i) the standard Gibbs energies of reaction used in the analysis, ii) the span of values within which the metabolite concentrations are assumed to vary, iii) the pH, ionic strength and pMg assumed to prevail in the cytosol, iv) the dissociation constants and the magnesium complexation constants of all the metabolites involved, and v) the assumptions concerning the precise nature of the reacting species. In view of the fact that no experimental data is available concerning these factors for most of the metabolites in a live cell, it can be concluded that systems biology in combination with TFA is still very far away from being able to predict the feasibility of a new, as yet unknown pathway. For a metabolic network known to function in nature, such as the metabolism of a well known bacterium, systems

biology and TFA may be used to gain insight concerning unknown metabolite concentrations or pathway alternatives, but only if most of the information cited above is known with considerable reliability. An experimental program measuring this information at a genome scale is urgently needed.

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S11P1

THERMODYNAMICS OF MICELLE FORMATION OF A GEMINI SURFACTANT IN THE ABSENCE AND IN THE PRESENCE OF MICELLE BUILDER AND MICELLE BREAKER ADDITIVES

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The number average molecular weight and the polydispersity index of the nonionic gemini surfactant Surfynol 465 (S465) were found to be 703 and 1.03, respectively, using electrospray ionization mass spectrometry.

The calorimetric enthalpies of micelle formation $\Delta_{mic}H_{cal}$ of S465 were measured in the temperature range 298-348 K by thermometric titration.

In water, the van't Hoff enthalpies of micelle formation $\Delta_{mic}H_{vH}$, calculated from the temperature dependence of the cmc, were found to be in excellent agreement with $\Delta_{mic}H_{cal}$. The micellization of S465 changes from endothermic to exothermic as the cmc passes through a minimum at elevated temperature. Thermodynamic analysis indicated that micelle formation is favoured by entropy and opposed by enthalpy up to this inversion temperature, after which aggregation is favoured both by entropy and enthalpy.

The cmc of S465 increases with increasing the concentration of urea. The cmc increases by a factor of 2 at 5 M urea. The temperature dependence of $\Delta_{mic}H_{cal}$ in 5 M urea is less negative than that in pure water and the positive entropy term $T\Delta_{mic}S$ increases slightly with the increase of temperature.

The cmc of S465 decreases with increasing the concentration of *n*-hexanol. The cmc decreases by a factor of 2 at 50 mM n-hexanol. $\Delta_{mic}H_{cal}$ and $T\Delta_{mic}S$ in 20 mM *n*-hexanol show little variation with temperature as compared with those in water and in urea solution.

For urea solution, $\Delta_{mic}H_{vH}$ vs T agree very well with $\Delta_{mic}H_{cal}$ vs T. This ideal behaviour indicates that micelle composition is not significantly affected by the presence of urea; the role of urea is restricted to increase the polarity of the solvent. For *n*-hexanol solution, however, significant deviation between $\Delta_{mic}H_{cal}$ and $\Delta_{mic}H_{vH}$ occurs indicating that *n*-hexanol is incorporated in the micelles via solubilization.

Enthalpy-entropy compensation results in a slight decrease in the Gibbs free energy with the increase of temperature, irrespective of the composition of the solvent.