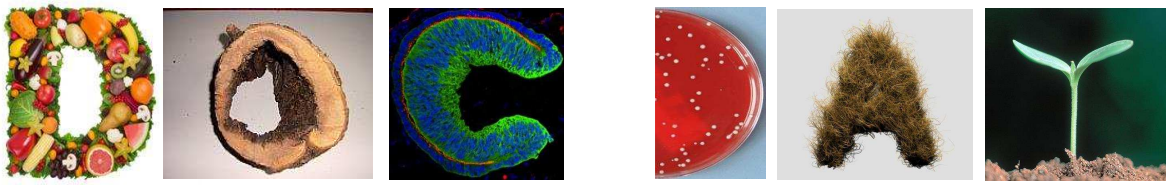
1st DocDay

@ Tulln



October, 10th 2013

Program 1. DocDay Tulln

10th October 2013

9.00 Welcome words by Univ. Doz. DI Dr. Georg Haberhauer, Vice-Rector

9.15 Key note lecture

Prof. Gianluca Ciardelli: Dep. of Mechanical and Aerospace Engineering (DIMEAS), Politecnico di Torino, Italy

"Materials design at the nanoscale for biomedicine"

10.00 *Session 1: VIRIS lab* *Chair:* Andreas Kreuzeder

Monika Sturm: "Age determination of plutonium for nuclear forensics"

Johanna Irrgeher: "Strontium isotopes as tracers of biological migration"

Monika Horsky: "Application of Sr isotope ratio measurements for the determination of origin of prehistoric wood"

11.00 – 11.30 Coffee break + Posters

11.30 *Session 2: Wood, grass and cellulose* *Chair:* Benjamin Lauterböck

Stefan Pinkl: "Fibrillated lignocellulose biomass"

Lucy Montgomery: "Enzymatic treatment of grass silage"

Martin Siller: "Functionalisation of viscose fibres"

12.30 – 13.30 Lunch break

13.30 – 14.00 Poster session

14.00 *Session 3: Food and medical related topics* *Chair:* Barbara Thallinger

Gerald Ebner: "Adding function to cellulosic tissue"

Doris Schiffer: "Advanced wound management and infection prevention in chronic wounds"

Katharina Köstlbauer: "A targeted proteomics approach for the elucidation of peptide markers for the detection of lupin allergens in food products"

15.00 -15.30 Coffee break + Posters

15.30 *Session 4: Analytics* *Chair:* Katrin Greimel

Irene Hahn: "Determination of metabolites formed by enzymatic degradation of ergot alkaloids"

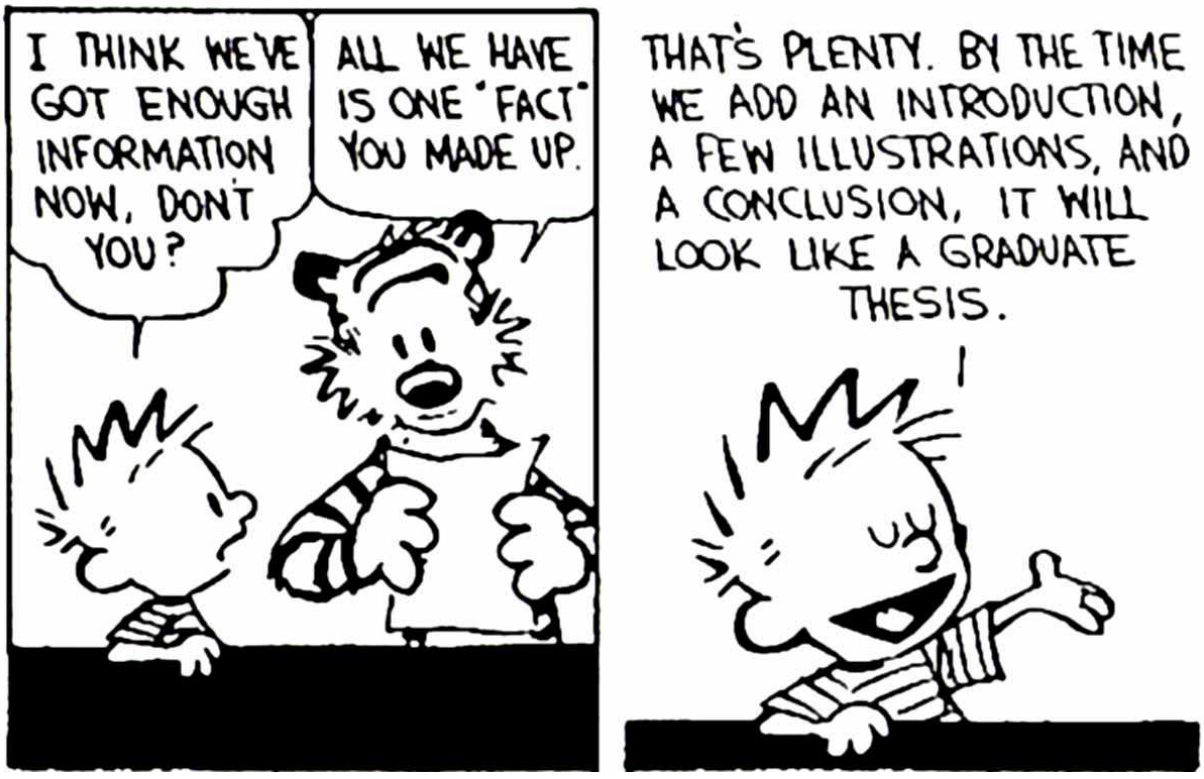
Christoph Höfer: "Development and improvement of novel approaches in 2D chemical imaging of rhizosphere processes"

Christoph Büschl: "Development and application of a work flow to find known and unknown metabolisation products of xenobiotics using stable isotope labeling and LC-HRMS"

16.30 Closing remarks + Poster/Talk awards

17.00 Get together at the IFA/UFT Oktoberfest ☺

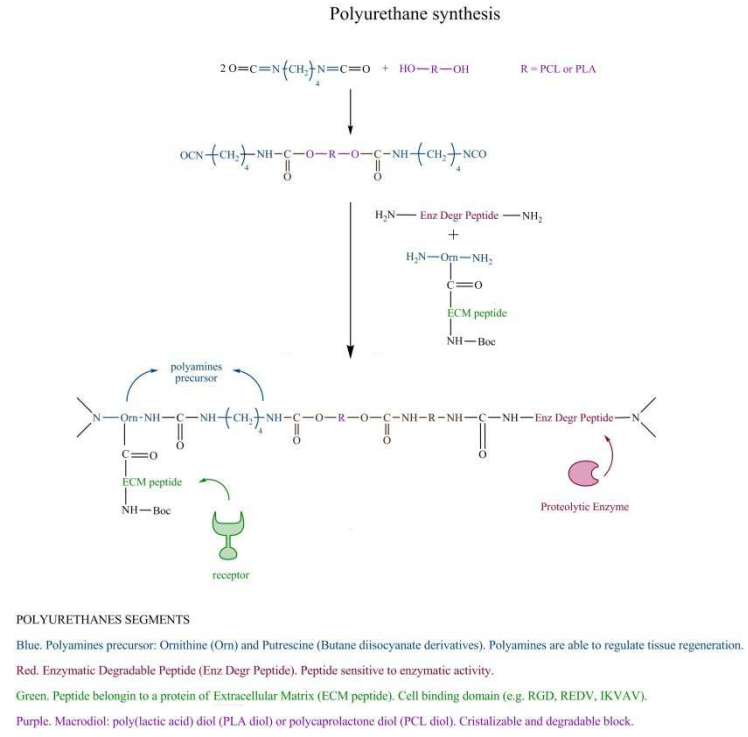
Abstracts for Talks



Materials design at the nanoscale for biomedicine

Gianluca Ciardelli
Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy

Demand for medical implants is estimated to increase 9.3% annually to US$43.6 bln in 2011, according to a report by Freedonia Group. Cardiac implants are expected to remain the top-selling group, led by stents and defibrillators, with demand expanding 9% pa to almost US$20 bln in 2011. Biodegradable plastics, that have found until now application in packaging, are slowly capturing newer markets, particularly medical products. Applications of biodegradable plastics have gained market acceptance in the medical sector in: medical implants, drug delivery, hernia repair devices. One reason for the rise in implant use is the performance and outcome advantages over alternative treatments, such as drugs. Another reason is the constant improvement and innovation of devices that keep getting smaller.
However, materials which were approved (e.g. polymers belonging to the polylactic or polyglycolic acids family) were originally designed for other applications and then proposed for medical use. This approach usually results in various drawbacks in the final application, e.g. the release of acidic compounds during the degradation of polylactic acid results in a lowering of the local pH at the implant site, with consequent danger of inflammation reaction and adverse body response. Consequently, it is clear that a precise design at the nanoscale of the chemical and morphological structure, can open the way to a new generation of biomaterials tailor-made to the challenging applications of biomedicine and bionics, such as tissue regeneration, advanced diagnostics, cancer treatment.
In this context, this contribution will present the more recent research results of the Biomedical Laboratory at Politecnico di Torino on the application of proprietary, degradable block copolymers in the regeneration of the cardiac, nervous and bone tissue and in nanomedicine.
The rationale for polymer design illustrated in the figure below, where the different building blocks are used to provide the final product with the expected mechanical properties during use, cell-adhesion and -targeting motifs, hydrolytic and enzymatically activated degradation (enhanced in the presence of pathological conditions), providing non toxic and bioactive degradation products. The polymers can be processed then in the suitable form (tubular structures, anisoptropic scaffolds, nanoparticles, injectable gels) for the final application.



POLYURETHANES SEGMENTS

Blue. Polyamines precursor: Ornithine (Orn) and Putrescine (Butane diisocyanate derivatives). Polyamines are able to regulate tissue regeneration.

Red. Enzymatic Degradable Peptide (Enz Degr Peptide). Peptide sensitive to enzymatic activity.

Green. Peptide belongin to a protein of Extracellular Matrix (ECM peptide). Cell binding domain (e.g. RGD, REDV, IKVAV).

Purple. Macrodiol: poly(lactic acid) diol (PLA diol) or polycaprolactone diol (PCL diol). Cristalizable and degradable block.

Age determination of plutonium for nuclear forensics

Sturm, M[a], Richter, S[b], Aregbe, Y[b], Wellum, R[b], Mayer, K[c], Prohaska, T[a]

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b European Commission, Joint Research Centre, Institute for Reference Materials and Measurements, Geel, Belgium
c European Commission, Joint Research Centre, Institute Transuranium Elements, Karlsruhe, Germany

Nuclear forensics investigations focus on the origin and the history of seized nuclear material in order to discover its latest legal owner and production place. The chemical composition and the physical appearance of the sample provide information on the production process and the intended use. The "age" of the material helps to limit the number of possible nuclear facilities such as reprocessing plants to those, which were active at the time of the production of the material. The "age" of a nuclear material is defined as the time elapsed since the last chemical separation of mother and daughter nuclides during enrichment or reprocessing. Different "clocks" (i.e. parent/daughter nuclide pairs) can be used for the age determination of plutonium: $^{241}Am/^{241}Pu$, $^{238}Pu/^{234}U$, $^{239}Pu/^{235}U$ and $^{240}Pu/^{236}U$. At present there are no certified nuclear reference materials available for the separation date of plutonium.

In order to meet this need of laboratories involved in nuclear forensics, the Institute for Reference Materials and Measurements (EC-JRC-IRMM) is cooperating with the Institute for Transuraniumelements (EC-JRC-ITU). The presented doctorate thesis has been carried out in cooperation between BOKU and both institutes of the European Commission mentioned above and was conducted in the nuclear laboratories of the IRMM.

The isotopic reference material SRM 946 (NBL CRM 136) was dated as part of a feasibility study on plutonium reference materials certified for the separation date. The results of the $^{238}Pu/^{234}U$, $^{239}Pu/^{235}U$ and $^{240}Pu/^{236}U$ clocks measured by isotope dilution thermal ionization mass spectrometry yielded ages of about 41.1 years (reference date 18.10.2011) and match within their uncertainties. These findings are in good agreement with the reported dates for the production of NBS 946 in 1970.

An abstract with similar content was submitted for an oral contribution to the 35th Annual ESARDA Symposium on International Safeguards 27- 30 May 2013, Bruges, Belgium.

Strontium Isotopes as Tracers of Biological Migration

Irrgeher, J[a], Zitek, A[a], Teschler-Nicola, M[b] and Prohaska, T[a]

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b Natural History Museum Vienna, Department of Anthropology, Burgring 7, 1010 Vienna, Austria

The analysis of Sr isotope ratios is regarded as a key approach to investigate biological migration, mobility and movement phenomena due to its outstanding properties of regional difference and natural variability.
In its first stage, the presented PhD work aimed at developing and validating an analytical method to determine accurate Sr isotope ratios by (laser ablation) - multi collector - inductively coupled plasma mass spectrometry ((LA)-MC-ICPMS) in Ca-rich archaeological and modern biological tissues such as bio-apatite matrices (e.g., tooth, bone) as well as calcium carbonate matrices (e.g. fish otoliths) to survey biological migration phenomena. Therefore, a number of essential parameters (e.g. blank, interferences and instrumental mass fractionation), which significantly influence the metrological quality and accuracy of the data and which are of paramount importance when absolute Sr isotope amount ratios are assessed in Ca-rich samples, were investigated in detail.
The developed method was subsequently applied to case studies, where Sr isotopes were used as tracers to address various research questions. This was achieved either by making use of the natural variation of the Sr isotopic system (by investigating human remains excavated from two Austrian excavation sites) or by introducing isotopically enriched Sr tracers into natural biological organisms (by transgenerational marking of fish). [1-3] The latter studies included the development of a detailed protocol for comprehensive data evaluation (i.e. isotope pattern deconvolution) applicable in cases, where enriched Sr stable isotopes are used. [4]
Furthermore, a candidate matrix reference material for Sr isotope amount ratios in a biological tissue was successfully characterized for its potential use as certified reference material, including full method validation. The material was considered as homogeneous for its use as isotope CRM and recommended values of all natural isotope amount ratios were published.[5]

[1] Irrgeher, J., Kern, D., Teschler-Nicola, M., Prohaska, T. (2012): Late Neolithic Graves from the Traisen Valley, Lower Austria. Archaeology and $^{87}Sr/^{86}Sr$ Isotope Analysis by MC-ICP-MS; in: *Population Dynamics in Pre- and Early History. New Approaches by Using Stable Isotopes and Genetics*, pages 199-212, De Gruyter**.**
[2] Zitek, A., Irrgeher, J., Kletzl, M., Weismann, T., Prohaska, T. (2013): Transgenerational marking of a freshwater fish species, Salmo trutta f.f. L., using an ^{84}Sr spike. *Fisheries Management and Ecology,* 20 (4), 654-361.
[3] Zitek, A., Irrgeher, J., Cervicek, M., Horsky, M., Kletzl, M., Weismann, T., Prohaska, T. (2013): Individual-specific transgenerational marking of common carp *Cyprinus carpio* L. using a $^{86}Sr/^{84}Sr$ double spike. Submitted to *Canadian Journal of Fisheries and Aquatic Sciences*.
[4] Irrgeher, J., Zitek, A., Cervicek, M., Prohaska T. (2013): Analytical factors to be considered for the application of enriched strontium spikes to monitor biological systems. Submitted to *Journal of Analytical Atomic Spectrometry.*
[5] Irrgeher, J., Prohaska, T., Sturgeon, R.E., Mester, Z., Yang, L. (2013): Determination of Strontium Isotope Ratios in Biological Tissue Using MC-ICPMS. *Analytical Methods*, 5 (7), 1687 – 1694.

Application of Sr isotope ratio measurements for the determination of origin of prehistoric wood

Horsky M[a], Tintner J[b], Grabner M[b], Kowarik K[c], Reschreiter H[c], Kern A[c], Prohaska T[a]

a University of Natural Resources and Life Sciences Vienna, Department of Chemistry, Division of Analytical Chemistry, VIRIS Laboratory, Konrad-Lorenz-Str. 24, 3430 Tulln

b University of Natural Resources and Life Sciences Vienna, Department of Material Sciences and Process Engineering, Institute of Wood Science and Technology, Konrad-Lorenz-Str. 24, 3430 Tulln, Austria

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Wood artefacts from prehistoric times have been preserved in a salt mine environment in Hallstatt, Austria, for more than 3000 years. These artefacts represent a unique archive of information on past mining industry. $^{87}Sr/^{86}Sr$ isotopic ratios are a recognised geochemical tool for the determination of origin in various fields as natural variations of the isotopic composition between regions are directly reflected in plants growing in these regions.

In this case, however, the storage conditions within the mine present a challenge with respect to inorganic contamination by the repository material. The extent of penetration of salt into the wood tissue was screened using laser ablation - inductively coupled plasma mass spectrometry (LA-ICPMS). A decontamination strategy based on acid leaching was developed and successful separation of contamination and natural strontium could be achieved. This was shown by measurement of $^{87}Sr/^{86}Sr$ in leaching solutions and digests of wood using multiple collector ICPMS. The assumption of non-exhaustive removal of secondary salts containing repository Sr was included into the evaluation by adoption of a mixing curve, which allows the mathematical extraction of biogenic Sr isotope ratios of the wood samples within an estimated uncertainty.

As a basis for the determination of possible origins of wooden prehistoric artefacts made of oak (which are assumed traded), modern trees from seven selected regions in Austria were analysed for their Sr isotopic ratios. The regions were chosen based on archaeological knowledge of settlements in the time period of interest and due to geological and silvicultural considerations. Four tree species, which are also present in the archaeological finds, were sampled. This set of data sets the basis for a map (‘isoscape’) of Sr isotopic signatures bioavailable to different trees in Austria.

An abstract with similar content was submitted for a poster presentation at the European Winter Conference on Plasma Spectrochemistry 2013, February 10 - 15, 2013 in Krakow, Poland.

Fibrillated lignocellulose biomass

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a Kompetenzzentrum Holz GmbH, Division Wood Materials Technologies, Konrad Lorenz Strasse 24, 3430 Tulln, Austria

b University of Natural Resources and Life Sciences, Department of Material Sciences and Process Engineering, Institute of Wood Technology and Renewable Resources, Konrad Lorenz Strasse 24, 3430 Tulln, Austria

The fibrillation of cellulose macro-fibers into smallest elementary fibrils, which in compound have mechanical properties comparable to steel, is scientific knowledge[1]. However, in plant cell walls a matrix of lignin and hemicellulose covers the cellulose-fibrils [2]. The effect of this compound cell wall structure on the fibrillation of plant biomass in the homogenizer and differences between the single lignocelluloses (spruce and beech wood, brewer's grain) are shown. The morphological and mechanical properties of dried suspensions containing fibrillated particles and fibers were characterized. Besides the chemical composition and anatomical structure of plant biomass, chemical pre-treatments and the choice of an appropriate fibrillation technology are of importance with regard to the quality and efficiency of fibrillation. One step before the fibrillation of cell walls, raw materials are grounded into smaller dimensions. Therefore many different machines are available on the market. One very useful and industrial well implemented is a disc refiner. Steam pressure and mechanical disruption between milling discs contribute for a well preliminary comminution [3]. Basic aspects of this technology will be shown in this presentation.

[1] Walker, C. (2012): Thinking small is leading to big changes. Paper360 7(1): 8-13.
[2] Klemm, D., Kramer, F., Moritz, S., Lindström, T., Ankerfors, M., Gray, D. und Dorris, A. (2011): Nanocelluloses: A new family of nature-based materials. Angewandte Chemie - International Edition 50(24): 5438-5466.
[3] Karande, V. S., Mhaske, S. T., Bharimalla, A. K., Hadge, G. B. und Vigneshwaran, N. (2012): "Evaluation of two-stage process (refining and homogenization) for nanofibrillation of cotton fibers." Polymer Engineering and Science.

Enzymatic pre-treatment of grass silage

Montgomery LFR, Xia H, Fuchs W, Bochmann G

Department of Agrobiotechnology, Institute of Environmental Biotechnology, Konrad Lorenz Strasse 20, 3430 Tulln a. d. Donau

The aim of this work is to produce enzyme mixtures (e.g. xylanases, cellulases, pectinases, acid esterases, etc.) using filamentous fungi grown in solid-substrate fermentation on grass silage and other agricultural residues. These enzyme mixtures can be used for breaking down grass silage to produce bulk chemicals or to improve biogas production from grass silage.

Various fungi including *Aspergillus niger*, *Trichoderma reesei* were grown by solid-substrate fermentation to produce enzyme mixtures suitable for hydrolysing grass silage. Pure untreated grass silage (~pH 4, 22 g lactic acid per kg fresh grass silage, 15 g/kg acetic acid, 7g/kg ethanol and other unknown inhibitors) was found to be unsuitable for solid-substrate fermentation of all tested fungi. Neither increasing the pH, increasing the water activity nor adding other carbon sources allowed fungal growth. However, washed grass silage (pH 4.3, 0.6g/kg lactic acid, 0.4 g/kg acetic acid and 0.2 g/L ethanol) was suitable for fungal growth and enzyme production. The addition of pressed sugar beet pulp, a residue from sugar production, to the washed grass silage was shown to enhance fungal growth but only to have a limited effect on enzyme production. In contrast, the addition of brewers' spent grains (BSG), a hemicellulose-rich residue from beer brewing, greatly increased enzyme yields. However, when more BSG than grass silage was used (ratio > 1:1), grass-degrading enzyme activity decreased for most tested fungi. Overall, the use of co-substrates gave more reproducible enzyme production.

Results demonstrate that it is possible to use grass silage as a substrate for enzyme production, but only when inhibitors are first removed by washing. As pilot-scale biorefinies using grass silage wash water / press water for acid production already exist, this research demonstrates a potential use for the solid residue from these processes, both for enzyme production and for enzymatic hydrolysis for further biorefinery.

Functionalisation of Viscose fibres

Siller M [a], Walter, R[c], Rosenau, T[b], Potthast, A[b]

a Kompetenzzentrum Holz GmbH and Department of Chemistry, University of Natural Resources and Life Sciences, UFT Campus Tulln, Konrad-Lorenz-Straße 24, 3430 Tulln, Austria
b Department of Chemistry, University of Natural Resources and Life Sciences, UFT Campus Tulln, Konrad-Lorenz-Straße 24, 3430 Tulln, Austria
c Kelheim Fibres GmbH, Regensburger Straße 109, 93309 Kelheim, Germany

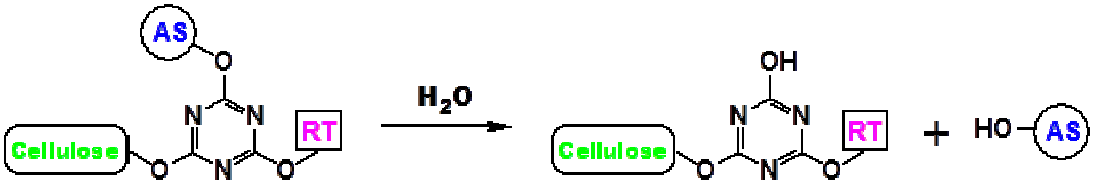
We are investigating chemical modifications of Viscose fibres to generate fibres with new functionalities for new fields of applications. In a first step, an anchor group is introduced which in a subsequent reaction is used for attachment of different functional molecules, e.g. fluorophores, biocides or proteins. For that purpose, we have generated aldehyde functionalities by periodate oxidation (which are also present as hydrates or semiacetals): this mild oxidation fully preserves molecular weight and mechanical fibre properties at low degrees of oxidation. Periodate oxidation causes ring opening of anhydroglucose unit between the 2 vicinal hydroxyl groups at C2 and C3 and causes formation of "2,3-dialdehyde cellulose". The oxidation kinetics was studied in more detail, providing a full parameter set of T-, t and c-dependencies. Carbonyl group contents in the range of mmol/g cellulose were achieved within some minutes of oxidation at high temperatures and periodate concentrations.
Aldehyde groups in rayon fibres are used for covalent attachment of functional molecules, possibly via suitable linkers to reduced steric effects. Of particular interest are reactions with N-nucleophiles, such as primary and secondary amines, hydrazides or semicarbazides. We have used dansyl hydrazide as a model compound to show formation of fluorescent hydrazones. The binding of the fluorophore was highly increased by pre-oxidation of Viscose fibres.
Another possible application of aldehyde-containing cellulosic fibers is utilization of the reducing activity toward metal ions. We demonstrated this by reaction of periodate-oxidized Viscose with silver ions (Tollens reagent), producing Ag-loaded fibers. The initial silver content can be in the range of percent, but significant amounts of silver are lost in reaction and washing solutions. Such fibres containing silver or copper are of interest because of their antimicrobial activity.

Adding function to cellulosic tissue

Ebner, Gerald, Potthast, A., Rosenau, T.

Department of Chemistry, Devision of Chemistry of Renewable Resources, Konrad Lorenz Strasse 24, 3430 Tulln a. d. Donau

Tri-functional triazines have been developed to implement a controllable release of various active substances from cellulosic substrates. The triazine reagent carries the desired active component, a tuner molecule, in order to govern the release behavior, and an anchor group to facilitate fixation of the reagent onto cellulose. While these compounds are completely stable under dry conditions, the release of the active agent is simply triggered by the surrounding humidity.



The release kinetics of 10 active substances together with 12 different reactivity tuners has been determined to demonstrate the prospects of this approach.
Complementary, an encapsulation method with all-sustainable materials was established which allows the formation of self-assembling casings out of two cheap bio-based waste products, namely chitosan and lignosulfonate. It is a facile approach that permits retarded release of an encapsulated active substance. The impact of some production parameters on the mechanical stability of the capsules has been investigated.
Chitosan was furthermore studied towards its applicability as material for antibacterial coatings of cellulosic tissues. In order to reduce the required amount of substance, the optimal degree of acetylation and average molecular weight were ascertained. Additionally, seven chitosan derivatives have been synthesized with the aim to amplify the antimicrobial activity of the starting material. Those substances have subsequently been evaluated towards their biocidic potential with four different microorganisms. Some promising substances have been identified which will be further investigated.

Advanced wound management and infection prevention in chronic wounds

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Wound infection is a global problem that affects 5-10% of post-surgical wounds and 25% of chronic wounds. The human immune system reacts on infection with excessive stimulation of neutrophils and consequent release of proteolytic enzymes into the wound environment.

The enzyme activities of HNE, MPO and matrix metalloproteinases (MMPs) were directly monitored in wound fluids of affected patients via hydrolysis of chromogenic or fluorescent biopolymer based substrates. Infected wound fluids led to significant higher substrate conversion compared to non infected wound fluids [1]. In addition, the gelatinolytic activity from both- MMPs and bacterial proteases were investigated in different types of wounds for the development of an biopolymer based enzyme-responsive detection method. Upon incubation of dyed gelatin based devices with infected wound fluids, an incubation for 30 minutes led to a clearly visible dye release. We furthermore found possibilities to trap cleaved or released colour particles in relation to applications in wound care systems.

Natural polyphenolic compounds recently received a great deal of attention in medicine owing to their antioxidant, antimicrobial, anti-inflammatory and consequently wound healing promoting properties. Therefore we immobilised phenolic compounds on biopolymers to achieve a partly inhibition of elevated enzymes present in wounds. To allow integration of sensors in typical bandage materials we successfully immobilized enzyme substrates various surfaces and silica gel. These immobilised substrates were converted only by infected wound fluids, thus allowing on-line monitoring of wounds due to different colour stages of the bandage. The combination of these rapid and simple diagnostic methods provide a powerful instrument in consideration of early stage warning of wound and additional promoting wound healing.

[1] A. Hasmann, G. M. Guebitz, and E. Wehrschuetz-Sigl, *Exp Dermatol*. **2011** *20(6):*508-13

A targeted proteomics approach for the elucidation of peptide markers for the detection of lupin allergens in food products

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Currently, the application of lupin flour increases in the European food industry, because it is a rich source of low cost protein. Additionally, lupin promises health benefits and nutraceutical properties such as hypocholesterolemic and hypoglycaemic effects. But the downside of this fabulous little bean is that individuals, who are allergic to other legumes, can suffer from severe allergic reactions against lupin. As a consequence, the European Commission decided that food products containing lupin - even in trace amounts - must be compulsory declared on food labels in the EU.

Objective of this study was to find lupin specific peptide markers, which can be used for the identification and quantification of lupin allergens in food products via HPLC-MS/MS. For this purpose, targeted proteomics was used to predict the proteolytic peptides. Afterwards, different lupin species and varieties were searched for those peptides which could be used as markers for lupin allergens. To examine the influence of processing and that of the food matrix, different food products, that should contain lupin flour according to their labelling, were cooked and screened for peptide markers.

It could be shown that targeted proteomics is a valuable tool that facilitates the identification of allergens in food. The method described above will help to protect allergic people from hidden lupin allergens in food products.

Determination of metabolites formed by enzymatic degradation of ergot alkaloids

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Ergot alkaloids are mycotoxins that are mainly produced by fungi of the genus *Claviceps* or by grass endophytes like *Neotyphodium*. The occurrence of ergot alkaloids remains a problem in animal nutrition and has increased during the last years [1]. The use of microorganisms capable of degrading ergot alkaloids represents one strategy of elimination. An ergopeptine-degrading *Rhodococcus erythropolis* strain was isolated from soil and the involved enzymes were identified. The α/β hydrolase ErgA is responsible for the metabolisation of different ergopeptines to ergine. The amidase ErgB catalyses the degradation of ergine to lysergic acid. The aims of this work were the determination and structure elucidation of metabolites formed during degradation of different ergopeptines by the purified enzyme ErgA, the *Rhodococcus erythropolis* strain and its lysate to provide insights into the intermediate and final products of the enzymatic degradation. An LC-MS/MS based method for the analysis of the investigated ergopeptines, their corresponding epimers and the metabolites was developed on a 4000 QTrap system (AB Sciex). Measurements of the degradation experiment samples resulted in information about molecular weight as well as fragment ions of the newly formed metabolites. Accurate mass measurements (6550 iFunnel QTOF, Agilent Technologies) as well as ^{1}H-, ^{13}C- and 2D-NMR measurements of isolated metabolites were conducted for structure elucidation.

Two main groups of metabolites were observed during microbial and enzymatic degradation of ergopeptines: Ergine hydroxy carboxylic acids still contain the ergoline ring system, are very unstable and degrade to ergine. Diketopiperazines are cyclic dipeptides which are probably non-toxic. Structure elucidation of the formed metabolites is essential to explore the mechanism of the enzymatic degradation. The analytical method will be applied for monitoring the enzymatic reaction and the identified metabolites will be needed for studies on enzyme kinetics.

[1] Strickland, JR, Looper, ML, Matthews, JC, Rosenkrans, CF, Flythe, MD, Brown, KR (2011): St. Anthony's fire in livestock. J Anim Sci 89: 1603-1626.

Development and improvement of novel approaches in 2D chemical imaging of rhizosphere processes

C. Höfer[1,*], M. Puschenreiter[1], J. Santner[1] and W. W. Wenzel[1]

1 University of Natural Resources and Life Sciences Vienna (BOKU), Department of Forest and Soil Sciences, H911, Rhizosphere Ecology and Biogeochemistry Group, A-3430 Tulln, Austria

**corresponding author, christoph.hoefer@boku.ac.at*

Two dimensional chemical imaging of root processes is a novel *in situ* method to investigate and map labile plant (micro)nutrients and/or contaminants at a high spatial resolution (sub-mm). The visualization of root processes demonstrates new insights in soil biogeochemistry and plant nutrition.

Here, chemical images are derived by using data from DGT-LA-ICP-MS (Diffusive Gradients in Thin Films and Laser Ablation Inductively Coupled Plasma Mass Spectrometry) and POS (Planar Optode Sensors). Both technologies have shown promising results but need to be refined and improved for imaging in the soil-plant interface. Combined approaches of both DGT and POS technologies, methodological challenges and the development of new sensors are in our focus.

DGTs are smart and thin (<0.4 mm) hydrogels; containing a binding resin for certain target analytes (e.g. trace metals, phosphate, sulphide or radionuclides). The measurement principle is passive and diffusion based. The present analytes are diffusing into the gel and are bound by the resin. Thereby, the resin acts as zero sink. After application, DGTs are retrieved, dried, and measured using line scans by LA-ICP-MS. The data is normalized by an internal standard (e.g. ^{13}C), calibrated and transferred into a chemical picture of the target area.

POS are thin sensor foils containing a fluorophore coating depending on the target analyte. The measurement principle is based on excitation of the flourophore by a specific wavelength of light and quenching of emitted light by the presence of the target analyte. POS measurements can be performed continuously during the application time by a simple and inexpensive modification of a DSLR camera and an excitation light source. Both semi-quantitative techniques allow us to visualize chemical processes directly at the soil-plant interface.

We present results from rhizotron experiments with willows and maize plants in metal contaminated and agricultural soils.

We conclude that these emerging techniques are visualizing rhizosphere processes and biogeochemical dynamics and thus provide a better understanding of plant root and soil interaction.

Development and application of a workflow to find known and unknown metabolisation products of xenobiotics using stable isotope labelling and LC-HRMS

Bernhard Kluger [a], Christoph Bueschl [a], Alexis Nathanail [a], Elisabeth Varga [a], Nora Neumann [a], Mark Lemmens [b], Gerhard Adam [c], Franz Berthiller [a], Rudolf Krska [a], Rainer Schuhmacher [a]

a Department of Agrobiotechnology, Center for Analytical Chemistry;

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Xenobiotics (mycotoxins, antibiotics, drugs, pesticides,..) are chemicals that are not produced by an organism (plants, bacteria, mammalians,..) but to which the organisms are exposed (food, medicine, contamination, ...). In many cases the living organism alters (biotransforms) the chemical, which can lead either to their activation or inactivation (e.g. detoxification).

To facilitate global, quick and reliable detection of biologically derived metabolite signals, an *in vivo* labelling-assisted LC-HRMS based metabolomics platform has been successfully implemented. This workflow for both the experimental part involving the application of ^{12}C and ^{13}C labelled xenobiotics to the organism of interest and a software tool [1] for automatic data evaluation, which recognizes the distinct isotopologue pattern present only for ^{12}C and ^{13}C labelled metabolites [2], have been adapted to help studying the biotransformation of xenobiotics in various organisms. The presented untargeted metabolisation approach has the potential to discover novel, and unexpected metabolites as well as known biotransformation products originating from exposure to xenobiotics. Furthermore, with the additional knowledge about remaining labelled carbon atoms in the metabolisation product, novel metabolite identification is aided and improved over non-labelling assisted approaches. Following MS/MS fragmentation experiments and data analysis with FragXtract [3] of both the ^{12}C and ^{13}C labelled metabolisation products further helps in unknown identification.

For workflow performance demonstration, an experiment involving the detoxification of the mycotoxin deoxynivalenol in wheat plants [4] is presented. The plants were incubated with ^{12}C and U-^{13}C isotopologues of deoxynivalenol for 48 hours and measured with an LTQ Orbitrap XL. Subsequent data processing revealed the presence the unprocessed tracer and 9 metabolisation products from which 7 were unknown at the time of the study. All new metabolisation products showed the same number of labelled carbon atoms as the unprocessed tracer leading to the assumption that the detoxification is mainly performed through conjugation.

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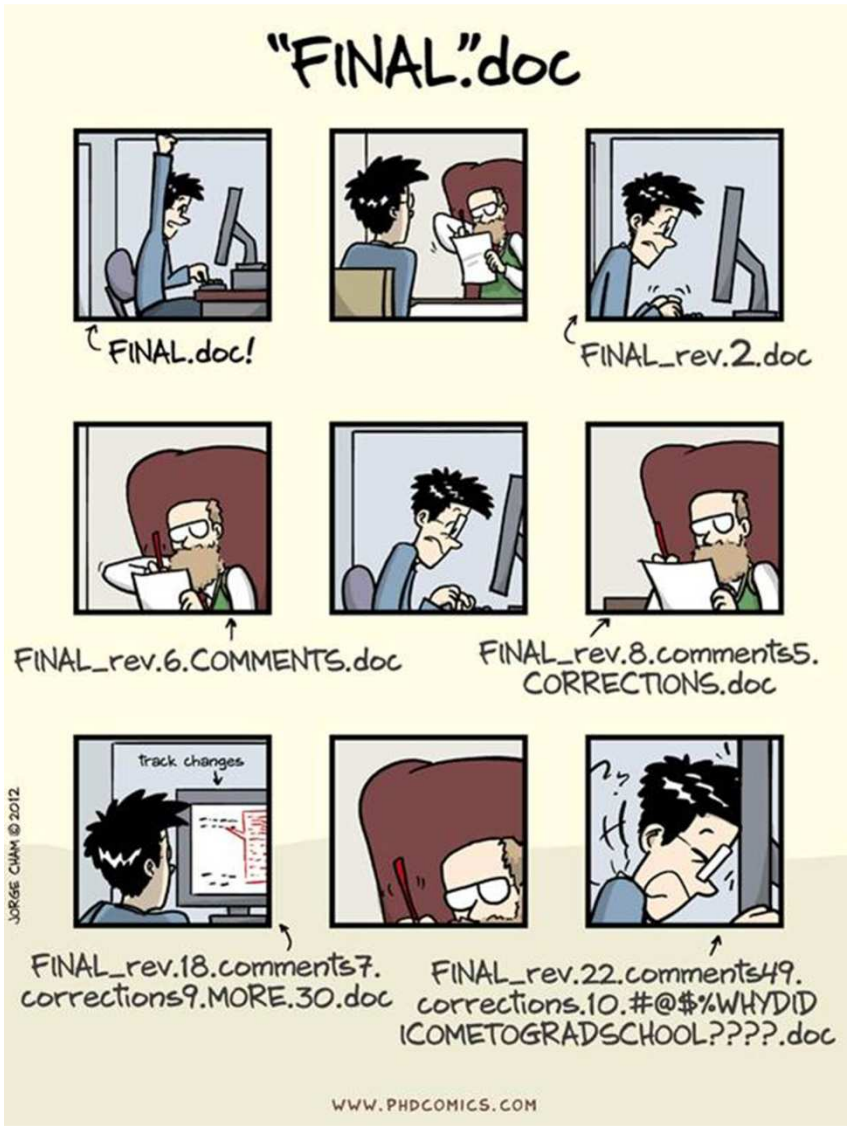
Abstracts POSTER



Expression QTL mapping for Fusarium Head Blight resistance in Wheat

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Breeding for Fusarium head blight (FHB) resistance is a challenging task for breeders worldwide, also due to the quantitative nature of resistance in wheat. To date more than 200 QTL on almost all chromosomes have been reported to contribute to resistance, with most of them encoding for minor effect genes. The application of genome-wide approaches to analyse quantitative traits has yielded relevant genes, involved in the expression of related traits [3]. The analysis of expression Quantitative Trait Loci (eQTL) is a method to identify novel QTL by employing transcriptome variation. In this study we exploited eQTL mapping that correlate microarray gene expression data and genetic markers data to identify genes involved in the specific response of wheat to *Fusarium graminearum* in a population of 200 doubled haploid lines segregating for FHB resistance. This population derives from the resistant line CM-82036 (progeny of Sumai 3) and the susceptible European spring wheat cultivar Remus [1,2]. Six central spikelets were inoculated with a Fusarium spore suspension at anthesis and samples were harvested at two time points (30 and 50 hours) after inoculation. RNA was hybridized onto a custom-build microarray (Agilent 8x60k), comprising 44.000 wheat unigenes, several hundred wheat candidate genes, that have been reported responsive to Fusarium in literature and the entire transcriptome of *Fusarium graminearum* (ca. 14.000 genes). In total, we hybridized about 500 microarrays. eQTL mapping was carried out by interval mapping analysis. We discovered more than 20,000 significant eQTL (based on high LOD score), distributed throughout all chromosomes. In a next step we detected “eQTL hotspots”, which describe gene-rich regions potentially co-regulated by eQTL. We further identified cis and trans-eQTL, based on the distance of the eQTL map position to the location of the target genes controlled by the respective eQTL.

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Novel Enzymes for Polyesters Synthesis and Modification

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Although traditional chemical catalysts for polyester synthesis have enabled the generation of important commercial products with poly-condensation and ring-opening polymerization as main routes for the production, shifting society´s dependence away from petroleum and heavy metals chemistry to renewable biomass resources and sustainable chemistry is generally viewed as an important contributor to the development of a sustainable industry [1, 2].
In vitro polymer synthesis by using enzymes as catalyst (enzymatic polymerization) was initiated in the late 1980s and extensively developed in the following decades because of the great potential of those catalysts as substitutes of the traditional heavy metals - such as antimony oxides, titanium oxides, tin and zinc - used for the polyester production [3,4].

The main aim of this study is to produce and engineer novel α/β hydrolases class enzymes in order to enhance the choice of “green catalysts” available for bio-catalyzed synthesis and modification of a various selection of polyester materials.

We acknowledge funding of this project by the FP-7 Marie Curie REFINE Project.

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Hormone based stress signalling in a plant-pathogen-symbiont interaction

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Sedentary plant-parasitic cyst nematodes, such as *Heterodera schachtii*, induce severe plant damage during invasion, intracellular migration, feeding site induction and nutrient and water withdrawal. Thus, cyst nematode infection may provoke severe loss of economically important crops such as soybean, wheat or potato. Presently, there is only little information about plant defence and signalling mechanisms during nematode invasion that may determine if the host plant is susceptible or resistant. On search for cost extensive and environmental friendly pest management strategies the application of plant symbionts if widely studied. The beneficial endophytic fungus *Piriformospora indica* colonizes the roots *Arabidopsis thaliana* what promotes plant growth, development, and seed production as well as resistance to various biotic and abiotic stresses.
In the present work the interaction between *A. thaliana-H. schachtii-P. indica* was selected as research model to elucidate potential hormone related stress signalling in a multi-organism cross-talk. Plant-pathogen/symbiont interactions have been reported to underlie changes in phytohormone levels such as salicylic acid, jasmonic acid and ethylene, determining plant resistance or susceptibility. First, the effect of *P. indica* plant colonisation on *H. schachtii* attraction, infection and development was tested. Second, the effects of the application of different plant hormones on *A. thaliana* leaves was studied 1) on the plants alone, 2) in the plant-pathogen, 3) plant-symbiont, and 4) plant-pathogen-symbiont interactions by analysing expression levels of related marker genes and hormone levels of the treated plants. Further, nematode attraction, infection and development assays were performed using the single treatments to study the potential changes in plant susceptibility.

Investigation of degradation products of novel implant materials used in orthopaedic trauma (degIMMAT)

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Newly developed biodegradable magnesium alloys show great benefits compared to implants made of e.g. steel or titanium. The investigated implant materials fully degrade in the body and are therefore thought to be of great advantage in orthopaedic trauma because no second surgery step to remove the implant is needed. With outstanding mechanical properties, controlled full degradation [1] and similar mechanical behaviour to natural bone material [2], these magnesium alloys can be used for osteosynthesis implants. However, the degradation rates and the related distribution of the alloying elements in the tissues are not fully investigated, yet.
The spatial distribution of Mg, Ca, P, Mn, Zn, Zr, and Yb in bone is investigated by laser ablation inductively coupled mass spectrometry (LA-ICPMS). Solid in-house matrix matched calibration standards were developed by co-precipitation of the desired alloying elements (Mg, Ca, Mn, Zn, Zr, Yb) in hydroxyapatite (HAp). The capability of this quantification approach was validated by comparative measurements of certified reference materials (SRM 1486, pressed into pellets for direct LA-ICPMS analysis) and natural bone material.
The adapted HAp preparation method [3] led to stable solid pellets. XRD measurements proved a perfect matrix matching of the precipitated HAp standards, whereas at high elemental concentrations, different phases could be observed. The calibration curves give a linear correlation within the working range (e.g. $R^2 \geq 0.99$ for Mg). The developed quantification strategy is further applied to bone material to enable the quantitative evaluation of the spatial distribution of trace elements in bone after selected degradation times of the implant material. Multi-layer imaging capabilities of Origin® were finally successfully applied to produce spatially resolved images. This analytical approach can be used for directly monitoring and visualizing the mobilization of elements of the implant material into the bone tissue in order to derive elemental resident times and diffusion profiles.

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An abstract with similar content was submitted for an oral contribution to the 9th ASAC JunganalytikerInnen Forum, 21-22 June 2013, Vienna, Austria.

Anaerobic Digestion of Microalgae

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There are several reasons why microalgae may be interesting in the field of environmental biotechnology. On the one hand microalgae synthesize a variety of interesting compounds (lipids, proteins, carbohydrates, pigments); on the other hand they can be seen as an important alternative biomass and have therefore been in scientific focus since the early 60ies. In this study, two green-algal strains, namely *C. vulgaris* (SAG 211-1b) and *S. obliquus* (SAG 276-1) were grown in sleeve-bag PBRs, harvested and total contents of proteins, carbohydrates and lipids determined. Microalgal suspensions were centrifuged to final volatile solid-concentrations of 79.36 g L^{-1} (*C. vulgaris*) and 68.96 g L^{-1} (*S. obliquus*) which is a concentration of 96- and 101-fold compared to the originally grown biomass. The substrate characterization revealed higher lipid contents in *C. vulgaris* (31.1 % DM^{-1}) compared to *S. obliquus* (25.7 % DM^{-1}) and similar protein contents (44.0 % and 45.1 % DM^{-1}). Parts of the biomass were afterwards thermally pre-treated (T = 140° C and T = 160° C) and the biochemical methane potential (BMP) measured in comparison to the untreated biomass. Highest CH_4-productivities were reached by untreated *C. vulgaris* biomass (293 Nm^3 t^{-1} VS) and untreated *S. obliquus* biomass (286 Nm^3 t^{-1} VS). Thermally pre-treated biomass did not increase in CH_4 productivity, in fact, led to lesser productivities per VS; pre-treated *C. vulgaris* biomass led to decreased CH_4 productivities (T140° C = -20.8 %; T160° C = -9.2 %) as did *S. obliquus* biomass (T140° C = -31.1 %; T160° C = -16.1 %) compared to untreated controls. Our first findings are inconsistent with numbers derived from literature [1] that proposed thermal pre-treatment to be effective in terms of microalgal cell wall degradation and concomitant higher CH_4-productivities.

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Investigation of sulphur mass balances in ecosystems by sulfur isotope ratio measurements using MC-ICPMS

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Strong regulations of SO_2 emissions applied in Austria during last four decades led to a significant decrease in sulphate mass fraction in rain water and dust particles. Recent studies showed that the stream water output of sulphur has already exceeded the sulphur input in many monitored catchments. Possible sources of this excess sulphur are desorption of sulphur adsorbed in the past, weathering of sulphur bearing rocks or mineralization of organic sulphur. Apparently, mineralization and desorption from past sulphur deposition are mainly contributing to the sulphur source. $^{34}S/^{32}S$ isotope ratio analysis of sulphur input (rain water) and output (soil solution) have the potential to distinguish between different contributions to the bulk sulphur mass balance [1].

Rain water and soil solution samples were collected at 3 sites in the Vienna Woods, Austria and at 3 sites in Kobernaußerwald, Austria from May 2010 to April 2012 and from May 2012 to April 2013, respectively. Sulphate concentration was determined by ion chromatography. $^{34}S/^{32}S$ isotope ratios have been measured by multi - collector inductively coupled plasma mass spectrometry (MC-ICPMS) in edge resolution mode using a method originally developed for food analysis [2], adapted for water samples.

The method was validated using reference materials IAEA-S-1 and IAEA-S-2. Short-time repeatability (<0.01 %, 1 sd, n=5), within lab-reproducibility (0.03 %, 1 sd, n=10) and the combined standard uncertainty of the measurement (0.10 %, k=1) show, that the method is fit for purpose [1]. Isotope ratios of the sulphur input ($\delta^{34/32}S_{VCDT}$ = 4,30 ± 0,32 ‰) were found in expected range with low seasonal variation. In sulphur output, they seem to be dependent on the distance from the tree stem and the sampling depth.

An abstract with similar content was submitted for an oral contribution at the 9th ASAC JunganalytikerInnen Forum 21 - 22 June 2013, Vienna, Austria.

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An enzymatic strategy to replace toxic heavy-metal catalysts from coatings

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Alkyd resins are a widely used type of paints containing unsaturated fatty acids. During the curing process the unsaturated groups are oxidized and subsequently cross-linked. Currently the chemical drying of these alkyd resins is accelerated by heavy-metal complexes. Cobalt based complexes are the most effective driers but they are suspected to be cancerogenic [1]. Due to environmental and health awareness of the market the coating industry is actively looking for a replacement.
In this work we present an enzyme based strategy for the drying of alkyd resins [2]. The potential of a laccase from *Trametes hirsuta* in combination with two different electron mediators was evaluated regarding its capability to crosslink the unsaturated fatty acids and consequently harden the resin. It could be shown that the enzyme reaction works not only in aqueous media, but also in films where diffusion of the enzyme is limited. Gel permeation chromatography confirmed a molecular weight increase of the laccase mediator treated resin. As mentioned before, during the chemical drying of alkyd resins the unsaturated fatty acid moieties are oxidized and subsequently cross-linked. Consequently, the concentration of triglycerides decreases. This decrease was monitored using gas chromatography.
During the drying of the alkyd film the oxidation of the unsaturated groups O_2 is consumed. Therefore a fluorescence based method to measure oxygen in drying alkyd resin films was developed. It allowed monitoring of the reaction progress and compare the effectiveness of the different mediators. Additionally the curing process was followed over time via FTIR spectroscopy. The drying of the alkyd resin was correlated to a clear decrease of the band assigned to double bonds (3010 cm^{-1}). Furthermore, drying recorder results demonstrated the potential of laccase mediator systems to replace cobalt based siccatives.

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Counteracting ammonia inhibition in anaerobic digestion by removal with a hollow fiber membrane contactor

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The aim of the current study was to investigate the feasibility of membrane contactors for continuous ammonia (NH_3-N) removal in an anaerobic digestion process and to counteract ammonia inhibition. Two laboratory anaerobic digesters were fed slaughterhouse wastes with ammonium (NH_4^+) concentrations ranging from 6 to 7.4 g/L. One reactor was used as reference reactor without any ammonia removal. In the second reactor, a hollow fiber membrane contactor module was used for continuous ammonia removal. The hollow fiber membranes were directly submerged into the digestate of the anaerobic reactor. Sulfuric acid was circulated in the lumen as an adsorbent solution. Using this set up, the NH_4^+-N concentration in the membrane reactor was significantly reduced. Moreover the extraction of ammonia lowered the pH by 0.2 units. In combination that led to a lowering of the free NH_3-N concentration by about 70 %. Ammonia inhibition in the reference reactor was observed when the concentration exceeded 6 g/L NH_4^+-N or 1 to 1.2 g/L NH_3-N. In contrast, in the membrane reactor the volatile fatty acid concentration, an indicator for process stability, was much lower and a higher gas yield and better degradation was observed. The chosen approach offers an appealing technology to remove ammonia directly from media having high concentrations of solids and it can help to improve process efficiency in anaerobic digestion of ammonia rich substrates.

Optimized alternative fuel production (biogas) in Alpine regions

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Alpine grasslands are currently being abandoned due to the lack of economic viability. This tendency can have important negative ecological and social consequences. The cultivation of grassland biomass in an extensive way for its further utilization in a biogas-based biorefinery concept can be an option to increase incomes and turn this trend.

Agriculture has the responsibility to produce food, feed, fuels and fiber in sustainable way. In the last decades, the conditions for agricultural production have changed: among other factors, an increasing societal interest in the production of renewable energy carriers as well as an improved understanding of the effects of climate change have highlighted the need to adapt to a new situation in agriculture. Adaptation has both ecological and technical aspects; the proposed project aims at optimizing agricultural production with a focus on technical aspects.

The main objective is to optimize the production of an alternative fuel (biogas) for agricultural machines. Grass, agricultural residues and industrial organic wastes will be studied as inputs. The biodegradability of these organic materials will be increased through pretreatment with the steam explosion technology. The biodegradability of the pre-treated organic materials will be analyzed and optimized in the laboratory. The final step of the second objective will be an analysis of different biogas utilization concepts, such as the liquefaction of biogas or peak load power production in cluster biogas plants.

Following a holistic approach, this concept will be assessed in selected tourism regions to demonstrate the potential of renewable energy production. In addition, the project will also investigate the impact of climate change on the productivity of grassland.

The Project CO2USE – Utilisation of purified carbon dioxide from flue gas for the production of polyhydroxybutyric acid by phototrophic fermentation

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In this project, purified carbon dioxide from the flue gas of a power plant is used for the cultivation of phototrophic microorganisms. Many plants and microalgae have evolved so that their biochemical pathways of CO_2-fixation have adapted to atmospheric CO_2-concentrations. Their growth rate cannot be influenced decisively by increased CO_2-concentrations. However, with cyanobacteria and purple bacteria much higher growth rates can be achieved by increased CO_2-concentrations if sufficient light is available. Therefore, they are the target organisms in this project. Especially cyanobacteria (aerobic), but also purple bacteria (anaerobic) are able to produce PHB (polyhydroxybutyric acid) as energy reserves.
The state-of-the-art production process for PHB is quite complex and expensive, the price of PHB lies currently between 3.7 and 15 € /kg. PHB is mainly produced via fermentation where sugars or sugar-rich substrates are used. With the increasing global demand for these substrates (food, feed, biofuel, etc.), the utilization of CO_2 as substrate – as intended in this project – could improve the performance of PHB production. In addition, over 250 million t of plastics are produced per year in the world. To replace even a small amount of these with PHB as a biodegradable polymer would improve sustainability and pollution problems.
Such a photo-fermentation process should not only produce a valuable product, but also provide energy and the demanded nutrients. Therefore, the residual biomass will be used in an anaerobic digestion process for the generation of energy, for a closed and efficient production cycle. In addition, by anaerobic digestion the nutrients are mineralised and will be reused as nutrients for the growth of the phototrophic microorganisms in order to become independent from unsustainable fossil nutrient addition. Any loss and separation of nutrients from the process can be replaced by adding co-substrates to the biogas fermentation.

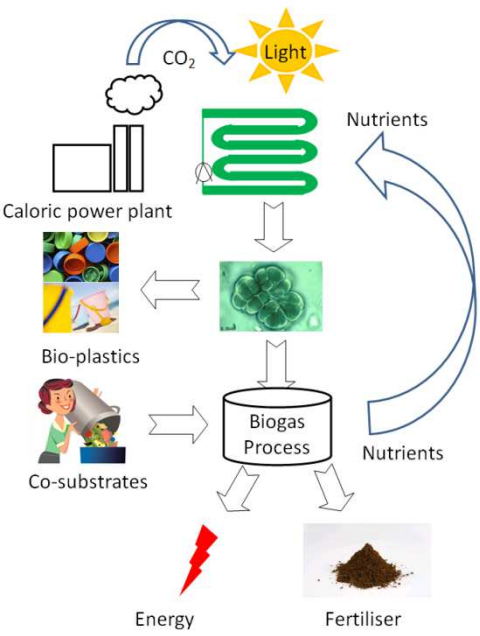

Figure 1: Overall production process investigated in the project CO2USE

Cutinase based degradation of polyesters

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The legislative and environmental pressure to reduce polymer and packaging waste is increasing. For this reason there is a strong demand to find and improve polyesters that are not only biodegradable but also meet the requirements of expected material properties. Ecoflex® is a polyester which shows promising material features and different studies have already proven its biodegradability. Nevertheless, there is considerably less known about enzymatic hydrolysis of Ecoflex that plays a crucial role during the degradation process.

In the here presented study, the enzymatic degradation of Ecoflex and oligomeric Ecoflex model substrates was mechanistically studied. On this account the substrate specificities of two enzymes namely a Cutinase from *Humicula insolens* (*Hi*C) and Cutinase 1 from *Thermobifida cellulosilytica* (*Thc*_Cut1) were compared and analyzed. The hydrolysis of the substrates was followed over time by means of HPLC-MS analysis and reaction products were quantified. It is remarkable that the two enzymes show a distinct hydrolysis mechanism for Ecoflex and the model-substrates.

Promising Fusarium head blight resistance in durum wheat

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Cultivated tetraploid wheat, especially durum wheat (*Triticum durum*), is highly susceptible to the wide-spread disease Fusarium head blight (FHB). While many resistance QTL have been reported in hexaploid wheat (*Triticum aestivum*) the QTL identified in tetraploid wheat do not provide satisfactory FHB resistance. To overcome *T. durum* susceptibility attempts have been made to introgress resistance alleles from wild and cultivated relatives. In this study, back-cross lines derived from crosses of *T. durum* and FHB resistance sources including *Triticum dicoccum* (cultivated emmer), *Triticum dicoccoides* (wild emmer) and *Triticum aestivum* (bread wheat) have been used as resistant parental lines in several bi- and multi-parental crosses with *T.durum.* A large population has been developed allowing the evaluation of FHB resistance derived from relatives in an agronomically acceptable durum background.

This population was evaluated in 2012 in disease nursery through artificial inoculation at BOKU University in Tulln (Austria). FHB disease symptoms were visually scored, morphological (plant height) and phenological (flowering date) traits were recorded. The population showed a large genetic variation for the different traits. More interestingly, a large spectrum of response for FHB resistance was observed among the lines ranging from highly resistant to susceptible. These first results are promising and need to be confirmed in following trials.

A subset of 500 lines will be analysed through both linkage and genome-wide association mapping. The lines will be genotyped in high-density at INRA Clermont-Ferrand (France) using *GENTYANE* platform and phenotyped at two locations: Florimond-Desprez in Cappelle-en-Pévèle (France) and BOKU University in Tulln. Through this project, we expect to unveil QTL linked with resistance and/or increased susceptibility and to evaluate the importance of epistatic interactions for FHB resistance in durum wheat.

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H_2S and NH_3 tolerance of acidophilic sulfur-oxidizing bacteria

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H_2S and NH_3 are toxic, corrosive and odorous gases that are released as byproducts during anaerobic digestion. Although biological techniques for the treatment of the resulting biogas are available, high H_2S and NH_3 contents have been reported to inhibit the microbial activity of sulfur-oxidizing bacteria that are involved in the removal process [1]. In biological methods H_2S is oxidized to either elemental sulfur or, at elevated O_2 concentrations, to sulfuric acid. This work investigated the tolerance of acidophilic sulfur-oxidizing bacteria towards increasing ammonia and sulfide concentrations during microbial conversion of H_2S to sulfuric acid. The study consisted of two sets of bacteria incubations with either increasing ammonia or sodium-thiosulfate concentrations of 1.5, 3.0, 5.0, 7.5 and 10.0 g/L. Sodium-thiosulfate was used as model substrate mimicking H_2S. Results show that even 10.0 g/L ammonia or sodium-thiosulfate did not completely inhibit H_2S oxidation. Nevertheless, a prolonged adaptation phase was observed for both compounds at concentrations above 5.0 g/L. At ammonium concentrations of 1.5, 3.0 and 5.0 g/L the pH dropped from initially 4.5 to 1.1 on day 5 indicating the production of sulfuric acid, whereas with 7.0 g/L ammonium the same pH was reached only after 12 days. With 10.0 g/L pH dropped to a final minimum of 1.3 on day 16. The same trend was observed with increasing sodium-thiosulfate concentration. When incubated with 7.5 and 10.0 g/L sodium-thiosulfate pH slightly increased during the first 9 days of incubation and was followed by a sharp drop until a final value of ~1.7 on day 13. At 1.5 and 3.0 g/L no such adaptation phase occurred. Using the example of sodium-thiosulfate it was proven that although an inhibitory effect does occur at higher ammonium and sulfide concentrations, the sulfur-oxidizing bacteria efficiently convert toxic H_2S to sulfuric acid after a short adaptation phase.

[1] Lee, EY, Cho, K-S, Ryu, HW (2005): Simultaneous Removal of H2S and NH3 in Biofilter Inoculated with *Acidithiobacillus thiooxidans* TAS. Journal of Bioscience and Bioengineering 99/6: 611-615.

Functional analysis of semi-dwarf genes in relation to *Fusarium* head blight response in wheat

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Semi-dwarf genes have been introduced to elite wheat varieties since the late 60s as the development and higher application of fertilizers and the development of higher yielding varieties led to lodging (falling over of plants)

One of the most important semi-dwarf genes *is Rht-D1b* that is able to down regulate the effective power of the growth hormone gibberelic acid. This negative regulation leads to dwarfed plants.

Fusarium head blight (FHB) is a devastating disease of wheat leading to high economic losses and a highly decreased yield. Problematic for animal and human health are also toxins produced by the fungi responsible for the infection with FHB (mainly *F. graminearum*). Different wheat varieties show a different resistance level towards FHB and this is due to the presence or absence of multiple quantitative trait loci (stretches of DNA that are responsible for a certain trait).

The advantage of semi dwarf genes for productivity is decreased by a higher susceptibility to FHB. The mutated allele *Rht-D1b* is strongly associated with enhanced FHB susceptibility. Why is this? There are two hypotheses. One is linkage drag meaning that *Rht-D1b* is linked with a nearby susceptibility conferring allele. The other possibility is pleiotropy meaning the gene is responsible for several traits.

To shed light on this crucial question for wheat breeders we want to give answers with a transgenic approach. We have generated transgenic plants harbouring the gene *Rht-D1b* disconnected from its natural genetic background. These plants will be crossed with medium resistant, tall lines. For comparatistics we will in addition generate near isogenic lines harbouring the same gene at its natural genetic locus. If we then after the inoculation with *F. graminearum* see no differences between transgenic and non-transgenic lines it is rather pleiotropy and not linkage drag.

Multi-mycotoxin applications with QqQ and QTOF-instruments

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Mycotoxins are secondary fungal metabolites occurring in a wide range of food and feed matrices. They have the capability of causing acute toxic, carcinogenic, mutagenic, teratogenic, and immunotoxic effects in animals and humans.

Two approaches of how various mycotoxins can be determined simultaneously in food and feed samples using liquid chromatography coupled to mass spectrometry (LC-MS/MS) are presented. Both of them are based on a simple sample preparation using an acidified acetonitrile-water mixture and a generic LC-method for the separation of the analytes. The first one is a targeted approach using highly sensitive triple quadrupole mass spectrometers (QqQ). For each analyte, precursor and product ions, as well as collision energies and other compound-dependent parameters have to be optimised prior to the implementation into the LC-MS/MS method. In general, two more or less specific transitions are chosen per compound. The second approach is based on the acquisition of high resolution spectra gained by a quadrupole-time of flight (QTOF) instrument. For this application, an exact mass LC-MS/MS library for mycotoxins was created which then allows an unambiguous identification. With this approach also post-acquisition data analysis is possible because in contrast to the QqQ application, full scan data are gathered. Concluding, both applications are powerful tools for the screening of various mycotoxins in food and feed samples.

Immobilisation of Cellobiose Dehydrogenase as an antibiofilm agent on silicon catheters

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Catheter associated urinary tract infections (CAUTI) are one of the most frequent healthcare associated infections which are caused by biofilm forming pathogens colonizing the catheter surface. One of the main reasons for the resistance of microorganisms living in biofilm communities is a protective network of exopolysaccharides. In this study cellobiose dehydrogenase (CDH) was used as an antimicrobial system to prevent the colonization and formation of biofilms on urinary catheters by microbial pathogens. CDH has the ability to reduce molecular oxygen to the effective antimicrobial agent hydrogen peroxide (H_2O_2) using a broad range of di-, oligo- and polysaccharides as electron donors. *In vitro* liquid studies showed that 0,3 U of CDH in combination with 2 mM of cellobiose have a strong antibiofilm effect against *E. coli* and *S. aureus* inhibiting the formation of biofilm by 60 and 95 % respectively. Interestingly, the CDH was also able to use *E. coli* and *S. aureus* exopolysaccharides as substrates for the production of H_2O_2 in the additional presence of amylase. Further studies aimed at covalently immobilizing CDH on to the silicon polymer showed that pre-activation with plasma was important to activate methyl groups on the silicon yielding reactive hydroxyl groups. When glutaraldehyde was used as a cross-linker/spacer arm in coupling of CDH to the generated hydroxyl groups, hydrogen peroxide production by the immobilized CDH was demonstrated. This modified polymer surface could be an answer to ongoing problems concerning multi-resistant biofilm forming bacteria with a variety of applications in the biomedical field.