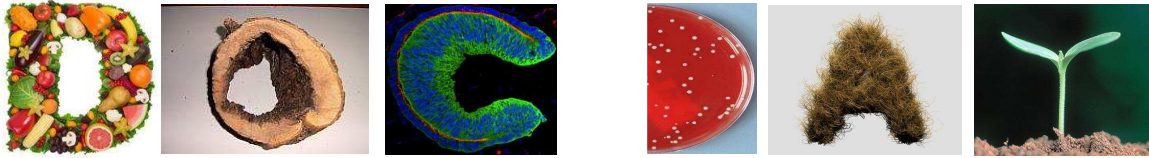


2nd



PhD Conference

UFT Seminar Centre, Tulln, Austria
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University of Natural Resources
and Life Sciences, Vienna



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DocDay 2014, Tulln

2nd DocDay- Book of Abstracts

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Conference Program

08:30 – 09:00	Conference opening and Registration Entrance of Seminar Centre UFT, Tulln
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09:40 – 11:00	Session 1: Plant and Soil 09:40 – 10:00 Johannes Kisser Selection, application and metal recovery from hyperaccumulating plants grown on waste incineration residues 10:00 – 10:20 Imran Hussain Stimulated phytoremediation of petroleum hydrocarbons in a freshly spiked soil 10:20 – 10:40 Ana Bejarano Inoculation of maize seeds with plant growth promoting bacteria by the Layer-by-layer method 10:40 – 11:00 Lukas Kühnen From Mitigation towards Adaptation: A Socio-economic Perspective on Sustainable Carbon Sequestration
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15:30 – 16:30	<p>Session 4: Novel Approaches 15:30 – 15:50 Anastassiya Tchaikovsky “Elemental and isotopic map of the Danube”- Analysis of the elemental composition and $^{87}\text{Sr}/^{86}\text{Sr}$ from source to mouth using (MC) ICP-MS 15:50 – 16:10 Katharina Meixner Cultivation of <i>Synechocystis cf. salina</i> in a tubular photobioreactor at pilot scale for photoautotrophic PHB-production 16:10 – 16:30 Marina Smerilli Production of lactic acid from biomass residues</p>
16:30 – 17:00	<p>Closing remarks and Award Ceremony Best Talk / Best Poster</p>
17:00 – 19:00	<p>Oktoberfest for PhD Students in Tulln- Get together with Weißwurst, Brezn and Weißbier</p>
19:00	<p>Official End of Conference</p>

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Abstracts Session 1:

Plant and Soil

Selection, application and metal recovery from hyperaccumulating plants grown on waste incineration residues

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Abstract

Sewage sludges as well as ashes and slags from waste incineration plants are known accumulation sinks of many elements that are either important nutrients for biological organisms (phosphorus, potassium, magnesium, etc.) or valuable metals in pure form (nickel, chrome, zinc, etc.); they are also serious pollutants when they occur at anthropogenic end- of-stream points. Often more than 90% of these same metals have to be imported from abroad for technological use. These primary resources are becoming more expensive as they become more scarce and remaining deposits more difficult to mine, which is a serious concern for industrialized nations.

The project BIO-ORE aimed to explore new pathways to concentrate metals from diluted sources such as sewage sludge and wastewater by using highly efficient biological absorption and transport mechanisms. These enzymatic systems from plants work with very little energy input. The process is called bioaccumulation and can be most effectively observed in hyperaccumulating metalophytes, which are studied for its suitability to be incorporated in metal recovery processes.

In a systematic series of tests under laboratory conditions the accumulation behaviour for a variety of metals of a selection of candidate plants growing on different waste streams was assessed (quantitatively and qualitatively). The results provide the groundwork for further research that may bring to practical implementation a technological option with potentially huge benefits:

- The recovery of valuable metal resources from waste by environmentally friendly and low energy means
- Simultaneous decontamination of the input substrates from heavy metals
- Simultaneous generation of biomass, which can yield usable energy surplus

All in all, the results look very promising to keep up the work and stretch it to other waste streams. Therefore a national follow-up research project started in 2014, where two years of field trials with hyperaccumulative plants and further phytomining strategies will be developed.

Stimulated phytoremediation of petroleum hydrocarbons in a freshly spiked soil

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Abstract

Remediation of organic contaminants in soils is a challenging problem in environmental science and engineering. Phytoremediation is a promising technology that uses plants and associated microorganisms for the remediation of petroleum hydrocarbons (PHC). *Lolium multiflorum* and *Lotus corniculatus* were used in a pot experiment in the greenhouse to compare phytoremediation rate in a freshly spiked soil having different amendments. The effect of simple plant growth in comparison to additional treatments with nutrients and biochar as well as inoculation of plants with PHC-degrading microorganisms was investigated. Plant performance was compared between the non-contaminated soil and freshly spiked soil.

Spiked soil was made by mixing sand and loess in a proportion of 9:1 to obtain a uniform mixture. The spiking concentration for diesel was 22.65 ml/kg soil. To stimulate phytoremediation nutrients, biochar and PHC-degrading microbial strains were added. Nutrients NH_4Cl (150 mg/100 g soil) and KH_2PO_4 (20 μg /100 g soil) were added, biochar was mixed with soil (5% w/w) and the microbial consortium (mixture of strains: *Pantoea* sp. strains, ITS110 and *Pseudomonas* sp. strain, MixRI75) was added by seed inoculation before sowing.

Germination percentage (GP) was monitored weekly until three weeks after seed sowing. Biometric parameters (plant height, fresh and dry weight of shoots) and leaf chlorophyll content were recorded in periodic intervals. Soil samples were taken in regular intervals and PHC content was measured by GC-FID. The pot experiment started in spring 2014 and is planned until autumn 2015. The current results show that the germination rate of both plant species was reduced on spiked soil compared to the non-contaminated control. However 49 days after sowing biomass was significantly lower on spiked soil. Amendment of nutrients and nutrients + microbial inoculation increased the biomass of *Lolium multiflorum*, but not in *Lotus corniculatus* in spiked soil. During the application of this abstract only three plant harvest was performed. In the presentation we will report about the development of plants and the degradation of PHCs in the first 8 months of the experiment.

Inoculation of maize seeds with plant growth promoting bacteria by the Layer-by-layer method

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Abstract

Pollution problems generated by current agricultural practices may be moderated considering better agricultural practices as for example the application of biotechnological processes that included bacteria that promote plant health.

Seed coating is a general technique for inoculation of plants and is the most reliable way to apply biocontrol/biofertilizer agents in close proximity with germinating seeds. However, commercialization of preinoculated seeds still encounters limiting factors, mainly due to poor bacteria survival.

Hence the main aim of our work was to create “artificial spores” [1] based on multilayer coatings [2] with good mechanical stability and selective permeability in order to develop environmentally friendly formulation technologies with increased product shelf life and efficient release and activation of encapsulated biomaterials.

To address this challenge we have developed new surface coating solutions based on polyelectrolyte complexes and inorganic carriers for field application of plant growth promoting bacteria (PGPB) such as *Burkholderia phytofirmans* PsJN [3]. As plant model maize was chosen, due to its nutritional importance.

Coating formulations were non-toxic and biodegradable and included adhesives like gelatin, celluloses, alginate, and xanthan and inorganic carriers, such as fumed and precipitated silica or talc. Bacteria concentration used for encapsulation was 10^9 cfu/mL of coating agent. Single and multilayer coatings were prepared. Multilayer coatings were fabricated by alternating coating with positively (e.g. chitosan) and negatively charged polyelectrolytes (e.g. gelatin). Characterization of bacteria-seed interfaces and bacteria-capsules was carried out by Scanning Electron Microscopy. Cell viability and release kinetics were determined by plating serial dilutions in Luria-Bertani agar. The effect of coating on seed germination was investigated in agar plates.

Results compiled up to now showed that viability of bacteria included in the coating was reduced by up to 40% during the processing step, while most of bacteria present in the formulations were released within the first hours after submersion. Favorable effects in germination were observed, such as germination of seeds treated with bacteria was enhanced by up to 60% compared with the untreated controls.

1. Yang et al. (2012). Artificial spores: cytocompatible encapsulation of individual living cells within thin, tough artificial cells. *Small*. 9: 178-86.
2. Domnanich et al. (2011). Xanthan/chitosan gold chip for metal enhanced protein biomarker detection. *Biosens. Bioelectronics*. 26:2559-65.
3. Mitter B. et al. (2013). Genome analysis, ecology and plant growth promotion of the endophyte *Burkholderia phytofirmans* strain PsJN. In *Molecular microbial ecology of the rhizosphere*, F.J. de Bruijn (Eds.), Wiley-Blackwell publishing (USA).

From Mitigation towards Adaptation: A Socio-economic Perspective on Sustainable Carbon Sequestration

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Abstract

Human actions on land resource systems strongly influence a variety of environmental factors such as the functions provided by ecosystems. Soil management has an imminent important role especially for maintaining the basis of a sovereign biological food production as well as the recycling of carbon and oxygen into natural processes. However, beside all different kinds of ways in modern agriculture advance further, farming practices still lack substantial contributions to diminish the negative effects of soil depletion. But different strategies of unconventional approaches that try to improve their influence on the environment are numerous, though they often get easily confronted with a range of socioeconomic barriers. Addressing just and equal shares of expenditures and gains are always difficult to become determined. To find solutions for these hindrances, it is necessary to overlook the dynamic complexity of socio-ecological and economic processes that are connected to them. A screening of literature in relevant natural and social disciplines shows a picture of a very heterogeneous field of approaches and theories, as well as techniques that are being used resulting in diverging outcomes. Research on sustainability and global warming in regard to soil subjects are demanding especially trans-disciplinary designed approaches which help to close this gap.

Therefore a multifaceted, multi-scale and multi-stakeholder approach is emphasised to transform and install a transparent set up, which also enables the integration of people from different sides related to the topic. The aim is to foster a more democratic process of decision making, where stakeholders are enhanced to evaluate defined options of agricultural practices, under different criteria (e.g.: effectivity, durability etc.) and different dimensions (ecologic, economic). Opportunities are numerous (e.g.: no till, cover crops, manure and compost or other constituents for soil restoration like bio char applications), but their sustainable development from strict mitigation towards integral adaptation remains challenging.

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Abstracts Session 2:

Biogas and Bioenergy

Reed as an alternative biomass source for biogas production

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Abstract

The utilization of crops for biofuel production competes directly with their use as food. Using lignocellulosic residues as a raw material for the production of biofuels such as biogas, can alleviate this competition. Common reed is a lignocellulosic perennial grass, recognized as a promising source for renewable energy due to its high regional potentials. Lignocellulosic biomass like reed requires a pretreatment step prior to anaerobic digestion, in order to allow the hydrolysis of polysaccharides into fermentable sugars. One efficient pretreatment method for hard degradable biomass is steam explosion. It consists of heating the biomass at high temperatures under high pressure achieved by direct steam injection, followed by a sudden pressure drop, which leads to mechanical disruption of the biomass fibers. This pretreatment allows an increase of methane yields and degradation speed. The aims of this study were to determine the specific methane yields of steam-exploded reed as well as to identify how the different pretreatment conditions influence its physico-chemical characteristics. Thus, reed was pretreated in a steam explosion unit, with temperatures ranging from 160 °C to 230 °C and residence times of 5, 10 and 15 min, and its effect on the methane yields was analyzed in batch experiments. For every pretreatment, scanning electron microscopy (SEM) pictures and detailed chemical analyses of the substrates were carried out. The results show that with increasing the pretreatment intensity up to 200 °C for 15 min, the methane yield improves around 90 %. Then, it decreases under harder conditions, which may be due to the formation of inhibitors. Optimizing the pretreatment process can help decrease current competition between biofuels and food production for raw material, by ensuring good methane yields from lignocellulosic residues.

Microalgae as Source of Biogas: Anaerobic Digestion of Untreated and Pretreated Biomass

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Abstract

Bioenergy production is strongly dependent on biomass. Since microalgae can be grown on wastewater or non-arable land, and, as theoretical yields per hectare are much higher than for energy crops, they have the potential to become an additional and/or alternative feedstock for biogas plants. In this study, two green-algal strains, namely *S. obliquus* (SAG 276-1) and *C. emersonii* (SAG 2334) were grown in a sleeve-bag photobioreactor system (production volume: 0.7 m³). Mean productivity ranged between 21.2 mg TSS (=total suspended solids) L⁻¹ d⁻¹ for *S. obliquus* and 25.5 mg TSS L⁻¹ d⁻¹ for *C. emersonii*. Microalgae were concentrated with a disc stack separator to final volatile solid-concentrations (VS) of 51.7 g VS L⁻¹ (*C. emersonii*) and 90.5 g VS L⁻¹ (*S. obliquus*). Referred to total solids (TS) total lipid content, being the most important substance class for anaerobic digestion, was higher in *C. emersonii* (n=2, 29.1 % TS⁻¹) compared to *S. obliquus* (n=2, 25.7 % TS⁻¹). *Scenedesmus* is known to have thick cell-walls; cells are difficult to decompose, and therefore, a screening of different pretreatments was conducted with *S. obliquus* biomass and coherently tested for biochemical methane potential (BMP). Most significant methods were ultrasonication (+52.6 % CH₄ yield VS⁻¹_{untreated}) and milling (51.2 % CH₄ yield VS⁻¹_{untreated}); these pretreatments were applied in a follow-up experiment to *S. obliquus* and *C. emersonii* biomass.

For both, *S. obliquus* (+40.3%, 321 Nm³ t⁻¹ VS_{untreated}) and *C. emersonii* (+32.8%, 307 Nm³ t⁻¹ VS_{untreated}), ultrasonication led to significantly higher methane yields compared to untreated controls (pairwise t-tests, p-values ≤ 0.05, n=3, bonferroni-adjusted values). Further, pretreatment was evaluated by analyzing the solubilized chemical oxygen demand (sCOD) which showed a significant positive correlation (r = 0.72, p ≤ 0.05). The results underline the importance of pretreatment of microalgal biomass prior to anaerobic digestion and verify the sCOD as useful parameter for pretreatment evaluation.

Closing the nutrient cycle in anaerobic digestion of industrial waste streams

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Abstract

Industrial wastes or side-products provide a valuable substrate alternative for anaerobic digestion as substrate shortage is hindering the expansion of renewable energies such as biogas. Especially the brewing industry and abattoirs could profit as their in-house demand for process heat and energy can be partly covered with biogas produced from waste streams. In the case of slaughterhouse waste the conversion from waste to energy even saves disposal costs in addition to the provided heat for the necessary substrate sanitation. To fully exploit the energy potential of these wastes pre-treatment and/or desulfurization of resulting biogas are required before further utilization.

High levels of toxic, corrosive and odorous hydrogen sulfide (H₂S) are present in biogas produced from both industrial wastes due to acidic pre-treatment of brewer's spent grain – the major side-product in brewing industry – or the high content of protein in slaughterhouse waste. Therefore, microbiological oxidation of H₂S was performed in a continuous desulfurization column with immobilized sulfur-oxidizing bacteria. Results demonstrate that specialized acidophilic bacteria are able to convert H₂S aerobically to sulfuric acid until pH below 1 which corresponds to acid concentrations applied during substrate pre-treatment. Additionally, the extensive requirement for fresh water during microbiological H₂S conversion can be reduced by replacing up to 50% with the liquid fraction of digestate, a residue of anaerobic digestion rich in nitrogen, phosphorous and trace elements. By introducing the liquid fraction of digestate as substitute for cultivation medium in the desulfurization process the demand for fresh water and synthetic medium can be minimized in combination with a sustainable nutrient recycling.

Summing up, industrial residues and side-products provide a good substrate alternative for anaerobic digestion. Specifically the energetic conversion of brewer's spent grain and slaughterhouse waste can contribute to energy self-sufficiency within industry. The combination of substrate pre-treatment and biogas desulphurization enables closing the nutrient cycle in anaerobic digestion. Thus, the base for an efficient utilization of industrial waste streams is created.

Abstracts Session 3:

Human and Environment

Cellobiose Dehydrogenase – antimicrobial functionalization of polydimethylsiloxane

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Abstract

Polydimethylsiloxane (PDMS) is a polymer widely used in biomedical devices especially in urinary catheters due to its favorable biocompatibility properties. Even though PDMS lowers the risk of infections due to its low surface tension and high hydrophobicity, catheters are usually colonized by microorganisms in most patients leading to urinary tract infections. In order to decrease the risk of infection, an antimicrobial enzyme, namely cellobiose dehydrogenase (CDH) was successfully grafted onto PDMS surface. The system is based on the ability of CDH to use oxygen as electron acceptor and different oligosaccharides (e.g. cellobiose) as electron donors to produce H₂O₂. Several approaches of immobilizing CDH on PDMS surface were exploited including surface activation using oxygen plasma followed by covalent linkage of CDH as well as layer by layer coating techniques. Success of the immobilization process was monitored by analyzing the change in the functional groups on the surfaces by FTIR measurements as well as measuring the ability of grafted CDH to produce H₂O₂. CDH was successfully immobilized on the surface of PDMS as evidenced by H₂O₂ production in the presence of cellobiose. The CDH modified PDMS catheters could help to prevent current problems of microbial colonization and multidrug resistant bacteria associated with catheters.

Gut microbiota composition associated with different degrees of impaired glucose metabolism

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Abstract

The prevalence of overweight and obese people has increased dramatically in the last 20 years. Associated metabolic and cardiovascular disorders negatively affect the quality of life and may lead to premature death. Recently, the human intestinal microbiota has been implicated an important role in the host energy metabolism and in the development of metabolic diseases. To gain a better understanding of the underlying host-microbiota relationship, we examined the composition of the intestinal bacterial communities in overweight or obese individuals showing varying degrees of impaired glucose metabolism.

For this pilot study, we selected 20 patients aged 58 to 71 years with normal glucose metabolism, prediabetes, or type 2 diabetes mellitus, respectively, among a cohort that was recruited in Salzburg and Vienna. Clinical parameters related to energy metabolism were assessed, and microbial DNA was isolated from stool samples using the Maxwell® 16 Tissue DNA Purification Kit together with the Maxwell® 16 Instrument with additional lysozyme treatment. The composition of the gut microbiota was analyzed through barcoded 454 sequencing of 16S rRNA amplicons (V1-V3).

Members of the Prevotellaceae and Erysipelotrichaceae bacterial families were enriched in the microbiota communities of individuals with impaired versus “healthy” glucose metabolism. Several clinical parameters of the glucose-metabolism were associated with differences in the gut microbiota composition among the study participants, as revealed in a canonical correspondence analysis. Leptin, an inflammatory marker and indicator of elevated body fat mass, appeared to be linked to the intestinal bacterial community composition specifically in women. The alpha-diversity of the gut bacteria communities, however, was not affected by the degree of metabolic impairment.

An improved understanding of the interactions of the gut microbiota and host metabolism in health versus metabolic disease may contribute to the future development of novel, microbiota-based pharmacological and dietary approaches to controlling obesity and associated metabolic disorders.

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

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Abstract

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.

Abstracts Session 4:

Novel Approaches

**“Elemental and isotopic map of the Danube”-
Analysis of the elemental composition and $^{87}\text{Sr}/^{86}\text{Sr}$ from source to
mouth using (MC) ICP-MS**

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Abstract

The Danube is the second largest river in Europe and connects- from its source in Germany to its mouth at the border of Rumania and the Ukraine- 10 countries. The composition of dissolved elements and their isotopic ratios reflect hereby the changing environmental and anthropogenic factors along the course of the river. In addition, site specific “elemental pattern” can be used as tracer for ecological studies, like habitat and migration studies of fish or birds.

During the Joint Danube Survey 3 in 2013 – the world’s largest scientific river expedition so far- water samples from 68 sites in the Danube and its most important tributaries were collected from source to mouth. The water samples were filtered and analyzed for the composition of dissolved macro and micro elements using ICP-QMS and the $^{87}\text{Sr}/^{86}\text{Sr}$ using MC-ICP-MS.

The results show pronounced regional variations of the elemental and $^{87}\text{Sr}/^{86}\text{Sr}$ composition along the course of the river as well as significant differences of these parameters between the Danube and its tributaries. These results allow amongst others conclusions on regional morphologies, weathering rates (e.g. Ca/Mg and Ca/Na) and microorganism activity (e.g. Ca/Si). For instance, a significant decrease of Si was observed in the region of the two largest dams in the Danube (Iron Gate I and Iron Gate II – in Serbia and Romania), which could be induced by a large population of diatoms in the reservoir.

Future efforts will focus on the integration of the data in a geographic information system (GIS) allowing for the first time the creation of an elemental map and subsequently the investigation of the eco-geochemical status of the entire Danube catchment.

An abstract with similar content was submitted for an oral contribution at the ICPMS Usermeeting 14th -18th September 2014 in Geestacht, Germany.

Cultivation of *Synechocystis cf. salina* in a tubular photobioreactor at pilot scale for photoautotrophic PHB-production

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Abstract

Currently, alternatives for non-biodegradable petroleum based plastics are searched for. One of these alternatives are biodegradable polyhydroxyalkanoates (PHA), including polyhydroxybutyrate (PHB). In the state of the art PHB-production process sugar is used as carbon source for heterotrophic bacteria. As an alternative, CO₂ from exhaust gas can be used in photoautotrophic PHB-production which reduces global demand for crops (food, feed, biofuel, etc.).

For evaluating photoautotrophic PHB-production a 200L tubular photobioreactor was constructed (see Figure). It consists of 20 glass tubes (3m, ID 60mm) in two layers, a compensation vessel and a pump, circulating the cyanobacteria through the reactor. The CO₂ injection is coupled to the pH-value of the medium. O₂-concentrations, pH-values, temperature and light intensity are measured online. The photobioreactor is situated in a greenhouse and was, in addition, artificially illuminated with a day/night cycle of 16:8. In the reactor the cyanobacteria *Synechocystis cf. salina* (CCALA 192) was cultivated in modified BG11 media. Cultivation conditions were a pH-value of 6.7, temperature of 22°C, salinity of 1‰ and a light intensity of 10 W*m⁻² which had been determined as optimal parameters in previous laboratory experiments.

The pilot-scale fermentation could be demonstrated successfully. An optical density (750nm) of 8.74 was achieved within 21 days. The cyanobacteria achieved final PHB-concentrations of 3.8%*TS⁻¹ which equals 77 mg*L⁻¹. During the exponential phase a TS productivity of 0.2 g*L⁻¹*d⁻¹ and an O₂-concentration of max 25 mg*L⁻¹ was reached, whereas TS productivity and O₂-concentration decreased in the stationary phase to 0.06 g*L⁻¹*d⁻¹ and 9 mg*L⁻¹, respectively. At the end of the exponential phase, when nitrogen was limited, the culture started to turn from dark green to orange, which is an indicator for the starting PHB-production. In average the photobioreactor was illuminated with 11 W*m⁻², on sunny days maxima of 25 W*m⁻² were reached.



Figure Tubular photobioreactor at pilot scale for photoautotrophic PHB-production by *Synechocystis cf. salina*.

Production of lactic acid from potato residues from food processing

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Abstract

In the region of Navarra (Spain) 2 kt of waste potato peels and puree accumulate as food processing residues every year. They are collected from the local food industries between July and September and currently represent only a disposal burden.

Potato residues are rich in starch (46 % in dry matter), which constitutes a valuable carbon source for many microbial products. Among them lactic acid (LA) is particularly promising, since the worldwide production capacities are continuously increasing since two decades and producers are interested in origins not in competition with food and feed. Furthermore, variable amounts (6 ± 2 % in dry matter) of LA are found already in the potato by-products.

The potato mash was treated with commercial amylases at 60-70 °C for one day in order to release glucose (167 g/l) from starch. The hydrolysate was used as the substrate in 3 l batch fermentations with *Lactobacillus casei* at 37 °C and pH 5.5. After 66 hours all the glucose was used up and the final LA concentration was ca. 100 g/l, with a yield of 92 %. The optical purity of the product (L-LA) was only 89%, mainly due to the amounts of D-LA already present in the potato by-product. The produced LA is not suitable for the PLA industry, but it meets the current standards for technical grade LA.

Poster Presentations

1. Diversity of secondary metabolite gene clusters - lipopeptides and polyketides from plant-associated *Bacilli* and *Pseudomonas* spp.

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Abstract

Plant-associated *Bacilli* and *Pseudomonas* produce structurally diverse secondary metabolites, mostly lipopeptides (LPs) and polyketides (PKs). These secondary metabolites are extensively studied for their plant growth promotion and biocontrol application in agriculture. Lipopeptides and polyketides are non-ribosomally synthesized via large enzymes known as non-ribosomal peptide synthetases (NRPS) and polyketide synthases (PKS), respectively. The encrypted sequence information available in the gene clusters encoding for these enzymes will provide us access to the potential of the production of structurally diverse secondary metabolites. Despite the fact that next generation technology (NGS) technology has rapidly amplified amount of the whole genome sequence data and increased the sequence resources, yet little is accomplished to explore the sequence data, to identify novel NRPS and PKS gene clusters, to the production of novel secondary metabolites. In this study, we will use secondary metabolite prediction tools to predict secondary metabolites production, based on sequence information. Using next generation sequencing, we will study diversity and novelty of NRPS and PKS gene sequences from the uncultivated, to estimate the potential of LPs and PKs for these two genera in plant associated environments.

2. The role of VELVET in communication between potential mating partners

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Abstract

Sexual development in the filamentous ascomycete *Trichoderma reesei* was described only a few years ago. The pheromone system of this fungus comprises the two pheromone receptors *hpr1* and *hpr2* as well as the alpha type peptide pheromone precursor gene *ppg1* and the unusual, named h-type peptide pheromone precursor gene *hpp1*, which assumes a-type function. Regulation of sexual development by light and the photoreceptors BLR1, BLR2 and ENV1 was shown. Hence we investigated the function of VEL1 (VELVET1), another factor potentially involved in regulation of light dependent development in *T. reesei*.

Vel1 is regulated by ENV1 in light and was found to be essential for conidiation and cellulase gene expression. Strains lacking *vel1* show a growth defect and are not able to mate in darkness. In light, *vel1* is essential for female fertility in both mating types, while male fertility remains unaffected.

VEL1 does not influence transcript abundance of *env1* but impacted regulation of the pheromone system (*hpr1*, *hpr2*, *hpp1*, *ppg1*) in a mating type dependent manner and depending on the presence of a mating partner. Additionally we found that strains lacking *vel1* respond differentially to wild-type and *vel1*-deletion strains. A partner effect was also found for the mating type gene *mat1-2-1*. Analysis of secondary metabolite patterns further revealed an involvement of secondary metabolites in partner recognition in *T. reesei*.

3. Characterization of a new alpha/beta hydrolase from *Pelosinus* sp. for polyesters hydrolysis

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Abstract

A new alpha/beta hydrolase gene from *Pelosinus* sp. (*PfL1*) was optimized against *Escherichia coli* codon usage and cloned into the pET26b(+) vector, containing a 6xHis-Tag downstream the polylinker, and transformed into the bacterial cells. *PfL1* is a member of the serine hydrolases containing a modification in the common –GxSxG– motif in the first glycine to have the –AxSxG– motif. The protein expression was induced with 0.08 mM isopropyl- β -D-thiogalactopyranoside (IPTG). Western blotting was carried out for the revelation of the enzyme with an antibody against the His-Tag and further purification was carried out by means of Immobilized Ion Metal Affinity Chromatography (IMAC) with Nickel-coated columns for the binding of the His-Tagged enzyme, following by buffer exchange against 0.1 M Tris-HCl pH 7. The *PfL1* purified was tested in hydrolase activity assay with *para*-nitrophenylbutyrate (*p*-NPB) (Herrero Acero E, 2013). *PfL1* has the pH optimal at 8 (0.1 M sodium phosphate buffer). Phylogenetic analysis and model analysis show similarities with structures containing an extra domain, called “lid”, characteristic feature for lipases, which covers the active site, containing serine, aspartic acid and histidine. The enzyme was able to hydrolyze polyesters such as polybutylene-adipate-co-terephthalate (PBAT), based on HPLC quantification of the solubilized hydrolysis products.

4. Production of PHB from chicory roots

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Abstract

Chicory roots from hydroponic salad cultivation are an abundant food residual in Navarra (Spain) which are underutilized to date. Aiming at a holistic utilization of resources, a process using chicory root hydrolysate for the production of polyhydroxybutyrate (PHB)-containing packaging material made for the locally produced vegetables was designed.

In the first step a hydrolysis protocol was developed with various parameters optimized. The solid fraction, pH, addition of enzymes, effect of autoclaving, as well as temperature were studied. The best results, taking into account the envisaged industrial production process, were obtained with a solid fraction of 10% at pH 4 and 50°C for 6h, adding 3600 U Inulinase mix (Megazyme, Ireland) per kg dry roots. Autoclaving had no negative effect on the sugar concentration. The dried chicory root material is composed of 36% carbohydrates and 78% of this amount are extractable using the designed protocol. Fructose is the main constituent (80% of the extractable carbohydrates), followed by glucose (15%) and some other sugars present in smaller quantities. The hydrolysate contains 34 g/L sugars, 0.43% protein.

Fermentation experiments were conducted in fully controlled bioreactors with approx. 0.5 L working volume using two of the best established strains for PHB-production from fructose. *Cupriavidus necator* DSM 428 reached a biomass concentration of 11.4 g/L with a PHB content of 70% PHB after 6 days. At this point, the fructose is used up and from then on PHB is used slowly as carbon source for maintaining cell viability. The second strain investigated was *Cupriavidus necator* DSM 531, which yielded 8.4 g/L dry biomass containing 64% PHB. Not all the available free fructose is consumed, but the fermentation is slowing down and has not finished after 10 days. The polymer was extracted using chloroform and several physico-chemical parameters determined, which are in good accordance to published results.

Compared to industrial processes, the present process has a comparable yield, but the productivity and cell density still need to be improved in order to be competitive.

5. Environmental Effects of a Novel Pre-treatment Technology for Maize Stover as a Substrate in a Typical Austrian Biogas Plant

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Abstract

In a baseline scenario, modeling current biogas technology, a 500 kW_{el}-biogas plant with off-heat utilization was assumed to be operated with a typical Austrian substrate mix. The system includes upstream production of the maize silage, as well as auxiliary inputs such as chemicals and machinery. The future scenario was modeled with a similar system based on an identical biogas plant, however the sole substrate in this scenario is maize stover. Additionally, the system includes an innovative steam explosion pre-treatment technology.

The potential environmental and energy impacts of both scenarios were quantified with a life-cycle assessment (LCA) approach, and modeled with open-source LCA software. The main functional unit is 1 kWh of electricity generated. Heat, as well as manure or digestate fertilizer were added to the scenarios as needed to ensure equivalent system functionality, particularly with respect to emissions from nutrient management in both systems.

Results for greenhouse gas (GHG) emissions are expressed as a 100-year global warming potential (GWP). Overall GHG emissions were 293 g (baseline) CO₂eq per kWh and 257 g (future) CO₂eq per kWh electricity generated. Of these, up to 60 percent stem from the gas engine making it the most important contributor. Consequently, 60-70 percent of the total GHG emissions are due to lost methane, and only 30-36 percent and about 3 percent are due to carbon dioxide and nitrous oxide emissions, respectively. Further results include other impacts such as on acidification, eutrophication, and land occupation, as well as a detailed assessment of the steam explosion technology's effects.

Conclusions will discuss the relative environmental benefits and improvement potentials of this substrate-pretreatment combination with respect to current biogas technology. This will inform manufacturers of biogas technology, plant operators and other decision-makers as to the potential of pre-treated secondary agricultural substrates.

6. Chemical imaging of phosphorus dynamics in the rhizosphere

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Abstract

Phosphorus (P) is one of the most limiting nutrients to plant growth and crop yield. To increase P uptake, plants can actively solubilise P by releasing e.g. protons or organic anions. Innovative approaches for 2D chemical imaging of rhizosphere processes can be used to investigate the complex interactions of plant roots and soil. Diffusive gradients in thin films (DGT) combined with laser ablation – inductively coupled plasma mass spectrometry (LA-ICP-MS) allow for the 2D visualisation of the distribution of cationic and anionic solute species in the rhizosphere. Furthermore, pH-sensitive planar optodes can map the rhizosphere pH.

The aim of this work was the development and testing of a combined setup of DGT-LA-ICP-MS and pH-sensitive planar optodes to investigate biogeochemical processes controlling P solubility in the rhizosphere. This was achieved by simultaneous imaging of P, Al, Ca, Fe and pH to assess if P solubilisation is related to the dissolution of P sorption sites (Fe- and Al-(oxy)-hydroxides) and of soil P minerals such as Ca-, Fe-, Al-phosphates. To this end, a plant experiment using *Triticum aestivum* L., *Fagopyrum esculentum* L., and *Lupinus albus* L. on calcareous and non-calcareous soils was conducted.

We show the applicability of simultaneous 2D chemical imaging of soluble Ca-, Fe-, and Al together with P using DGT-LA-ICP-MS as well as 2D mapping of plant induced pH-changes based on planar optodes. We conclude that this combined imaging approach offers great potential for investigating P depletion around roots, mineral dissolution due to root activities as well as plant induced pH-changes at sub-mm resolution.

This contribution is also presented at the 5th International Symposium on Phosphorus in Soils and Plants (2014), Montpellier, France.

7. Spice plants under mercury ions stress condition - antioxidants and defense proteins

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Plants during the vegetation process are exposed to plenty of unfavorable environmental factors both abiotic (variation of the temperature, drought, minerals deficiency, salinity, presence of heavy metal ions) and biotic (pathogens) which initiate the activation of defense mechanisms. Direct contact of a plant with microorganisms, injury or environmental stress factors results in an activation of the local resistance response. This response consequently triggers defense mechanisms affecting the whole plant commonly termed *Systemic Resistance* (Kozłowska & Konieczny 2003; Breiteneder & Radauer 2004). *Systemic Resistance* is a physiological state of each living plant maintaining its ability to protect itself against potential pathogens like viruses, bacteria, fungi and insects (Vallad & Goodman 2004). The main objective of systemic defense mechanism activation is the limitation of pathogens spreading and even blocking them in the infected area. *Systemic Resistance* may be reached either by a direct transport of resistance metabolites from infected area to all plant tissues or by synthesis of compounds in not infected tissues (Heil & Ton, 2008).

The purpose of our research is to determine the concentration of plant's metabolites which are involved in plant's defense mechanisms. Presented study considers plants usually used as spice additives in polish cuisine for which there is a risk of presence of defense metabolites responsible for inducing the immunological response in humans. Examined plant materials were representatives of two different plant families from which several species were analyzed. Apiceae family included following plants: coriander (*Coriandrum sativum*), dill (*Anethum graveolens*) and parsley (*Petroselinum crispum*), whereas Lamiaceae family included basil (*Ocimum basilicum*), peppermint (*Mentha piperita*) and oregano (*Origanum vulgare*).

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8. Enzyme based degradation of aliphatic aromatic polyesters

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Abstract

One of the primary goals of polymer development was to design materials of high stability and durability. Today, these features lead to major environmental problems. That is why society is confronted with the need to reduce packaging waste as well as to find and enhance polyesters that are biodegradable and show required material properties. Several studies have clearly demonstrated the biodegradability of the aliphatic-aromatic copolyester PBAT (poly(butylene adipate-co-butylene terephthalate)). Nevertheless, there exists hardly any information about the enzymes that play the major role during PBAT hydrolysis.

In this research study, enzymatic degradation pattern of different hydrolases was tested on polymeric and oligomeric PBAT model substrates. The substrate specificities of a bacterial cutinase from *Thermobifida cellulosilytica* (*Thc_Cut1*), a fungal cutinase from *Humicola insolens* (HiC) and a polyhydroxyalkanoate depolymerase (ePhaZmcl) from *Pseudomonas fluorescens* was evaluated. The degradation process was followed over time and hydrolysis products were analyzed and quantified via HPLC-MS. The difference between the temperature where the degradation takes place and the melting temperature (T_m) of the polymer was reported to be a crucial factor for enzymatic hydrolysis. Consequently a TG-DSC analysis was performed for PBAT and all tested model substrates and T_m values were taken into account during the evaluation of the degradation results. It is notable that the enzymes show a distinct mechanism for the model substrates and for PBAT.

9. Development of electrically conductive biopolymer materials by means of unmodified carbon nanotubes

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Abstract

Based on recent data bioplastic production capacities in Europe have been forecasted to increase by 400% between 2012 and 2017. Bioplastics are mainly based on biopolymers which properties are modified or enhanced by blending them with small amounts of other polymers or with special fillers and additives. An important property to enhance for polymers is the electrical conductivity. Polymers are usually known for their high electrical resistance, which often causes electrical charge or dust accumulation at the surface of final products. To address this issue permanently different additives in various shapes have been applied: conductive polymers, metallic particles, or carbon based particles. Recent research has been focusing on carbon-based nanofillers such as graphene, carbon nanofibres and carbon nanotubes. Distribution and interaction of these nanofillers are crucial in building an electrical network, which is in the position to improve electrical conductance. Different production methods have been assessed, and easy to up-scale ones for industry were selected. In our research, we have processed unmodified carbon nanotubes with polylactic acid.

The repartition of the nanotubes has been assessed by Atomic-Force-Microscopy. Effects of carbon nanotubes on the electrical conductivity, on the crystallization ratio, on mechanical properties and on various thermal properties of the composite have been studied. In the presentation, first results and interpretations will be presented.

10. Investigation of protein phosphatases in *Trichoderma reesei*

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Abstract

Perception of external environment changes and detection of intracellular energetic status allows the balance of requirements for growth and cell survival. Cellular responses are regulated by different processes as post-translational modifications by phosphorylation and dephosphorylation. In the biotechnological workhorse *Trichoderma reesei*, signaling pathways transmitting light signals and nutrient signals were shown to influence expression of plant cell wall degrading enzymes. Therefore we aimed to characterize protein phosphatases of *T. reesei* in order to assess their potential for improvement of enzyme expression.

We performed hierarchical cluster analysis of transcript patterns of phosphatase genes in different mutants upon growth on cellulose and on inducing or repressing carbon sources. The profiles in photoreceptor mutants still showed light specific clustering, suggesting that the photoreceptors influence light dependent transcription of phosphatase genes. In agreement with literature, also the G-protein beta and gamma subunits and the phosphatase like protein PhLP1 have a light dependent influence. Additionally, the transcript profiles of the phosphatases largely clustered according to the inducing or repressing effect of the carbon source used. Functional category analysis of coregulated genes enable us to assign putative functions to groups of phosphatases. Thereby we could distinguish between phosphatases predominantly involved in metabolic functions or in energy supply and protein synthesis. Analysis of deletion strains revealed functions in development (conidiation, fruiting body formation or ascosporeogenesis), growth on different carbon sources and formation of cellulases in light and darkness. In summary we provide first insights into the functions of protein phosphatases in *T. reesei*.

11. Phytomining of metals from waste incineration residues using hyperaccumulator plants

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Abstract

The overall aim of this project is to recover metals from waste incineration slags (bottom ash) by growing metal hyperaccumulating plants on this substrates and use the metal enriched biomass as a form of bio-ore. As a first stage, material from Vienna's waste incineration plants was sampled and analyzed. Residues from municipal wastes as well as residues from hazardous waste incineration and sewage sludge incineration were analyzed. In general, the slags can be characterized by a very high pH, salinity and high heavy metal concentrations. The target metal species in this project are amongst others Cr, Co, Mg, Ni, V and rare earth elements. This so-called critical raw materials are present in the slags at moderate to low concentrations.

In order to optimize the substrate for plant growth the high pH and salt content as well as the low nitrogen content in the slags need to be controlled. Thus, different combinations of amendments, such as material from mechanical biological waste treatment (MBT), compost, nitric acid and waste water from flue gas cleaning were tested for the conditioning of the substrate. Washing the slags with 5% HNO₃, as well as the leaching with waste water from flue gas cleaning turned out to be promising for lowering the pH. The acid treated substrate in combination with material from MBT was tested in a first pot experiment for plant toxicity.

In a next step a selection of potential hyperaccumulating plants is tested on the substrate to gain a proper understanding of all important interactions and end up with a selection of promising plants for a 2-year field trial on the landfill Rautenweg in Vienna.

12. Strategies for infection detection in wounds based on enzymes of the human immune system

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Abstract

Wound infection is a global problem that affects 5-10% of post-surgical wounds and 25% of chronic wounds and furthermore delays or prevents the healing process. The diagnosis is currently based on the classical clinical signs of infection as redness (*rubor*), heat (*calor*), swelling (*tumor*), pain (*dolor*) and impairment of function (*functiolaesa*) or microbiological analyses which take several days.

It is known that infection is characterized by an excessive stimulation of neutrophil granulocytes, resulting in the release of proteolytic enzymes like human neutrophil elastase (HNE), myeloperoxidase (MPO), lysozyme (Lys) and also matrix metalloproteinases (MMP) into the plasma.

The enzyme activities of HNE, Lys, MPO and MMPs were directly monitored in wound fluid of affected patients via hydrolysis of chromogenic and fluorescent substrates or of peptidoglycan (PG) respectively (1). MPO was measured via oxidation of guaiacol. Infected wound fluids led to significant higher substrate conversion compared to non-infected wound fluids (2). The lysozyme present in infected wound fluids led to an increased hydrolyses of PG, visible as decrease of turbidity compared to non-infected fluids (3).

In addition, the gelatinolytic activity from both- matrix metalloproteinases (MMPs) and bacterial proteases were investigated in different types of wounds for the development of an enzyme-responsive detection method.

An electrochemical sensor for fast and simple detection of MPO activity as marker for infection was investigated. The MPO-chlorination activity - the formation of hypochlorous acid (HOCl) - in different wound fluids was used to differentiate between infected and non-infected wounds.

To furthermore allow integration of sensors in typical bandage materials we successfully immobilized enzyme substrates on collagen, modified collagen, polyamide, polyesters and silica gel. These immobilized substrates were converted only by infected wound fluids, thus allowing on-line monitoring of wounds due to different colour stages of the bandage.

13. Plant-associated microbes as biocontrol agents against common ragweed

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Abstract

Ragweed is rapidly spreading in Europe and naturalized in many areas mainly through contaminated sunflower- and bird seeds. This noxious weed produces pollen which is highly allergenic and can cause allergenic rhinitis and seasonal asthma. Effective agents to control this weed of the *Asteraceae* family are limited.

Novel approaches for more environmental friendly and effective agents to combat this weed are demanded. Application of plant-associated bacteria as bioherbicides could be a successful strategy to win the battle against the invasive common Ragweed (*Ambrosia artemisiifolia* L.). Such bacteria could either be rhizobacteria or endophytes, which are in close relationship with ragweed. Because of their selectiveness of association with the host plant, such bacteria can be applied on agriculture land, without harming the crops. Another aspect of using natural occurring bacteria is the low risk of non-target effects which can be a great issue when introduction exotic biocontrol agents into a new environment.

In frame of this research project we are looking for rhizobacteria and endophytes from ragweed, which can reduce germination, growth and pollen production. Therefore we collected ragweed from different sites in Austria and isolated around thousand bacteria and taxonomic classified them through 16S rRNA sequencing. In the screening for bioherbicidal effects we include the production of hydrogen cyanide, indole acetic acid and antimetabolite toxins like tabtoxin and coronatine. Positive tested isolates will be tested on ragweed seeds and seedlings as well as non-target plants. Simultaneously an effective inoculating technique should be established. Additionally, the allergen production of treated- compared to non-treated plants will be evaluated.

Up to this point, the abundance and diversity was established for the sample site of the federal state Burgenland. Furthermore, approximately 15% out of 80 *Pseudomonas* isolates were tested positive for antimetabolite production.

Key words: Ragweed, bioherbicide, plant-associated bacteria

14. Light dependent regulation of protein secretion in *Trichoderma reesei* upon growth on cellulose

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Abstract

Changing light conditions, caused by the rotation of earth resulting in day and night or growth on the surface or within a substrate, result in considerably altered physiological processes in fungi. For the biotechnological workhorse *Trichoderma reesei* (syn. *Hypocrea jecorina*), regulation of glycoside hydrolase gene expression, especially cellulase expression was shown to be a target of light dependent gene regulation on a transcriptional level. Since transcription of genes doesn't necessarily correlate with expression of proteins, we were looking at the secretome of *T. reesei* under different light conditions upon growth on cellulose. Several secreted proteins could be identified by mass spectrometry, the majority of which have a signal peptide for secretion. Many members of the glycoside hydrolase families, like *cbh1*, *cel3a*, *cel61b*, *xyn2* and *xyn4*, were detected.

Microarray analysis of these genes revealed up-regulation under cellulose-inducing conditions. SDS-PAGE of the secreted proteins showed down-regulation of most proteins after growth in constant light. In the light receptor knock-out strains $\Delta blr1$ and $\Delta blr2$ this effect is much less severe.

Measurements of cellulase activity in culture filtrates of the wildtype strain QM6a revealed no detectable activity in light, independent of the light intensity. In darkness cellulase activity was present. In the light receptor mutant strains $\Delta blr1$ and $\Delta blr2$ cellulase activity was modulated in response to light.

We conclude that protein secretion is subject to regulation by light with differential effects on individual proteins.

15. „Bio-refinery of food waste - production of liquid and gaseous biofuels“

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Abstract

The city of Vienna collects bio-waste such as food and canteen waste and utilizes it in a local anaerobic digestion plant for biogas production.

Within the frame of an industry supported project, the bio-waste is used for an innovative bio-refinery concept in order to produce gaseous (bio-methane) and liquid biofuels (ethanol, butanol).

The bio-refinery concept consists of three fermentative processes, whereas the core process is an ABE-fermentation with continuous in situ removal of products.

During the first process (acidification and hydrolysis) liquid and gaseous intermediates such as butyric acid, acetic acid as well as hydrogen and carbon dioxide will be produced. The liquid intermediates serve as a feedstock for the second process (ABE fermentation), whereas hydrogen separated by membrane technique functions as a co-substrate for the third process (anaerobic digestion) in order to utilize waste CO₂ and increase the methane yield.

Products of the ABE-fermentation process are mainly butanol and ethanol generated by clostridia strains.

One of the main challenges of ABE-fermentation is the product inhibition caused by butanol and ethanol, accumulated during the process. In order to overcome this limitation in-situ solvent recovery technique based on selective adsorption and pervaporation technology will be investigated. Furthermore, Clostridia strains also tend to lose their solventogenic ability at prolonged cultivation time. The reason of strain degeneration is still under investigation but various fermentation techniques have been established to minimize this phenomenon.

16. Detection of wound infection using enzyme-responsive materials

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Abstract

Timely detection of an incipient wound infection still constitutes a challenging issue. To date, wound infection can only be diagnosed until becoming already evident which subsequently complicates the treatment of the patient. Especially medical facilities struggle with the treatment of infections, like those of postoperative wounds. Highly elevated enzyme activities were found in wound fluids of these wounds, most notably myeloperoxidase, lysozyme and elastase. Detection of increasing enzyme activities is an opportunity to enable a fast and simple evaluation of the wound's contamination level. Therefore enzyme-responsive materials are synthesized in order to detect wound infection at an early stage. These functional materials visualize an emerging infection by color change caused by release of a dye. A corresponding detection system, incorporated in a wound dressing, informs both patient and therapist about the wound status, thus directing towards the following therapeutic step.

17. Immobilisation of heavy metals to enhance biological degradation of organic soil contaminants

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Abstract

According to the Austrian Environment Agency (UBA) there are more than 2000 contaminated sites in Austria in need of remediation. Mixed contaminations (organic plus inorganic pollutants) represent a frequently occurring contamination type. Conventional remediation techniques like “dig and dump” are costly and limited in scale. Plant- and microbe-based alternatives, e.g. phytoremediation options, offer a cheap and environmentally friendly approach that can be applied on larger areas. However, the application of phytoremediation techniques to mixed contaminated sites may be tricky due to the potential inhibition of biodegradation processes by the presence of heavy metals in soil. Therefore, the objective of this study is to test the hypothesis that the degradation of organic pollutants can be enhanced by immobilising interfering heavy metals.

As part of the ISOMON project (“Isotope application for remediation, aftercare and monitoring of contaminated sites”), this 3 year study aims to identify the influence of heavy metal immobilisation on the degradation of organic pollutants, and to determine chemical, physical and biological measures further accelerating these processes. The influence of heavy metals on organic pollutant degradation will be assessed by using ¹³C-phospholipid fatty acid analysis (¹³C-PLFA). Application of ¹³C-labeled phenanthrene will allow the identification of microbial groups responsible for the degradation process. The influence of heavy metal immobilisation on the degradation of organic pollutants will first be analyzed in a laboratory batch trial, followed by a greenhouse experiment and a field trial. For metal immobilisation and enhanced biodegradation, distinct mineral and organic soil amendments (iron oxides, gravel sludge, biochar) are deployed, partly in combination with fast-growing and pollution-tolerant woody plants (willow, black locust and alder).

