PHYTO2ENERGY NEWSLETTER #1

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Editorial

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Dear Readers,

Welcome to issue no. 1 of our newsletter. As coordinator of the Phyto2Energy project, together with my colleagues, I would like to introduce our project and its activities to you. In particular, we would like to share with you what we have achieved during the first year of our collaboration and how these achievements were worked out jointly by fellows from research units and industry. But first a few words about the project.

Phyto2Energy is a four-year initiative which aims to develop and validate in field conditions an innovative, complex approach combining phytoremediation of heavy metal contaminated sites with production of energy crops and their conversion to energy using gasification. Easy renewability of the resource is one of the biggest advantages of biomass from energy crops. It makes it a source of energy constantly gaining an increasing interest. This interest, however, should not compete with food crops production on arable land, especially that some of the energy species like miscanthus, virginia mallow, switchgrass or cordgrass can not only perfectly grow on degraded, contaminated sites but also help remove the contaminants such as heavy metals from soil.

To properly explore this potential an in-depth knowledge is needed to understand what happens in the plant-soil environment and how the growth processes can be stimulated and the phytoremediation effect successfully achieved. Here the interactions between plants and microbes play a crucial role, especially at sites where plant growth is affected by contaminants like heavy metals. Therefore, Phyto2Energy will attempt to identify plant growth promoting microorganisms as investigating the beneficial partnership between plants and their associated microorganisms may become a key in developing a strategy to accelerate plant biomass production and clean-up of the contaminated areas. It will be translated into appropriate agritechnical measures as well as a suitable formula of an inoculum which could be used for large scale phytoremediation driven energy crops production. Obviously, the use of energy plants for cleaning soils contaminated with heavy metals as a way of producing biomass for energy purposes raises a number of environmental concerns as on one hand the polluted soil becomes cleaner but on the other an environmentally safe way must be found to convert the biomass with heavy metals content into energy. A promising way of doing so is gasification process. Although there are a number of technologies for contaminated biomass utilization, however gasification has this beneficial feature that it enables on site conversion of the biofuel into both electricity and heat minimizing the transport costs. Moreover, gasification process has many other advantages as it leads to a multi-use high-quality flammable gas that can be combusted for the generation of electricity or support such processes as drying or co-combustion in boilers to reduce emission of air pollutants such as nitrogen oxides. However, to take advantage of the benefits offered by the gasification process it is important to investigate how the fact that the biomass contains heavy metals and additives used to promote its growth will affect the gasification process itself, the quality and composition of the end gas and the end products such as ash or char as well as the entire installation. These issues will be also investigated in Phyto2Energy project. In our project we would like to demonstrate that this approach may become an alternative for managing agricultural areas and postindustrial sites contaminated with heavy metals while delivering an environmental and economic added value.

All the scientific and technological objectives of the Phyto2Energy project will be achieved thanks to the transfer of knowledge between the project partners from research sector and industry. This happens through the exchange of staff members (secondments) from the research organizations involved in the project: Institute for Ecology of Industrial Areas (PL), Silesian University of Technology (PL) and the Helmholtz Zentrum München Deutsches Forschungszentrum für Gesundheit und Umwelt GmbH (DE) and the industrial partners: ProBiotics (PL), VITA34 Business Unit BioPlanta (DE) and Institute for Studies and Power Engineering (RO). Thus the Phyto2Energy project, beside the anticipated advancements in science and technology, offers the staff members a unique opportunity to learn how innovation is done from the perspective of science and the business. The scheme enabling such cooperation is Maria Skłodowska-Curie Action Industry Academia Partnerships and Pathways of the 7. Framework Programme of the EU, under which the project is implemented.

I encourage you to learn from the articles in this newsletter what we have achieved in the first year of the project, as well as to contact us if you find something of special interest to you. Please visit our project web site **www.phyto2energy.eu** or contact us directly by e-mail.

Must waste land be a wasted potential?

By Marta Pogrzeba, Jacek Krzyżak (IETU) and Kristina Ziegler (VITA34)

While energy from biomass is promoted by current EU law, mainly in order to deliver greenhouse gas emission savings, the use of land for energy crops production in the EU has become the subject of considerable debate. What raises the biggest concerns is the competition between using land for food and for energy crops production. Already now solutions are available to reconcile these priorities without harming food crops production while addressing environmental challenges. The potential to be taken advantage from is in waste land: either idle arable land or post-industrial sites. Without much discussion there is a great deal of currently underused land on European farms that could be mobilized quickly to produce biomass by planting energy crops. Across the EU, land remains out of cultivation for a variety of reasons. These include economic and market forces; topographic, bioclimatic and edaphic considerations; contamination or pollution factors; and a variety of institutional factors. At the same time remediation of polluted soils is still a challenge not only in scientific and technical terms but also as a societal challenge (rehabilitation of former industrial sites, restoration of ecosystem services) and an economic issue (markets of soil rehabilitation; production of plant biomass for feedstock on contaminated soils integrated in the biobased-knowledge for bioeconomy). In that context finding a proper way for managing the use of heavy metal contaminated (HMC) soils in a way that would generate environmental added value and provide economic benefits gains high importance. It is estimated that across the Europe about 800 000 km^2 are identified as contaminated or potentially contaminated. The share of heavy metal contaminated areas is about

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30%. Despite that fact, little has been investigated on combining the production of energy crops on the contaminated areas with phytoremediation of these sites. Whereas HMC soils are unsuitable for food production, energy crops can allow the commercial exploitation of these soils by establishing biofuel feedstock production systems. In addition, the cultivation of the plants offers opportunities for site stabilization and phytoremediation of contaminated soils.

A simple method is missing that would allow for making a selection of an optimal energy crop species with respect to the biomass production potential, plant robustness and the goal of a site management i.e. which species are capable of producing large biomass yield but uptake slightly less amounts of heavy metals i.e. are more suitable for biomass production oriented site management option, and which absorb more metals but deliver a slightly lower biomass yield i.e. are suitable for the remediation oriented management option of a HMC site. These is one of the key challenges and at the same time objectives of the Phyto2Eneregy project. Among the candidate plant species are typical energy crops such as: miscanthus, virginia mallow, switchgrass and cordgrass. The field experiments conducted within the Phyto2Energy project are aimed at testing their new, two-fold function as

Aerial view of the German test site - Biotope Schladitz

heavy metal accumulators and biomass providers. The candidate species were pre-selected for the field experiments based on some previous multi-year testing done by IETU. In 2014 IETU and VITA 34 elaborated jointly a test plan detailing how the four-year field experiments with the pre-selected energy crop species will be conducted taking also into account the tests related to the understanding of the plants growth stimulation by microorganisms and the targeted use of the produced biomass as feedstock for gasification. Based on the plan, in 2014 experimental plots on heavy metal contaminated sites in Poland and in Germany were established by the partners.

In Germany, a post-industrial site which is a former sewage sludge dewatering plant located in the north of Leipzig was chosen as a test area. After the closure of the plant in 1990 about 800,000 tons of sewage sludge contaminated inter alia with heavy metals remained in several basins. The pollutant concentration was reduced to natural occurring background concentrations, however, heavy metals such as lead, cadmium and zinc are still detectable in the soil in high amounts.

The Polish test site is located in the outskirts of Bytom, an industrial city about 15 km from Katowice, Southern Poland in the proximity of the closed - down large lead/zinc/cadmium works consisting of the

Miscanthus test plots in Poland at the end of the first vegetation season

ore mining, enriching and smelting facilities. This metallurgical complex was in operation for more than 100 years and contributed significantly in pollution of local soils. During the last 30 years the area was used for agricultural purposes. Recently, the land has been used for grain crops farming, especially for wheat production. Soil contamination with lead, cadmium and zinc exceed permissible limits for agricultural soil in Poland.

Once the plots were established initial soil sampling and analyses were performed to determine basic soil parameters and metals bioavailability. Afterwards seedlings of miscanthus, virginia mallow, switchgrass and cordgrass were planted. At the end of the vegetation season plant samples were taken for chemical analyses to check the amount of metals taken up by the plants. The aim here is to get some initial data which of the chosen energy species represent potential in terms of environmentally save biomass i.e. do not accumulate large amounts of heavy metals and which are able to cleanup the soils.

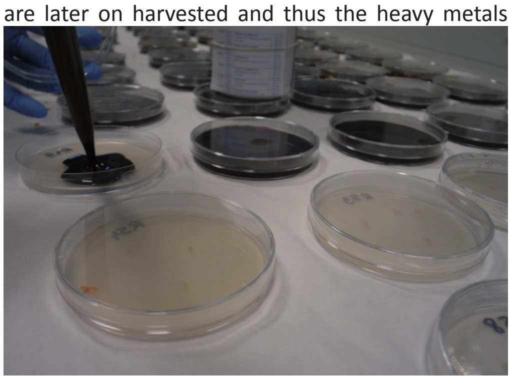
Establishment and maintenance of the plot experiment involved secondments of fellows from IETU and VITA34 during which relevant knowledge was transferred and experiences exchanged. VITA34 hosted 3 experts from IETU for a total of 5.5 month secondments to train VITA34 engineers on how to plan, set up and care of a field trial under environmental conditions. Later these gained skills were put into practice when establishing the plots and running the experiment in Germany. The main challenge here was that for a field experiment there are no standard conditions given like in a lab-scale test. Furthermore, the secondments were accompanied by a series of outreach activities like lectures for students and stakeholders as well as meetings organized in Germany to disseminate the project aims and capacity building.

Also a young researcher from VITA34 benefited from a one month secondment to IETU that was an opportunity to gain some knowledge and practical skills on the technical aspects of a field trial like implementation of good agritechnical practices in dealing with energy crops cultivation on contaminated sites, sampling methods and preparation of soil and plant material for analyses. Since VITA34 is an engineering company, this knowledge will find application in their future activities related to phytoremediation combined with energy crops production.

How can microbes help energy crops to grow on heavy metal contaminated sites

By Barbara Cania (ProBiotics Poland), Joseph Nesme and Michael Schloter (HMGU)

Plants, just like animals, are full of microbes. Some of these microorganisms can cause plant diseases and thus should be termed pathogens. Other microorganisms that colonize plant tissues can have a positive effect on the fitness of their host plants. They form a large and diverse functional group of "plant-growth promoting microorganisms" (PGPM) that consists of belowground microbial plant associates called mycorrhizal fungi, rhizobia and rhizosphere bacteria (rhizobacteria), internally living bacteria and fungi (endophytes) and microbes living on the surface of aboveground plant parts (epiphytes). Most importantly, the presence of microorganisms in and on plants must be considered to be a rule, rather than an exception. It is up to scientists to research plant interactions with different microorganisms in order to learn how to eliminate the threat of "bad" pathogens and make use of "good" plant-growth promoting microbes. A promising field to exploit plant–microbe partnerships is remediation (clean-up) of soils contaminated with heavy metals. Phytoremediation (remediation by plants) may be inhibited because the plants experience toxic effects from heavy metals. However, many plant-associated microorganisms can assist their host plant to overcome these contaminant-induced stress responses, thus providing improved plant growth. For phytoremediation of toxic metals, plant-associated microorganisms possessing a metal-resistance/sequestration system can lower metal phytotoxicity and affect metal translocation to the aboveground plant parts. If the phytoremediation process is conducted using energy crops, the plants are later on harvested and thus the heavy metals



Preparation of test material

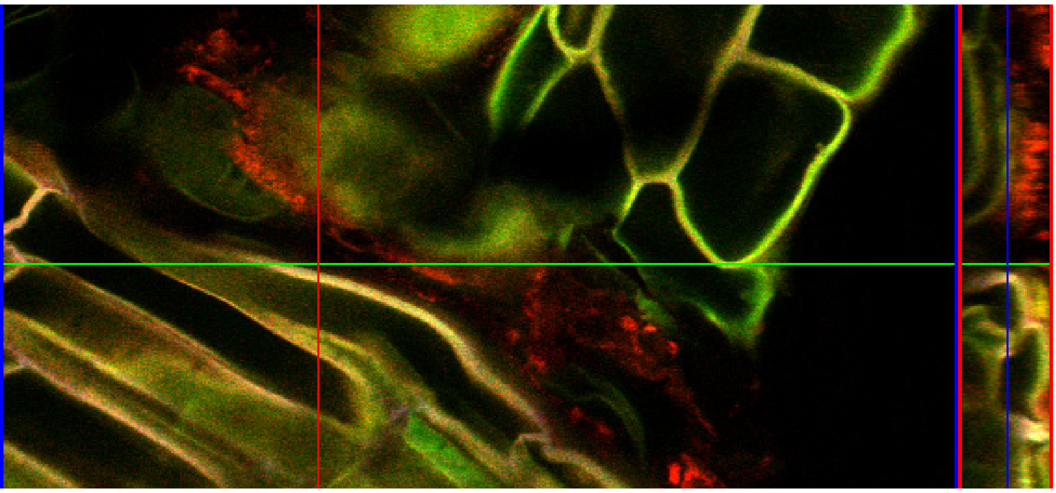


Photo of bacteria in plant cells

are effectively removed from the contaminated soil. Furthermore, plants, like all living beings, need nutrients to grow. The lack of these nutrients may be another cause of inhibition of phytoremediation. Iron, phosphorus and nitrogen are one of the most essential mineral nutrients for biological growth and development. They are also often the limiting mineral nutrients for biomass production in natural ecosystems. However, plant growth is usually not inhibited by the lack of these nutrients as such, but rather by the lack of their bioavailable forms that plants are able to take up and use for their needs. Some plant growth promoting microorganisms are able to enhance availability of minerals through production of organic acids, release of chelators or redox changes. Some of these microbes are also able to stimulate plant growth by producing plant growth hormones (phytohormones) that control plant development, or by protecting them from pathogens and herbivores. Plant growth promoting microorganisms best known for possessing such mechanisms are rhizobacteria, bacterial endophytes and mycorrhizal fungi.

The Phyto2Energy project aims at identification of microorganisms promoting growth of energy crops and accelerate clean-up of areas contaminated with heavy metals. To achieve this objective the already existing inoculum EmFarma Plus™ provided by ProBiotics Poland will be used as a starting point to develop a method to ensure healthy and sustainable phytoremediation driven energy crops production. Moreover, the functional diversity of the rhizobacteria and bacterial endophytes will be studied to define strategies to enhance the abundance and activity of plant beneficial microbes in situ. In this aspect, the changes in the composition of the microbial diversity will be investigated to reveal the response of keyplayers to the heavy metals and adaptations to stress conditions in the habitat. Furthermore, these data will be used for improving isolation strategies for potential plant growth promoting rhizosphere bacteria and endophytes. In addition, new bacterial strains will be isolated from the rhizosphere and endosphere of the respective plant species to design new and more targeted formulations. Finally, mycorrhizal strains with improved tolerance for heavy metals will be selected to micropropagate the plants before planting into contaminated soils. A robust indicator system will be developed to measure the success of the remediation process in situ. The indicator system shall be based on the abundance of bacterial resistance genes against heavy metals. The work will involve extensive knowledge of products commercially offered by ProBiotics as a basis for innovations and optimization with expertise on fungi pathogens offered by IETU and on environmental factors and individual genetic disposition offered by HMGU. As a result, a composition of a novel inoculum or inocula will be proposed to stimulate the phytoremediation driven energy crops production together with a set of indicators enabling to monitor the success of energy crops cultivation and the phytoremediation effect.

During the first year of the Phyto2Energy project new experimental plots in Bytom (Poland) and Leipzig (Germany) have been established and initial experiments have been performed using the old IETU plantations. In May a young researcher from ProBiotics Poland came to IETU for secondment to teach the IETU's team how to use ProBiotics' EmFarma Plus™ on the old and new plantations in Bytom. This newly obtained knowledge was later used also with the VITA34 during the secondment of IETU researchers in June to establish experimental plots at the German site and apply the inoculum. IETU and ProBiotics Poland continued to exchange knowledge during a workshop organized at IETU on the isolation and identification of plant-associated bacteria as part of planning activities towards the development of a new inoculum. These activities were put into practice during the secondments organised in September and October when ProBiotics and IETU researchers worked jointly to isolate plant associated microorganisms from energy crops collected from the established plantations. They lead to identification of over one hundred different microorganisms potentially able to promote growth of the Phyto2Energy crop species and assist in the clean-up of heavy metals contaminated areas. In order to select from such a large pool of microbes the ones most suitable to become a part of the novel inoculum their properties need to be carefully checked.

In the meantime HMGU researchers prepared a dedicated bioinformatic tool to help identify genetic elements conferring resistance to heavy metals in bacteria. In most cases, such genes are not essential for the bacteria hosting them. They're therefore located on accessory genetic elements, such as plasmids, that can be transferred horizontally, for example to plant growth promoting microorganisms. Yet, very little is known on the behavior of such genetic transfer events in soil. To better characterize this aspect of the problem a greenhouse experiment, where all variables can be controlled more easily, will be conducted at HMGU to study influences of heavy metals and inoculum on the dynamics of gene transfer events occurring in the chosen plants rhizospheres. Indeed, it is also known that such genetic elements are often carrying antibiotic resistance genes together with heavy metals resistance genes. We need to make sure that the inoculum to be developed under the project does not promote antibiotic resistance in microorganisms naturally occuring in soil, such as *Acinetobacter baumanii* or *Burkholderia spp*. that can cause diseases to humans.

Based on the obtained results the most promising candidate microbes will be chosen and further elaborated jointly by researchers from Probiotics and HMGU during her secondments in February and March. The plan is to carry our molecular experiments on the selected candidates towards determination of the final composition of the first version of the novel inoculum early 2015. Once developed, the novel inoculum will be tested in a field trial. During the next years of the Phyto2Energy project data from the field trials will be collected and the inoculum's composition and its application conditions will be optimized. The project's works are not limited only to the timeframes of the secondments though and everybody is just as busy during its whole duration working on reaching the common goal.

CONVERTING HEAVY METAL CONTAMINATED BIOMASS INTO ENERGY: A CHALLENGE OR AN OPPORTUNITY?

By Sebastian Werle (SUT), Daniela Burnete (ISPE)

View on Govora Power Plant over energy crop plantation

Biomass use for energy purposes, beside the positive impact that it has on the environment caused by the so-called zero-CO_2 emission, brings also other consequences resulting from the over-exploitation for the purposes of meeting energy needs. Excessive exploitation of agricultural biomass could contribute to its competition with the food market while the use of forest biomass could pose threat to the protected woodland. Taking account of these risks use of energy crops grown on degraded industrial land seems to be a good solution. Even more, crops can be used as a tool for their remediation. Biomass, which has been used in soil remediation process, due to the higher content of pollutants must be treated in a special way. One of them is thermal transformation so that, in addition to biomass disposal, recovery of the energy content is possible. The utilization of biomass can be generally done by two types of processes, bio-chemical conversion and thermal conversion. Bio-chemical conversion methods include fermentation and anaerobic digestion while thermal methods are combustion, gasification and pyrolysis. The approach to carry out the processes varies, depending on the desired products of the process, but the primary aim is the transformation of the biomass into fuel. For the Phyto2Energy project the gasification process was selected as it allows to generate a high quality gas which can then be used in a wide range of equipment in order to produce energy. From the installation viewpoint, the project focuses in particular on small scale installations as they represent a considerable potential for market penetration, especially in Central and Eastern Europe, which on one hand demonstrates high demand for biomass resources and on the other has many chemically degraded, post-industrial sites which require efficient management.

The Phyto2Energy project creates an opportunity to work together and exchange experience and knowledge between scientists involved in the research of biomass production (IETU), gasification and combustion installations (SUT) and practitioners in the area of design and economic analyses of the energy production installations (ISPE). We join our

General view on the SUT Laboratory of High Temperatures Processes

effort to study and develop innovative technological solutions that will be economically feasible and environmentally friendly. For example, the research includes analyses and evaluation of the impact of the composition of the produced biomass on the quality and composition of the gasification products: the end gas as well as ash and char. Thanks to the gasification tests and the analysis of the end products it will be possible to understand which char and ash fractions are formed, how heavy metals behave during this process and if and which mineral components used in agronomy to stimulate biomass growth may affect the gasification process. At the same time this knowledge becomes crucial providing contributions on how to develop appropriate stimulation methods for the biomass growth and the metal uptake by plants from the viewpoint of the gasification process and, reciprocally, how to optimize the process parameters from the viewpoint of the produced gas, ash and char so as to maximize their applications. For example, IETU and ISPE will also assess the applicability of the ash and char for land applications. As mineral fertilizer, ash could be used to improve the production of biomass by providing nutrients for the plants while reducing soil acidification. It can be also used as soil amendment in remediation process improving soil quality and diminishing metals bioavailability.

In the first year of the project gasification tests were carried out jointly by researchers from SUT and fellows from industrial partner – ISPE. Control biomass samples of the 4 tested energy crops: miscanthus, virginia mallow, switchgrass or cordgrass grown on an arable land with heavy metal concentrations exceeding the permissible levels by 5 times (Bytom site) were gasified using a fixed-bed gasification facility located in SUT laboratory. The initial results showed that as biofuel, miscanthus achieved the best result in terms of the amount of energy produced in gasification demonstrated by much higher temperature inside the gasifier during the testing, compared to the other three types of biomass which obtained significantly lower values. Analysis of the other gasification products such as fly ash, bottom ash and tar from the gasification trials was carried out jointly by ISPE and IETU. The results showed that the ash after gasification of the tested plants was rich in microelements typically used for fertilization purposes. However, as expected, the issue of heavy metal content becomes problematic. In the case of miscanthus and virginia mallow the content of lead was too high while in the case of cordgrass – the content of cadmium.

Only the ash from switchgrass demonstrated some potential to be used as fertilizer since the amount of heavy metals contained in it remained within the permissible levels for its direct land application.

Furthermore, in order to determine the impact of the biomass parameters on the quality and composition of the end gas from gasification, tests were performed at SUT using thermogravimetric analyzer (TGA) coupled with Fourier transform infrared (FTIR) spectrophotometer. These techniques allow to measure the change in the mass of a sample over a range of temperatures using the principle that as a sample is heated it's mass also changes. This change can be then used as to determine the composition of a material or its thermal stability.

The joint research conducted In 2014 was implemented by a series of secondments involving 2 researchers from SUT who stayed for a total duration of 3 months in ISPE, 2 researchers from IETU working for a total of 2 months with ISPE experts as well as 2 practitioners from industrial sector – ISPE working for a total of 2 months with scientists from SUT. Working together was an opportunity to learn each others' R&D workshop. The fellows during their stays at host organisations organised also a series of internal trainings and open seminars to facilitate the transfer of knowledge and exchange of experience.

1st Year Transfer of Knowledge Activities

12	**Researchers on Secondments:**
7	Experienced Researchers
5	Young Researchers
20	**Secondments between academia & industry**
20	**Personmonths spent by researchers on secondments**
21	**Internal trainings & seminars**
13	**Outreach & project promotion activities**

Project Details

Project full title:
Phytoremediation driven energy crops production on heavy metal degraded areas as local energy carrier

Project acronym: **Phyto2Energy**

Project scheme:
Industry-Academia Partnerships and Pathways

Grant agreement no.: **610797**

Call identifier: **FP7-PEOPLE-2013-IAPP**

Project start date: **February 1st, 2014**

Duration of the project: **48 months**

Project Consortium

Project co-ordinating unit:

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Deutsches Forschungszentrum für Gesundheit und Umwelt

Helmholtz Zentrum München, German Research Center for Environmental Health GmbH (DE)

Institute for Studies and Power Engineering (RO)

ProBiotics Polska

ProBiotics (PL)

Silesian University of Technology, Institute of Thermal Technology (PL)

VITA 34 THE STEM CELL BANK
BioPlanta

VITA 34, BioPlanta (DE)