PHYTO2ENERGY NEWSLETTER #2

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EDITORIAL

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Dear Readers,

Welcome to issue #2 of our newsletter. As coordinator of the Phyto2Energy - an Industry-Academia Partnership and Pathways project implemented under the flag of Maria Curie-Skłodowska Actions of EU FP7, together with my colleagues, we would like to share with you our work and its accomplishments in 2015.

We are a consortium of six organizations representing industry and research from Poland, Germany and Romania who committed to work jointly for 48 months in order to elaborate and test a novel approach to management of heavy metal contaminated sites such as post-industrial sites or arable land so as to restore their environmental and economic qualities. The innovation we are developing and testing in field conditions combines phytoremediation energy crops production and conversion of the produced biomass into energy using gasification. We build our know-how and practical experiences necessary to propose the approach to potential users as well as develop our own competences and extend commercial offer in the future based on the transfer of knowledge implemented by secondments or recruitment of fellows from research units of our consortium : Institute for Ecology of Industrial Areas - IETU (PL), Technical University of Silesia - SUT (PL), Helmholtz Zentrum München- HMGU (DE) to industrial partners : VITA34 Bioplanta (DE), Probiotics (PL) and Institute for Studies and Power Engineering (RO) and vice versa.

Based on 33 months of joint effort including 12 months of recruitment of an experienced researcher by HMGU and 21 months of secondment facilitated by other researchers from our organizations who ensure continuity of our research activities when no secondments are implemented in 2015 we managed to make a substantial advancement in project progress. Our experimental fields established in 2014 in Poland and Germany started providing interesting data on the phytoremediation and biomass production, the microbiological studies provided us with a knowledge to start working on a prototype innoculum to make its first application in 2016 while the gasification experiments of the collected biomass provided some initial data on the fate on how heavy metals behave in the process and affect its products. You can read more in this newsletter on these experiments and collaboration of our 12 fellows.

The work was also very abundant in trainings, seminars and presentation of project beyond the consortium. The fellows delivered 14 trainings and seminars in hosting organizations, participated in 4 other events to present the project . Worth mentioning is also a special Phyto2Energy session organized during the XXII International Symposium on Combustion Processes in September, Poland.

I cordially welcome you to read our newsletter, visit our website at www.phyto2energy.pl to see our advancements and meet our fellows and follow us on Facebook.

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 mail.

Our consortium and their contributions to the transfer of knowledge

IETU (academia) contributes with R&D expertise in two areas: phytoremediation plant species expertise and microbiology. In the area of phytoremediation IETU contributes with knowledge and test data concerning selection of species both typical energy crops and phytoremediation crops which may be successfully used as RES while simultaneously demonstrating phytoremediation properties. IETU expertise in the area of microbiology, especially expertise in investigation and tests of indigenous microbs supporting plant growth as well as pathogens affecting the crops combined with HMGU studies and Probiotics expertise will build knowledge on determining optimal conditions and methods to stimulate the growth (including composition of the inoculum/inocula to be developed and tested) and eventually the yield of the produced biomass using soil microorganisms. IETU will additionally contribute to SUT and ISPE knowledge with expertise related to gasification end-products management possibly as fertilizer in agriculture.

ISPE (large industry) contributes with extensive practical experience in bioenergy and RES field (development of energy generation using biomass, agricultural and non-agricultural residues, studies and analyses for promoting E-RES and H-RES, development of the biomass-coal co-combustion systems).This experience will be directly translated into setting up the control parameters for valorisation of biofuel feedstock for gasification and performance of analyses and tests of the HMC biomass according to this set of parameters. This will help elaborate in cooperation with SUT and IETU innovations in small scale installations (gasification) for their optimal operation taking into account the specificity of the HMC biomass and the use of the gasification end-products (e.g. ash). Additionally ISPE contributes with an extensive expertise in performing the cost-benefit analysis and environmental impact studies of the biomass gasification process from phytoremediation driven energy crops production as local energy carrier which will contribute to define the needed conditions for making this approach a competitive option on the market.

VITA34 (SME, industry) has practical engineering experience and provides complex consulting services on phytoremediation planning, crop management and agronomic practice including site investigations etc. but also cost-benefit analyses and assessment of environmental risks accompanying soil phytoremediation processes. This expertise will contribute to a joint IETU-VITA34 effort in designing and optimization of the combined phytoremediation/biomass production approach in particular in balancing the pollutant transfer as a basis for decontamination forecast using the selected plant species and the optimization of the integrated approach from the remediation viewpoint. Expertise in crop management and agronomic practices provided by VITA34 combined with IETU phytoremediation expertise will enable development of guidance on the optimal selection of species for phytoremediation driven energy crop production on HMC sites in real scale site management operations.

HMGU (academia) is a recognized center dealing with diseases which develop from the interaction of environmental factors and individual genetic disposition. HMGU provides well recognized experimental platforms (genomics, proteomics, metabolomics as well as environmental simulation) which will be used in the project to define and ensure an in depth promotion of plant growth by microbes and in particular to understand the impact on the development of microbes which carry gene clusters coding for multiple antibiotic resistance at the sites of investigation. Data from this investigation will be further explored by IETU and Probiotics in the project to work out an optimal composition of the inoculum/inocula to stimulate successful and sustainable biomass growth on heavy metal contaminated sites and minimize the negative effect of pathogenses.

Probiotics (SME, industry) is know-how owner (ProBiotechnology) and manufacturer of different types inocula ProBio Ems (70% of Polish market) used in agriculture and for soil functions improvement. They will provide the

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initial knowledge on the microbiological systems stimulating crops growth based on their commercial products offered on the market for agricultural applications Their knowledge and know-how on crops yield stimulating biosurfactants in a form of inocula combined with the transfer of knowledge on microbial communities facilitating energy crops growth and yield, metal uptake and plant pathogens provided by joint activities with HMGU and IETU should result in working out one of the expected project outputs – a novel inocula stimulating the phytoremediation driven energy crops production on heavy metal contaminated sites.

SUT (academia) contributes with extensive knowledge related to solid and liquid biomass utilization in energy sector, in particular thermochemical conversion of the organic substances in such processes as gasification, pyrolysis, combustion, TG-FTIR analysis etc. They will be also investigating pollutant emission and heavy metal transformations during thermochemical conversion processes of the biomass and the influence of process conditions and fuel composition. The gasification experiments will be carried out at SUT small scale installation. The experiments facilitated by ISPE knowledge will allow defining process and installation parameters guidance as well as biofuel feedstock parameters (contribution to the inocula composition and cultivation practices) making this type of installation a competitive solution on the market.

The XXII International Symposium on Combustion Processes

Sebastian Werle
Silesian Universitu of technology (SUT)

In September 2015, Daniel Bisorca and Andreas Phopol from ISPE, Romania come to SUT to work together with SUT team on the fixed bed gasification process and TG analysis of the plant material sampled in February/March 2015. They've analyzed the influence: of the addition to soil N, K fertilizer and the application of the commercial inoculum provided by ProBiotics on the biomass combustible properties, the quality of the achieved gasification gas, the properties of the ash in the context of its application as an unconventional fertilizer and the composition of liquid phase produced during gasification.

The results we achieved are very promising. It can be concluded that the inoculum and fertilizer application helps the absorption of organic components from the soil. It means, that such biomass is characterized by a high content of organic content. This is a very positive feature in the terms of the use such biomass as an energy carrier.

Taking into consideration the ash quality in the context of use it as an agricultural or forest land application, the data we got so far provde a solid backgroud to think about the feasibility of such solutions. It however needs further analyses.

The main conclusion of the liquid gasification end products shows that heavy metal content in tars is much lower in comparison to ash. It confirmed an advantage of the gasification that heavy metals from biomass are transformed into solid fraction.

WP3 core staff during the XXII International Symposium on Combustion Processes

During the their secondment at SUT, the ISPE fellows had an opportunity to participate in the XXII International Symposium on Combustion Processes which has been organized by the Institute of Thermal Technology, SUT. The Conference was held in Polish Jurassic Highland on 22-25 September 2015. This Symposium, organized by the Section of Thermodynamics and Combustion of the Polish Academy of Sciences, has a fifty-year tradition as a meeting place for distinguished scientists and leading engineers from major industrial companies all over the world. Number of participants of the conference were over 100 scientists.

During the XXIInd Symposium, Special Session of the Phyto2Energy Project has been organized. Six presentations were presented. The program of the session consists following papers:

- Izabela Ratman-Kłosińska: An introduction to the PHYTO2ENERGY project
- Daniel Bisorca: Cost benefit analysis of HMC biomass gasification as local energy carrier
- Andreas Pahopol: Lab test results of the 1styear biomass feedstock and solid gasification residue properties
- Marta Pogrzeba: Approach to energy crop cultivation on heavy metal contaminated sites - Polish and German case study
- Sebastian Werle: Laboratory test results of the gasification of heavy metal contaminated energy crops
- Sebastian Werle: TG/FT-IR analysis results of the gasification of heavy metal contaminated energy crops

The dedicated session was a forum for the presentation and discussion of current stage activities in the field of Phyto2Energy project.

Session participants asked many detailed questions. The discussion was an important element in organizing research results. The session was an important element to promote the project topic to broad group of the scientists and business representatives.

Heavy metals didn't stop us in the energy crop cultivation

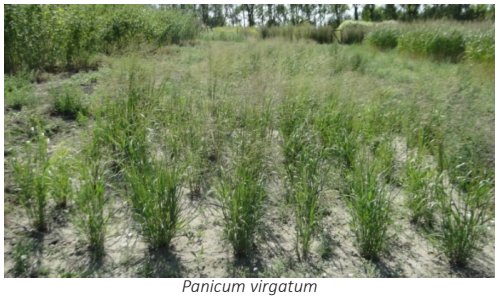
Marta Pogrzeba, Jacek Krzyżak
Institute for Ecology of Industrial areas (IETU)

We are coming back to you with news from the second year of our field experiment focused on energy crops production combined with phytoremediation of heavy metals contaminated sites. You may remember that in 2014 IETU team together with our colleagues from VITA 34 established two field experiments: one at arable land near Bytom, Southern Poland and one in the vicinity of Leipzig – a former sewage sludge disposal site (Schalditz). At each site we planted four preselected energy plant spices that also represented potential to be used for phytoremediation purposes: *Miscanthus gigantheus*, *Sida hermaphrodita*, *Spartina pectinata*, and *Panicum virgatum*. As in the case of any experiment, despite a detailed test plan, 2014 was a year of facing continuous challenges and unexpected situations like long dry weather season, struggle with dominating autochthonous vegetation and appetite of wild animals to eat our plantation as an early spring dainty. Together with our colleagues from IETU, Probiotics and HMGU dealing with the microbiological aspects of the project, we have applied a commercial inoculum. Now when the experiments are established and we see that the plants grow really well we can say that it was worth our effort and determination. That all created a solid basis to set up an extensive monitoring during project year two i.e. in 2015. Extensive monitoring means that we not only measured the physiological parameters of the plants such as rate

View of plant species grown at Bytom site during second vegetation season

Miscanthus x giganteus

Sida hermaphordita



Panicum virgatum

Spartina pectinata

Field trial at German site near Leipzig

of transpiration, rate of photosynthesis and plant growth parameter to see how the species behave in the contaminated environment and what is their potential in terms of biomass production, but we also analyzed plant material sampled at the end of the vegetation season in September/October 2014 and during harvest in February/March 2015 to determine the heavy metal content in the context of their phytoremediation properties. To get a full picture of relationships between plants and soil we also sampled and analysed soil from each site after each growing season as well as assessed total yield of the biomass per experimental plot. The numbers of samples speak for themselves. So far about 600 plant samples and 200 soil samples were taken, prepared and analyzed for each file trial site during six sampling campaigns carried out at Polish and German field trial sites. We took as many as 300 soil samples from the field trial sites. All these analyses led us to the following conclusions:

- The historical use and soil conditions of two sites is different which leads to a diverse mobility and bioavailability of metals, availability of nutrients and effects of the added amendments (i.e. innoculum and fertilizer).
- At the beginning of the experiment total cadmium and lead concentrations in soil were almost in the same range at both sites, however total zinc concentration was higher at the German site. In contrast the bioavailable concentrations at the experimental site in Poland exceeded the corresponding values for the German site , especially in the case of zinc.
- Bioavailable fraction is the most important factor influencing the uptake of heavy metals by plants. It has been confirmed by the results obtained from the 1st and 2nd vegetation season.
- Uptake of lead (content in plant biomass) and removal (content multiplied by yield) were the highest for Panicum virgatum.
- Sida hermaphrodita shows the highest uptake for cadmium and zinc. For zinc for example a removal of about 2.05 g per 16 m² only in one season have been observed. This comes up to 1,281 g zinc per hectare and year. For cadmium and lead it was about 4.9 and 65.9 g per hectare respective.
- Based on the results after the first growing season up to 3.2 t biomass can be obtained for Spartina pectinata but much more biomass yield is expected for the next growing seasons, due to the optimal plantation development from third growing season.

Plant sampling preparation in lab of Vita 34

- Besides that, lowest content of metals in plant biomass was also found for Spartina pectinata regardless of their bioavailability in soil. It means that this plant species can be used as a “safe biomass” produced on heavy metal contaminated soils.
- Till now there are no significant positive impacts of used additives, although the application of commercial available inoculum diminished cadmium and zinc uptake by Sida hermaphrodita. Results of the plant physiological parameters show higher photosynthesis rates for plants which were grown on the German site. It can be attributed to lower metal availability and higher initial nutrient content compared to the conditions at the Polish field trial site.

Secondment of Polish experienced scientist to Vita 34 and German expert and young researchers to IETU were used to share professional knowledge, long lasting experiences and established techniques as well new innovative approaches to insure quality of work. They provided German experienced and early stage researchers an opportunity to become familiar with measurement of plant physiological parameters developed on both field trials not only in theory but also in practice. Another aspect of knowledge transfer referred to statistical methods used for the conversion of data to results. Joint work enabled to synchronize them to ensure comparability of results gained from investigations carried out in Polish and German experiment.

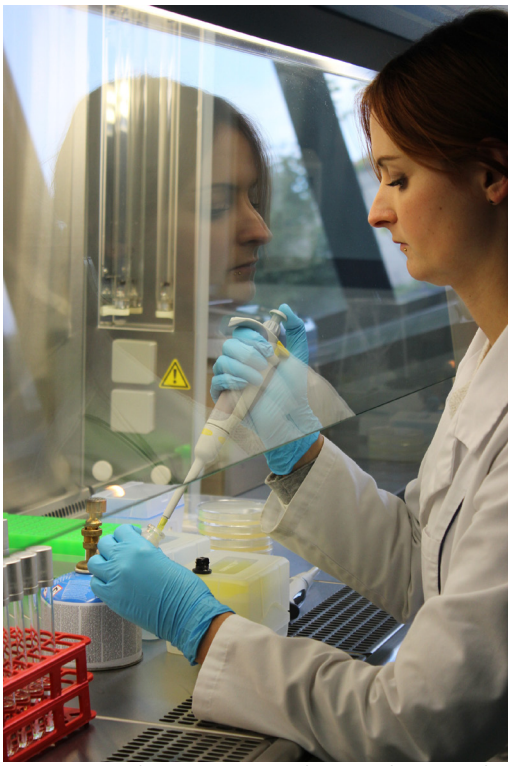
Soil sampling preparation in lab of Vita 34

To the advantages of joint project developed by scientific institutes and partners from industry belong that aspects on feasibility, economy, ecology as well as biodiversity. They are considered for all measures and during the whole duration of the project. These increase the prospects of success to investigate phytoremediation using an integral approach and develop an economically and sustainable method to deal with large area which are contaminated with metals caused by industry.

Discussion and presentations on project related issues increased knowledge not only of fellows also of colleagues and master students of team Engineering and R&D of Vita 34 who took part in training sessions organized in Leipzig. Beside working on ongoing project activities, partners work together on outreach activities. Several workshops were organized for example at the University of Leipzig; Environmental Research Institute and Biomass Research Center in Leipzig. These meetings involving partners outside the Phyto2Energy network are an excellent opportunity for future activities and hopefully for future projects .

Phyto2Energy from the perspective of a young researcher

Joanna Chojniak
Institute for ecology of Industrial Areas (IETU)



I am an early stage researcher working in the microbiology team of Professor Grażyna Plaza at the Institute for Ecology of Industrial Areas in Katowice, Poland.

Participation in the project Phyto2Energy I started from the plan what should be done and when in microbiology task. Firstly it was application the commercial inoculum of new seedlings of energy crops. Another task in the same year was to isolate endophytic and rhizosphere bacteria from some part of plant (leaves, roots, stems). Next the isolates were tested using plate methods to growth on

media with heavy metals. This part of experiments was made in my home organization.

Last year I went for a two- month secondment to ProBiotics under the Phyto2Energy project. I have never worked for a company before so I was quite interested how my activities there will look like. Our colleagues from ProBiotics Szymon Powałowski and Iza Gebler and the owner of the company Marta Górska took good care of me. Together with Szymon and Iza we were working optimization the process preparation new inoculum. Before my secondment in ProBiotics we selected strains to new inoculum. Three strains were tested on another media. Together with my colleagues from ProBiotics and with Professor Grazyna Plaza decided right media and method preparation the new inoculum. ProBiotics labs are very well equipped so the chance to use the infrastructure of a commercial lab was also an interesting experience. For example I used standard equipment to preparation bacterial culture: incubator, shaker. Growth on media was monitored using the spectrophotometer. Moreover I had a chance to work using a special machine for the preparation of freeze- dried bacteria, this kind of equipment was new to me. Another interesting experience was with a production line of the inoculum where I could participate in testing the quality of the product and work on real-time PCR – an equipment used for duplication of DNA and identification of the same genes in genetic material.

The main result of my secondment in ProBiotics was to select the right media and methods for the preparation of the new inoculum to be applied in the Phyto2Energy project. Together with my colleagues from ProBiotics we have been quite successful in achieving this result. Although important, but that was not the only outcome of my secondment. Working in a real commercial lab together and meeting new people who work on similar themes as me however in a business reality was an exceptional experience but also fun.

Our game with genes

Szymon Powałowski, Barbara Cania
ProBiotics Polska

In the beginning of last year, Barbara Cania and myslelf as ProBiotics fellows drove all the way down to Munich Helmholtz Center for Environmental Health (HMGU) bringing more than a hundred of bacteria strains isolated before on Petri dishes. Our objective for the two months secondment together was to give a name to each of these beasts. Indeed, from all the work already performed by ProBiotics and IETU in the Phyto2Energy project, we knew that some of them were producing this or that compound known to help plant growth or were capable of growing in presence of toxic heavy metals but we still couldn't figure who they really were. Indeed, nothing looks more similar to a bacteria growing on a plate than another bacteria growing in another plate. Before, one could spend months identifying a single specimen using a large variety of biochemical tests. But we only had a few weeks and considerably more samples to analyse! This is where sequencing becomes really practical and we had an opportunity to experience ourselves. The goal of that approach is to amplify a gene that is present in every bacterium and sufficiently conserved that it can be fished with the same hook in every microbe. So that's what we've done. As a first step, we took a little bit of each colonies growing on the surface of plates and cracked open the cells to release their genomic DNA. Joseph Nesme – our French colleague from HMGU and a fellow recruited by the Center to carry out research under the Phyto2Energy project provided us with the hooks: primers targeting conserved region of that gene and the fishpole: all reagents necessary to perform a PCR reaction and we went fishing that famous piece of DNA: the 16S rRNA gene. Like every piece of DNA, this gene is a sequence of four letters A, T, C and G. The purpose of sequencing is literally to read that sequence. That's what the Genomic Analysis Center of HMGU is really good at and that's where we had all our pieces of DNA read. The last step of our naming quest was to compare the sequence obtained for each bacterium with 16S rRNA sequence databases of the same gene and find which one resembles ours the most. We finally found that out of the 144 isolates brought by us to HMGU most belong to the Bacillus (50%) and Pseudomonas (18%) groups. And indeed, these two groups of bacteria are often found in association with plants and many have already been used for plant growth promotion. Now, in our work on the new inoculum we focus on the Pseudomonas isolates that we isolated as they demonstrated the most promising features for plant growth promotion and heavy metal resistance. Working with microbes is always fun. That was also the case of our secondment at HMGU. But speaking seriously our work was not about playing. We learned a lot about advanced and sophisticated methods for their identification and we also had a chance to use these methods in practice. And that is the most important game result.

2nd Year Transfer of Knowledge Activities

11	**Researchers on Secondments:**
7	Experienced Researchers
4	Young Researchers
24	**Secondments between academia & industry**
21	**Personmonths spent by researchers on secondments**
51	**Internal trainings & seminars**
51	**Outreach & project promotion activities**
3	Conferences
31	Open seminars for general public
4	Students sessions
3	Articles
7	Abstracts published
1	Broadcast
2	Short video clips

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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Institute for Studies and Power Engineering (RO)

ProBiotics (PL)

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

VITA 34, BioPlanta (DE)