BIORESCUE: GETTING HIGH ADDED VALUE PRODUCTS FROM MUSHROOM COMPOST

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ABSTRACT: Spent mushroom compost is the residual compost waste generated by the mushroom production industry. Annually 3 million tons of these residues are generated in Europe resulting in disposal costs of up to 150 million Euro. The BIOrescue project aims to develop an innovative biorefinery strategy to valorise this promising source of biomass together with other underutilised lignocellulosic feedstocks. This paper shows the main results obtained during the first half of BIOrescue project.

Keywords: agricultural residues, biobased products, circular economy, rural development, sustainability

1 INTRODUCTION

Spent mushroom compost is the residual compost remaining after fresh mushroom production. It generally consists of a combination of wheat straw, chicken litter, horse manure, water and gypsum, all composted together [1]. Due to its composition it is an excellent source of nutrients as well as a useful soil conditioner.

BIOrescue project which started in September 2016, aims to develop and demonstrate a novel biorefinery concept based on the cascading use of mushroom compost supplemented by wheat straw (and other seasonal underutilized lignocellulosic feedstocks). This new concept will avoid disposal costs and allow for the production of some biodegradable bio-based products and bioactive compounds, all the while helping to replace existing products based on fossil fuels.

The project consortium is composed of ten partners from seven European countries, each doing complementary tasks that will pursue the project objectives. Figure 1 shows the European dimension of the consortium.

Partner	Country
National Renewable Energy Centre (CENER) - Coordinator	Spain
Monaghan Mushrooms	Ireland
MetGen Oy	Finland
Universita degli Studi di Napoli Federico II (UNINA)	
Max-Planck-Institut für Polymerforschung (MPIP)	Germany
Celignis Limited	Ireland
Imperial College of London	United Kingdom
C-TECH Innovation Limited	United Kingdom
Greenovate Europe	Belgium
Zabala Innovation Consulting	Spain

Table 1. BIOrescue's consortium

BIOrescue is structured in seven work packages covering from feedstock supply to sustainability assessments, the following sections show the main outcomes reached by project partners during the first half of the project.

Figure 1: BIOrescue's consortium map

2 FEEDSTOCK SUPPLY, CULTIVATION AND ASSESSMENT

In this work package (WP2) the main goal is the characterization of the biomass feedstocks (spent mushroom substrate (SMS) and wheat straw (WS)), among other feedstocks, that would be available to the proposed biorefinery. Celignis is developing customized mathematical models that will allow the composition of biomass and the outputs of the BIOrescue technologies to be determined in seconds. These models only require that the samples be scanned using near infrared spectrophotometers, whereas the current standard methods of analysis involve several stages of chemical processing and require approximately 2 weeks to provide the same set of results. The models are being developed on samples in various stages of preparation, with a particular target being robust models for samples in their wet, unprocessed, form. Such models will facilitate the development of systems for the rapid at-line/inline analysis of inputs and outputs within a biorefinery based on the BIOrescue process.

A year-round assessment of wheat straw, mushroom compost and spent mushroom compost from a conventional mushroom cultivation farm, has been carried out by Monaghan and Celignis in order to define an optimum storage and supply strategy for the proposed biorefinery co-located on the mushroom cultivation farm. The samples were analysed via chemical and near infrared means by Celignis in order to give an understanding of any variability in composition over this period. The main results can be summarized as follows:

- A total of 48 samples of mushroom compost, 46 samples of SMS and 46 samples of wheat straw were collected and supplied by Monaghan to Celignis. All samples were scanned by NIR (1) (wet and dry) at Celignis' lab.
- 63 samples of SMS and compost and 32 straw samples were analysed chemically by Celignis.
- Accurate NIR models have been developed by Celignis and used to predict composition of all samples received allowing for examinations to be made regarding the variability of feedstocks (compost and straw) and output (SMS) over a year, also allowing comparisons between compost and SMS, i.e. what is happening to the compost during mushroom production stage.
- Not significant changes were observed in the composition of all three feedstocks over a period of 12 months, suggesting the feedstock composition is stable throughout the year.
- Some variability in the Klason lignin composition of SMS was observed, however this difference is also observed within samples belonging to the same month.
- A data base containing compositional data of all the analysed samples was generated.
- A gradual increase in the percentage of lignin is observed as the biomass changes from straw to compost and from compost to spent mushroom compost, this increase is due to the sugars being utilised, firstly by the microorganisms during the composting process and secondly by the mushrooms during the mushroom growing process; final lignin concentration in the SMS is 30%.
- Data base containing compositional data for all samples sent from Celignis to Monaghan has been generated.

The results obtained showed that were not significant differences in composition throughout the 12 month period in either of the substrates, wheat straw (figure 2), compost (figure 3) and spent mushroom compost (figure 4). Some differences were observed in the amount of Klason lignin present in SMS, however this variability does not seem to be season related as it was also present within the monthly set of samples.

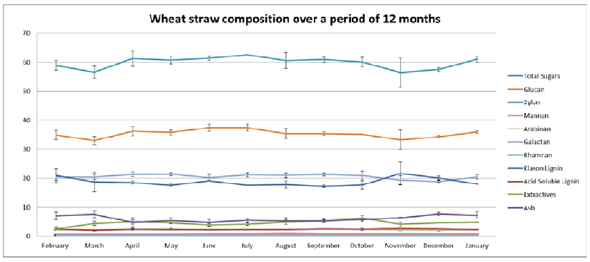

Figure 2: Wheat straw average total composition month by month, (dry weight %). Values are the mean of all data for each variable ± standard deviation.

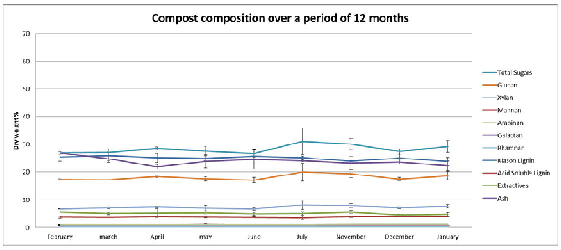

Figure 3:. Mushroom compost average total composition month by month, (dry weight %). Values are the mean of all data for each variable ± standard deviation.

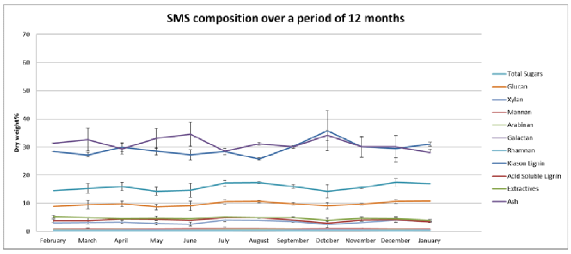

Figure 4: Spent mushroom compost average total composition month by month, (dry weight %). Values are the mean of all data for each variable ± standard deviation.

3 SEPARATION AND FRACTIONATION

CENER in collaboration with Monaghan has been working in the fractionation of mushroom compost, in combination with other agricultural residues, through the application of a two- step process based on the extraction of the soluble bioactive components and the thermochemical pretreatment of the insoluble fraction to get different side-streams to be used for the secondary conversion of the feedstock into different products.

The separation and fractionation process begins with the extraction step, which involves subjecting the SMS to a washing step followed by a mechanical separation using a filter press to get an extractives-free solid (matrix). This extracted SMS is combined with underutilised lignocellulosic substrates and subjected to a thermochemical pretreatment to break down the structure and fractionate the mixture to a largest extent. Thermochemical pretreatment has been carried out using mixtures of SMS at a ratio of 40% (wet weight) in the final blend and other underutilized feedstocks. These alternative feedstocks have been blended with SMS making out binary (SMS and Wheat Straw (WS) and ternary (SMS, WS and Oat or Barley Straw (OS or BS) blends. Likewise, the ratio used in binary blend was 40:60 in wet basis to SMS and WS respectively. In case of ternary blends, the ratio used was 40:30:30 in wet basis to SMS, WS and either OS or BS respectively. Binary and ternary blend slurries composition showed no mayor differences within cellulose content. In contrast, soluble xylose obtained after thermochemical fractionation ranges greatly when varying the residence time (figure 5) even at the same temperature.

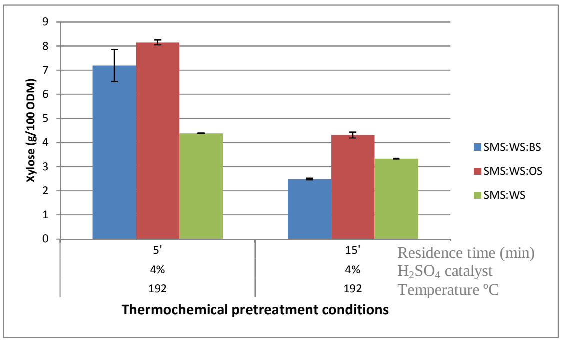

Figure 5: Soluble Xylose content (g/100g ODM) for each mixture of SMS combined with wheat straw (WS) and barley (BS) or oat straw (OS). ODM: Oven dried matter.

Likewise, CENER has conducted the analysis for quantifiying compounds that are generated during thermochemical pretreatment, and they are well known to have a inhibiting effect over enzymatic hydrolysis [2] and over fermentation [3] either. These compounds (such as furans (addition of 5-Hydroxymethylfurfural and furfural) and acetic acid) increased in ternary blends when longer the residence time was, as opposed to binary blend where the residence time seemed not to have a big impact (figures 6 and 7).

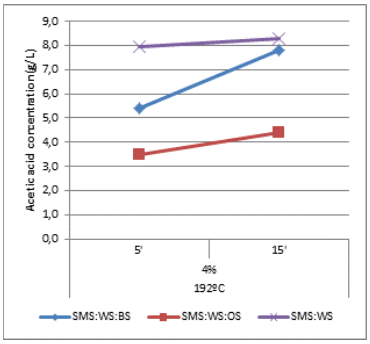

Figure 6: Acetic acid content (g/L) quantified in the slurry for each mixture of SMS combined with wheat straw (WS) and barley (BS) or oat straw (OS) at different thermochemical pretreatment conditions.

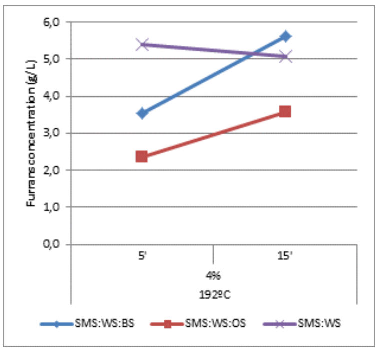

Figure 7: Soluble Furans content (g/L) quantified in the slurry for each mixture of SMS combined with wheat straw (WS) and barley (BS) or oat straw (OS) at different thermochemical pretreatment conditions.

Additionally, Monaghan Mushrooms has been working on the analysis of the aqueous phase for nutrient content and bioactive enzymes determination.

4 ENZYMATIC HYDROLYSIS

MetGen has conducted an extensive screening of tailored enzyme solutions (MetZyme® SUNO™) together with hydrolysis conditions for the optimal saccharification efficiency of pre-treated biomasses. Depending on the type of the biomass and pre-treatment conditions, best enzyme formulations showed high reducing sugar yields and 80-100% glucan to glucose conversion ratios (compared to theoretical maximum) already within the first 24 h of hydrolysis. Figure 8 shows example of the conversion results for selected enzyme cocktails. On top of the technical performance, aspects like processability and production cost of the enzyme cocktails have been considered, which will enable the selection of the most techno-economical feasible solution for the subsequent pilot-scale hydrolysis assays

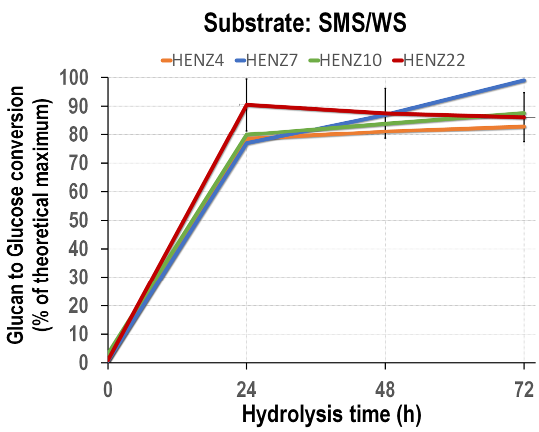

Figure 8: Glucan to Glucose conversion with selected MetZyme® SUNO™ solutions (HENZ4, HENZ7, HENZ10 and HENZ22). Conditions: pH 5, T = 50°C. Substrate: a mix of spent mushroom substrate (SMS) and wheat straw (WS).

Additionally, UNINA has selected one cellulase to be subjected to directed evolution by developing recombinant expression systems and characterising two new *Streptomyces* cellulases of family GH5. The strategy for diversity generation and screening was set up using an automated workstation. This strategy was validated by applying it to generate and screen 5000 cellulase mutants and demonstrating that 6 of these new mutants show higher activity than wild type cellulase. Moreover, this strategy was applied to obtain another set of 25,000 transformants to generate a total of 30,000 mutants.

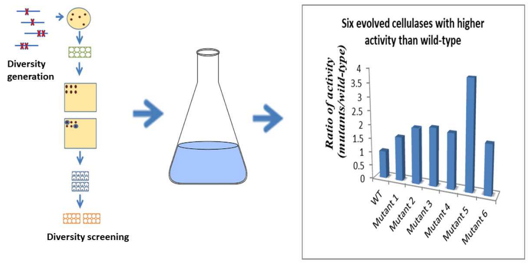

Figure 9: Directed evolution strategy and results

Furthermore, Monaghan Mushrooms has been providing cellulases for the compost saccharification trials and is currently involved in the enzymatic hydrolysis of spent mushroom compost at a medium scale for the optimization of the saccharification process.

5 CHEMICAL CONVERSION

5.1. Synthesis of nanocarriers

The Max Planck Institute for Polymer Research is developing a versatile and scalable approach for the large-scale synthesis of polymer nanocarriers from the soluble fraction obtained after thermochemical pretreatment. The nanocarriers are prepared by an emulsion polymerization and previously already different pure biopolymers (polysaccharides, lignin and proteins) could be transformed into well-defined nanoparticles [4, 5]. Within BIOrescue a protocol for preparing biodegradable nanocarriers based on the soluble fractions from the mushroom waste was developed. Biodegradable nanocarriers with different morphologies (solid nanoparticles, core-shell structures, porous nanoparticles) can be produced by a combination of miniemulsion polymerization and a solvent evaporation process. They may find useful application as novel drug delivery vehicle in agriculture or as carbon materials for water purification.

6 SUSTAINABILITY ASSESSMENT

Imperial College London through the Centre of Environmental Policy is coordinating the Sustainability Assessment of the project. In collaboration with CENER, Imperial has defined the methodology and environmental indicators to be used in a Life Cycle Assessment for the biorefinery. Imperial will be applying a Social Life cycle assessment and policy assessment and has already started to identify the stakeholders and indicators for the assessment. C-TECH has also identified the methodology and indicators for the economic assessment. The final sustainability integration of all issues will describe the most sustainable pathways among the value chains compared to all reference systems.

In addition, a technical assessment of selected feedstocks has been conducted by Celignis in predetermined regions: the Southern region (comprising Italy, France, and Spain) where prunings from olive trees, vineyards, almond trees, and peach trees were tested, selecting prunings from vineyards as the best option ; the Western region (comprising the UK and Ireland) where barley straw, oat straw, and poultry litter were tested with the outputs of this analysis leading to the selection of oat straw and barley straw as the chosen feedstocks; and the Northern region (comprising The Netherlands and Germany) where tobacco residues and sugar beet pulp were tested and sugar beet pulp was considered to have a reasonable lignocellulosic composition and became the chosen feedstock for this region

The Eastern region was represented by Poland, where pomace samples were analyzed and selected as the chosen feedstock due to its lignocellulosic composition and its high level of abundance in Poland

A full socio-economic analysis will be conducted for these selected feedstocks.

7 CONCLUSIONS

The project BIOrescue has reached half of its life and the main results obtained are summarized below:

- A wide variety of underutilized feedstocks from different European regions have been analyzed to select the best combinations with mushroom compost. Based on these results, an optimal feedstock mixture of compost, wheat, oat and barley straw has been proposed for a mushroom-farm-based biorefinery located in the Western region of Europe.
- Preliminary extraction assays on mushroom compost have been carried out and shown the release of a high amount of soluble compounds. The solid and liquid fractions are being characterized to determine the presence of: sugars, organic acids or furfurals, proteins and nutrients. Moreover, enzymatic activities such as cellulase and xylanase are being measured in the extracted fraction although low values have been identified.
- A set of thermochemical pretreatment tests have been carried out on mushroom compost alone and in combination with wheat straw using different catalysts and process conditions.
- The best enzyme cocktails for the hydrolysis of combinations of mushroom compost and wheat straw have been selected. Results from the enzyme cocktail screening experiments were then used to assess techno-economically most suitable enzyme cocktail candidates for further pilot-scale hydrolysis assays. Moreover, an optimization of the enzymatic hydrolysis conditions has also been carried out. Furthermore, different cellulases, have been successfully cloned, expressed and characterized for future mutant library screen.
- The liquid fraction obtained after the thermochemical pretreatment has been used as feedstock to produce two different types of biodegradable, enzyme-responsive lignin nanocarriers by miniemulsion polymerization for drug delivery.
- An initial definition of the system boundaries and settings for the environmental, techno-economic and social impact assessments of the future biorefinery has been carried out.

8 NOTES

(1) NIR: Near Infrared Spectroscopy

9 REFERENCES

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11 LOGO SPACE

More information about BIOrescue:
http://www.biorescue.eu