BIORESCUE: ENHANCED BIOCONVERSION OF AGRICULTURAL RESIDUES THROUGH CASCADING USE

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ABSTRACT: Mushroom production generates three million tonnes of compost each year in the European Union which is currently landfilled or used for landscaping purposes even though it contains valuable organic components. In view of transforming this compost into a new income stream for mushroom producers, the BIOrescue project aims to develop and demonstrate a new innovative biorefinery concept based on the cascading use of spent mushroom substrate (SMS) supplemented by wheat straw (and other seasonal underutilized lignocellulosic feedstocks). This new concept will avoid disposal and allow for the production of some biodegradable bio-based products and bioactive compounds that will help to replace the existing ones based on fossil resources.
The research will help to expand the business opportunities of the mushroom cultivation farms, and the know-how and business opportunities of all the partners involved.
The main scientific innovations are: improved methods for the lab-based rapid (NIR) (1) analysis of biomass, innovative two step fractionation of SMS, synergistic effects for complete SMS glucan hydrolysis, innovative enzyme immobilisation strategy, development of highly efficient glucan-enzymes, novel lignin based nano- and micro-carriers and biopesticide production from monomeric sugars derived from SMS and their packaging into nanocarriers.

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. It is an excellent source of humus, although much of its nitrogen content will have been used up during the composting and mushroom cultivation processes. It remains, however, a good source of general nutrients (0.7% N, 0.3% P, 0.3% K plus a full range of trace elements), as well as a useful soil conditioner. However, due to some components (e.g. gypsum), it may be alkaline, and should not be used on acid-loving plants, nor should it be applied too frequently, as it will overly raise the soil's pH levels.

Total fresh mushroom production in Europe in 2013 amounted to approximately one million tonnes [1]. For every ton of mushroom harvested, approximately three times the amount of mushroom compost (or spent mushroom substrate) is produced. This ratio means a disposal of three million tons of compost per year. Mushroom compost is currently collected from farms for a fee associated with transport and disposal, creating significant economic and logistical problems for farmers. Moreover, in some cases, it is stored temporarily before being disposed of or spread on land or sold as soil amendment or conditioner product. According to European mushroom producers, the current cost for mushroom compost disposal vary across Europe from 10 to 50 €/t. This means that the mushroom industry is spending up to 150,000,000 €/year for compost disposal and this cost is expected to increase in the short and medium term. This situation has led the mushroom industry to take advantage of the existing positive bioeconomy environment and to look for valorisation alternatives for these residues to retrofit the current business into more sustainable and efficient activities.

2 THE BIOrescue CONCEPT

In view of transforming this compost into new income streams for mushroom producers, the BIOrescue project aims to develop and demonstrate a novel biorefinery concept based on the cascading use of mushroom compost supplemented by wheat straw (and other seasonal underutilised 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.

By designing a solution to upgrade up the majority of mushroom compost, the BIOrescue project will help creating further value from mushroom by-products. In that sense, the novel concept will contribute to the extension of products' lifecycles through the development of greater re-use and recycling processes. This objective is in direct agreement with Communications from the European Commission referencing a circular economy; "Towards a circular economy: A zero waste Programme for Europe"[2].

The research will help to expand the business opportunities for the mushroom farmer, and significantly increase the technical know-how and business opportunities of all the partners involved.

The main scientific innovations to be developed within the project are:

- Deployment of improved methods for the lab-based rapid (NIR) analysis of biomass that could be applied for on-line real-time evaluation of biomass feedstocks and process outputs.
- Application of an innovative two-step fractionation process of mushroom compost with synergistic effects for complete glucan hydrolysis.
- New improved enzymes for lignocellulose conversion for formulating optimized enzymatic cocktails for hydrolysis of SMS with increased monosaccharide yield.
- Development of an innovative enzyme immobilisation and recovery strategy that will include the use of mCLEAs.
- Formulation of novel lignin based nano- and micro-carriers and a biopesticide from monomeric sugars derived from the compost and their packaging into nanocarriers.
- Fermentation of the sugars obtained from the compost for the production of enzymes to be used within the biorefinery .

The BIOrescue project brings together 11 partners from 8 different European countries, each doing complementary tasks that will pursue the project objective. Six of the entities are companies (of which five are SMEs) with recognised experience in the field having clear focus commercialisation of the results, in addition to four scientific partners (two research centres and two universities) for the development of the cutting-edge technology, as well as an EEIG (2) for project communication and dissemination. The following figure shows the European dimension of the consortium.

Figure 1: BIOrescue's Consortium map

3 NOVEL BIO-BASED PRODUCTS FROM MUSHROOM COMPOST

In a more detailed way, BIOrescue aims to produce the following products:

- **Mushroom-derived enzymes and other bioactive compounds.**
- **Nutrient rich horticultural fertiliser** which can be used by mushrooms cultivars.
- **Highly efficient glucan-enzymes** that can improve enzymatic hydrolysis of lignocellulosic feedstocks.
- **Biopesticides** that are naturally occurring bioactive substances, and therefore less toxic than conventional ones and very specific to the target pest.
- **Nanocapsules,** which are nanoscale shells (nanocarriers) made out of a nontoxic polymer membrane encapsulating an inner liquid. They have a myriad of uses, including medical applications for drug delivery, biopesticide encapsulation, food enhancement, nutraceuticals, and self-healing of materials. The benefits of encapsulation methods are related to the protection of these substances in adverse environments, for controlled release and precision targeting.

The following subsections describe in more detail each of the individual developments that will be carried out by BIOrescue

3.1 Mushroom-derived enzymes and other bioactive compounds.

SMS contains a large amount of mushroom mycelium which colonises the substrate during mushroom growth. This network of mycelium secretes extracellular enzymes, mainly cellulases, xylanases and laccases to release nutrients from the substrate for mushroom growth. These valuable extracellular enzymes contained within the SMS are lost when the SMS is landfilled or used for other purposes. During the BIOrescue project these enzymes will be recovered through an aqueous extraction process and their activity quantified. Recovered enzymes will in turn be tested, along with other glucan enzymes, for their ability to hydrolyse other BIOrescue biomass substrates. These activities will be carried out by Monaghan, CENER and Celignis.

3.2 Nutrient rich horticultural fertiliser which can be used by mushrooms cultivars.

As well as the lignocellulosic and enzyme content of SMS, the substrate also contains macronutrients such as nitrogen (N), phosphorus (P) and potassium (K). Although the presence of these nutrients has seen SMS used for landscaping and land spreading applications, the high variability and low nutrient to mass ratio makes SMS difficult to use for these applications in practice. During the BIOrescue project the macronutrient content of aqueous extracts of SMS will be measured by Monaghan and Celignis. The NPK content of SMS extracts will be compared to commercially available liquid fertiliser products and the application of these extracts for mushroom production will be assessed.

3.3 Highly efficient glucan-enzymes

MetGen will test and develop MetZyme® SUNO™ enzymatic solutions and further optimise their performance for the specific BIOrescue biomass substrates. This is due to the fact that the processes, feedstock, and especially the pre-treatment create a variance to the substrate that has to be addressed case-by-case. Application material tests will include a screening of large matrix of enzyme formulations with process-specific conditions to identify suitable enzyme formulations for designing a material specific MetZyme® SUNO™ enzyme solution with optimal properties. During BIOrescue, MetGen will utilized two lines of products within MetZyme® SUNO™ family:

• MetZyme® SUNO™ BOOSTER, a high performance enzyme formulation custom designed to significantly improve saccharification capabilities of hydrolysis enzymes used by Monaghan and UNINA.

• MetZyme® SUNO™ COMPLETE, a competitive, high performance solution tailored to significantly improve saccharification yields to replace existing commercial hydrolysis solutions.

Similarly, as mentioned above, Monaghan will supply two different groups of enzymes to be tested in combination with MetGen enzyme solutions:

• A novel cellulase enzyme cocktail developed for the hydrolysis of lignocellulosic substrates.

• Cellulase, xylanase and laccase enzymes recovered from aqueous extracts of SMS described above. These enzymes will be used to supplement the Monaghan cellulase cocktail or be used in combination with any of the other enzyme solutions if they prove to be effective on the BIOrescue biomass substrates.

UNINA will create the library of cellulase variants obtained by directed evolution, in order to increase biomass conversion rate in the enzymatic cocktail formulation.

New cellulases from a cellulolytic strain *Streptomyces,* previously identified by UNINA starting from lignocellulosic biomasses of *Arundo donax*, *Eucalyptus camaldulensis* and *Populus nigra* will be cloned into expression vectors and their production optimized into an appropriate heterologous host. The enzyme stability and efficiency will be improved through directed evolution. Libraries of 30000 random mutants of cellulase will be created using error-prone PCR. A strategy of high-throughput screening will be specifically set up and the mutant libraries will be subjected to high-throughput screening to identify variants with higher cellulolytic activity using semi-synthetic/chromogenic compounds on solid and liquid media, using an UNINA automated workstation.

The improved enzymes will be delivered to the other project partners to be mixed with other cellulases (Monaghan) and laccases (MetGen) to define the best combination in the biomass conversion.

3.4 Lignin-derived nanocarriers

Max Planck Institute of Polymers (MPIP) is working in the production of lignin-derived nanocarriers from the soluble lignin fraction obtained after feedstock pretreatment. These nanocarriers can be loaded with drugs (herbicides, antifungals, pesticides) and cleaved by natural occurring enzymes (laccases). This is a very innovative solution as it is consider the first use of lignin as a modular building block for the design of nanocarriers.

3.5 Biopesticides from carbohydrate fermentation

Microbial pesticides are a type of biopesticides which consist of a microorganism (e.g., a bacterium, fungus, virus or protozoan) as the active ingredient. They can control many different kinds of pests, although each separate active ingredient is relatively specific for its target pest/s.

The most widely used microbial pesticides are subspecies and strains of Bacillus thuringiensis, or Bt. It is bacteria that can metabolize a carbon source (i.e. monomeric sugars) and produces a different mix of proteins and specifically kills one or a few related species of insect larvae.

It has been proved that this type of biopesticides are also effective in small quantities, resulting in lower operator exposure, and therefore decompose quickly, and are less likely to have resistance issues.

However, one of the major problems of commercial biopesticides is its low persistence in the field, since the exposure of proteins to ultraviolet light causes their effectiveness to decrease markedly. Therefore, in this project besides producing a biopesticide from the monomeric sugars obtained after the enzymatic hydrolysis of the feedstocks, the lignin derived nanocarriers will be used as natural containers for the biopesticide produced from sugar fermentation. The fermentation process will be carried out by CENER and the encapsulation activities by Max Planck Institute of Polymers.

4 FEEDSTOCK SUPPLY AND SUSTAINABILITY ASSESSMENTS

Besides the production of different biobased products, BIOrescue is evaluating the application of the innovative biorefinery concept based on the cascading use of biomass to a conventional mushroom farm.

Therefore, some additional assessments focused on the availability of feedstocks and the process sustainability will also be carried out. More detail is included in the following subsections.

4.1 Feedstock supply assessment

Monaghan is supplying Celignis with a large number of samples of mushroom compost and wheat straw over the course of 12 months. These samples will be characterized for relevant lignocellulosic constituents and the consistency and/or variations over time evaluated. Celignis will also develop custom rapid analysis models for each feedstock that will allow its composition to be determined using its near infrared spectrum. This will reduce the analysis time from weeks to seconds. Separate models will be developed for feedstocks in their wet unprocessed states, as well as after they have been dried and ground.

Celignis is also evaluating the composition of a range of other potential lignocellulosic feedstocks for processing in the BIOrescue conversion technologies.

These are all agricultural residues or wastes from the processing of crops and are often seasonal in their nature, with low current market values. Their compositions will be determined using Celignis' existing NIR models, as well as chemical analysis methods where necessary. The data will be evaluated in the context of the feedstock being a suitable co-feed with mushroom compost and straw. The selection of suitable residues for conversion in the BIOrescue technologies could greatly enhance their revenue potential for farmers and processors and also help to address concerns regarding the wastage of biomass.

4.2 Sustainability assessment of the biorefinery

It is led by Imperial College and with the participation of CTECH Innovation, CENER, Monaghan and MetGen.

The work to be carried out comprises a sustainability assessment that includes the techno-economic performance as well as an analysis of environmental, economic, social and political issues of the new multi-product biorefinery in comparison with a conventional mushroom production farm.

The partners involved are providing the definitions and settings that will be used as well as the system boundaries for the assessments.

The sustainability assessment will include an analysis with different methodologies comprising LCA (3), Social LCA, environmental and social impact assessment and sustainability assessment. The assessments will comprise the whole value chain of the biorefinery.

In the economic assessment the economic implications of the identified value chains and the most promising reference systems will be analysed. A micro-economic assessment will be conducted for all selected and reference systems to determine the costs and benefits of individual process chains. The most critical components will be identified by sensitivity analyses e.g. using possible price ranges. A macro-economic assessment will be also conducted to identify and quantify the economic impacts the project will produce at regional, national and European / Global scales. A Life Cycle Costing (LCC) methodology will be implemented, whereby costs will be calculated on the basis of the whole life-cycle of the supplies, services and works included in the biorefinery system. Investment, operation, maintenance and end-of-life disposal expenses in the biorefinery system will be also taken into account.

An integrated sustainability assessment will be conducted as the final assessment integrating the results of the separate assessment of the economic, environmental and social issues. In the integrated assessment, the results from the previous analyses will be put together to identify and describe the most sustainable pathways among the value chains compared to all reference systems. Different methodologies will be used including multi-criteria evaluation and sensitivity analyses to identify the main parameters influencing the economic and environmental performance of the systems under investigation and to show optimisation potentials of these systems

5 CONCLUSIONS

An innovative biorefinery concept based on the cascading use of mushroom compost supplemented by other lignocellulosic feedstocks will be demonstrated by the project BIOrescue. This new concept is aligned with the existing circular bioeconomy approach as it will avoid the disposal of high amounts of compost and the production of a series of novel biodegradable bio-based products.

6 NOTES

(1) NIR: Near-infrared
(2) EEIG: European Economic Interest Grouping
(3) LCA: Lifecycle Analysis

7 REFERENCES

[1] Monaghan Mushrooms. Ireland.

[2] COMMUNICATION FROM THE COMMISSION TO THE EUROPEAN PARLIAMENT, THE COUNCIL, THE EUROPEAN ECONOMIC AND SOCIAL COMMITTEE AND THE COMMITTEE OF THE REGIONS Towards a circular economy: A zero waste programme for Europe /* COM/2014/0398 final */

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9 LOGO SPACE

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