BIO RESCUE

A NOVEL BIOREFINERY CONCEPT FOR MUSHROOM COMPOST

ENHANCED BIOCONVERSION OF AGRICULTURAL RESIDUES THROUGH CASCADING USE

CONTENTS

The BIOrescue project has developed a novel biorefinery process to convert used mushroom compost and other underutilised agricultural feedstocks into valuable bio-based materials with multiple applications in the agro-food industry and beyond.

Why?
Moving towards a circular economy in the agro-food industry

The BIOrescue concept: making new added value products from waste material 3
A circular bioeconomy is what Europe needs 4
New value chains and opportunities for agro-food businesses: the BBI Joint Undertaking 6
A more circular mushroom industry 8

How?
The innovations step-by-step

The BIOrescue process explained 10
Rapid biomass analysis: the key to a successful biorefinery process 12
A parallel process for optimised biomass pretreatment 14
Tailor-made and improved enzymatic solutions 16

What?
From waste to new products

Wonders you can do with mushroom compost 18
A replicable concept for thriving rural economies 20

© BIOrescue 2019

Copyright Monaghan Mushrooms

LET'S "BIORESCUE" **MUSHROOM COMPOST**

Mushrooms are well known as ingredients in delicious dishes, and for being rich in important nutrients, such as B vitamins, selenium, potassium, copper, vitamin D and antioxidants.

But how many of us are familiar with the mushroom production process? Mushroom growing needs a dedicated compost, which can only be used for a limited amount of time. After that farmers have to pay to get rid of it. **For every tonne of mushrooms harvested, around three tonnes of mushroom compost are generated**. Considering that total fresh mushroom production in Europe amounts to about one million tonnes a year, it is easy to understand the dimension of the problem.

BIOrescue is developing processes and technologies to **give new life to used mushroom compost**. The novel waste conversion system will extract the valuable organic components left in the compost and transform them into new products such as **biodegradable pill cases and enzymes** that can be used in other industrial bioprocesses. The concept will eventually be implemented at a mushroom farm in Ireland operated by Monaghan Mushrooms, one of the world's largest mushroom producers, which will be retrofitted to become a sustainable and efficient biorefinery.

But **this research does not stop at mushrooms**. Project partners are assessing the possibility to extend its application to other crop systems in Europe such as vineyard prunings, oats and barley straw, sugar beet pulp and apple pomace. So BIOrescue could be a step towards a truly circular bioeconomy.

A CIRCULAR BIOECONOMY **IS WHAT EUROPE NEEDS**

Population growth, climate change, resource scarcity: Europe needs to develop new ways to live within its boundaries.

The bioeconomy is part of the solution. The sustainable production and exploitation of biological resources has the potential to 'make more with less', including turning waste into business opportunities, while helping the transition towards a carbon-free and circular (bio) economy.

Bioeconomy is not a niche area. An annual turnover of two trillion euros and the employment of 22 million people[1] make it one of the Union's biggest and most important sectors. It includes agriculture, forestry, fisheries, food, biofuels, textiles, paper manufactures and bio-based chemicals used for a great variety of products.

The bioeconomy has huge growth potential. European Commission estimates show that each euro invested under the Horizon 2020 programme could generate ten euros of added value in the different bioeconomy sectors by 2025. And this is just considering the investment in the EU research and innovation programmes.

While the bioeconomy focuses on biological rather than fossil resources, in a circular economy resources are **reduced, reused, recycled or recovered**. Combined together, both concepts are looking at the development of sustainable products recovering valuable components from biowaste materials. Their joint application will enable the replacement of fossil-based products with low carbon and natural alternatives, while relieving pressure of human activities on the environment.

[1] "Innovating for sustainable growth. A bioeconomy for Europe" European Commission Action Plan (2012)

"For each euro invested under Horizon 2020, ten euros of added value can be generated in the different bioeconomy sectors by 2025"

Bioeconomy: what does it mean?

The bioeconomy refers to the production of **renewable biological resources** and the conversion of these resources and waste streams into **value added products**, such as food, feed, bio-based products and bioenergy. Bio-based products and bioenergy can be "bio-based versions" of **traditional products, or novel products** with entirely new functionalities and potential for existing or innovative markets.

Circular bioeconomy includes:

WHAT DOES IT TAKE **FOR THE BIOECONOMY TO THRIVE?**

We asked Philippe MENGAL, Executive Director of Bio-based Industries Joint Undertaking, to tell us about how public-private collaboration can foster the bioeconomy.

How is BBI-JU promoting industrial cooperation, also among different sectors, to develop new bio-based value chains and products?

Bio-based industries and their value chains are faced with complex and substantial technology, innovation and financial challenges – challenges which n**o single company, industrial sector or Member State can tackle alone**. As a public-private partnership, BBI JU is instrumental in bringing together bio-based activities under one pan-European structure, pooling national and regional assets, strengths and skills. The initiative aims at connecting key sectors, creating new value chains and producing a range of innovative bio-based products, and I am happy to say that we are already delivering well beyond the 2020 targets set in our Strategic Innovation and Research Agenda. By 2020, BBI JU projects expect to create **113 new bio-based value chains and 65 new bio-based consumer products** (the targets were 10 and 30 respectively). Furthermore, the structuring and mobilising effect were the two main impacts of BBI JU underlined in the interim evaluation performed by the European Commission.

What are the most important benefits coming from financing projects like BIOrescue?

BBI JU's projects such as BIOrescue are developing the potential of waste as well as agricultural and forestry residues. They are perfect examples of a bioeconomy in action, meaning **sustainable, resource-efficient and largely waste-free** utilisation of Europe's renewable raw materials for industrial processing.

Everyone benefits from a strong European bio-based industrial sector that can significantly reduce Europe's dependency on fossil-based products, and help Europe meet its climate change targets. Among other things, the creation of a competitive bio-based infrastructure in Europe will help primary producers (farmers), boost employment as well as support regional development, especially in rural areas.

What are the next steps for the European Union to support a 'circular bioeconomy'?

In October 2018 the European Commission adopted the **updated bioeconomy strategy**, which aims at accelerating the deployment of a sustainable European bioeconomy. The strategy proposes concrete actions aimed at scaling up and strengthening the bio-based sectors, unlocking investments and markets, deploying local bioeconomies across Europe, and understanding the ecological boundaries of the bioeconomy. Key actions in the updated strategy include facilitating the development of **new sustainable biorefineries** and intensifying the **mobilisation of public and private stakeholders**, areas in which the BBI JU plays a key role.

The Bio-based Industries Joint Undertaking (BBI JU) is a public-private partnership aiming at increasing investment in the development of a sustainable bio-based industry sector in Europe. The mission of this EU body is to implement, under Horizon 2020 rules, the Strategic Innovation and Research Agenda developed by the industry and validated by the European Commission.

TOWARDS A MORE CIRCULAR **MUSHROOM INDUSTRY**

Back in 2014, Monaghan Mushrooms, one of the world's largest mushroom production companies, opened a dedicated research division, Monaghan Biosciences – to exploit the power of enzymes to extract high-value products from biomass. Since then, the start-up has launched several research initiatives, including BIOrescue, one of their first international collaboration projects.

Darragh Gaffney, Research and Development Manager at Monaghan Biosciences, outlines what the project brings to the mushroom industry.

Copyright Monaghan Mushrooms

How could circular economy processes such as BIOrescue benefit the mushroom industry?

Mushroom growing is already a **semi-circular process**, requiring the preparation of a **dedicated compost, often made from agricultural by-product materials** such as wheat straw, poultry manure and gypsum, mixed with spawn and ammonium sulphate. This compost, covered with a layer of peat, can only be used for a limited number of mushroom harvests.

With the increasing landfilling costs and the implementation of the Nitrate directive restraining possibilities to spread mushroom compost on lands, this situation creates a significant economic problem but also an opportunity for our industry. New circular bioeconomy processes, such as the ones developed within the BIOrescue project, would enable us not only to **avoid these costs**, but also to **create new value** from what was previously considered as waste.

How does BIOrescue fit into Monaghan Mushrooms' general sustainability strategy?

At Monaghan, sustainability is one of our core concerns and we were one of the first companies to become **Origin Green Certified**, a sustainability programme run by the Irish Food Agency to make Ireland a world leader in high quality, sustainably produced food and drink.

Natural resources are central to the mushroom production process, but they are limited. **Peat reserves**, for instance, declined by almost 40% in Ireland between 1995 and 2012, according to a BBC report. That is why we have decided to cut peat usage by one third in all of our farms, and are currently developing new strategies to reuse the peat layer.

Another of our targets, fully in line with the BIOrescue project, is to **reduce our waste** to landfill by 20% by 2019. On top of this, we have already **curbed our energy usage** by 17% per tonne of mushroom produced and intend to reduce it by a further 2.5% in the coming years. All of our energy supply comes from **renewable sources**, and we installed **rainwater capture systems** in our compost yards and mushroom farms to reduce press ure on water resources. As we are looking at the full process, we also try to use **less and less plastic in packaging**, and our mushrooms are delivered in **fuel-efficient lorries**, powered with renewable fuels.

What are the main advantages of international cooperation projects like BIOrescue?

As a company with specialist technical knowledge in certain areas, we do not have the full solutions at out fingertips. But thanks to the **complementary and highly-specialised competences of BIOrescue partners**, we are now able to test a completely new process in state-of-the-art facilities, while assessing its environmental, social and economic impacts. For Monaghan, BIOrescue is an investment in the future.

In parallel to BIOrescue, we have launched another research project, **Funguschain**, also funded by the Bio-based Industries Joint Undertaking. Funguschain partners are working on another biorefinery process, this time using **mushrooms as the feedstock**. Both projects will help to reduce waste in the mushroom industry, while contributing to the development of a truly circular European bioeconomy.

A NOVEL BIOREFINERY PROCESS **FOR THE AGRO-FOOD INDUSTRY**

Bridging the gap between rural entrepreneurs and the biotech industry, BIOrescue has developed a new biorefinery concept to transform used mushroom compost and other types of underutilised agricultural waste into high value products.

Before the process starts, feedstock is first analysed using **novel dynamic modelling tools** (pp. 12-13) to determine the raw materials' potential and adapt technical conditions for their transformation.

As in every biorefinery concept, the biomass undergoes dedicated pretreatment processes that separate its main components and prepare it for the next steps. BIOrescue is seeking to extract two main components: lignin and sugars. To maximise extraction yields, the project developed **two innovative biomass pretreatment processes** (p. 14) that are carried out in parallel. An **organosolv pretreatment** is used to obtain the maximum amount of lignin, while sugars are retrieved through **thermochemical pretreatment**.

To solubilise the sugars obtained, an enzymatic hydrolysis is applied using **tailor-made and improved enzymatic solutions** (p. 16). The sugars are then fermented to produce **sustainable and low cost biopesticides** (p. 19), as well as **new enzymes** (p. 18) to be employed in other biomass conversion processes.

On the side of the lignin, the fractions obtained from the organosolv and thermochemical pretreatment are mixed and submitted to a chemical conversion process to transform lignin into polymers. The polymer membranes are used for the development of **biodegradable nanocapsules** (p. 17), which are then loaded with the biopesticides produced within BIOrescue.

"From biomass analysis to conversion, innovation is driving each step of this biorefinery process. By generating low carbon bio-based products that can be used directly in the agricultural sector, the BIOrescue project is closing the loop towards a truly circular agro-food industry"

Inés Del Campo, from BIOrescue project coordinator CENER

THE NOVEL **BIOREFINERY PROCESS**

STEP-BY-STEP **INNOVATIONS**

A. **Rapid biomass analysis:** the key to a successful biorefinery process

Feedstock composition is a critical factor for the success of biomass transformation processes. However, precise data on a wide range of feedstocks is still missing. Companies have to subcontract laboratories to **chemically analyse biomass samples**. This process is time consuming and expensive: taking approximately **two weeks per sample**, and costing hundreds of euros.

Looking for solutions to improve this process, BIOrescue partner Celignis Analytical created a novel methodology for biomass analysis, which models samples' composition according to the results of a **Near Infrared spectroscopy analysis**. Following the analysis of hundreds of different feedstocks from across the world, Celignis developed unique algorithms to predict with high accuracy and precision the composition of biomass samples. And **this in only one day**, for less than a hundred euros per sample.

This process has been instrumental in the successful development and implementation of the BIOrescue biorefinery concept. First of all, it **analyses and predicts the composition of used mushroom compost samples**. Using complementary chemical analysis when necessary, Celignis could determine the type and amounts of sugar, lignin and ash present in the compost. Secondly, the models were also used to **select the most suitable combination of used mushroom compost and other agricultural residues**.

This method will facilitate the possibility to explore the employment of new types of underutilised biomass, and can therefore foster the development of new and innovative bio-based processes.

What is Near Infrared Spectroscopy?

When you put your hand next to a fire, you can feel the heat on your skin. This is due to the near infrared radiations emitted by the fire, which are absorbed by water molecules in your skin, thus raising their temperature.

The principle is the same for molecules: when scientist send them infrared light, the liaisons **within each molecule react in different ways**, depending on the type of molecule. The reaction of these molecules is observable and recognisable. In fact, the frequency of this light signal emitted differs according to the type of molecules, which allows scientists to identify which molecules are present in a material by analysing these parameters with a spectroscope.

The data collected is then interpreted through **tailor-made algorithms**, which make the link to the type of molecules and the amount present in the sample.

B.
Parallel process for optimised biomass pretreatment

To recover the vast majority of the valuable components contained in mushroom compost and other agricultural waste, the National Renewable Energy Centre of Spain (CENER) has developed two parallel pretreatment processes, one being oriented towards sugars extraction and the other at lignin valorisation.

THERMOCHEMICAL PRETREATMENT FOR SUGARS

On one hand, a mixture of mushroom compost and other underutilised feedstocks, such as wheat straw, is submitted to a thermochemical pretreatment to solubilise the sugars contained in these raw materials. During this operation, the biomass is **heated with an acid catalyst and water** for an average of fifteen minutes. The sugars isolated through this process are then broken down into monosugars through an **enzymatic hydrolysis process** and fermented for the production of new enzymes and biopesticides.

ORGANOSOLV PROCESS FOR LIGNIN

In parallel, partners applied an organosolv process on used mushroom compost, **heating the biomass up to 250°C with water and solvent** to extract the maximum amount of lignin. The residual solid fraction obtained after enzymatic hydrolysis following thermochemical pretreatment is also subjected to the same organosolv process to maximise lignin recovery yields. Organosolv pretreatment is widely used in the bio-based industry for lignin production. In BIOrescue, it was selected to retrieve soluble lignin that can be employed directly for the development of polymer membranes as a basis for the production of nanocapsules. From the tests carried out in the project, CENER managed to **extract up to 40% of soluble lignin** from the total amount of lignin contained in mushroom compost.

In addition to addressing the sugars, BIOrescue is increasing the value of mushroom compost through the valorisation of lignin, the “gold dust” in biomass.

Upscaling biopesticides production using BIOrescue’s sugar hydrolysate

C.
Tailor-made and improved enzymatic solutions

Enzymes are used to break down the long-chains of sugar and lignin polymers present in the biomass into smaller pieces. Typically the market offers only a general combination of enzymes for any kind of feedstock, without considering the specific characteristics of different biomass. This makes the hydrolysis process less efficient. But BIOrescue has some answers to this challenge.

Finnish company MetGen has created and **patented tailored enzymatic solutions** (MetZyme® SUNO™) **to extract the sugars** from the mushroom compost in an optimal way. With an optimised SUNO™ solution, they found out the best conditions to break down the molecules obtained from pre-treated mushroom compost into monosugars with a maximal conversion yield (>95%). In addition, MetGen utilised its **innovative enzymatic lignin fractionation technology** (MetZyme® PURECO™) to produce highly reactive depolymerised lignin in a very sustainable and efficient manner. This technology has the benefits of avoiding the use of heavy metals or toxic solvents, and operating in mild process conditions (temperature below 60°C) to effectively break down and at the same time preserve the beneficial properties of lignin.

MetZyme® SUNO™ cocktail tailored for mushroom compost has already **reduced the time and resources needed** for the hydrolysis **by at least 20%**, thus improving the overall competitiveness of mushroom compost conversion.

Partners are also using cross-linking and immobilisation processes to improve the ability of enzymatic components to **resist higher temperatures** and **lower the dosage of enzymes** needed in biorefineries. This will facilitate their storage, reduce logistical and operational costs and increase the efficiency of the conversion.

Looking into enzymes DNA

In parallel, the University of Naples tested an **enzyme called cellulase**, which has around three times more efficient sugar extraction yield than commercial enzymes. The production costs of cellulase is the most expensive factor in the overall industrial conversion of lignocellulosic biomass. Because industrial scale lignocellulose hydrolysis requires a huge amount of enzymes, this has an adverse effect on the competitiveness of biochemicals. To solve this the University of Naples introduced DNA modifications to **obtain 30,000 variations of the cellulase enzyme**. They screened and tested all the modified enzymes to **finally select the best performing ones**. This can increase the extraction yield of sugars, improving the conversion process and significantly reducing the cost.

Example of the "Hedgehog" fraction from MetGen's lignin valorisation process. This particular fraction and its modifications could be a match for applications such as foams and composites (e.g. furniture, construction material, car tires).

THE PRODUCTS:
WONDERS YOU CAN DO WITH MUSHROOM COMPOST

1.
New enzyme cocktails for a more efficient biorefinery process

Enzymes have **multiple applications** in the bio-based industries, as they can be used to produce bio-based polymers and materials for **any kind of product** (from yoga mats to loud speakers), as well as for paints, biochemicals, cosmetics, bioplastics and biofuels.

The novel tailor-made enzyme cocktails and the genetically enhanced enzymes created in BIOrescue show a clear improvement in the conversion of lignocellulosic biomass compared to generic and non-tailored enzymes currently available commercially.

Enzymes are **necessary in all the steps of the biorefinery process** (from pre-treatment and fractionation, to organosolv and biochemical conversion) and for any kind of feedstock. Therefore, the improved performance of enzymes in BIOrescue will reduce the overall time and costs of the biomass conversion.

2.
Low cost and sustainable biopesticides

Every year more than two million tonnes of agricultural pesticides are sprayed across the world, and demand for bio-based pesticides is increasing by almost 10%. Within BIOrescue, researchers from CENER developed low cost and sustainable biopesticides, which are **less toxic but equally efficient** as their fossil-based alternatives on the market. To produce the biopesticides, the monosugars obtained from the enzymatic hydrolysis are submitted to a dedicated fermentation process. The new pesticide created in the project is **highly concentrated and particularly effective** as insect repellent, which makes it a product with very good potential on the market.

Copyright Max Planck Institute for Polymer Research

Test of nanocapsules to treat plants diseases.

3.
Biodegradable nanocapsules for enhanced drug delivery

One of the flagship products of the BIOrescue project are microscopic capsules made out of polymer membranes, which can be used as a **targeted drug delivery system for plants**. With the soluble lignin obtained after the pretreatment, the Max Planck Institute for Polymer Research carried out a chemical conversion process, transforming lignin into tiny biodegradable capsules.
Once created, the capsules can be loaded with the biopesticides produced within the project as well as other drugs such as herbicides or fungicides. Thanks to a natural enzymatic degradation process, this product can be employed for **controlled and progressive drug release** and even as a potential new plant vaccine targeting specific diseases. Beyond the agricultural sector, the capsules have a **myriad of other potential applications** from food enhancement to pharmaceutical products.

UNLOCKING THE POTENTIAL OF THE BIOECONOMY **IN RURAL AREAS**

The agro-food industry is responsible for 10% of European greenhouse gas emissions. To achieve the European Union's climate targets, the sector is in crucial need of new technological solutions and products to achieve the transition towards a circular bioeconomy. With the new bioprocesses developed within the project, BIOrescue partners are helping to **close this technology gap** in the mushroom industry and beyond, turning what was previously considered as waste into added value for rural economies.

Landfill charges for mushroom compost vary from €3 to €100 per tonne across the European Union, and are set to increase as Member States strive to curb waste disposal.

With its new biorefinery concept, BIOrescue will not only reduce these costs, but will also **generate additional revenues for farmers**.

In addition, the integrated biorefinery system will allow **for a local treatment and valorisation of waste streams** from the agro-food industry, and thus boost **high skilled job creation** in rural areas. Around 70% of costs in biorefineries are linked to pretreatment and enzymatic hydrolysis. The innovations developed in BIOrescue reduce these costs by making the process more efficient, therefore providing competitive products for the agro-food industry.

A SUSTAINABLE AND **REPLICABLE CONCEPT**

The potential of BIOrescue goes far beyond mushrooms. Its biorefinery system is **very flexible and can be replicated** not only in other mushroom farms but also with other agricultural by-products. In fact, the full title of the project is "Enhanced bioconversion of agricultural residues through cascading use".

Copyright Monaghan Mushrooms

Partners carried out preliminary analysis to evaluate potential combinations of mushroom compost with other underutilised agricultural feedstocks such as cereal straw, hops and sugar beet residues, apple pomace, citrus peels and olive tree prunings. They found out that wheat straw, oat straw and barley straw combine most efficiently with used mushroom compost. Finally, they assessed the availability of these feedstocks during the entire year in different regions, which is a key element towards the development of **local biorefinery processes**.

The BIOrescue concept could therefore be **expanded to other agricultural sectors** after the project and lead to the development of new bio-based alternatives for all the fossil-based products existing on the market.

WHO MADE IT HAPPEN:
BIORESCUE PARTNERS

The BIOrescue consortium is a multidisciplinary team bringing together industrial players and research centres from seven different European countries, co-ordinated by the National Renewable Energy Centre of Spain (CENER) with the support of Monaghan Mushrooms as technical co-ordinator. Together, the project partners optimised the processes and technologies available for biomass conversion while adapting them to the transformation of mushroom compost.

CENER NATIONAL RENEWABLE ENERGY CENTRE ADItech — Spain

MONAGHAN MUSHROOMS — Ireland

UNIVERSITÀ DEGLI STUDI DI NAPOLI FEDERICO II — Italy

METGEN — Finland

zabala innovation consulting — Spain

Greenovate! EUROPE — Belgium

Max-Planck-Institut für Polymerforschung, Max Planck Institute for Polymer Research — Germany

Celignis Analytical — Ireland

Imperial College London — United Kingdom

C-Tech INNOVATION — United Kingdom

The present booklet introduces the main outcomes of the BIOrescue project which has received funding from the Bio-Based Industries Joint Undertaking under the European Union's Horizon 2020 research and innovation programme, under Grant Agreement N°720708.

The views expressed in this booklet are those of the consortium and cannot be attributed in any way to the European Commission.

All material featured in this booklet is free to use for educational purposes, but may not be sold or distributed for commercial gain.

COORDINATION

Inés del Campo, CENER,
idelcampo@cener.com

COMMUNICATION AND DISSEMINATION

Bénédicte Julliard, Greenovate! Europe,
b.julliard@greenovate-europe.eu

Valeria Mazzagatti, Greenovate! Europe,
v.mazzagatti@greenovate-europe.eu

Written and edited by Valeria Mazzagatti, Bénédicte Julliard and James Ling of Greenovate! Europe, with contributions from consortium partners.

Graphic design: Formas do Possivel (www.formasdopossivel.com)

© BIOrescue 2019

BIO RESCUE

www.biorescue.eu

@BIOrescue_BBI

BBI JU

Bio-based Industries Consortium

Horizon 2020
European Union Funding
for Research & Innovation

The BIOrescue project has received funding from the Bio-Based Industries Joint Undertaking under the European Union's Horizon 2020 research and innovation programme, under Grant Agreement N°720708.