The valorisation of mycelium waste streams for cost-efficient production of mycelium materials and operationalisation of the circular bioeconomy.

Thesis Research Project
MSC Industrial Ecology
CML & Faculty of Technology, Policy and Management
Leiden University & TU Delft

STUDENT

Jelte van Mil	Leiden University	S2636522
	TU Delft	5190878
Date of Submission	June 16th, 2022	

SUPERVISORS

First supervisor:	Dr. José Mogollon – Institute of Environmental Sciences (CML), LU
Second supervisor:	Prof. dr. Erik Jan Hultink – Faculty of Industrial Design Engineering, TUD

Universiteit Leiden

TU Delft

ACKNOWLEDGEMENTS

Ever since I was a kid, I have been fascinated by nature. Enjoying it, observing it, noticing how everything is connected and perfectly balanced, naturally. However, we, as humans, have made an end to this perfect balance by disrupting Earth's natural cycles. We have been, and are, polluting and destroying much of the beautiful environment that surrounds us and provides us everything we need. Nevertheless, I believe we have the capacity to make a change and to restore much of what has been lost. Before you is my thesis that allows me to graduate from the MSc Industrial Ecology and to provide a small contribution to the solutions for the environmental problems of our time.

For the past fifteen months, I have been working on this thesis. This period has not always been easy for me. The process of my thesis was not a straight race to the end. Initially, part of this research focussed on assessing the strategic advantages that could be provided to a specific mycelium producer through the valorisation of its waste streams. I read two complete books about strategic market planning and product design only to realise that this was not the way to go after three months of working on this project. At that point I was not really sure which way to go, and I tried several different directions only to find out that these were neither the way to go by July 2021. At this point I had already thought of quitting several times. Additionally, the world got struck by a terrible pandemic of which the consequences weighted heavier on me than I initially thought. Being in the same environment constantly had a serious impact on my motivation to study and joy of everyday life. The graduation of my masters marks the end of this difficult period. However, I would not have been able to come to this point without the support of the many helpful people around me.

First of all, I would like to thank my two supervisors José Mogollón and Erik-Jan Hultink. José, you really helped me in finding the final direction of my thesis that I needed so much. The meetings we had made me think critically of what I needed to do throughout my thesis and I absolutely could not have missed your advice on how to approach several difficulties during this thesis. EJ, although you had less experience and knowledge of the specific topic of my thesis, you provided me the necessary support that I needed at times and taught me valuable things with regards to my academic writing. You made me learn more than I had hoped for at the beginning of this thesis. As a return, I hope to have provided you with some additional knowledge of my own field of research.

Next, I would like to thank Simon Hoenderop and Bert Rademakers of Mycelium Materials Europe for providing me the initial idea of this thesis and for providing me valuable insights into the world of mycelium production.

Then, I would like to thank my friends and fellow students who supported me at difficult times and were always there for some good fun or good advice. I want to make a special acknowledgement to Eline Willems. You really helped me with questions throughout this thesis that were more difficult to me than it may have seemed. Your help is appreciated.

Of course, I cannot forget about my parents who have always supported me throughout my entire educational career and made it possible for me to study. My sister, Nanne. We have been working on our master's theses simultaneously and although you were able to finish way earlier than me your support is very much appreciated and really helped me to reach my goal.

Finally, I want to thank my partner Lisanne for always being there for me when I needed you. Your doubtless patience with me is one thing I could not do without.

During my thesis, I learned far more than could be presented in this document. The value of helpful people in your surroundings reminding you of your own capabilities is one to mention.

Jelte van Mil
Amsterdam, June 2022

EXECUTIVE SUMMARY

Pure mycelium materials are a novel type of biobased materials that provide an alternative to otherwise polluting leather products and meat. Pure mycelium is considered a sustainable material as it is being produced through biofabrication while making use of renewable biomass resources or waste streams. However, the production of this pure mycelium results in a waste stream itself. This waste stream consists mostly of the spent mycelium substrate (SMS) used for its production along with a smaller share of pure mycelium waste (PMW) which cannot be used for its intended purpose due to contamination with substrate particles. The current knowledge gap with regards to the lack of appropriate options for its valorisation results in these waste streams to be composted and provides a serious burden to producers of mycelium materials as this disposal is costly. Additionally, composting of these mycelium waste streams is considered unsustainable according to the principles of the circular bioeconomy (CBE). The CBE is suggested as an alternative economic system that is to overcome the problems that are inherent to our present linear economic systems e.g., resource depletion and environmental degradation. This thesis aims to determine the potential for valorisation of these mycelium waste streams to support operationalisation of the CBE and to improve the cost-efficiency of pure mycelium materials.

To assess this potential, the characteristics of the mycelium waste streams are synthesized from the literature and various options for its valorisation are identified from the literature based on these characteristics. The environmental impacts (GWP, water- and energy consumption) and economic viability of the identified valorisation options are determined by studying available lifecycle impact assessments and techno-economic assessments of processes comparable to those for the valorisation of the mycelium waste stream. Additionally, the eco-efficiency, or the relation between the environmental costs per monetary unit benefit of these valorisation options is calculated with the idea that this provides a more straightforward answer as to what would be the most favourable options for this valorisation based on the separate assessments of environmental impacts and economic viability.

The results of this study suggest that there are several economically viable options that theoretically provide the potential to support operationalisation of the CBE through the valorisation of mycelium waste streams. These are the production of mushrooms, cellulase and fuel pellets for the valorisation of the SMS and extraction of dietary fibers and phenolic compounds from the PMW. The production of fuel pellets is considered the preferred option for valorisation of the SMS due to its fast ROI. However, it does not provide the possibility for optimalisation of biomass resource utilisation. Furthermore, the identified options for valorisation of the PMW are not desirable due to their extremely high environmental impacts. Nonetheless, the production of fuel pellets from the SMS provides the opportunity to improve the cost-efficiency of pure mycelium production as it makes up the vast majority of the mycelium waste streams.

Additionally, the novel synthesis of mycelium waste characteristics provides the potential to study additional options for valorisation of mycelium waste streams in the future. These options could potentially provide the possibility to improve the cost-efficiency of pure mycelium production while simultaneously allowing an optimisation of the utilisation of the resources used for its production. Furthermore, the findings of this study suggest that a different approach in the decision-making of appropriate options for valorisation of biomass waste streams, including mycelium waste streams, is required. An initial idea for a potential decision-making tool is therefore provided.

Lastly, the findings of this study provide the first indication of the possibility to capture economic value from a waste stream that provides a growing burden to the mycelium materials industry. This will allow reductions in the cost price of pure mycelium, making it available to a larger group of consumers and allowing the release of their full potential through increased substitution of otherwise polluting materials and meat.

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1 Introduction

1.1 Research context and problem statement

Pure mycelium materials are a novel type of biobased materials that provide an alternative to otherwise polluting leather products and meat (Jones et al., 2021; Karana et al., 2018; Vandelook et al., 2021). Pure mycelium is considered a sustainable material as it is being produced through biofabrication while making use of renewable biomass resources or waste streams (Elsacker et al., 2019). However, the production of this pure mycelium results in a waste stream itself (Fig. 1). This waste stream consists mostly of the spent mycelium substrate (SMS) used for its production along with a smaller share of pure mycelium waste (PMW) which cannot be used for its intended purpose due to contamination with substrate particles (MME, 2021). These waste streams are generally composted due to a lack of options for appropriate valorisation (Butu et al., 2020; MME, 2021). In Europe, the costs of this disposal range from €10 to 50 per ton and attribute significantly to the costs of pure mycelium production which is considered a serious concern to producers of pure mycelium (Beckers et al., 2019; MME, 2021).

Additionally, composting of these mycelium waste streams is considered unsustainable according to the principles of the circular bioeconomy (CBE). The CBE is suggested as an alternative economic system that is to overcome the problems that are inherent to our present linear economic systems e.g., resource depletion and environmental degradation, through the integration of circular economy (CE) and bioeconomy principles (Geldermann et al., 2018). It uses biomass as its primary renewable resource for the production of food, energy and materials and optimises the utilization of these resources through application of waste hierarchies and cascading (Stegmann et al., 2020). Biomass resources should initially be (re)utilized at high levels of resource quality before being cascaded to lower levels of resource quality (Carus & Dammer, 2018). They should only be used for the production of energy, heat, or compost when maintaining the resources at any higher level of resource quality is no longer considered sustainable (Kourmentza et al., 2018). This approach minimizes the need for virgin biomass inputs and is how the CBE operates sustainably (Stegmann et al., 2020). However, it also suggests that the current disposal of mycelium waste streams is unsustainable as it results in an underutilisation of the resources used for pure mycelium production (Ubando et al., 2020).

Pure mycelium materials provide a sustainable substitute for leather and meat and could play an important role in the operationalisation of the CBE. However, their current cost price and the underutilisation of the resulting waste streams prevents the release of their full potential (Hahn, 2020). These waste streams will become a growing burden to the industry of pure mycelium materials as they will parallel the increase of pure mycelium production that is expected in the nearby future (Roshitsh, 2021; Vandelook et al., 2021). Hence, identification of profitable valorisation options for the mycelium waste streams is required to overcome the economic and environmental issues relating to its disposal. Consequently, this will allow the release of the full potential of pure mycelium materials and support successful operationalization of the CBE.

Figure 1. The mycelium waste. Left: initial waste after harvesting of pure mycelium. Middle and right: separated pure mycelium waste (PMW) and spent mycelium substrate (SMS), respectively (MME, 2021).

1.2 Goal and research questions

The existing knowledge gap regarding the lack of options for profitable and sustainable valorisation of mycelium waste streams is to be addressed by researching the potential for its valorisation. This valorisation is to improve the cost-efficiency of pure mycelium production and to optimise the utilization of the resources used for this production. Therefore, the aim of this thesis is to identify valorisation options that can reduce the costs of and optimise the utilisation of the resources used for pure mycelium production. This will allow the release of the full potential of pure mycelium materials and support operationalisation of the CBE. The resulting information can be used by industrial ecologists, designers, engineers, or producers of mycelium materials that work to optimise the utilization of these resources in their specific context or to motivate their choice for working with mycelium materials. The following central research question will be the point of focus during this thesis:

'What is the potential for alternative valorisation of mycelium waste streams to reduce the costs of pure mycelium production and to support operationalisation of the circular bioeconomy?'

To support operationalisation of the CBE it is required that the valorisation of the mycelium waste streams provides an optimisation of resource utilisation i.e., utilisation of the waste streams at appropriate levels of resource quality (Donner et al., 2021). Therefore, an assessment of the environmental impacts of the identified valorisation options is required. Additionally, it is the economic viability of the valorisation options which determines their potential to reduce the costs of pure mycelium production, The following sub research questions will allow to answer the main research question while taking into account these considerations:

1) What are the characteristics of the mycelium waste stream?
2) What are promising options for mycelium waste valorisation?
3) What are the environmental impacts of the identified valorisation options for mycelium waste?
4) Are the identified options for valorisation of mycelium waste economically viable?

1.3 Relevance to Industrial Ecology and thesis outlook

Circular bioeconomy activities find their inspiration in the philosophy of Industrial Ecology (IE). According to IE, the resource throughput of our economies is to resemble the balanced material flows of natural ecosystems where no waste exist as the outputs of one process provide the inputs to another which creates a closed system (Blomsma & Brennan, 2017). This approach of a closed, circular system provides the basis for the CBE in which the value of products, materials and resources is maintained in the economy for as long as possible, and the generation of waste is minimized (European Commission, 2015). Attention is focused on the efficient use of resources (economic and ecological) and the treatment of waste as a resource (Carus & Dammer, 2018). This thesis is concerned with exactly these aspects of the CBE that find their inspiration in IE and can therefore be considered highly relevant to the field of IE.

This chapter has provided the research context, the problem statement and the goal of this thesis and has shown why this research is relevant to the field of industrial ecology. Additional information with regards to mycelium materials, their applications and production is provided in Appendix A. Figure 2 provides the research flow diagram of this thesis. As mycelium has a potential important role to play in the CBE and since its principles provide the theoretical framework for the methods of this thesis, the next chapter will elaborate on CBE theory. Chapter three provides the methods of this research. The results of this thesis are presented in chapter four and will be discussed and concluded in chapter five. As a whole, this thesis allows a greater understanding of the CBE and the importance of (biomass) waste valorisation. This information provides product designers, developers, engineers and industrial ecologists with the opportunity to use this information in the transition towards a sustainable economic system.

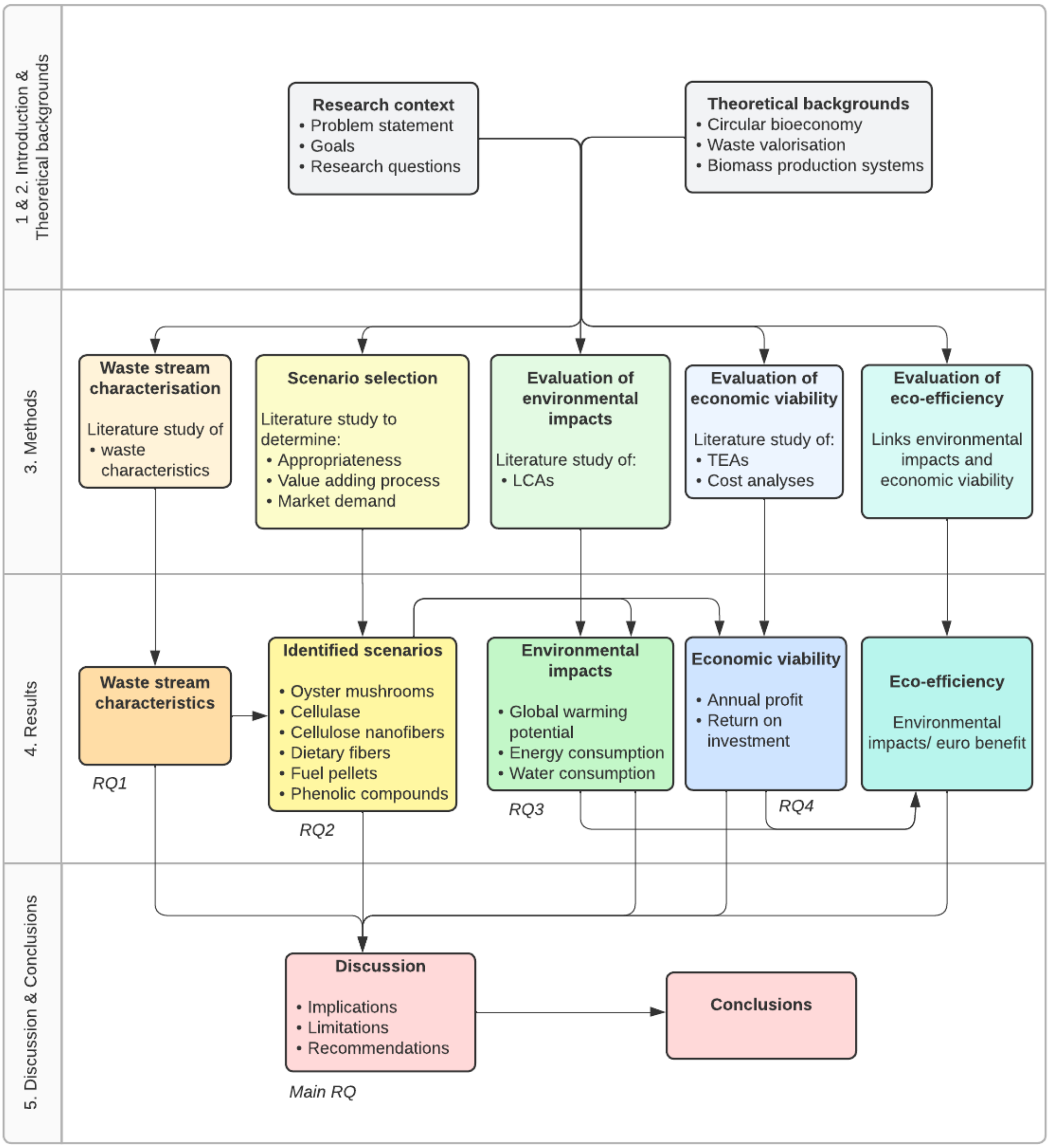

Figure 2. Research flow diagram. The research flow diagram shows the different steps of this thesis and how they tie together.

2 Theoretical framework: The Circular Bioeconomy

Our present linear economic and industrial systems rely mainly on fossil fuels and non-renewable resources to provide products and services. The overexploitation of these resources has led to environmental degradation and resource depletion which undermines sustainability (Del Borghi et al., 2019). This chapter provides a brief overview of how resources could be managed more sustainably according to the principles of the CBE. It elaborates these principles and shows why careful assessment of waste valorisation is required. The information presented here provides a framework for the critical assessment of the mycelium waste valorisation options in later stages of this thesis.

2.1 The circular bioeconomy

The CBE (Fig. 3) is increasingly acknowledged by the EU and its member states as an alternative economic system that is to overcome the problems related to our present linear economic system (Schipfer et al., 2017). It integrates the concepts of the bioeconomy, an economic system based on biomass as its primary renewable resource (Carrez & Van Leeuwen, 2015), and applies the principles of circular economy (CE) to create an efficient and sustainable resource system (McCormick & Kautto, 2013). This results in a circular system that aims to optimise the utilisation of biomass resource utilisation over time through recycling or cascading. Such an optimisation can focus on economic, environmental, or social aspects of sustainability and ideally includes all three. Sustainably sourced biomass is used as the primary resource for the production of food, feed, energy and materials. Circular and durable product design are required to provide efficient resource use throughout a products or materials lifecycle and to facilitate recycling or cascading at their end-of-life. Most bio-based products and materials are produced in biorefineries. Here, biomass, wastes and residues provide the resources for integrated, multi-output production of both high-value e.g., bio-based chemicals, and low-value products e.g., bioenergy). The value of these products is to be maintained throughout their lifecycle. At their end-of-life, resources can be recovered and re-entered into the economic system through recycling or cascading (Stegmann et al., 2020).

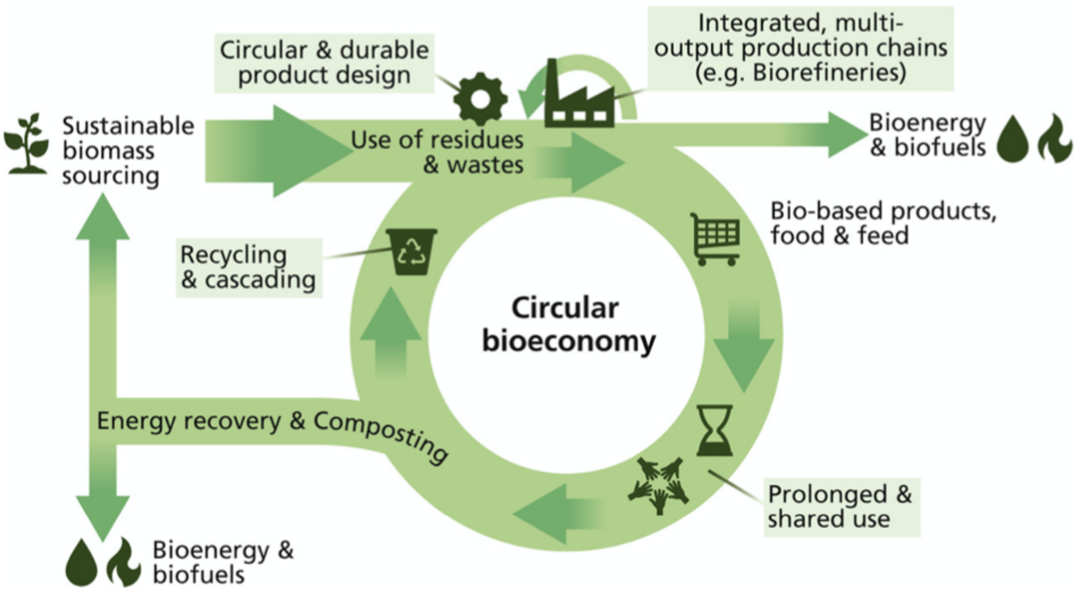

Figure 3. Visual representation of the circular bioeconomy. Taken from Stegmann et al. (2020).

2.2 Waste valorisation in the circular bioeconomy

An important aspect for operationalisation of the CBE is optimal utilization of biomass resources, including the valorisation of biomass waste residues (Banu et al., 2020). Ideally, the concept of 'waste' no longer exists as it is to be considered a resource for the production of materials or recovery of valuable compounds (Kourmentza et al., 2018). Optimal utilization of biomass resources can be realized through cascading (Fig. 4). Cascading refers to the consecutive utilization of resources in multiple use phases (Geldermann et al., 2016). Biomass is to be used for high-value purposes initially, and to be cascaded to lower-value purposes as its resource quality diminishes over time (Carus & Dammer, 2018). This cascading of biomass resources suggests a movement down the bio-based value pyramid as shown in Figure 3. According to this pyramid, a direct cascade of biomass to energy recovery would prevent its consecutive utilisation and increase the demand for biomass inputs which is considered inefficient and unsustainable according to the CBE principles (Stegmann et al., 2020). However, endlessly maintaining a resource within the economic system is not necessarily favourable from an environmental perspective due to its potential energy or resources requirements (Daioglou et al., 2014; Ghisellini et al., 2016). Therefore, an evaluation of the environmental impacts of valorisation options for biomass or waste residues by using environmental tools is required (Del Borghi et al., 2019).

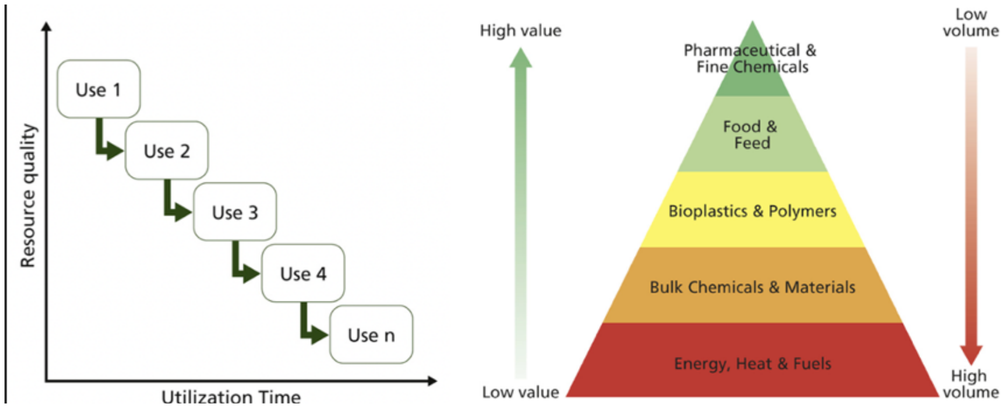

Figure 4. Resource optimisation in the CBE. Optimisation of resource utilisation in the CBE is to be achieved through cascading (left): the consecutive use of a resource in different utilisations with varying resource quality. This is to be guided by the bio-based value pyramid (right). Taken from Stegmann et al., 2020.

2.3 Biomass production systems

The CBE should in theory provide a sustainable economic system through substitution of non-renewable resources with bio-based alternatives and by optimisation of biomass resource utilization through cascading (Carrez & Van Leeuwen, 2015). The production of biomass can be considered the 'biological motor' of the CBE and is of paramount importance for its operationalisation (Stegmann et al., 2020). However, the production of biomass requires healthy (agro)ecological systems that provide water, energy, and a stable climate (Muscat et al., 2021). These prerequisites for biomass production have been connected in the food-energy-water-climate (FEWC) nexus (Fig. 5). Although it was originally designed for food production, the nexus could also be applied to biomass production as food is principally a form of biomass which relies on similar production systems (Geldermann et al., 2016). The nexus suggests a connection between its separate systems and how activities in each of them influence and are influenced by each other. This suggests that mitigation of environmental impacts in one subsystem might have adverse effects on another (Laso et al., 2018). Although operationalisation of the CBE is considered to reduce environmental pressures on these systems in general (Kardung et al., 2021), it remains important to consider the impacts of any biomass valorisation option on any of these subsystems (Laso et al., 2018).

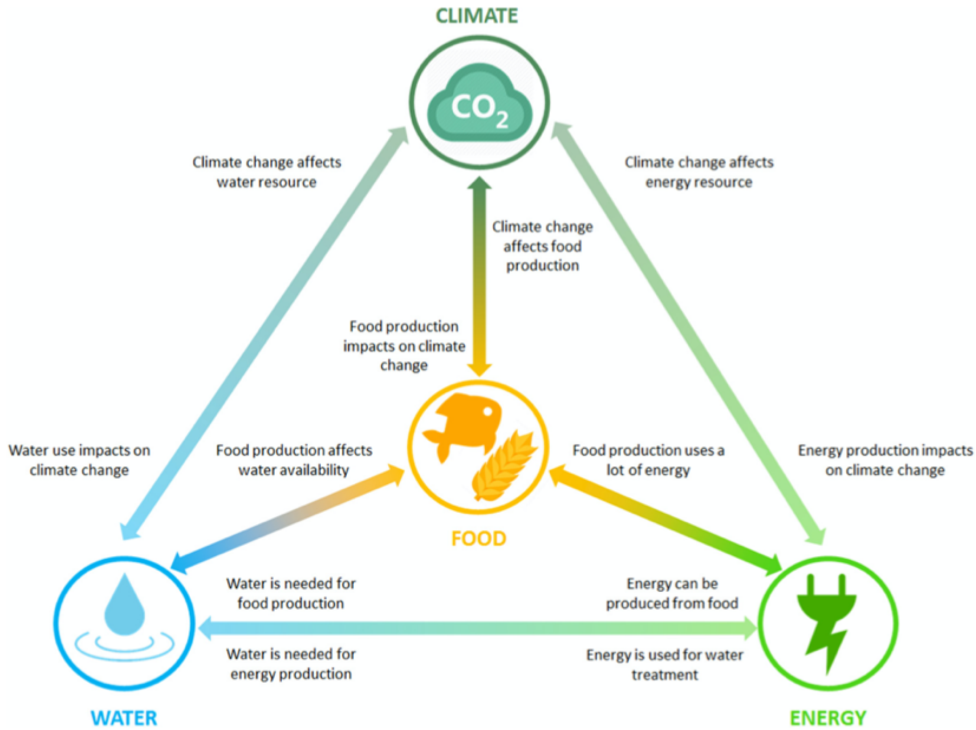

Figure 5. The water-energy-food-climate nexus. The nexus shows all relevant systems for the production of food and how they influence and are influenced by each other. The nexus could alternatively be applied to the production of biomass. Taken from Laso et al. (2018).

The information presented in this chapter suggests the importance of assessing the economic viability and environmental impacts when researching options for valorisation of the mycelium waste streams. Vice versa, this chapter suggests the potential important role for mycelium to play in the CBE as it supports the optimisation of biomass resource utilisation through the conversion of biomass waste streams into bio-based materials (Cerimi et al., 2019). The presented CBE theory provides a framework that supports the methods of this thesis and will guide the interpretation of the results in the discussion.

3 Methods

As mentioned in Section 1.2, the goal of this thesis is to identify valorisation options that can reduce the costs of mycelium materials production and support operationalisation of the CBE. This thesis follows an exploratory approach to research the potential of mycelium waste stream valorisation to achieve this goal by drawing on data and information available in the literature. The first step of this research consists of a characterization of the waste streams. Secondly, these waste stream characteristics are used to identify valorisation options. Thirdly, the environmental impacts and economic viability will be assessed to determine the potential of the selected valorisation options to reduce the costs of mycelium materials production sustainably. This chapter further explains the steps taken and the assumptions made to come to the final conclusions of this thesis.

3.1 Waste stream characterization

To begin with, a better understanding of the characteristics and composition of the waste streams is required. Many studies have explored options to valorise various organic or inorganic waste streams (Babaei et al., 2019; Ledesma & Beltramone, 2021; Maina et al., 2017). All these highlight the importance of the characteristics of the waste stream as the starting point for exploring valorisation options as the presence of specific waste stream components either suggests or limits its specific utilization. For this reason, options for valorisation of the SMS and PMW will be studied separately as their external appearance suggests major differences in their characteristics and composition.

It appears that information about the characteristics of the SMS is currently absent from scientific literature due to the novelty of mycelium materials. However, similar information about spent substrates of edible mushrooms production is available. As their production process is similar, except for the absence of light and presence of high carbon dioxide concentrations to stimulate mycelium growth and prevent mushroom formation (Elsacker et al., 2020; Gurung et al., 2012), it is assumed that the degradation of the fresh substrates, and thus the resulting spent substrates, are also similar. Therefore, this information can be used as a proxy to circumvent the absent information regarding the SMS specifically. Table 1 shows the similarity of the substrate feedstock, moisture- and pH level used for production of mycelium materials and mushrooms for the four most frequently used fungal species.

The following characterization of the SMS, using information about spent substrates of mushroom production as a proxy, will focus on the protein, fat, carbo(hydrate), energy (kcal/100g), nitrogen, lignocellulosic and ash content in dry weight percentage (dwt%). The moisture content will be given in percentage of wet weight while the carbon/nitrogen (C/N) ratio is unitless. These characteristics are similar to studies by Antunes et al. (2020) and Mahari et al. (2020), who researched options for valorisation of spent mushroom substrates.

The characterisation of the PMW will again focus on the four most frequently used species of fungi for pure mycelium production. Similar characteristics to Antunes et al. (2020) and Cohen et al. (2014) who researched valorisation options for waste streams (e.g., mushroom caps and stalks) from the edible mushrooms industry e.g., protein, fat, total carbohydrate, energy (kcal/100g), dietary fibre, phenolic content, moisture, and ash content in dwt% will be determined. An initial literature review (see Appendix A) suggested the extraction of beta-glucans and chitin as potential interesting valorisation options and their content will therefore also be assessed (Chilanti et al., 2021; Nitschke et al., 2015).

The information for this part of this thesis will be sourced from Scopus by snowballing of the following search terms: 'characteristics mycelium substrate' 'chemical composition of spent mushroom substrate' 'spent mushroom compost' 'nutrient content spent mycelium substrate' 'nutrient content spent mushroom substrate' 'fungal mycelium recipe patent' 'g lucidum substrate composition' 'trametes spp substrate composition' 'lentinula edodes substrate formula' 'pleurotus ostreatus mycelium' 'mycelial cell wall extracts' 'pleurotus mushroom mycelia' 'ganoderma mushroom mycelia' 'beta glucan content mushroom mycelium' 'fruiting bodies and mycelia glucan contents'.

Table 1. Fresh substrate characteristics of mycelium and mushrooms. The similarity in characteristics and production processes suggests similar substrate degradation, resulting in similar spent substrate characteristics.

Substrate variable	Mycelium production	Reference	Mushroom production	Reference
Fungal species	*Ganoderma* lucidum. *Lentinula* edodes. *Pleurotus* spp. *Trametes* spp.	1-2, 4, 6, 9-13	*Ganoderma* lucidum, *Lentinula* edodes. *Pleurotus* spp. *Trametes* spp,	14-25
Feedstock	Cotton (fibre/ seed hulls); flax; gypsum/chalk powder; (hemp; kenaf; rice (bran/hull/straw); wheat (bran/straw); wood (chips/ sawdust/shavings); mixtures of feedstocks.	1-2, 4, 6-13	Cotton (fibre/ seed hulls); flax; gypsum/chalk powder; hazelnut husk; hemp; rice (bran/hull/straw); sugarcane bagasse; tea waste; wheat (bran/ straw); wood (chips/ sawdust/shavings).	14-25
Moisture (%)	60 - 90	1-2, 5-6, 9, 11	60 – 75	14-17, 20-25
pH	5.0 – 7.0	2, 9	4.5 – 7.0	14, 18, 20-25
Production (days)	5 – 30	1-3, 6, 12	13 – 60	14, 17-19, 25

References: 1) Appels et al., 2019; 2) Attias 2020; 3) Bruscato et al., 2019; 4) Cerimi 2019; 5) Elsacker et al., 2019; 6) Elsacker et al., 2020; 7) Girometta et al., 2019; 8) Jones et al., 2020; 9) Manan et al., 2021; 10) O'Brien et al., 2020; 11) Ross, 2016; 12) Ross et al., 2020; 13) Tacer-Caba et al., 2020; 14) Atila, 2020; 15) Cobos et al., 2021; 16) Gaitán-Hernández et al., 2006; 17) Gaitán-Hernández et al., 2011; 18) Gurung et al., 2012; 19) Özçelik & Peksen, 2007; 20) Peksen & Yakupoglu, 2009; 21) Peksen et al., 2011; 22) Philippoussis et al 2003; 23) Thakur & Sharma, 2015; 24) Thiribhuvanamala & Krishnamoorthy, 2021; 25) Xiong et al., 2019.

3.2 Exploring valorisation options

This section elaborates on the process of valorisation option identification and selection of the production methods for their resulting products.

3.2.1 Identification and selection of valorisation options

As valorisation at higher levels of the biobased value pyramid (Fig. 4) is not necessarily the better option from an environmental or economic perspective (Daoiglu et al., 2014), various options at different levels of resource quality will be considered. Therefore, the initial exploration of valorisation options will be based on a literature study guided by the levels of the biobased value pyramid and extended with the specific characteristics of the waste streams. The following terms will be used to explore potential valorisation options through Scopus: 'valorisation spent mushroom substrate' 'reuse spent mycelium substrate' 'spent mushroom substrate AND pharmaceuticals' '… AND animal feed' '… AND bioplastics' '… AND polymers' '… AND hemicellulose' '… AND cellulose' '… AND lignin' '… AND enzyme extraction' '… AND biofuel' 'mycelium AND food' 'mycelium component extraction' '… AND chitin' '… AND beta-glucans' 'lignocellulosic biomass valorisation'.

Only those options that are considered appropriate for valorisation of the mycelium waste streams will be selected for further research. The determination of this appropriateness will be based on the accordance of the waste stream characteristics and the specific requirements of the identified valorisation options. This means that the values of the waste stream characteristics should match the specific requirements for e.g., animal feed or materials production. As there is no uniform selection criterium to assess the appropriateness of all the identified valorisation options, their appropriateness will be discussed and determined using the requirements for each individual option. However, one requirement that could be considered critical for any of the identified options is the inclusion of a value-adding process. Options that allow direct reutilization of the mycelium waste streams without such a value-adding process will not be selected for further research. Additionally, there needs to be a demand for the resulting products of the identified valorisation options. Therefore, available information about market developments of these products will be used to determine their demand.

3.2.2 Selection of production methods

There will likely be various methods to produce or extract the products resulting from the valorisation options, each having their specific pros and cons. However, the restricted time and scope of this thesis do not allow a full assessment of the environmental impacts and economic viability of all these methods. Therefore, one method will be selected for each of the valorisation options. This selection procedure aims to identify the preferred method for the production of the resulting products. Again, there is no single selection criterium to determine the preferred production method. What is the preferred production method will rather be determined by using information about the technological maturity, yield, environmental sustainability and ease of operationalization of the available methods. Scientific literature concerned with these methods, such as review articles discussing this kind of information, will be used to determine the preferred production methods for the identified options.

3.3 Evaluation of environmental impacts of identified valorisation options

This section provides information about the methods that will be used to determine the environmental impacts of the selected valorisation options.

3.3.1 Assessment of environmental impacts

The environmental impacts of the selected valorisation options will be evaluated through lifecycle impact assessment, or LCA. LCA is an ISO 14040/14044 standardized tool to assess the environmental impacts of a product or service, or in this case a valorisation option (Parascanu et al., 2019). It is used to measure and evaluate the energy and resource consumption and emissions over the life cycle of a product, process, or service (Gonzalez-Garcia et al., 2017). LCAs are typically comprised of four stages: 1) goal and scope definition; 2) lifecycle inventory analysis; 3) lifecycle impact assessment; and 4) results interpretation and presentation in which, respectively, the objective, system boundary and functional unit (FU) are defined; the used data is explained and presented; the impact categories are selected; and the results are discussed (Lam et al., 2018).

LCA data available in literature will be used to determine the environmental impacts of the selected valorisation options. This data will come from studies concerned with the production of the products and their corresponding production methods selected in previous steps (Sections 3.2.1 and 3.2.2). Additionally, the feedstocks used for the production processes in these studies should be similar to the mycelium waste streams for them to be valid.

3.3.2 Functional unit and system boundaries

To be able to compare the environmental impacts between the various options, it is important to define the FU and system boundaries. The FU is a description of the specific function of a product, service or process that serves as the reference basis for all calculations regarding the environmental impact assessment (Arzoumanidis et al., 2020). The FU chosen for this thesis is the 'valorisation of one ton mycelium waste', as this allows a comparison of the environmental impacts associated to the valorisation of the mycelium waste streams through the various options which is in line with the goal of this thesis.

However, the FU alone is not sufficient for a valid comparison of the environmental impacts of the various options. Such a comparison also needs a definition of the system boundaries. This definition determines which contribution processes are included or excluded in an LCA (Li et al., 2014). For this thesis it was chosen to include all impacts related to the production of the products resulting from the valorisation options in a cradle-to-gate approach. Here, cradle-to-gate refers to processing of the mycelium waste streams into the final products leaving the mycelium production facilities and includes all associated impacts and emissions during this process. The cradle-to-gate approach is chosen as it are the environmental impacts associated to the value-adding processes that are at the concern of this thesis.

3.3.3 Selection of environmental impact indicators

Several environmental impacts of the selected valorisation options will be compared. The results of LCA studies are an assessment of environmental impacts on specific impact categories. Endpoint categories suggest the environmental impacts at the level of e.g., human-health, biodiversity, or resource scarcity. They are aggregates of a broader set of midpoint indicators that have been normalized and weighted to allow evaluation of these impacts. Midpoint indicators correspond to more specific impacts such as global warming potential (GWP) or water- and energy consumption. Midpoint indicators are aggregates of life cycle inventory data such as the amount of greenhouse gases emitted in different processes of a product's life cycle (Lam et al., 2018).

Laso et al. (2018) describe the indicators that should be considered in assessments of environmental impacts on biomass production nsystems. The nexus suggests the dependence of biomass production on climate, water, and energy systems. According to Laso et al. (2018), the environmental impacts on these systems could be evaluated by assessing the global warming potential (GWP) and the water (C_W) and energy (C_E) consumption of the valorisation options, respectively. The GWP is used to determine the climate change potential of the valorisation options and is measured in kgCO2-equivalent (kgCO2-eq.) per FU. Water and energy consumption are measured in m^3 and MJ per FU, respectively. Although these are midpoint indicators, their assessment and evaluation provides information about the impacts on these systems and they are therefore considered sufficient for this thesis.

3.3.4 Data collection and calculations

As mentioned before, the information required to assess the environmental impacts of the selected valorisation options will be provided through available LCA studies concerned with feedstocks, production methods and resulting products that correspond to these options. However, this data is not necessarily directly applicable to the valorisation of mycelium waste streams. Differences in system boundaries might require the exclusion of specific processes. Therefore, inventory data for the specific processes involved in the valorisation of one ton SMS or PMW will be used to calculate the GWP, and water- and energy consumption of this functional unit. The following formula will be used to determine the impacts on the climate, water, and energy systems of the selected valorisation options:

$$\textit{Environmental impact indicator } (y) = P_{FU} * (I_d + I_i)$$

The impacts on these systems are described by the impact indicator y, which refers to the GWP, C_W, or C_E in $kgCO_2$-eq., m^3 water, or MJ. The indicator values are calculated by multiplication of the product output P resulting from the valorisation of one ton SMS or PMW in the specific options, by the cumulative impacts resulting from the production of these products. These impacts are the sum of the direct (I_d) and indirect (I_i) impacts of the corresponding production processes for the specific options which will be provided by the inventory data or midpoint indicator values of the available LCA studies.

3.4 Evaluation of economic viability of identified valorisation options

The economic viability of the identified valorisation options will be used to determine the preferred options from an economic perspective. This section elaborates on its assessment and evaluation.

3.4.1 Data collection and calculation of return-on-investment

The economic viability of the selected valorisation options will be determined through calculation of the return-on-investment (ROI) that can be achieved by selling the resulting products of these options. An option will be considered economically viable if its annually returns are positive. The annual return is usually calculated by subtraction of raw material costs and annual operating costs from the annual revenue (Demichelis et al., 2018; Dimou et al., 2016). The annual return of the valorisation options will be calculated using the following formula:

$$\textit{Annual return (EUR)} = S - (C_{mat.} + C_{op.})$$

in which *S* is the annual revenue created through sales of the resulting products, $C_{mat.}$ are the annual costs of materials required to produce the resulting products, and $C_{op.}$ are the annual operating costs. Finally, the ROI will be calculated by dividing the capital costs by the annual return:

$$ROI = C_{cap.} / Annual\ return$$

Data to determine the capital costs, annual materials and operating costs, and the annual revenue will be sourced from techno-economic or cost analyses of production processes similar to those used for evaluation of the environmental impacts that are available in the literature. An exchange rate of 0.93 will be used to convert costs and prices in USD to EUR (ECB, 2022).

3.4.2 Economic viability applied to Mycelium Materials Europe

Section 3.4.1 explained how the annual return and ROI will be calculated. However, these cannot be calculated without data about the annual waste production of a reference case. Mycelium Materials Europe (MME) is a Dutch company and considered one of the world's leading pure mycelium producers. MME produces pure mycelium for Bolt Threads, a major producer of mycelium materials, in its state-of-the-art production facility in the Netherlands (Yarns and Fibers, 2022). MME is considered a good representative for the mycelium industry as major industrial producer of pure mycelium. MME produces 902.5 tons of SMS and 47.5 tons of PMW annually. These numbers will be used to calculate the annual return and ROI as described in section 3.4.1.

3.5 Calculation of eco-efficiency indicator

The resulting information from the environmental and economic evaluation of the selected valorisation options provides two indicators, the environmental impacts and the economic viability. However, Bello et al. (2018) argue that it is the relation between these two indicators that is of actual interest to companies as this provides information about the environmental impacts per monetary unit earned. The eco-efficiency indicator expresses the environmental impacts of each indicator per unitary benefit potentially achieved through a process or, in this case, the selected valorisation options. Optimization of the eco-efficiency minimizes the environmental impacts while maximizing the economic benefits. Therefore, the eco-efficiency indicators of the selected valorisation options will be determined and used for comparison of the selected valorisation options. It will be calculated as follows:

Eco-efficiency (unit impact/€ benefit) = environmental indicator / (annual return / total weight)

This chapter has elaborated on the methods that will be used to determine the potential for valorisation of mycelium waste streams to support the operationalisation of the CBE. The next chapter of this thesis will provide the results of this research.

4 Results
This chapter provides the results for the characterization of the SMS and PMW, the selection of valorisation options and an evaluation of the environmental impacts and economic viability of these options. Finally, the eco-efficiency indicator values will be given to link the environmental impacts with the economic value created through valorisation of the mycelium waste streams.

4.1 Waste stream characteristics
This section provides the results of the literature study into the characteristics of the SMS and PMW. These characteristics provide the basis for the selection of valorisation options and determination of their resulting product yields in sections 4.3 and 4.4 of this thesis.

4.1.1 Characteristics of spent mycelium substrates
As discussed in Section 3.1, characteristics of spent mushrooms substrates are used as a proxy to determine the characteristics of the SMS. Table 2 shows the results of the literature study into the characteristics of the SMS following similar combinations of fungal species and substrate mixtures as mentioned in Section 3.1. All variables, except pH level, moisture level (wet) and C/N ratio are given in SMS dry weight percentage (dwt%). All SMS appear to be acidic (pH < 7) and to have a moisture content between 53.0 and 72.1% (Gaitán-Hernández et al., 2006; Economou et al., 2017). Carbon contents range between 21.6 and 44.7 dwt%, while nitrogen contents range between 1.0 and 10.1 dwt% (Lou et al., 2017; Xiao et al., 2018), resulting in C/N ratios between 19.00 and 97.6 (Economou et al., 2017; Lou et al., 2017). The carbohydrate content ranges from 63.57 to 70.42 dwt% (Abd Rasib et al., 2015). Fat and protein levels vary from 6.60 to 25.56 dwt% (Picornell-Buendia et al., 2016; Abd Rasib et al., 2015) and 16.1 to 62.4 dwt% (Abd Rasib et al., 2015; Owaid et al., 2017), respectively. The fibre content (i.e., cellulose, hemicellulose and lignin) ranges from 38.0 to 45.3 (Asada et al., 2011; Gaitán-Hernández et al., 2006), 9.9 to 18.0 (Gaitán-Hernández et al., 2006 Asada et al., 2011), and 9.0 to 20.5 (Abd Rasib et al., 2015; Asada et al., 2011) dwt%, respectively. Lastly, the content of ash, or non-organic residues, ranges between 3.18 and 20.50 dwt% (Gaitán-Hernández et al., 2006; Owaid et al., 2017). Although the hemicellulose content is lower, the cellulose, hemicellulose and lignin ratio (30.8 – 45.5; 9.9 – 18.6; 7.1 – 20.5) of the SMS essentially make it a form of lignocellulosic biomass (35 – 50; 20 – 35; 10 – 25) (Ubando et al., 2020; Wyman et al., 2018).

Table 2. Characteristics of spent mycelium substrates for *G. lucidum, L. edodes and Pleurotus species*. All values except pH level, moisture level and C/N ratio are given in dry weight percentage.

	G. lucidum	*L. edodes*	*Pleurotus spp.*	References
Protein	36.6	41.6 – 49.8	16.1 – 62.4	Abd Rasib et al., 2015; Gaitan-Hernandez et al., 2006; Owaid et al., 2017
Fat	25.6	7.8 – 14.7	6.6 – 23.8	Abd Rasib et al., 2015; Gaitan-Hernandez et al., 2006; Picornell-Buendia et al., 2016
Carbon/ carbohydrate	41.9 – 44.7/ 63.6 – 70.4	-	21.6 - 37.1	Abd Rasib et al., 2015; Lou et al., 2017; Peksen et al., 2011; Owaid et al., 2017
Nitrogen	8.3 – 10.1	-	1.0 – 10.0	Lou et al., 2017; Owaid et al., 2017; Peksen et al., 2011; Picornell-Buendia et al., 2016
C/N ratio	41.3 – 53.3	45.7 - 97.6	19.0 – 30.0	Economou et al., 2017; Leong et al., 2022; Owaid et al., 2017; Peksen et al., 2011
Cellulose	-	30.8 – 40.9	37.5 – 45.3	Asada et al., 2011; Gaitan-Hernandez et al., 2006; Picornell-Buendia et al., 2016; Rajavat et al., 2020
Hemicellulose	-	9.9 – 18.0	18.6	Asada et al., 2011; Gaitan-Hernandez et al., 2006; Rajavat et al., 2020
Lignin	-	9.0 -16.7	20.5	Asada et al., 2011; Gaitan-Hernandez et al., 2006; Rajavat et al., 2020
Ash (%)	5.3 – 5.6	10.6 -13.8	5.3 – 20.5	Abd Rasib et al., 2015; Gaitan-Hernandez et al., 2006; Owaid et al., 2017; Picornell-Buendia et al., 2016

Moisture (%)	-	53.0 – 66.9	68.7 – 72.1	Economou et al., 2017; Gaitan-Hernandez et al., 2006; Picornell-Buendia et al., 2016
pH	-	-	4.7 – 6.5	Economou et al., 2017; Owaid et al., 2017; Picornell-Buendia et al., 2016

4.1.2 Characteristics of pure mycelium waste

The results of the characterization of the PMW are summarized in Table 3. All values are based on the weight percentage of dry mycelium (dwt%), except for the energy and total phenolic content which are given in kcal per 100 grams and mg gallic acid equivalent (GAE)/g, respectively. The protein content of mycelium ranges from 5.0 – 25.20 dwt%, depending on the species (Cohen et al., 2014; Ghada et al., 2014; Laforteza et al., 2020). The mycelia show low fat content ranging from 0.1 dwt% for *P. ostreatus* to 2.8 dwt% for *G. lucidum* (Cohen et al., 2014; Laforteza et al., 2020). The total carbohydrate content ranges between 61.0 and 83.6 dwt% (Cohen et al., 2014; Ghada, 2011; Laforteza et al., 2020) which has an energy content of 380 – 386 kcal/100g (Cohen et al., 2014). Moisture and ash contents range from 2.8 to 18.0 dwt% and from 1.0 to 12.0 dwt%, respectively (Cohen et al., 2014; Ghada, 2011; Laforteza et al., 2020). PMW also contains several biological compounds with potential interesting applications. Beta-glucans and chitin are present at 2.58 – 27.09 dwt% and 1.35 – 21.8 dwt%, respectively (Bak et al., 2014; Di Mario et al., 2008; Nitschke et al., 2011; Vetter, 2007). The total dietary fibre (TDF) and phenolic content (TPC) range between 49.9 – 55.0 dwt% (Cheung, 1996; Laforteza et al., 2020) and 4.2 – 29.0 dwt% (Chilanti et al., 2021; Helno et al., 2012), respectively.

Table 3. Characteristics of PMW of the four most frequently used species of mushrooms. All values, except energy content (kcal/100g) and phenolic content (mgGAE), are given in dry weight percentage (dwt%).

	G. lucidum	***L. edodes***	***Pleurotus spp.***	***T. versicolor***	**References**
Protein	25.2	19.6 – 20.4	5.0	8.6	Cohen et al., 2014; Ghada, 2011; Laforteza et al., 2020
Fat	2.8	-	0.1	2.0	Cohen et al., 2014; Laforteza et al., 2020
Total carbohydrate	63.6	63.4 – 69.5	61.0	83.5	Cohen et al., 2014; Ghada, 2011; Laforteza et al., 2020
Energy (kcal/100g)	380	-	-	386	Cohen et al., 2014
Beta-glucans	-	2.6 – 27.1	3.0 – 26.7	3.3 – 12.6	Bak et al., 2014; Di Mario et al., 2008; Nitschke et al., 2011
Chitin	-	2.5 – 21.8	2.2 – 15.3	1.4 – 11.1	Cheung, 1996; Di Mario et al., 2008; Nitschke et al., 2015; Vetter, 2007
TDF	-	49.9	55.0	-	Cheung, 1996; Laforteza et al., 2020
TPC (mgGAE/g)	29.0	4.2 – 24.1	8.2 – 16.0	-	Chilanti et al., 2021; Helno et al., 2012; Weihuan et al., 2011; Wu & Hansen, 2008
Moisture	5.7	11.8 – 12.2	18.0	2.8	Cohen et al., 2014; Ghada, 2011; Laforteza et al., 2020
Ash	2.7	7.6 – 9.2	12.0	3.1	Cohen et al., 2014; Laforteza et al., 2020

4.2 Identified valorisation options of the mycelium waste streams

This section provides a description of the identified options for valorisation of the mycelium waste streams following a literature study based on the results of section 4.1. Their potential as interesting valorisation option will be based on the applications of and demand for their resulting products and the suitability of the mycelium waste to produce these resulting products. Additionally, this section elaborates on the preferred methods for production of the resulting products. This information will be used for the assessment of the environmental impacts and economic viability in sections 4.3 and 4.4.

4.2.1 Identified valorisation options for spent mycelium substrates

This section provides the results of the literature study for identification of valorisation options for the SMS.

4.2.1.1 Production of mushrooms

A promising option at the second level of the biobased value pyramid is valorisation of the SMS through reutilization for the production of mushrooms. It is known from the edible mushrooms industry (e.g., shiitake and oyster mushrooms) that the degradation of the substrates during mushroom growth is not complete after one round of production (Cunha Zied et al., 2020). Research has shown that reusing spent mushroom substrates, either for the production of the same or different mushrooms, does not negatively affect the yield of these mushrooms (Noonsong et al., 2016; Wu et al., 2020). Moreover, it has been demonstrated that the reutilization of unamended spent substrates resulted in higher mushroom yields compared to spent substrates that were supplemented with fresh feedstock ingredients (Economou et al., 2017). As such, these substrates are often reused for a second or even third round of production which increases their efficiency and reduces costs and environmental impacts (Estrada et al., 2009; Leong et al., 2022).

Several important substrate characteristics for the production of mushrooms should be considered for this valorisation option. Table 4 shows the optimal values of the carbon, nitrogen and moisture content, the pH level and the C/N ratio for mushroom production (Mahari et al., 2020). Comparison of these optimal values with the data from Table 2 suggests that only the spent *Pleurotus spp.* substrates are suitable for mushroom production. Although its carbon content (21.6 – 37.1 dwt%) is lower than the optimum range (approximately 40 dwt%), it is known that mushrooms grow well at somewhat lower carbon content levels as long as the C/N ratio is in agreement (Mahari et al., 2020). As for the values shown in Table 2, the C/N ratio of the spent substrates of *Pleurotus spp.* (19.0 – 30.0) is within this optimum range. The spent *Pleurotus spp.* substrates can be used for the successive production of *Pleurotus spp.* itself or other mushrooms including *G. lucidum* and *L. edodes* (Cunha Zied et al., 2020; Economou et al., 2017). However, the highest biological efficiency (BE = 48.9%), a measure of the fruiting body output per substrate input, was achieved for successive production of *P. ostreatus* using the typical 'baglog' method (Leong et al., 2022; Pardo-Giménez et al., 2010). Thus, spent *Ostreatus spp.* substrates are best used for the successive production of *P. ostreatus* in this valorisation option.

Table 4. Optimum substrate characteristics for *P. ostreatus* production (Mahari et al., 2020) vs spent *Pleurotus spp.* substrates (data from Table 2).

Substrate characteristic	Optimum	Spent *Pleurotus spp,* substrates
Carbon (dwt%)	~ 40 %	21.6 – 37.1
Nitrogen (dwt%)	1.8 – 2.1	1.0 – 10.0
C/N ratio	19.1 – 22.1	19.0 – 30.0
pH	5.0 – 7.0	4.7 – 6.5
Moisture (wt%)	65 - 80	68.7 – 72.1

4.2.1.2 Production of cellulose nanofibres

At the third level of the biobased value pyramid stands the production of bioplastics and polymers. The (hemi)cellulose and lignin polymers, still present in the SMS, provide a resource for the production of biodegradable and sustainable alternatives for otherwise polluting petroleum-based materials and chemicals (Isikgor & Becer, 2015; Liao et al., 2020). A high-value, directly marketable product that can be produced from these polymers is that of cellulose nanofibres (CNF). CNF finds applications in food packaging, drug delivery, electronics and building materials (Gu et al., 2015; Ma et al., 2022). It is nothing but cellulose in the form of fibres with lengths up to a few micrometres and a diameter below 100 nanometres that can be extracted from crude cellulose fibres (Sharma et al., 2018). The production of CNF is preferred over the production of 'crude' cellulose or any of its derivatives as it expands the downstream application potential due to its improved properties and reduced heterogeneity (Ma et al., 2022; Tian et al., 2017). The global market value for nanocellulose is projected to reach USD 661 million by 2023 and suggests a strong demand (Dhali et al., 2021).

CNF is produced in a two-stage production process that includes pre-treatment of the cellulose feedstock and purification of CNF for which numerous enzymatic, chemical, and mechanical methods exist (Foroughi et al., 2021; Gallo Stampino et al., 2021). However, enzymatic methods remain yet to be optimized while purely mechanical methods are extremely energy intensive and purely chemical methods use considerable quantities of chemicals and water (Michelin et al., 2020; Turk et al., 2020). A combination of chemical and mechanical production methods can significantly reduce these requirements while still providing high yields and is therefore the preferred production method for CNF (Haroni et al., 2021; Li et al., 2013).

4.2.1.3 Production of cellulase

Bulk chemicals and materials are at the fourth level of the biobased value pyramid. A group of high-value bulk chemicals that could be produced from the spent mycelium substrates are the lignocellulolytic enzymes that are excreted by the mycelium to digest the substrates (Lim et al., 2014). These enzymes are used for various applications including pre-treatment of lignocellulosic biomass for sustainable bioethanol production (Siqueira et al., 2020), waste treatment (Chukwuma et al., 2020), and (animal) food- and drink processing (Sadhu & Maiti, 2013; Toushik et al., 2017). The sales of lignocellulolytic enzymes account for 20% of the total global enzyme market, which is estimated to reach USD 7.0 billion in 2023, and therefore represents a clear demand (Jaramillo et al., 2015; Leite et al., 2021).

Although many different lignocellulolytic enzymes could be produced from the spent substrate, it is important to consider the (hemi)cellulose and lignin fractions of the substrates as these can inhibit or stimulate the production of specific lignocellulolytic enzymes i.e., substrates high in lignin provide the appropriate feedstock for ligninolytic enzyme production but are less appropriate for cellulolytic enzyme production (Leite et al., 2021). Therefore, the high share of cellulose (30.8 – 45.3%) in comparison to hemicellulose (9.9 – 18.6%) and lignin (7.6 – 20.5%) (see Table 2) suggests that the spent mycelium substrates are best used for the production of cellulolytic enzymes, such as cellulases. Cellulases are often produced through submerged aerobic fermentation (SmF) with the help of the fungus *Trichoderma reesei* that feeds on lignocellulolytic substrates with a high cellulose fraction (Ellilä et al., 2017; Gilpin & Andrae, 2017). Although other methods exist, this is the preferred method due to its mature technological development and ease of operation (Ramesh et al., 2019).

4.2.1.4 Production of fuel pellets

At the bottom of the biobased value pyramid is the production or extraction of energy, heat, and fuels. A promising valorisation option for the recovery of energy from the SMS is that of fuel pellets production. Fuel pellets are a form of solid biofuels. They are combusted for heat- and power generation in both industrial power plants and small-scale residential heating systems (Pradhan, Mahajani & Arora, 2018). The technology for conversion from feedstock to product is highly effective and provides the most efficient feedstock energy recovery at low production costs (Guo, Song & Buhain, 2015). Therefore, the production of fuel pellets is preferred over other options at this level of the biobased value pyramid. Although there are no exact numbers on the market value of wood fuel pellets available, their demand parallels the continuously growing demand for renewable energy sources and is therefore considered significant (Proskurina et al., 2017; Thrän et al., 2019).

The ash content of the feedstock is an important characteristic to consider for this option as it can negatively influence the combustion of fuel pellets. Da Silva Alves et al. (2021) have shown that spent *P. ostreatus* and *L. edodes* substrates with ash contents of 2.65 – 37.5 wt% can be used to produce good-quality fuel pellets for low-emission bioenergy systems. As the ash content of the SMS falls well within this range (5.29 – 20.50 wt%), it can be concluded that it can be used for the production of fuel pellets.

4.2.2 Identified valorisation options for pure mycelium waste

This section provides the results of the literature study for identification of valorisation options for the PMW.

4.2.2.1 Extraction of dietary fibres

Mushroom mycelia are rich in dietary fibres (Antunes et al., 2020). The total dietary fibre (TDF) content contributes to 49.9 – 55.0 % of the dry mycelial weight (Cheung, 1996; Laforteza et al., 2020). It is hard to define dietary fibres as they comprise a mixture of non-starch polysaccharides such as cellulose and pectin but also beta-glucans (Fuller et al., 2016). Nevertheless, adequate intake of these dietary fibres provides significant benefits to human health e.g., lowering blood pressure, cholesterol levels and reduces risk of colon cancer (Maphosa et al., 2016). Dietary fibres are of high interest to the food industry to improve nutritional value, colour and texture of various food types (Tejada-Ortigoza et al., 2016; Yang et al., 2017). Increased attention for healthy diets provides a high demand for dietary fibres. The global market value for dietary fibres was projected to reach USD 5.32 billion by 2020 (Salmas et al., 2017) and therefore provides a promising option for valorisation of the PMW.

Many methods exist for the extraction of dietary fibres from biomass. Amongst them are chemical, mechanical, and biological extraction methods, each having their specific pros and cons (Tejada-Ortigoza et al., 2016). The most promising of these methods is that of ultrasound assisted extraction (UAE). As the name suggests, UAE makes use of ultrasound generation to disrupt the biomass cell-structure to allow better penetration of extraction solvents into the biomass solids (Kumar et al., 2021). This increases the yield of dietary fibre extraction while reducing reaction time, temperature, and chemical solvent requirements in comparison to other extraction methods (Aguiló-Aguayo et al., 2017). Therefore, UAE is considered the preferred method for extraction of dietary fibres from the PMW.

4.2.2.2 Extraction of phenolic compounds

A different group of biological compounds with known benefits to human health are phenolic compounds (Antunes et al., 2020). These compounds exhibit anti-allergenic, anti-inflammatory, anti-microbial, antiviral, antioxidant, cardioprotective and vasodilatory effects (Montenegro-Landívar et al., 2021). Phenolic compounds are therefore of specific interest to the pharma- and nutraceutical industries where they are used for various purposes (Ghitescu et al., 2015). Although a total phenolic compound (TPC) content of 4.2 - 29.0 mgGAE/100 g dry mushroom mycelia might seem low, these mycelia have shown to be an excellent source for the extraction of phenolic compounds (Heleno et al., 2012). The demand for phenolic compounds has been growing over the past years due to its beneficiary health effects and reached a global market value of USD 1.28 million in 2018 (Siacor et al., 2020). There are many methods for extraction of phenolic compounds. However, UAE is again the preferred method for reasons similar to the extraction of dietary fibres (Dzah et al., 2020).

4.3 Environmental impacts of identified valorisation options

This section provides the results of the environmental impact assessment of the identified valorisation options for the mycelium waste streams. The presented information was found in available life cycle assessment studies that used comparable production processes, feedstocks, and conditions as the production processes of the identified valorisation options. The results of these available studies have been adjusted to the FU of ‘the valorisation of one ton mycelium waste (SMS or PMW)’ and are shown in Table 5. The required data and calculations for these conversions are given in Appendix B.

4.3.1 Environmental impacts of mushroom production

The highest BE (48.9%) for *P. ostreatus* production from spent substrates was achieved by Pardo-Giménez et al. (2010) by using the common ‘cylindrical baglog’ cultivation method. According to Pardo-Giménez et al. (2010), 489 kg of *P. ostreatus* could be produced from one ton of spent *Pleurotus spp.* substrates through this method. The baglog method involves substrate preparation, sterilization, and inoculation with fungal spores after which the mushrooms grow in cylindrical bags in vertical farms similar to those of mycelium production (Mahari et al., 2020). Dorr et al. (2021) assessed the environmental impacts of each stage in the production of one kg *P. ostreatus* using the baglog method. Based on the results of Dorr et al. (2021), the production of 489 kg of *P. ostreatus* results in a GWP of 190 kgCO_2-eq. and has an energy and water consumption of 43 * 10^3 MJ and 817 m^3, respectively.

4.3.2 Environmental impacts of cellulose nanofibres production

The preferred method for CNF pre-treatment and purification is a combination of chemical and mechanical methods. Haroni et al. (2021) assessed the environmental impacts of four combinations of chemical and mechanical production methods for the production of CNF from sugarcane waste streams. The cellulose content of this waste stream (34.6 dwt%) is comparable to that of the SMS (30.8 – 45.3 dwt%). The preferred method, due to high yields with the lowest environmental impacts, is that of alkaline pre-treatment followed by lime juice hydrolysis and high-pressure homogenization. According to the results by Haroni et al. (2021) it is possible to produce 300 kg of CNF from one ton of spent mycelium substrate. This production of CNF results in a GWP of 951 kgCO_2-eq. with a C_e and C_w of 3.5 * 10^3 MJ and 3 * 10^3 m^3, respectively.

4.3.3 Environmental impacts of cellulase production

Gilpin and Andrae (2017) researched the life cycle impacts of different feedstocks for the production of cellulase using the SmF method of which softwood biomass appeared to be the most sustainable. This lignocellulosic feedstock was pre-treated using ammonia and lime and then degraded by *Trichoderma reesie* bacteria to produce cellulase in a bioreactor. Based on the results of Gilpin and Andrae (2017), it is possible to produce 80 kg of cellulase from one ton of spent mycelium substrate with a water and energy consumption of 5 m^3 and 4.2 * 10^3 MJ, respectively. The contribution to global warming of this valorisation option is 633 kgCO_2-eq. per ton SMS.

4.3.4 Environmental impacts of energy production

The production of fuel pellets from SMS is a rather simple process. Biomass feedstocks are treated, processed, and pelletized. Laschi et al. (2016) researched the environmental impacts of fuel pellets production from wood through an LCA. Based on their results, 885 kg of fuel pellets could be produced from one ton of SMS. The processes relevant for fuel pellets production from SMS are limited to grinding, drying and finally pelletisation. The valorisation of one ton SMS through this process results in a GWP of 335 kgCO^2-eq. and has an energy demand of 2.2 * 10^3 MJ. No water is involved in the production process of fuel pellets and its demand is therefore determined at zero m^3.

4.3.5 Environmental impacts of dietary fibre extraction

The preferred method for extraction of dietary fibres from the PMW is through ultrasound assisted extraction (UAE). Following this method and a maximum yield of 27.81% under optimal conditions (Bagherian et al., 2011), 40 kg dietary fibre can be extracted from one ton PMW. Balicki et al. (2020) studied the water and energy requirements for the extraction of dietary fibres using UAE. Based on their results, the water and energy requirements for the valorisation of one ton PMW are 9.6 * 10^3 m3 and 3.3 * 10^3 MJ, respectively. The study did not involve the assessment of the GWP of dietary fibre extraction by using UAE. As the required data is not available in other literature, the GWP could not be determined.

4.3.6 Environmental impacts of phenolic compound extraction

UAE is also the preferred method for extraction of polyphenols from the PMW. Based on the results of Section 4.1.2 and a maximum attainable extraction yield of 35.1% of TPC (Priyadarshini et al., 2022), 1.6 kg GAE of phenolic compounds can be extracted from one ton PMW through UAE. Barjoveanu et al. (2020) researched the environmental impacts of this extraction. Based on their results, the extraction of 1.6 kg GAE phenolic compounds has an energy and water requirement of 67 * 10^3 MJ and 220 * 10^{3} m^3, respectively. The GWP of the valorisation of one ton PMW through UAE of phenolic compounds is 12.8 * 10^6 kgCO_2-eq.

The results of the assessment of environmental impacts are summarized in Table 5. These results suggest that the production of CNF has the highest GWP (951.0 kgCO_2-eq./FU) for valorisation of one ton SMS while mushrooms production has the lowest GWP (335.1 kgCO_2-eq./FU). However, mushrooms production shows to have the highest energy consumption with 43.7 * 10^3 MJ/FU. The production of fuel pellets has the lowest energy- and water consumption (2.2 * 10^3 MJ/FU and 0 m^3/FU, respectively). The production of CNF shows to have the highest water consumption (3.0 * 10^3 m^3/FU) for the valorisation of the SMS. Dietary fibre extraction shows to have the lowest environmental impacts for the valorisation of one ton PMW. However, the GWP for this valorisation option is not available in the literature. Extraction of phenolic compounds shows the highest environmental impacts for valorisation of the PMW with an extremely high GWP (12.8 * 10^6 kgCO_2-eq./FU), energy- (67.0 * 10^3) and water consumption (220 * 10^3 m^3) per FU.

Table 5. The product yield and related environmental impacts (global warming potential and energy- and water consumption) of the selected options for valorisation of one ton spent mycelium substrate (SMS) or pure mycelium waste (PMW).

Valorisation option	**Product** (kg)	**GWP** (kgCO_2-eq.)	C_e (MJ)	C_w (m^3)	**Reference**
SMS					
Cellulase	80	633	4.20 * 10^3	5	Gilpin & Andrae (2017)
CNF	300	951	3.50 * 10^3	3.0 * 10^3	Haroni et al. (2021)
Fuel pellets	885	335	2.20 * 10^3	0	Laschi et al. (2016)
Mushrooms production	489	190	43.7 * 10^3	817	Dorr et al. (2021)
PMW					
Dietary fibre	40.0	-	33.0 * 10^3	9.60 * 10^3	Balicki et al. (2020)
Phenolic compounds	1.6	12.8 * 10^6	67.0 * 10^3	220 * 10^3	Barjoveanu et al. (2020)

4.4 Economic viability of identified valorisation options

This section provides the results for the assessment of the economic viability of the identified valorisation options. The costs and potential revenues were assessed by using data from comparable production processes available in the literature. The required data and calculations for these conversions are given in Appendix C.

4.4.1 Economic viability of mushrooms production

Following the bioconversion efficiency of Pardo-Giménez et al. (2010), 441 tons of *P. ostreatus* could be produced from MME's annual production of SMS. Mushroom production does not require complicated technologies. However, construction of the production facilities and acquisition of the equipment to grow mushrooms does require significant investment costs. Akritidis (2018) determined the costs of setting up a mushroom production unit with an annual production volume of 350 tons. Based on the results of Akritidis (2018) and linear scaling, the capital investment for construction of a mushroom production facility and additionally required equipment amounts to 1.7 million EUR. The operating costs, including the raw material costs, are one million EUR/year. The revenue resulting from the sales of all mushrooms, based on a price of 3.33 EUR/kg, amounts to 1.5 million euros EUR. Altogether this results in a positive annual return of 450 thousand which allows a return of investment within three years and ten months. Thus, valorisation of the SMS through the production of *P. ostreatus* can be considered economically viable.

Table 6. Economic viability of mushrooms production.

	Value	**Unit**	**Reference**
Capital costs	1.7 * 10^6	EUR	Akritidis (2018)
Operating costs (incl. raw materials)	1.0 * 10^6	EUR/year	Akritidis (2018)
Revenue	1.5 * 10^6	EUR/year	Akritidis (2018)
Annual return	450 * 10^3	EUR/year	-
ROI	3.8	Years	-

4.4.2 Economic viability of cellulose nanofibers

MME could produce a total amount of 271 tons of CNF per year if all SMS is used for CNF production. Based on a techno-economic analysis of CNF production using an alkaline pretreatment followed by mechanical homogenization by Bondancia et al. (2020), the required capital investment for a CNF production unit that allows the production of such amounts is 3.5 million EUR. The costs of the biomass feedstock used for this analysis are 700 EUR/ton. Subtraction of these costs due to replacement by the SMS feedstock results in an annual operating cost of 3.5 million EUR, including additional raw material requirement e.g., solvents. The CNF could be sold for 8.84 EUR/kg which results in a total annual revenue of 2.4 million EUR (Nechyporchuk et al., 2016). However, this results in a negative annual return of 1.1 and thus a negative ROI. Therefore, the valorisation of the SMS through the production of CNF is not considered economically viable.

Table 7. Economic viability of CNF production

	Value	Unit	Reference
Capital costs	$3.5 * 10^6$	EUR	Bondancia et al. (2020)
Operating costs (incl. raw materials/ excl. feedstock)	$3.5 * 10^6$	EUR/year	Bondancia et al. (2020)
Revenue	$2.4 * 10^6$	EUR/year	Nechyporchuk et al. (2016)
Annual return	$-1.1 * 10^6$	EUR/year	-
ROI	Negative	-	-

4.4.3 Economic viability of cellulase production

A total of 72 tons of cellulase could be produced from MME's annual SMS production. Based on a cost-analysis of an anaerobic cellulase production unit with an annual cellulase production of 40 tons, the total capital investment for this valorisation option is 8.3 million EUR (Hong et al., 2013). The operating costs are based on a comparable production unit using *T. reesei* bacteria for production of cellulase. The operating costs include the costs of operation and the raw materials. As the biomass feedstock contributes to 50% of the total operating costs, these costs will be halved when utilizing SMS (Elilä et al., 2017). Hence, the total annual operating costs including the raw materials for production of cellulase are 150 thousand EUR. The total annual revenue, based on a sales price of 16.72 EUR/kg, of 1.2 million results in a positive annual return of 1.1 million EUR/year and an ROI of seven years and 11 months. Thus, the valorisation of the SMS through production of cellulase is economically viable.

Table 8. Economic viability of cellulase production.

	Value	Unit	Reference
Capital costs	$8.3 * 10^6$	EUR	Hong et al. (2013)
Operating costs (incl. raw materials/ excl. feedstock)	$150 * 10^3$	EUR/year	Elilä et al. (2017)
Revenue	$1.2 * 10^6$	EUR/year	Liu et al. (2016)
Annual return	$1.1 * 10^6$	EUR/year	-
ROI	7.9	Years	-

4.4.4 Economic viability of fuel pellets production

Wang et al. (2020) assessed the economic viability of a biomass fuel pellets production facility with an annual pellet production of 47 thousand tons. Based on their results and a total annual fuel pellet production of 799 tons by MME, the required capital investment for this valorisation option is relatively low at 85 thousand EUR. No additional raw materials are required for the production of fuel pellets and the annual operating costs are therefore limited to 78 thousand EUR. The annual revenue created through the sales of all fuel pellets amounts to 140 thousand EUR. This results in a positive annual return of 60 thousand EUR and an ROI of one year and five months. Thus, the production of fuel pellets can be considered an economically viable option for valorisation of the SMS.

Table 9. Economic viability of fuel pellets production.

	Value	Unit	Reference
Capital costs	$85 * 10^3$	EUR	Wang et al. (2020)
Operating costs	$78 * 10^3$	EUR/year	Wang et al. (2020)
Revenue	$140 * 10^3$	EUR/year	Wang et al. (2020)
Annual return	$60 * 10^3$	EUR/year	-
ROI	1.4	Years	-

4.4.5 Economic viability dietary fibre extraction

Assuming 350 annual eight hour working days (Priyadarshini et al., 2022), a 5L UAE unit would be sufficient for a full extraction of all the dietary fibre content from the PMW annually produced by MME. The capital costs of such a unit are 10,000 EUR (Prado et al., 2017). Recovery of the ethanol used for extraction of the dietary fibres is 95%, meaning that every extraction run requires an ethanol supplementation of 5% (Barjoveanu et al., 2020). This brings the total annual raw material costs to 415 EUR (Vieira et al., 2013). The operating costs are based on a cost-analysis of a 125L UAE unit by Prado et al. (2017) and amount to 44 euro per year, based on linear scaling. The sales of all the dietary fibre as functional food supplements creates an annual revenue of 11 thousand EUR (Wenger et al., 2018). Subtraction of the production costs gives an annual annual return of roughly 11 thousand EUR, resulting in a positive ROI of only eleven months. Although the annual revenue is rather low, the valorisation of the PMW through extraction of dietary fibres can be considered economically viable.

Table 10. Economic viability of dietary fibre extraction.

	Value	Unit	Reference
Capital costs	$10 * 10^3$	EUR	Prado et al. (2017)
Raw materials costs	415	EUR/year	Vieira et al. (2013)
Operating costs	44	EUR/year	Prado et al. (2017)
Revenue	$11 * 10^3$	EUR/year	Wenger et al. (2018)
Annual return	$11 * 10^3$	EUR/year	-
ROI	0.9	Years	-

4.4.6 Economic viability phenolic compound extraction

The extraction of phenolic compounds from the PMW using UAE follows largely the same process as that of dietary fibre extraction. It uses the same equipment and extraction solvents by which a similar feedstock input can be processed at equal extraction duration. The final products are distinguished mainly due to differences in the filtration process (Priyadarshini et al., 2022). Therefore, the capital, raw materials and operating costs are equal to that of dietary fibre extraction and are 10,000 EUR, 415 EUR and 44 EUR, respectively (Prado et al., 2017; Vieira et al., 2013). Sales of all extracted polyphenols results in an annual revenue of 2.2 thousand EUR at a sales price of 28 EUR/kg for crude polyphenolic extracts (Uyttebroek et al., 2018). This results in a low annual profit of 1.7 thousand EUR/year and a positive ROI of six years. Thus, the extraction of phenolic compounds from the PMW is economically viable although it provides a rather low revenue.

Table 11. Economic viability of phenolic compound extraction.

	Value	Unit	Reference
Capital costs	$10 *10^3$	EUR	Prado et al. (2017)
Raw material costs	415	EUR/year	Vieira et al. (2013)
Operating costs	44	EUR/year	Prado et al. (2017)
Revenue	$2.1 *10^3$	EUR/year	Uyttebroek et al. (2018)
Annual return	$1.7 * 10^3$	EUR/year	-
ROI	6.0	Years	-

The results of the economic viability assessment show large variations between the different valorisation options. The positive annual return for valorisation of the SMS ranges from 60 thousand EUR for fuel pellets production to 450 thousand EUR for mushrooms production. Each option for valorisation of the SMS can be considered economically viable, except for the production of CNF due to its negative annual return of 1.1 million EUR. Of these options, the production of cellulase shows to have the highest annual return (1.1 million EUR) and an ROI of 7.9 years. Although the annual return of fuel pellets production is much lower (sixty thousand EUR) it shows the fastest ROI (1.4 years). Extraction of dietary fibres results in the highest annual return (ten thousand EUR) and fastest ROI (0.9 years) for valorisation of the PMW. The extraction of phenolic compounds yields a rather low annual return of 1.5 thousand EUR with an ROI of 6.0 years.

4.5 Eco-efficiency of the selected valorisation options

The results of the eco-efficiency indicators are shown in Table 12. What can be noticed from these results is the large difference in environmental impacts per monetary unit benefit between the valorisation options of the PMW (dietary fibre and phenolic compound extraction) and SMS (others). The options for valorisation of the PMW show the highest environmental impacts per monetary unit benefit for every indicator. Of these two, extraction of phenolic compounds shows the highest values for every indicator. Although the GWP for dietary fibre extraction is unknown, its extraction procedure is similar to that of phenolic compound extraction. It is assumed that the GWP is also comparable and the GWP of phenolic compound extraction is therefore used to calculate the GWP of dietary fibre extraction. The mushroom production option shows the lowest GWP (0.38 kgCO_2-eq./EUR) while cellulase production shows the lowest values for energy consumption (3.59 MJ/EUR) and fuel pellets production shows the lowest values for water consumption (0 m^3/EUR) for the valorisation options of the SMS. The highest impacts per monetary unit benefit of the options for valorisation of the SMS belong to the production of fuel pellets for climate change (0.54 kgCO_2-eq./EUR) and to the production of mushrooms for energy consumption (88.2 MJ/EUR). The required data and calculations for determination of the eco-efficiency indicators are given in Appendix C.

Table 12. Eco-efficiency indicators showing the environmental impacts per monetary unit benefit of the selected valorisation options.

Valorisation option	**Climate change** (kgCO_2-eq/EUR)	**Energy consumption** (MJ/EUR)	**Water consumption** (m^3/EUR)
Cellulase production	0.54	3.59	1.30
CNF production	-	-	-
Fuel pellets production	5.07	32.8	0
Mushroom production	0.38	88.2	1.65
Dietary fibre extraction	$55.5 * 10^3$	145	41.6
Phenolic compound extraction	$3.65 * 10^6$	$1.92 * 10^3$	$6.29 * 10^3$

This chapter has provided the results following the research methods that have been previously described. These results will be interpreted and discussed in the next chapter to synthesize the final conclusions of this research.

5 Discussion & conclusions

The goal of this thesis was to determine the potential for valorisation of the two waste streams resulting from pure mycelium production i.e., spent mycelium substrates and pure mycelium waste, to improve the cost-efficiency of pure mycelium production and to optimise the utilisation of the resources used for this production. This will allow the release of the full potential of pure mycelium materials and support operationalisation of the CBE. This operationalisation is necessary to overcome issues of resource depletion and environmental degradation that are inherent to our current linear economy (Geissdoerfer et al., 2017). This chapter provides an interpretation of the results of this thesis in relation to the research questions. Additionally, the broader implications of these results for science and practitioners, the limitations of the study, and recommendations for implementation and future research will be discussed to finally conclude this research.

5.1 Interpretation of results

The results of this study provide new insights into the possibilities for the valorisation of mycelium waste streams. The SMS can be valorised through the production of mushrooms, cellulase and fuel pellets. The production of mushrooms and cellulase will result in additional waste streams resulting from their own production but that is precisely what the CBE is about: the optimisation of biomass utilization through its consecutive utilization in intermediate steps before cascading it to the lowest level of resource quality (Stegmann et al., 2020). The production of mushrooms or cellulase allows the extraction of economic value in intermediate steps as the biomass remaining after their production can be used to create value in consecutive utilization steps e.g., the production of fuel pellets and lignocellulolytic enzymes after mushrooms production or cellulase production, respectively (Chukwuma et al., 2020). The results of this thesis suggest that the environmental impacts of these valorisation options are higher than those of fuel pellets production. However, according to the principles of the CBE, it is preferred to use biomass waste streams, such as the SMS, where possible and adequate and to reduce the need for virgin biomass inputs consequently (Stegmann et al., 2020). The point of this is that the valorisation of waste streams should be allowed if the environmental impacts of such a valorisation do not exceed the impacts from the conventional production of the resulting products. As the results of this thesis show similar environmental impacts for the production of mushrooms and cellulase from the SMS and conventional production (Dorr et al., 2021; Gilpin-Andrae et al., 2017), it can be said that the valorisation of the SMS through the production of cellulase or mushrooms supports adequate optimisation of biomass resource utilization. However, the significantly longer time for their ROI make the production of fuel pellets the preferred option over the production of mushrooms or cellulase. The production of fuel does not provide consecutive utilisation of biomass resources as it is considered its final utilisation. Nonetheless, it still supports operationalisation of the CBE as optimisation of biomass resource utilisation does not imply that these resources should be endlessly maintained within the economic system. The production of fuel pellets could also be interpreted as an optimisation as it provides the fastest ROI at the lowest environmental impacts. Therefore, the production of fuel pellets is considered the preferred option for valorisation of the SMS.

The PMW could be valorised through the extraction of dietary fibre or phenolic compounds. Both options are economically viable and therefore provide the potential to reduce the costs of pure mycelium production. Additionally, only little amounts of these compounds are extracted from the PMW allowing most of the waste to be utilized in consecutive utilization steps. Therefore, the valorisation of the PMW through extraction of dietary fibre or phenolic compounds does provide the option to reduce the costs of mycelium production and to improve the utilization of this resource. However, both options create only rather little value, and the ROI of phenolic compound is rather long especially when considering the low amount of value created. Moreover, the environmental impacts resulting from these valorisation options are extremely high. Therefore, the results of this study suggest that the valorisation of PMW through extraction of dietary fibres or phenolic compounds cannot be considered sustainable in any way and their implementation is therefore not desirable.

The results of the waste stream characterisation confirm the assumption that the waste streams should be valorised separately. The values for their common characteristics i.e., proteins, fats and carbohydrate suggest significant differences in their composition. Chitin, beta-glucans, dietary fibre, phenolic compounds and lignin show to be absent in one or the other. The results of this characterisation suggest that the SMS could potentially also be valorised through the extraction of proteins, fats, carbohydrates or lignin. The values of these and other compounds in the SMS show to be comparable for the different species of fungi used for mycelium production. Similar findings result from the characterisation of the PMW. Other than the extraction of dietary fibres or phenolic compounds, it shows to be a good source of chitin and beta-glucans for each of the species of fungi. The protein content is particularly interesting for the PMW resulting from mycelium production with Reishi or Shiitake fungi. These findings suggest additional options for valorisation of the PMW that could provide it with the potential to reduce the costs of pure mycelium and optimise the utilisation of this biomass resource, nonetheless. Again, the characteristics of the different types of PMW show to be comparable which means that the results of this study are applicable to the majority of the produced mycelium waste streams. The validity of these findings is supported by the large number of sources consulted for this characterisation.

The findings of this thesis show that mycelium waste streams are a valuable resource, both from an economic as well as an environmental sustainability perspective. The SMS can be used for profitable production of fuel pellets, cellulase or mushrooms. The biomass remaining after production of mushrooms or celullase allows the consecutive production of e.g., compost, fuel pellets or ligninolytic enzymes. Although the selected options for valorisation of the PMW in this study show not to be desired, the results also suggest that there are potential other interesting options for its valorisation. These findings confirm the expectations with regards to the current underutilisation of these wase streams as a resource in the pure mycelium industry. The findings of this study apply to the majority of the pure mycelium producers due to the generalisability of the characteristics for the waste streams resulting from mycelium production with the most commonly used species of fungi. With that, this study provides the first indications for profitable and sustainable options for valorisation of mycelium waste streams that could improve the economic and environmental sustainability of this industry.

5.2 Implications to science and practice

Firstly, the findings of this study have some implications for science. For as far as the literature concerns, this is the first study to compare various options for valorisation of mycelium waste streams. Information from studies concerned with comparable feedstocks or processes were used as proxies to provide the first insights into the economic viability and environmental impacts of mycelium waste stream valorisation. Until this point, a synthesis of the mycelium waste stream characteristics as presented in this study was absent from the literature. The large number of sources consulted in the literature study adds validity to this novel synthesis. This information can be used to determine potential additional valorisation options for the waste streams resulting from pure mycelium production using Reisshi, Oyster, Turkey Tail and Shiitake mushrooms and various substrate mixtures. The current absence of this data explains the knowledge gap regarding mycelium waste valorisation as these characteristics are essential to explore potential options for valorisation of waste streams (Stone et al., 2019). With the identification of these characteristics and the assessments of the environmental impacts and the economically viability of the resulting options for valorisation, the findings of this study add valuable information to the science and practice of mycelium materials, the CBE and industrial ecology.

The results of the economic assessment show that the production of mushrooms and fuel pellets and the extraction of dietary fibres have similar values for return on investment as found in the literature which supports the validity of these results (Akritidis, 2018; Prado et al., 2017; Wang et al., 2020). However, the resulting ROI for extraction of phenolic compounds shows to be three to four years longer than most extraction processes using UAE (Prado et al., 2017). This could be explained by the rather low phenolic content of the SMS in comparison to other sources of phenolic compounds resulting in lower revenues and thus a higher ROI (Priyadarshini et al., 2022). Information regarding the ROI of cellulase production is currently absent from the literature. Therefore, the results of this study provide a first indication of the potential profitability of cellulase production from lignocellulosic biomass resources.

The production of cellulose nanofibers appears not to be economically viable which is unexpected as the production of CNF is suggested as an economically viable product in the literature (Ma et al., 2022). This unexpected finding could be explained by the methods used for the production of CNF. It was chosen to assess the economic viability of CNF production through a combination of chemical and mechanical methods as this was suggested to be the most sustainable production method for CNF currently available (Michelin et al., 2020; Haroni et al., 2021). However, this method did not provide the expected economic return. Nonetheless, CNF production could possibly be a valuable option for valorisation of the SMS provided that a different method is chosen for its production or that the economic performance of this combined production method will be improved.

Another unexpected finding is concerned with the difference in environmental impacts of the options for valorisation of the PMW. Although they make use of the same production method, ultrasound-assisted extraction at comparable temperature and duration, the results of this study suggest that the water and energy demand of phenolic compound extraction is roughly twenty-two and two times higher than that of dietary fibre extraction, respectively. This can be explained by the data used for this assessment. The study of Balicki et al. (2020) does not include all impacts of the entire lifecycle of this extraction method. Instead, it focuses on the direct cumulative water and energy demands of ultrasound-assisted dietary fibre extraction. On the other hand, the study by Barjoveanu et al. (2020) includes the full life cycle impacts of ultrasound assisted extraction of phenolic compounds which explains the much higher environmental impacts as it includes the impacts of all the processes and material inputs e.g., extraction solvents and energy production. The data by Balicki et al. (2020) were used nonetheless as the literature currently provides no other studies concerned with the environmental impacts of ultrasound-assisted extraction of dietary fibres from biomass sources. However, the similarity in extraction methods and procedures suggests that the environmental impacts of the full lifecycle of phenolic compound extraction are probably closer to those of phenolic compound extraction.

The assessment of eco-efficiency of the selected valorisation options was included in this research as its optimisation is suggested to stimulate optimisation of biomass resource utilisation (Bello et al., 2018). The results of the eco-efficiency assessment were therefore believed to be decisive in the selection of the preferred options for valorisation of the mycelium waste streams. However, the results of this assessment suggest that optimisation of eco-efficiency does not necessarily provide absolute sustainability. Some of the valorisation pathways with the highest absolute environmental impacts show to perform best for eco-efficiency. As such, optimisation of eco-efficiency only leads to improved sustainability through an absolute reduction of environmental impacts. Therefore, the results of this thesis suggest that eco-efficiency indicators alone provide little information about the sustainability of valorising specific waste streams and that their values should always be presented in combination with absolute environmental impacts to prevent unwanted outcomes of waste stream valorisations.

The results of this study support the findings by Ghisellini et al. (2016) that it is not necessarily preferred to maintain biomass resources at their highest possible resource level from an environmental or an economic perspective. This finding supports recent discussions about the application of waste hierarchies i.e., the biobased value pyramid, in the field of industrial ecology and CBE (Campbell-Johnston et al., 2020; van Ewijk & Stegemann, 2016). According to van Ewijk & Stegemann (2016), the intrinsic potential of such waste hierarchies to achieve absolute reductions in materials throughput and improve environmental sustainability is limited. Stegmann et al. (2020) suggest that the allocation procedure of biomass resources to certain options should focus on the optimisation of the value of these resources over time via cascading. It is thus important for industrial ecologists to not blindly follow waste hierarchies but to carefully assess the environmental impacts and the value created over the entire cascading sequence of biomass resources. Although the data presented in this study with regards to the environmental impacts and economic viability of the identified options for valorisation of mycelium waste streams focuses on only one step in a potentially larger cascading option, it provides initial information to determine the optimal cascade of mycelium waste streams.

The results of this thesis suggest that the production of cellulase results in the highest GWP for valorisation of the SMS, roughly three times higher than that of mushrooms production. On the other hand, its energy demand is roughly a factor ten lower than the energy demand of mushrooms production. This is unexpected as a high energy demand requires more energy production which often results in a higher GWP (Carus & Dammer, 2018). The rather low GWP of the energy required for the production of mushrooms can be explained by the specific location of the LCA study by Dorr et al. (2021) as a large share of the French energy mixture is contributed by nuclear energy. Although the sustainability of nuclear energy is subject of a different discussion, it has a rather low impact on climate change (Dorr et al., 2021). The outcome of LCA studies is specific to their spatial locations and the background information used in the assessments. The GWP of mushrooms production will therefore likely be higher than that of cellulase production if these mushrooms are produced in a spatial location other than France.

Sustainable product design is considered one of the pillars of the CBE as it provides efficient resource use throughout a products or materials lifecycle and to facilitate recycling or cascading at end-of-life (Stegmann et al., 2020). Therefore, designers are increasingly expected to consider sustainability in their materials selection and to have knowledge about the environmental impacts of their chosen materials (Camere & Karana, 2018). The findings of this thesis suggest that the waste streams resulting from pure mycelium production can be sustainably valorised through various options that support operationalisation of the CBE. This is valuable information for designers that would like to or already work with mycelium as it further justifies their choice for this material from a sustainability perspective.

Secondly, the results of this thesis have significant practical implications for different industries. It is expected that the production of pure mycelium will increase in the near future (Hahn, 2020; Roshitsh, 2021). According to Yarns and Fibers (2022) MME is ready to scale up their production facilities to produce pure mycelium similarly to the production of edible mushrooms. Such developments will significantly boost the production of pure mycelium and thus the resulting waste streams. The current lack of appropriate options for valorisation of these growing waste streams will result in an even larger underutilization of biomass resources if this remains unaddressed. Therefore, the identification of economically viable options for valorisation of SMS is all the more important and can significantly increase the contribution to a sustainable economic system by this industry through the optimisation of biomass resource utilisation. In contrast to the SMS, the results of this study suggest that the PMW is to be composted for the time being as the selected options for its valorisation are not desirable from an environmental sustainability perspective.

Additionally, the production of pure mycelium is currently still quite costly (Hahn, 2020; MME, 2021) and the disposal of its resulting waste streams is a major contributor to these costs (Beckers et al., 2019). The SMS provides 95% of this waste stream (MME, 2021). Thus, the identification of various economically viable options for valorisation of the SMS provides the opportunity to reduce its cost price even though no desirable options for valorisation of the PMW have been identified. With that, mycelium materials will become cheaper and available to a larger group of consumers. Unlike conventional meat, leather or plastics, mycelium-based alternatives are produced sustainably from renewable biomass resources or waste streams through biofabrication (Elsacker et al., 2019). The substitution of these materials with mycelium alternatives could be increased by the foreseen cost price reduction due to valorisation of the SMS which will have significant beneficial impacts. Therefore, successful valorisation of the SMS allows the release of the full potential of pure mycelium materials.

Valorisation of mycelium waste streams is not only interesting to mycelium producers. Other industries could potentially take advantage of the valorisation of mycelium waste streams as well. Dorr et al. (2021) observed that the highest environmental impacts of Oyster mushroom production are created by the production, preparation, and sterilisation of the substrate materials. Substitution of these substrates by the SMS resulting from mycelium production could significantly lower these environmental impacts. Similar findings have been suggested for the other options for valorisation of the SMS (Gilpin-Andrae et al., 2017; Wang et al., 2020).

The results of the waste stream characterisation suggest that the SMS is essentially a form of lignocellulosic biomass. On its own, this is not necessarily an interesting finding. However, it is when placed in relation to lignocellulosic biorefineries. These complicated industrial processing plants aim at retrieving maximum value from lignocellulosic biomass resources through the simultaneous or consecutive production of various products such as biofuels and biochemicals. Such biorefinery of lignocellulosic biomass is an important aspect of the CBE (Stegmann et al., 2020). However, the techno-economic viability of these biorefinery plants remains challenging due to high costs and energy requirements of the current pre-treatment of biomass (Vu et al., 2020). Interestingly, research has shown that the (partial) digestion of lignocellulosic biomass by fungi provides a promising biological alternative for this pre-treatment that could improve the economic viability and reduce the environmental impacts of lignocellulosic biomass biorefinery (Zhou et al., 2017). Thus, the SMS could potentially provide a valuable resource for lignocellulosic biomass refinery.

Finally, the findings of this study have implications to the field of industrial ecology specifically. In this field, scientists and practitioners aim to study and assess the resource throughput of our societies with the goal to identify solutions to issues regarding environmental degradation and resource depletion. Among other things, this is done by balancing the resource throughput of our societies by minimising the virgin material inputs and waste outputs (Blomsma & Brennan, 2017). The identification of options for valorisation of mycelium waste streams supports this balance through operationalisation of the CBE and can be considered a good demonstration of the work of an industrial ecologist.

5.3 Limitations of the research

This thesis was at the constrain of several limitations with regards to the methods and data availability. This section will discuss these limitations, how they were addressed and what can and cannot be concluded from the results. Following the flow of this research, the first point to mention is the absence of data regarding the characteristics of the SMS which could be attributed to the novelty of mycelium materials. Producers of mycelium materials work with patented substrate mixtures or have developed their own substrate mixtures that they are not willing or allowed to share information about (Cerimi et al., 2019). This initial limitation was addressed by using information about spent substrates from mushrooms production under the assumption that the similarity in fresh substrates would translate in similar spent substrates after completion of a production cycle. Despite the good motivation for this assumption in Section 3.1, it could be that the actual SMS characteristics would yield slightly different results. Nevertheless, the results of this study, which are based on this assumption, provide a good initial exploration of options for valorisation of the SMS.

Six potential options for valorisation of the two mycelium waste streams were studied in this thesis. However, additional interesting options were identified but their environmental impacts and economic viability could not be determined due to a lack of data. Consequently, this study does not provide all the possible options for valorisation of mycelium waste streams and the options presented are therefore not the best options per se. Therefore, the presentation of potential options for valorisation of mycelium waste streams in this study is limited. Nevertheless, it provides a best estimate considering the data available and has shown that it is possible to improve the cost-efficiency of pure mycelium production and to support operationalisation of the CBE through optimisation of resource utilisation.

Another limitation regarding the identification and selection for the valorisation options and production methods is the limited input of selection criteria by mycelium producers. The selection of the valorisation options mainly focused on the appropriateness of the options for valorisation of the waste streams. This appropriateness was based on the accordance of the waste stream characteristics and the requirements for the specific options. Additionally, the valorisation options had to include a value-adding step and a demand for their resulting products. Although these selection criteria can be considered reasonable, it is not known whether these are the criteria that matter most to mycelium producers. The selection of production methods for the resulting products of the selected valorisation options faces a similar limitation. Information about the technological maturity, yield, environmental sustainability, and ease of operationalization available in the literature was used to determine the preferred production method. Likewise, this did not involve direct inputs of practitioners. Nevertheless, this study provides several economically viable options for valorisation of mycelium waste streams that could be adopted by practitioners if desired.

Under the ideal circumstances, the selection of production methods for the products resulting from the identified valorisation options in Section 4.2 would be based on a full comparison of their environmental impacts and economic viability prior to their selection as was done afterwards in Sections 4.3 and 4.4 However, this was not allowed due to the time available for this thesis. Consequentially, the selection of these methods was based on information about their sustainability and ease of operation available in the literature. This limitation suggests that other production methods could potentially be more sustainable if they were to be fully analysed prior to their selection.

Due to the limited availability of time, it was also not possible to perform life cycle (LCA) or techno-economic assessments (TEA) for each valorisation option specifically. Such assessments are time consuming and could be the subject of an entire thesis. Instead, information from studies available in the literature that were concerned with such assessments of processes similar to the identified options for valorisation of the mycelium waste streams were used as proxies and adjusted to the situation of this study. This was done under the assumption that these proxies provide a good representation for the economic viability and environmental impacts of the selected options for valorisation of the mycelium waste streams. Although there are good reasons for these assumptions, the data remains a representation and deviations from the actual economic viability and environmental impacts are therefore possible. Furthermore, the availability of this information in the literature is scarce and the assessments were therefore mostly based on the results of single proxies. The inclusion of primary data, by case specific LCAs and TEAs, or a higher availability of sources regarding this type of information would increase the certainty of the results presented in this study. Therefore, the findings of this study with regards to the environmental impacts and economic viability of the identified valorisation options provide a best estimate of this information about these options but should be taken with caution.

The assessment of economic viability of the identified valorisation options should be taken as an indication. Although the consulted studies for this assessment are a good source of information, they do not include information about labour costs or potential subsidies. Additionally, the capital-, raw material-, and operational costs of the production units concerned in the available studies deviates from the specific situation of this thesis due to difference in size of production capacity. This problem was circumvented by linear scaling of the costs to the ratio of the difference in production capacities. However, costs do not follow a linear scale but tend to decrease with increased production capacity in reality (Gilpin-Andrae et al, 2017). Again, this implies that the results of the economic viability assessment should be interpreted as an indication rather than an absolute certainty.

The environmental impact assessments of this study are based on the food-, water-, energy-, climate-nexus. As suggested by Laso et al. (2018), the production of food (or biomass) requires healthy (agro)ecological systems that provide water energy and a stable climate and the impacts on these systems should therefore be considered as was done in this study. However, there are other important environmental indicators that are not taken into account by the FEWC-nexus framework. These indicators e.g., freshwater eutrophication, ozone depletion, land-use change, are additional important determinants of the sustainability of waste valorisation options and should be determined to provide a complete assessment of environmental impacts (Dorr et al., 2021).

As previously mentioned, maximisation of resource utility through the consecutive cascading of biomass resources is of paramount importance for successful operationalisation of the CBE (Stegmann et al., 2020). This study did not involve an assessment of the possibilities for consecutive reutilization of the products and waste streams resulting from the valorisation options as this was beyond the scope of this thesis. Nonetheless, the valorisation options were among other things selected for their environmentally sustainable character. None make use of environmentally harmful substances or reagents. Therefore, it can be assumed that a good potential for consecutive reutilization of the resulting products and waste streams exists.

5.4 Recommendations for implementation and future research

Section 5.3 already suggested several methodological improvements to increase the certainty of the results presented in this study. This section will therefore mainly focus on suggestions for future research that build on the knowledge and insights gained from this thesis and address some practical recommendations for implementation.

Several options for valorisation of the mycelium waste streams appeared to create only little economic value or not to be economically viable at all i.e., the extraction of phenolic compounds and dietary fibres and the production of cellulose nanofibers. Nonetheless, these products are considered promising and interesting for various reasons such as their significant health benefits (Maphosa et al., 2016) or application potential (Ma et al., 2022). It should be noted that the methods for production or extraction of these products are still under development (Barjoveanu et al., 2022). Recommendations for future research are therefore focused on improving the extraction or production yields of these products or to reduce the costs of their extraction or production methods.

The novel synthesis of mycelium waste stream characteristics as presented in this study provides information about potentially other interesting options for valorisation of these waste streams e.g., chitin or beta-glucans. However, data regarding the environmental impacts and economic viability of these potential valorisation options is currently not available. It is therefore recommended to future research to perform LCAs or TEAs that will provide this required information to discover the full potential of mycelium waste stream valorisation.

A recommendation to the industry of mycelium materials would be to provide openness about the characteristics of spent mycelium substrates. The absence of this data in the literature is a serious constrain for detailed assessments of options for valorisation of this waste stream. Making this data available to science allows a much better assessment of the exact economic viability and environmental impacts of options for valorisation of the SMS that could benefit the waste management sector and mycelium producers specifically.

The appropriate selection of options for valorisation of the mycelium waste streams ideally includes a complete assessment of the environmental impacts and the economic returns of the various production methods for all the possible options for its valorisation. However, LCA sand TEAs are costly and time-consuming and such a complete comparison is therefore not always possible, as was demonstrated in this thesis. It can be assumed that this problem is not unique to the valorisation of mycelium waste streams but to any biomass waste stream in different industries. A solution to this problem could be found in a tool that can support the decision-making in the selection of appropriate options for valorisation of biomass waste streams. The tool provides the economic returns and environmental impacts that will result from various options for the valorisation of specific waste streams and the consecutive utilisation of the remaining biomass resources from this initial valorisation. It also suggests when energy recovery or composting should be considered at the point where additional utilisation of these resources is no longer considered desirable from an economic or sustainability perspective. Essentially, the tool provides the foresight that is required for optimisation of biomass resource utilisation in economic and environmental terms through computation of the total created value and environmental impacts of different valorisation options and their production methods. This allows easier selection of the appropriate option for valorisation of biomass waste streams. Ideally, the decision-making also includes social aspects e.g., job creation or health aspects that are provided by the potential cascading options (Stegmann et al., 2020). The realisation of such a tool will rely on a vast amount of data with regards to the environmental, economic, and social aspects of various valorisation options. The data presented in this thesis could provide a valuable contribution to this tool, but future research will be needed to provide the additionally required data to build this tool.

The implementation of the options for valorisation of mycelium waste streams presented in this study might be challenging to realise in practice. Some options require large production facilities e.g., mushrooms production, or expertise e.g., cellulase production, that might not be available to producers of mycelium materials themselves. Investments in new business relations could provide a solution to this issue. Additionally, intensification of cooperation between different stakeholders within the biobased materials sector is believed to provide the required infrastructure for the optimisation of biomass resource utilisation (Stegmann et al., 2020). Such cooperation could be realised by applying a business ecosystem perspective. A business ecosystem is an overarching approach that aims to combine business models of separate companies to achieve a collective outcome (Konietzko et al., 2020), which would be the optimisation of mycelium waste utilisation and associated value creation in this context. Future studies should therefore focus on researching potential opportunities for business ecosystem realisation in which each company focuses on its own expertise to optimise the utilisation of the mycelium waste streams and optimise the overall value creation.

This research has provided several options for valorisation of the SMS that provide the opportunity to improve the cost-efficiency of pure mycelium production and optimisation of the resources used for this production. The production of cellulase and mushrooms provides the largest potential for the improvements in cost-efficiency and optimisation of resource optimisation. However, they also require the largest investments and have a long ROI. As the findings of this study are only a first initial exploration of possible options for valorisation of the SMS, it is recommended to mycelium producers to implement the production of fuel pellets in their production process for the valorisation of the SMS. This provides the option for profitable valorisation of the SMS with a fast ROI. Implementation of mushrooms or cellulase production for valorisation of the SMS is currently too risky due to their longer ROI and the infancy of this mycelium materials industry. Future research regarding additional options for valorisation of the SMS might prove that other options provide a similar optimisation of biomass resource utilisation at faster ROI. The results of this study suggest that the valorisation of the PMW through the production of dietary fibre or phenolic compounds is not desired due to their extremely high environmental impacts. Until other profitable options at lower environmental impacts for valorisation of the PMW have been identified, it is recommended to continue the current disposal of the PMW through composting. Producers of mycelium materials should therefore start separating the mycelium waste stream into the SMS and PMW. As the SMS makes up the majority of the mycelium waste stream i.e., 95 wt%, its valorisation will provide significant improvements in the cost-efficiency of pure mycelium production, nonetheless.

5.5 Conclusions

The existing knowledge gap with regards to the valorisation of mycelium waste streams currently results in its disposal through composting. This disposal is costly and considered unsustainable according to the principles of the CBE as it results in an underutilization of these biomass resources. Therefore, the goal of this thesis was to research options for the valorisation of mycelium waste streams. Consequently, this is supposed to improve the cost-efficiency of pure mycelium production and to optimize the utilization of the resources used for this production which allows the release of the full potential of pure mycelium materials and operationalization of the CBE.

The insights resulting from this research provide the first contributions to solving the knowledge gap with regards to the valorisation of mycelium waste streams i.e., spent mycelium substrates and pure mycelium waste. The SMS could be valorised through the production of mushrooms, cellulase or fuel pellets. The production of fuel pellets provides the fastest ROI and is therefore considered the preferred option for valorisation of the SMS. Although the production of mushrooms and cellulase could provide higher economic returns in the long run, their investment costs and ROI are significantly higher. Practitioners could therefore hesitate to invest in these valorisation options. Additional options e.g., the extraction of proteins, fats or lignin as suggested in this thesis, potentially provide the option to optimise the utilisation of the SMS at faster ROI. However, future research will be necessary to determine this potential. Therefore, it is suggested to valorise the SMS through the production of fuel pellets for now due to its fast ROI and low environmental impacts.

The valorisation of the PMW through the options identified in this study i.e., extraction of dietary fibre or phenolic compounds, is not desirable due to the extremely high environmental impacts resulting from this valorisation. This suggests that the PMW should continue to be composted unless better options for its valorisation e.g., the extraction of proteins, chitin or beta-glucans as suggested in this thesis, are found to be profitable at lower environmental impacts. Nonetheless, the PMW makes up only 5% of the total mycelium waste streams and the valorisation of the SMS through the production of fuel pellets will therefore significantly improve the cost-efficiency of pure mycelium production.

The novel synthesis of mycelium waste stream characteristics as presented in this thesis will allow future research to determine the potential of additional pathways for valorisation of the mycelium waste streams. This novel data, along with the first estimations of the environmental impacts and economic viability of the identified valorisation pathways could be the initial inputs to the database for a decision-making tool. This tool is to provide the foresight that is required to select the appropriate options and production methods for valorisation of specific biomass waste streams through computation of the total created value and environmental. Future research will be necessary to build the database that is required for operationalisation of this tool.

This thesis has also shown that implementation of the possible options for valorisation of the SMS in practice might be challenging due to the required investments in production facilities or expertise. It is therefore recommended to research the potential for business ecosystem creation that provides the required infrastructure for the optimisation of mycelium waste stream utilisation. Such a business ecosystem could potentially provide the possibility to benefit from the valorisation of mycelium waste streams to a larger group of stakeholders within the biobased sector and through optimisation of the overall value creation.

In conclusion, this study has demonstrated that mycelium waste streams can be considered a valuable resource and that its current disposal through composting can be considered wasteful. The valorisation of the majority of the mycelium waste through the production of fuel pellets provides a profitable alternative for this current costly disposal. The production of fuel pellets could be interpreted as an optimisation of resource utilisation due to its fast ROI and low environmental impacts, despite that it does not allow a consecutive utilisation. Therefore, the valorisation of mycelium waste streams through the production of fuel pellets provides the possibility to improve the cost-efficiency of pure mycelium materials and to support operationalisation of the CBE. Future research will have to determine whether other options identified in this study i.e., extraction of proteins, fats, chitin, or beta-glucans could also provide profitable options for valorisation of the mycelium waste streams. Nonetheless, the findings of this study provide the first indication of the possibility to capture economic value from a waste stream that provides a growing burden to the mycelium materials industry. This will allow reductions in the cost price of pure mycelium, making it available to a larger group of consumers and allowing the release of its full potential through increased substitution of otherwise polluting materials and meat.

6 References

Abd Rasib, N. A., Zakaria, Z., Tompang, M. F., Abdul Rahman, R., & Othman, H. (2015). Characterization of biochemical composition for different types of spent mushroom substrate in Malaysia. Malaysian Journal of Analytical Sciences, 19(1), 41-45.

Aguiló-Aguayo, I., Walton, J., Viñas, I., & Tiwari, B. K. (2017). Ultrasound assisted extraction of polysaccharides from mushroom by-products. Lwt, 77, 92-99.

Akritidis, N. (2018). Business Plan for the Establishment of a Mushrooms Production Unit. SCHOOL OF ECONOMICS, BUSINESS ADMINISTRATION & LEGAL STUDIES. International Hellenic University.

Antunes, F., Marçal, S., Taofiq, O., MMB Morais, A., Freitas, A. C., CFR Ferreira, I., & Pintado, M. (2020). Valorisation of mushroom by-products as a source of value-added compounds and potential applications. Molecules, 25(11), 2672.

Appels, F. V., Camere, S., Montalti, M., Karana, E., Jansen, K. M., Dijksterhuis, J., ... & Wösten, H. A. (2019). Fabrication factors influencing mechanical, moisture-and water-related properties of mycelium-based composites. Materials & Design, 161, 64-71.

Arzoumanidis, I., D'Eusanio, M., Raggi, A., & Petti, L. (2020). Functional unit definition criteria in life cycle assessment and social life cycle assessment: a discussion. In Perspectives on social LCA (pp. 1-10). Springer, Cham.

Asada, C., Asakawa, A., Sasaki, C., & Nakamura, Y. (2011). Characterization of the steam-exploded spent Shiitake mushroom medium and its efficient conversion to ethanol. *Bioresource technology*, *102*(21), 10052-10056.

Atila, F. (2020). Comparative study on the mycelial growth and yield of Ganoderma lucidum (Curt.: Fr.) Karst. on different lignocellulosic wastes. Acta Ecologica Sinica, 40(2), 153-157.

Attias, N., Danai, O., Abitbol, T., Tarazi, E., Ezov, N., Pereman, I., & Grobman, Y. J. (2020). Mycelium bio-composites in industrial design and architecture: Comparative review and experimental analysis. Journal of Cleaner Production, 246, 119037.

Babaei, M., Tsapekos, P., Alvarado-Morales, M., Hosseini, M., Ebrahimi, S., Niaei, A., & Angelidaki, I. (2019). Valorisation of organic waste with simultaneous biogas upgrading for the production of succinic acid. Biochemical engineering journal, 147, 136-145.

Bagherian, H., Ashtiani, F. Z., Fouladitajar, A., & Mohtashamy, M. (2011). Comparisons between conventional, microwave-and ultrasound-assisted methods for extraction of pectin from grapefruit. Chemical engineering and processing: Process Intensification, 50(11-12), 1237-1243.

Bajwa, D. S., Pourhashem, G., Ullah, A. H., & Bajwa, S. G. (2019). A concise review of current lignin production, applications, products and their environmental impact. Industrial Crops and Products, 139, 111526.

Balicki, S., Pawlaczyk-Graja, I., Gancarz, R., Capek, P., & Wilk, K. A. (2020). Optimization of Ultrasound-Assisted Extraction of Functional Food Fibre from Canadian Horseweed (Erigeron canadensis L.). ACS omega, 5(33), 20854-20862.

Barjoveanu, G., Pătrăuțanu, O. A., Teodosiu, C., & Volf, I. (2020). Life cycle assessment of polyphenols extraction processes from waste biomass. Scientific Reports, 10(1), 1-12.

Bano, Zakia, S. Rajarathnam, and Keith H. Steinkraus. "Pleurotus mushrooms. Part II. Chemical composition, nutritional value, post-harvest physiology, preservation, and role as human food." Critical Reviews in Food Science & Nutrition 27.2 (1988): 87-158.

Banu, J. R., Kavitha, S., Kannah, R. Y., Kumar, M. D., Atabani, A. E., & Kumar, G. (2020). Biorefinery of spent coffee grounds waste: Viable option towards circular bioeconomy. Bioresource technology, 302, 122821.

Beckers, S. J., Dallo, I. A., Del Campo, I., Rosenauer, C., Klein, K., & Wurm, F. R. (2019). From Compost to Colloids—Valorisation of Spent Mushroom Substrate. ACS Sustainable Chemistry & Engineering, 7(7), 6991-6998.

Beisl, S., Miltner, A., & Friedl, A. (2017). Lignin from micro-to nanosize: Production methods. International Journal of Molecular Sciences, 18(6), 1244.

Bello, S., Rios, C., Feijoo, G., & Moreira, M. T. (2018). Comparative evaluation of lignocellulosic biorefinery options under a life-cycle assessment approach. Biofuels, Bioproducts and Biorefining, 12(6), 1047-1064.

Bentsen, N. S., Larsen, S., & Stupak, I. (2019). Sustainability governance of the Danish bioeconomy—the case of bioenergy and biomaterials from agriculture. Energy, Sustainability and Society, 9(1), 1-14.

Blomsma, F., & Brennan, G. (2017). The emergence of circular economy: a new framing around prolonging resource productivity. Journal of Industrial Ecology, 21(3), 603-614.

Bondancia, T. J., de Aguiar, J., Batista, G., Cruz, A. J., Marconcini, J. M., Mattoso, L. H. C., & Farinas, C. S. (2020). Production of nanocellulose using citric acid in a biorefinery concept: effect of the hydrolysis reaction time and techno-economic analysis. Industrial & Engineering Chemistry Research, 59(25), 11505-11516.

Bruscato, C., Malvessi, E., Brandalise, R. N., & Camassola, M. (2019). High performance of macrofungi in the production of mycelium-based biofoams using sawdust—sustainable technology for waste reduction. Journal of Cleaner Production, 234, 225-232.

Butu, A., Rodino, S., Miu, B., & Butu, M. (2020). Mycelium-based materials for the ecodesign of bioeconomy. Digest Journal of Nanomaterials and Biostructures, 15(4), 1129-1140.

Camere, S., & Karana, E. (2018). Fabricating materials from living organisms: An emerging design practice. *Journal of Cleaner Production*, *186*, 570-584.

Campbell-Johnston, K., Vermeulen, W. J., Reike, D., & Bullot, S. (2020). The circular economy and cascading: towards a framework. *Resources, Conservation & Recycling: X*, 100038.

Carrez, D., & Van Leeuwen, P. (2015). Bioeconomy: circular by nature. The European Files, 38, 34-35.

Carus, M., & Dammer, L. (2018). The circular bioeconomy—concepts, opportunities, and limitations. Industrial biotechnology, 14(2), 83-91.

Cerimi, K., Akkaya, K. C., Pohl, C., Schmidt, B., & Neubauer, P. (2019). Fungi as source for new bio-based materials: a patent review. Fungal biology and biotechnology, 6(1), 1-10.

Cheug, P.C.K. (1996). Dietary fibre content and composition of some cultivated edible mushroom fruiting bodies and mycelia. Journal of agricultural and food chemistry, 44(2), 468-471.

Chilanti, G., Todescatto, K., Andrade, L. B., Branco, C. S., Salvador, M., Camassola, M., … & Dillon, A. J. (2021). Polyphenolic Content and Antioxidant Activity of Mycelia and Basidiomes of Oyster Mushrooms Pleurotus spp. (Agaricomycetes) from Brazil. International Journal of Medicinal Mushrooms, 23(6).

Chukwuma, O. B., Rafatullah, M., Tajarudin, H. A., & Ismail, N. (2020). Lignocellulolytic enzymes in biotechnological and industrial processes: a review. Sustainability, 12(18), 7282.

Cobos, J. D. V., Viejó, F. G., Cobo, J. C., Sulca, R. L., Verdugo, D. N., Moncayo, M. F. G., & Endara, A. G. (2021). Chemical and productivity characterization of parental and hybrid strains of Lentinula edodes cultivated in different agricultural residues. Emirates Journal of Food and Agriculture, 260-265.

Cohen, N., Cohen, J., Asatiani, M. D., Varshney, V. K., Yu, H. T., Yang, Y. C., ... & Wasser, S. P. (2014). Chemical composition and nutritional and medicinal value of fruit bodies and submerged cultured mycelia of culinary- medicinal higher Basidiomycetes mushrooms. International journal of medicinal mushrooms, 16(3).

Cunha Zied, D., Sánchez, J. E., Noble, R., & Pardo-Giménez, A. (2020). Use of spent mushroom substrate in new mushroom crops to promote the transition towards a circular economy. Agronomy, 10(9), 1239.

Daioglou, V., Faaij, A. P., Saygin, D., Patel, M. K., Wicke, B., & van Vuuren, D. P. (2014). Energy demand and emissions of the non-energy sector. Energy & Environmental Science, 7(2), 482-498.

Del Borghi, A., Moreschi, L., & Gallo, M. (2020). Circular economy approach to reduce water–energy–food nexus. Current Opinion in Environmental Science & Health, 13, 23-28.

Demichelis, F., Fiore, S., Pleissner, D., & Venus, J. (2018). Technical and economic assessment of food waste valorisation through a biorefinery chain. Renewable and Sustainable Energy Reviews, 94, 38-48.

Di Mario, F., Rapana, P., Tomati, U., & Galli, E. (2008). Chitin and chitosan from Basidiomycetes. International Journal of Biological Macromolecules, 43(1), 8-12.
Dimou, C., Vlysidis, A., Kopsahelis, N., Papanikolaou, S., Koutinas, A. A., & Kookos, I. K. (2016). Techno-economic evaluation of wine lees refining for the production of value-added products. Biochemical engineering journal, 116, 157-165.
Donner, M., Verniquet, A., Broeze, J., Kayser, K., & De Vries, H. (2021). Critical success and risk factors for circular business models valorising agricultural waste and by-products. Resources, Conservation and Recycling, 165, 105236.
Dzah, C. S., Duan, Y., Zhang, H., Wen, C., Zhang, J., Chen, G., & Ma, H. (2020). The effects of ultrasound assisted extraction on yield, antioxidant, anticancer and antimicrobial activity of polyphenol extracts: A review. Food Bioscience, 35, 100547.
Economou, C. N., Diamantopoulou, P. A., & Philippoussis, A. N. (2017). Valorisation of spent oyster mushroom substrate and laccase recovery through successive solid-state cultivation of Pleurotus, Ganoderma, and Lentinula strains. Applied microbiology and biotechnology, 101(12), 5213-5222.
Ecovative. (2021). Food – the ingredient for structure. Retrieved November 18, 2021, from https://ecovativedesign.com/food.
Ellilä, S., Fonseca, L., Uchima, C., Cota, J., Goldman, G. H., Saloheimo, M., ... & Siika-Aho, M. (2017). Development of a low-cost cellulase production process using Trichoderma reesei for Brazilian biorefineries. Biotechnology for biofuels, 10(1), 1-17.
Elsacker, E., Vandelook, S., Brancart, J., Peeters, E., & De Laet, L. (2019). Mechanical, physical and chemical characterisation of mycelium-based composites with different types of lignocellulosic substrates. PLoS One, 14(7), e0213954.
Elsacker, E., Vandelook, S., Van Wylick, A., Ruytinx, J., De Laet, L., & Peeters, E. (2020). A comprehensive framework for the production of mycelium-based lignocellulosic composites. Science of The Total Environment, 725, 138431.
El Knidri, H., Belaabed, R., Addaou, A., Laajeb, A., & Lahsini, A. (2018). Extraction, chemical modification and characterization of chitin and chitosan. International journal of biological macromolecules, 120, 1181-1189.
Estrada, A. E. R., del Mar Jimenez-Gasco, M., & Royse, D. J. (2009). Improvement of yield of Pleurotus eryngii var. eryngii by substrate supplementation and use of a casing overlay. Bioresource Technology, 100(21), 5270-5276.
Europese Centrale Bank (ECB). Referentiewisselkoersen van de euro. Retrieved April 25th 2022 from https://www.ecb.europa.eu/stats/policy_and_exchange_rates/euro_reference_exchange_rates/html/eurofxref-graph-usd.nl.html.
European Commission 2015: Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions. Closing the loop – An EU action plan for the Circular Economy. Brussels, 02.12.2015.
Ewijk van, S., & Stegemann, J. A. (2016). Limitations of the waste hierarchy for achieving absolute reductions in material throughput. *Journal of Cleaner Production*, *132*, 122-128.
Feldman, A. (2018). Bolt Threads Debuts New 'Leather' Made From Mushroom Roots. Forbes. Retrieved on June 30, 2021 from: https://www.forbes.com/sites/amyfeldman/2018/04/16/synthetic-spider-silk-maker-bolt-threads-debuts-new-bio-material-leather-made-from-mushroom roots/?sh=48e619a71837.
Foroughi, F., Rezvani Ghomi, E., Morshedi Dehaghi, F., Borayek, R., & Ramakrishna, S. (2021). A review on the life cycle assessment of cellulose: From properties to the potential of making it a low carbon material. Materials, 14(4), 714.
Fuller, S., Beck, E., Salman, H., & Tapsell, L. (2016). New horizons for the study of dietary fibre and health: a review. Plant foods for human nutrition, 71(1), 1-12.
Gaitán-Hernández, R., Esqueda, M., Gutiérrez, A., Sánchez, A., Beltrán-García, M., & Mata, G. (2006). Bioconversion of agrowastes by Lentinula edodes: the high potential of viticulture residues. Applied microbiology and biotechnology, 71(4), 432-439.
Gallo Stampino, P., Riva, L., Punta, C., Elegir, G., Bussini, D., & Dotelli, G. (2021). Comparative Life Cycle Assessment of Cellulose Nanofibres Production Routes from Virgin and Recycled Raw Materials. Molecules, 26(9), 2558.

Gama, N. V., Ferreira, A., & Barros-Timmons, A. (2018). Polyurethane foams: Past, present, and future. Materials, 11(10), 1841.
Garcia-Rubio, R., de Oliveira, H. C., Rivera, J., & Trevijano-Contador, N. (2020). The fungal cell wall: Candida, Cryptococcus, and Aspergillus species. Frontiers in microbiology, 10, 2993.
Geissdoerfer, M., Savaget, P., Bocken, N. M., & Hultink, E. J. (2017). The Circular Economy–A new sustainability paradigm?. Journal of cleaner production, 143, 757-768.
Geldermann, J., Kolbe, L. M., Krause, A., Mai, C., Militz, H., Osburg, V. S., ... & Westphal, S. (2016). Improved resource efficiency and cascading utilisation of renewable materials.
Ghada, M. M. (2011). Optimization of submerged culture conditions for mycelial biomass production by shiitake mushroom (Lentinus edodes). Res. J. Agric. Biol. Sci, 7(4), 350-356.
Ghisellini, P., Cialani, C., & Ulgiati, S. (2016). A review on circular economy: the expected transition to a balanced interplay of environmental and economic systems. Journal of Cleaner production, 114, 11-32.
Ghitescu, R. E., Volf, I., Carausu, C., Bühlmann, A. M., Gilca, I. A., & Popa, V. I. (2015). Optimization of ultrasound-assisted extraction of polyphenols from spruce wood bark. Ultrasonics sonochemistry, 22, 535-541.
Gilpin, G. S., & Andrae, A. S. (2017). Comparative attributional life cycle assessment of European cellulase enzyme production for use in second-generation lignocellulosic bioethanol production. The International Journal of Life Cycle Assessment, 22(7), 1034-1053.
Girometta, C., Picco, A. M., Baiguera, R. M., Dondi, D., Babbini, S., Cartabia, M., ... & Savino, E. (2019). Physico-mechanical and thermodynamic properties of mycelium-based biocomposites: a review. Sustainability, 11(1), 281.
Gonzalez-Garcia, S., Gullón, B., & Moreira, M. T. (2018). Environmental assessment of biorefinery processes for the valorisation of lignocellulosic wastes into oligosaccharides. Journal of Cleaner Production, 172, 4066-4073.
Grown.bio. (2021). Packaging & interior design products - Naturally grown. Retrieved on September 7, 2021 from https://www.grown.bio/.
Gu, H., Reiner, R., Bergman, R., & Rudie, A. (2015). LCA study for pilot scale production of cellulose nano crystals (CNC) from wood pulp. In Proceedings from the LCA XV Conference (pp. 33-42).
Gurung, O. K., Budathoki, U., & Parajuli, G. (2012). Effect of different substrates on the production of Ganoderma lucidum (Curt.: Fr.) Karst. Our nature, 10(1), 191-198.
Hahn, J. (2020). Major fashion houses will sell products made from mushroom leather by next year. De Zeen. Retrieved on November 18 2021 from https://www.dezeen.com/2020/10/08/mylo-consortium-adidas-stella-mccartney-lululemon-kering-mycelium/.
Haroni, S., Dizaji, H. Z., Bahrami, H., & Alriols, M. G. (2021). Sustainable production of cellulose nanofibre from sugarcane trash: A quality and life cycle assessment. Industrial Crops and Products, 173, 114084.
Heleno, S. A., Barros, L., Martins, A., Queiroz, M. J. R., Santos-Buelga, C., & Ferreira, I. C. (2012). Fruiting body, spores and in vitro produced mycelium of Ganoderma lucidum from Northeast Portugal: A comparative study of the antioxidant potential of phenolic and polysaccharidic extracts. Food Research International, 46(1), 135-140.
Hong, Y., Nizami, A. S., Pour Bafrani, M., Saville, B. A., & MacLean, H. L. (2013). Impact of cellulase production on environmental and financial metrics for lignocellulosic ethanol. Biofuels, Bioproducts and Biorefining, 7(3), 303-313.
Huang, W., Kim, J. S., & Chung, H. Y. (2011). Antioxidant activity and total phenolic content in shiitake mycelial exudates. Natural product communications, 6(6), 1934578X1100600622.
Huggins, T., & Whiteley, J. (2019). U.S. Patent Application No. 16/435,261.
Isikgor, F. H., & Becer, C. R. (2015). Lignocellulosic biomass: a sustainable platform for the production of bio-based chemicals and polymers. Polymer Chemistry, 6(25), 4497-4559.
Jaramillo, P. M., Gomes, H. A., Monclaro, A. V., Silva, C. O., & Edivaldo Filho, X. F. (2015). Lignocellulose-degrading enzymes: An overview of the global market. Fungal Biomolecules: Sources, Applications and Recent Developments, 6.

Jiang, L., Walczyk, D., McIntyre, G., & Chan, W. K. (2016). Cost modeling and optimization of a manufacturing system for mycelium-based biocomposite parts. Journal of Manufacturing Systems, 41, 8-20.

Jones, M., Bhat, T., Huynh, T., Kandare, E., Yuen, R., Wang, C. H., & John, S. (2018). Waste-derived low-cost mycelium composite construction materials with improved fire safety. Fire and materials, 42(7), 816-825.

Jones, M., Mautner, A., Luenco, S., Bismarck, A., & John, S. (2020). Engineered mycelium composite construction materials from fungal biorefineries: A critical review. Materials & Design, 187, 108397.

Jones, M., Gandia, A., John, S., & Bismarck, A. (2021). Leather-like material biofabrication using fungi. Nature Sustainability, 4(1), 9-16.

Karana, E., Blauwhoff, D., Hultink, E. -J., & Camere, S. (2018). When the material grows: A case study on designing (with) mycelium-based materials. International Journal of Design, 12(2), 119-13.

Kardung, M., Cingiz, K., Costenoble, O., Delahaye, R., Heijman, W., Lovrić, M., ... & Zhu, B. X. (2021). Development of the circular bioeconomy: Drivers and indicators. Sustainability, 13(1), 413.

Konietzko, J., Bocken, N., & Hultink, E. J. (2020). A tool to analyze, ideate and develop circular innovation ecosystems. Sustainability, 12(1), 417.

Kourmentza, C., Economou, C. N., Tsafrakidou, P., & Kornaros, M. (2018). Spent coffee grounds make much more than waste: Exploring recent advances and future exploitation strategies for the valorisation of an emerging food waste stream. Journal of Cleaner Production, 172, 980-992.

Kumar, K., Srivastav, S., & Sharanagat, V. S. (2021). Ultrasound assisted extraction (UAE) of bioactive compounds from fruit and vegetable processing by-products: A review. Ultrasonics Sonochemistry, 70, 105325.

Laforteza, J., Reyes, R., & Trinidad, T. (2020). Total, Insoluble, Soluble Dietary Fibre Contents and its Fermentability In Vitro of Pleurotus ostreatus cv. Florida Mycelia (Agaricomycetes).

Lam, C. M., Iris, K. M., Hsu, S. C., & Tsang, D. C. (2018). Life-cycle assessment on food waste valorisation to value-added products. Journal of Cleaner Production, 199, 840-848.

Laso, J., Margallo, M., García-Herrero, I., Fullana, P., Bala, A., Gazulla, C., ... & Aldaco, R. (2018). Combined application of Life Cycle Assessment and linear programming to evaluate food waste-to-food strategies: Seeking for answers in the nexus approach. Waste Management, 80, 186-197.

Ledesma, B., & Beltramone, A. (2021). Revalorisation of agro-industrial waste as a catalyst source for production of biofuels. Renewable Energy, 174, 747-757.

Lee, K. H., Oh, J. I., Chu, K. H., Kwon, S. H., & Yoo, S. S. (2017). Comparison and evaluation of large-scale and on-site recycling systems for food waste via life cycle cost analysis. Sustainability, 9(12), 2186.

Leite, P., Sousa, D., Fernandes, H., Ferreira, M., Costa, A. R., Filipe, D., ... & Salgado, J. M. (2021). Recent advances in production of lignocellulolytic enzymes by solid-state fermentation of agro-industrial wastes. Current Opinion in Green and Sustainable Chemistry, 27, 100407.

Leong, Y. K., Ma, T. W., Chang, J. S., & Yang, F. C. (2022). Recent advances and future directions on the valorisation of spent mushroom substrate (SMS): A review. Bioresource technology, 344, 126157.

Li, Q., McGinnis, S., Sydnor, C., Wong, A., & Renneckar, S. (2013). Nanocellulose life cycle assessment. ACS Sustainable Chemistry & Engineering, 1(8), 919-928.

Li, T., Zhang, H., Liu, Z., Ke, Q., & Alting, L. (2014). A system boundary identification method for life cycle assessment. The International Journal of Life Cycle Assessment, 19(3), 646-660.

Liao, J. J., Abd Latif, N. H., Trache, D., Brosse, N., & Hussin, M. H. (2020). Current advancement on the isolation, characterization and application of lignin. International journal of biological macromolecules, 162, 985-1024.

Lim, S. H., Lee, Y. H., & Kang, H. W. (2013). Efficient recovery of lignocellulolytic enzymes of spent mushroom compost from oyster mushrooms, Pleurotus spp., and potential use in dye decolorization. Mycobiology, 41(4), 214-220.

Liu, G., Zhang, J., & Bao, J. (2016). Cost evaluation of cellulase enzyme for industrial-scale cellulosic ethanol production based on rigorous Aspen Plus modeling. Bioprocess and biosystems engineering, 39(1), 133-140.
Lou, Z., Sun, Y., Zhou, X., Baig, S. A., Hu, B., & Xu, X. (2017). Composition variability of spent mushroom substrates during continuous cultivation, composting process and their effects on mineral nitrogen transformation in soil. Geoderma, 307, 30-37.
Ma, T., Hu, X., Lu, S., Liao, X., Song, Y., & Hu, X. (2022). Nanocellulose: a promising green treasure from food wastes to available food materials. Critical Reviews in Food Science and Nutrition, 62(4), 989-1002.
Mahari, W. A. W., Peng, W., Nam, W. L., Yang, H., Lee, X. Y., Lee, Y. K., ... & Lam, S. S. (2020). A review on valorisation of oyster mushroom and waste generated in the mushroom cultivation industry. Journal of hazardous materials, 400, 123156.
Maina, S., Kachrimanidou, V., & Koutinas, A. (2017). A roadmap towards a circular and sustainable bioeconomy through waste valorisation. Current Opinion in Green and Sustainable Chemistry, 8, 18-23.
Manan, S., Ullah, M. W., Ul-Islam, M., Atta, O. M., & Yang, G. (2021). Synthesis and applications of fungal mycelium-based advanced functional materials. Journal of Bioresources and Bioproducts.
Maphosa, Y., & Jideani, V. A. (2016). Dietary fibre extraction for human nutrition—A review. Food Reviews International, 32(1), 98-115.)
McCartney, S. (2018). "Recycled nylon and polyester", available at: www.stellamccartney.com/ experience/en/sustainability/materials-and-innovation/recycled-nylon-polyester/ (Accessed 18 November 2021).
McCormick, K., & Kautto, N. (2013). The bioeconomy in Europe: An overview. *Sustainability*, *5*(6), 2589-2608.
Michelin, M., Gomes, D. G., Romaní, A., Polizeli, M. D. L., & Teixeira, J. A. (2020). Nanocellulose production: exploring the enzymatic route and residues of pulp and paper industry. Molecules, 25(15), 3411.
MME. (2021). Mycelium Materials Europe – personal communication with owners of MME.
Moktadir, M. A., Rahman, T., Rahman, M. H., Ali, S. M., & Paul, S. K. (2018). Drivers to sustainable manufacturing practices and circular economy: A perspective of leather industries in Bangladesh. Journal of Cleaner Production, 174, 1366-1380.
Montenegro-Landívar, M. F., Tapia-Quirós, P., Vecino, X., Reig, M., Valderrama, C., Granados, M., ... & Saurina, J. (2021). Polyphenols and their potential role to fight viral diseases: An overview. Science of the Total Environment, 801, 149719.
Muscat, A., de Olde, E. M., Ripoll-Bosch, R., Van Zanten, H. H., Metze, T. A., Termeer, C. J., ... & de Boer, I. J. (2021). Principles, drivers and opportunities of a circular bioeconomy. Nature Food, 2(8), 561-566.
Nechyporchuk, O., Belgacem, M. N., & Bras, J. (2016). Production of cellulose nanofibrils: A review of recent advances. Industrial Crops and Products, 93, 2-25.
Newcomb, T. (2021). How Adidas Is Using Mushrooms To Create A Line Of Stan Smith Mylo Sneakers. Retrieved November 21 2021 from https://www.forbes.com/sites/timnewcomb/2021/04/22/creating-adidas-mushroom-based-stan-smith-mylo-sneakers/?sh=58c8d6f37c0d.
Nitschke, J., Modick, H., Busch, E., Von Rekowski, R. W., Altenbach, H. J., & Mölleken, H. (2011). A new colorimetric method to quantify β-1, 3-1, 6-glucans in comparison with total β-1, 3-glucans in edible mushrooms. Food chemistry, 127(2), 791-796.
Noonsong, V., Puttakun, N., Tinsirisuk, M., & Seephueak, P. (2016). Recycling of spent Pleurotus compost for production of the Agrocybe cylindracea. Mycosphere, 7(1), 36-4
O'Brien, M. A., Carlton, A., & Mueller, P. (2020). U.S. Patent Application No. 16/773,272.
Ongpeng, M. C., Inciong, E., Sendo, V., Soliman, C., & Siggaoat, A. (2020). Using Waste in Producing Bio-Composite Mycelium Bricks. Applied Sciences, 10(15), 5303.
Owaid, M. N., Abed, I. A., & Al-Saeedi, S. S. S. (2017). Applicable properties of the bio-fertilizer spent mushroom substrate in organic systems as a byproduct from the cultivation of Pleurotus spp. Information Processing in Agriculture, 4(1), 78-82.

Özçelik, E., & Pekşen, A. (2007). Hazelnut husk as a substrate for the cultivation of shiitake mushroom (Lentinula edodes). Bioresource technology, 98(14), 2652-2658.

Parascanu, M. M., Sánchez, P., Soreanu, G., Valverde, J. L., & Sanchez-Silva, L. (2019). Mexican biomasses valorisation through pyrolysis process: environmental and costs analysis. Waste Management, 95, 171-181.

Pardo-Giménez, A., Picornell Buendia, M. R., de Juan Valero, J. A., Pardo-González, J. E., & Cunha Zied, D. (2010, August). Cultivation of Pleurotus ostreatus using supplemented spent oyster mushroom substrate. In XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010): International Symposium on 933 (pp. 267-272).

Payne, C. M., Knott, B. C., Mayes, H. B., Hansson, H., Himmel, M. E., Sandgren, M., ... & Beckham, G. T. (2015). Fungal cellulases. Chemical reviews, 115(3), 1308-1448.

Peksen, A., Yakupoglu, G., Yakupoglu, T., Gulser, C., Ozturk, E., & Ozdemir, N. (2011). Changes in chemical compositions of substrates before and after Ganoderma lucidum cultivation. World Journal of Microbiology and Biotechnology, 27(3), 637-642.

Pelletier, M. G., Holt, G. A., Wanjura, J. D., Bayer, E., & McIntyre, G. (2013). An evaluation study of mycelium based acoustic absorbers grown on agricultural by-product substrates. Industrial Crops and Products, 51, 480-485.

Philippoussis, A. N., Diamantopoulou, P. A., & Zervakis, G. I. (2003). Correlation of the properties of several lignocellulosic substrates to the crop performance of the shiitake mushroom Lentinula edodes. World Journal of Microbiology and Biotechnology, 19(6), 551-557.

Picornell Buendía, M. R., Pardo-Giménez, A., & de Juan-Valero, J. A. (2016). Reuse of degraded Pleurotus ostreatus (Jacq.) P. Kumm. substrate by supplementation with wheat bran. Quantitative parameters. Mycology, 7(2), 53-63.

Pradhan, P., Mahajani, S. M., & Arora, A. (2018). Production and utilization of fuel pellets from biomass: A review. Fuel Processing Technology, 181, 215-232.

Prado, J. M., Veggi, P. C., & Meireles, M. A. A. (2017). Scale-up issues and cost of manufacturing bioactive compounds by supercritical fluid extraction and ultrasound assisted extraction. In Global food security and wellness (pp. 377-433). Springer, New York, NY.

Priyadarshini, A., Tiwari, B. K., & Rajauria, G. (2022). Assessing the Environmental and Economic Sustainability of Functional Food Ingredient Production Process. Processes, 10(3), 445.

Proskurina, S., Alakangas, E., Heinimö, J., Mikkilä, M., & Vakkilainen, E. (2017). A survey analysis of the wood pellet industry in Finland: Future perspectives. Energy, 118, 692-704.

Quijano, L., Speight, R., & Payne, A. (2021). Future fashion, biotechnology and the living world: microbial cell factories and forming new 'oddkins'. Continuum, 1-17.

Rajavat, A. S., Rai, S., Pandiyan, K., Kushwaha, P., Choudhary, P., Kumar, M., ... & Saxena, A. K. (2020). Sustainable use of the spent mushroom substrate of Pleurotus florida for production of lignocellulolytic enzymes. Journal of basic microbiology, 60(2), 173-184.

Ramesh, D., Muniraj, I. K., Thangavelu, K., & Karthikeyan, S. (2019). Knowledge update on bioreactor technology for cellulase production. In New and future developments in microbial biotechnology and bioengineering (pp. 181-193). Elsevier.

Roberts-Islam, B. (2021). Mushroom-Based 'Leather' Is Now A Scalable Alternative To Animal Leathers, Poised For Market Disruption. Forbes. Retrieved on September 7, 2021 from https://www.forbes.com/sites/brookerobertsislam/2021/04/01/mushroom-leather-is-now-a--scalable-alternative-to-animal-leathers-poised-for-market-disruption/?sh=60f1139a5b19.

Roshitsh, K. (2021). New York-Based Mycelium Innovation Firm Ecovative Nabs $60M in Funding. WWD. Retrieved on June 30, 2021 from https://wwd.com/sustainability/materials/new-york-based-mycelium-innovation-firm-ecovative-nabs-60m-in-funding-1234789733/.

Ross, P. (2016). U.S. Patent No. 9,410,116. Washington, DC: U.S. Patent and Trademark Office.

Sadhu, S., & Maiti, T. K. (2013). Cellulase production by bacteria: a review. British microbiology research journal, 3(3), 235-258.

Salmas, G., DeVries, J. W., & Plank, D. (2017). Challenges for dietary fibre: Benefits and costs of new US regulations. Cereal Foods World, 62(3), 88-94.

Schipfer, F., Kranzl, L., Leclère, D., Sylvain, L., Forsell, N., & Valin, H. (2017). Advanced biomaterials options for the EU28 up to 2050 and their respective biomass demand. Biomass and Bioenergy, 96, 19-27.

Sharma, A., Thakur, M., Bhattacharya, M., Mandal, T., & Goswami, S. (2019). Commercial application of cellulose nano-composites–A review. Biotechnology Reports, 21, e00316.

Siacor, F. D. C., Lim, K. J. A., Cabajar, A. A., Lobarbio, C. F. Y., Lacks, D. J., & Taboada, E. B. (2020). Physicochemical properties of spray-dried mango phenolic compounds extracts. Journal of Agriculture and Food Research, 2, 100048.

da Silva Alves, L., de Almeida Moreira, B. R., da Silva Viana, R., Pardo-Gimenez, A., Dias, E. S., Noble, R., & Zied, D. C. (2021). Recycling spent mushroom substrate into fuel pellets for low-emission bioenergy producing systems. Journal of Cleaner Production, 313, 127875.

Sim, K. Y., Liew, J. Y., Ding, X. Y., CHOONG, W. S., & Intan, S. (2017). Effect of vacuum and oven drying on the radical scavenging activity and nutritional contents of submerged fermented Maitake (Grifola frondosa) mycelia. Food Science and Technology, 37, 131-135.

Siqueira, J. G. W., Rodrigues, C., de Souza Vandenberghe, L. P., Woiciechowski, A. L., & Soccol, C. R. (2020). Current advances in on-site cellulase production and application on lignocellulosic biomass conversion to biofuels: a review. Biomass and Bioenergy, 132, 105419.

Sorvino, C. (2021). Maker Of Mushroom-Sourced Bacon Raises $40 Million To Reach Grocers At Scale. Forbes. Retrieved on September 7, 2021 from https://www.forbes.com/sites/chloesorvino/2021/04/15/maker-of-mushroom-sourced-bacon-raises-40- million-to-reach-grocers-at-scale/?sh=c3f1a272d1fc.

Stegmann, P., Londo, M., & Junginger, M. (2020). The circular bioeconomy: Its elements and role in European bioeconomy clusters. Resources, Conservation & Recycling: X, 6, 100029.

Stone, J., Garcia-Garcia, G., & Rahimifard, S. (2019). Development of a pragmatic framework to help food and drink manufacturers select the most sustainable food waste valorisation strategy. Journal of environmental management, 247, 425-438.

Tacer-Caba, Z., Varis, J. J., Lankinen, P., & Mikkonen, K. S. (2020). Comparison of novel fungal mycelia strains and sustainable growth substrates to produce humidity-resistant biocomposites. Materials & Design, 192, 108728.

Tejada-Ortigoza, V., Garcia-Amezquita, L. E., Serna-Saldívar, S. O., & Welti-Chanes, J. (2016). Advances in the functional characterization and extraction processes of dietary fibre. Food Engineering Reviews, 8(3), 251-271.

Thakur, R., & Sharma, B. M. (2015). Deployment of indigenous wild Ganoderma lucidum for better yield on different substrates. African Journal of Agricultural Research, 10(33), 3338-3341.

Thrän, D., Schaubach, K., Peetz, D., Junginger, M., Mai-Moulin, T., Schipfer, F., ... & Lamers, P. (2019). The dynamics of the global wood pellet markets and trade–key regions, developments and impact factors. Biofuels, Bioproducts and Biorefining, 13(2), 267-280.

Tian, D., Hu, J., Bao, J., Chandra, R. P., Saddler, J. N., & Lu, C. (2017). Lignin valorisation: Lignin nanoparticles as high-value bio-additive for multifunctional nanocomposites. Biotechnology for biofuels, 10(1), 1-11.

Thiribhuvanamala, G., & Krishnamoorthy, A. S. (2021). Evaluation of different lignocellulosic substrates for cultivation of medicinal mushroom Ganoderma lucidum. Journal of Environmental Biology, 42(5), 1314-1319.

Toushik, S. H., Lee, K. T., Lee, J. S., & Kim, K. S. (2017). Functional applications of lignocellulolytic enzymes in the fruit and vegetable processing industries. Journal of food science, 82(3), 585-593.

Turk, J., Oven, P., Poljanšek, I., Lešek, A., Knez, F., & Rebec, K. M. (2020). Evaluation of an environmental profile comparison for nanocellulose production and supply chain by applying different life cycle assessment methods. Journal of Cleaner Production, 247, 119107.

Ubando, A. T., Felix, C. B., & Chen, W. H. (2020). Biorefineries in circular bioeconomy: A comprehensive review. Bioresource technology, 299, 122585.

Uyttebroek, M., Vandezande, P., Van Dael, M., Vloemans, S., Noten, B., Bongers, B., ... & Lemmens, B. (2018). Concentration of phenolic compounds from apple pomace extracts by nanofiltration at lab and pilot scale with a techno-economic assessment. Journal of Food Process Engineering, 41(1), e12629.

Vandelook, S., Elsacker, E., Van Wylick, A., De Laet, L., & Peeters, E. (2021). Current state and future prospects of pure mycelium materials. Fungal biology and biotechnology, 8(1), 1-10.
Vieira, G. S., Cavalcanti, R. N., Meireles, M. A. A., & Hubinger, M. D. (2013). Chemical and economic evaluation of natural antioxidant extracts obtained by ultrasound-assisted and agitated bed extraction from jussara pulp (Euterpe edulis). Journal of Food Engineering, 119(2), 196-204.
Vu, H. P., Nguyen, L. N., Vu, M. T., Johir, M. A. H., McLaughlan, R., & Nghiem, L. D. (2020). A comprehensive review on the framework to valorise lignocellulosic biomass as biorefinery feedstocks. Science of The Total Environment, 743, 140630.
Wang, Y., Wang, J., Zhang, X., & Grushecky, S. (2020). Environmental and economic assessments and uncertainties of multiple lignocellulosic biomass utilization for bioenergy products: case studies. Energies, 13(23), 6277.
Wenger, J., Stern. T., Schöggl, J.P., van Ree, R., De Corato, U., De Bari, I., Bell, G., Stichnothe, H. (2018). Natural fibres and fibre-based materials in biorefineries. IEA Bioenergy.
Wu, X. J., & Hansen, C. (2008). Antioxidant capacity, phenolic content, and polysaccharide content of Lentinus edodes grown in whey permeate-based submerged culture. Journal of food science, 73(1), M1-M8.
Wu, C. Y., Liang, C. H., & Liang, Z. C. (2020). Evaluation of Using Spent Mushroom Sawdust Wastes for Cultivation of Auricularia polytricha. Agronomy, 10(12), 1892.
Wyman, V., Henríquez, J., Palma, C., & Carvajal, A. (2018). Lignocellulosic waste valorisation strategy through enzyme and biogas production. Bioresource technology, 247, 402-411.
Xiao, Z., Lin, M., Fan, J., Chen, Y., Zhao, C., & Liu, B. (2018). Anaerobic digestion of spent mushroom substrate under thermophilic conditions: performance and microbial community analysis. Applied microbiology and biotechnology, 102(1), 499-507.
Xiong, S., Martín, C., Eilertsen, L., Wei, M., Myronycheva, O., Larsson, S. H., ... & Jönsson, L. J. (2019). Energy-efficient substrate pasteurisation for combined production of shiitake mushroom (Lentinula edodes) and bioethanol. Bioresource technology, 274, 65-72.
Yang, Y. Y., Ma, S., Wang, X. X., & Zheng, X. L. (2017). Modification and application of dietary fibre in foods. Journal of Chemistry, 2017.)
Yang, Z., Zhang, F., Still, B., White, M., & Amstislavski, P. (2017). Physical and mechanical properties of fungal mycelium-based biofoam. Journal of Materials in Civil Engineering, 29(7), 04017030.
Yarns and Fibers News Bureau. (2022). Bolt Threads partners with Mycelium Materials Europe to commercialize Mylo. Retrieved May 3rd 2022 from https://www.yarnsandfibers.com/news/textile-news/bolt-threads-partners-with-mycelium-materials-europe-to-commercialize-mylo/.
Zhou, S., Raouche, S., Grisel, S., Sigoillot, J. C., & Gimbert, I. (2017). Efficient biomass pretreatment using the White-rot Fungus Polyporus Brumalis. *Fungal Genomics & Biology*, *7*(1), 1-6.
Ziegler, A. R., Bajwa, S. G., Holt, G. A., McIntyre, G., & Bajwa, D. S. (2016). Evaluation of physico-mechanical properties of mycelium reinforced green biocomposites made from cellulosic fibres. Applied engineering in agriculture, 32(6), 931-938.

Appendix A - Mycelium materials: applications and production

This appendix provides a basic understanding about mycelium materials, their production and applications. Mycelium biofabrication is used for the production of two types of mycelium materials: mycelium composites and pure mycelium (Girometta et al., 2019; Jones et al., 2021).

Applications of mycelium materials

Mycelium composites and pure mycelium have different product applications which relate to their technical characteristics. Mycelium composites are the denser material of the two and its mechanical characteristics, fire resistance and acoustic absorption properties facilitate its application as packaging and construction materials, thermal and acoustic insulating panels, and various design objects e.g., flowerpots, lampshades, and furniture (Fig. A1; Girometta et al., 2019). Making use of mycelium in these applications, provides the opportunity to substitute many non-renewable and polluting materials including Styrofoam for packaging applications (Bruscato et al., 2019); polyurethane, rock- and glass wool for thermal and acoustic insulation panels (Jones et al., 2018; Pelletier et al., 2013); wood and bricks for construction purposes (Ongpeng et al., 2020; Ziegler et al., 2016); and plastics and metals in design applications (Girometta et al., 2019).

Figure A1. Examples of mycelium composites (top right; Girometta et al., 2019) in different applications; a mycelium insulation panel (left; grown.bio, 2021) and mycelium packaging (bottom right; grown.bio, 2021).

Pure mycelium is used for the production of other mycelium materials including mycelium leather and mycelium meat alternatives (Fig. A2 & A3; Jones et al., 2021; Huggins & Whitely, 2019). Additional post-processing of pure mycelium results in a material with leather-like properties which can be used for the production of apparel, upholstery and footwear (Quijano, Speight & Payne, 2021; McCartney, 2018). This provides mycelium the opportunity to substitute conventional leather which is associated with unsustainable livestock farming and environmental pollution (Moktadir et al., 2018). It is also considered a far better alternative than synthetic leathers in terms of sustainability (Gama et al., 2018).

Figure A2. Examples of mycelium leather products. Top left: A collaboration between Adidas and mycelium leather producer Bolt Threads has resulted in the first commercially available mycelium leather shoes (Newcomb, 2021). Bottom left: A sheet of mycelium leather produced by MycoWorks that can be used in any application similar to traditional leather (Roberts-Islam, 2021). Right: Mycelium apparel designed by Stella McCartney (Feldman, 2018).

Additionally, pure mycelium provides the possibility to produce whole cut meat alternatives such as bacon and steaks, which has so far not been succeeded by other meat substitutes (Ecovative, 2021). These whole cuts are simply produced through addition of flavour to sheets of pure mycelium that have been cut in the desired shape and size (Sorvino, 2021). The substitution of real meat by mycelium tackles the environmental problems relating to animal farming in a similar way as the substitution of leather (Moktadir, et al., 2018).

Figure A3. Examples of mycelium meat alternatives. Whole slabs of pure mycelium (bottom right) are easily processed into bacon (left) by cutting in the desired dimensions and addition of specific spices. The texture very much resembles that of meat, which is a result of how mycelium grows (upper right) (Sorvino, 2021). Images taken from Ecovative (left), and Atlast (right).

The production of mycelium materials

Both the production of mycelium composites as well as pure mycelium relies on mycelium biofabrication. The studies of Elsacker et al. (2020) & Karana et al., (2018) describe this biofabrication process (Fig. A4). The process starts by inoculating a sterilized substrate consisting of organic material with fungal spores. The substrate needs to be sterilized to prevent unwanted fungal or bacterial growth, which is often done through steaming or boiling (Jiang et al., 2016). Water and nutrients are added to the mixture to stimulate mycelial growth. The mycelium substrate mixture is then packed in containers, which can have any desired shape, and left to incubate in production rooms for ten to twelve days. Temperature, moisture and carbon dioxide levels are controlled while maintaining complete darkness to stimulate mycelial growth and prevent mushroom formation. During the incubation period, the mycelium colonizes the substrate through the formation of mycelial cells known as hyphae. From these hyphae, the mycelium excretes enzymes that break down lignin and cellulose into nutrients that are easily absorbed (Butu et al., 2020). These hyphae form a strong interconnected network through fusion, known as anastomosis, that cements and encapsulates the remaining substrate particles into mycelium composites (Fig A5). Alternatively, pure mycelium can be produced in a similar process with open containers resulting in the formation of a dense sheet of pure mycelium that can be harvested directly from the substrate. Finally, the mycelium composites as well as pure mycelium require oven drying to inhibit mycelial growth and stabilize the materials before further processing.

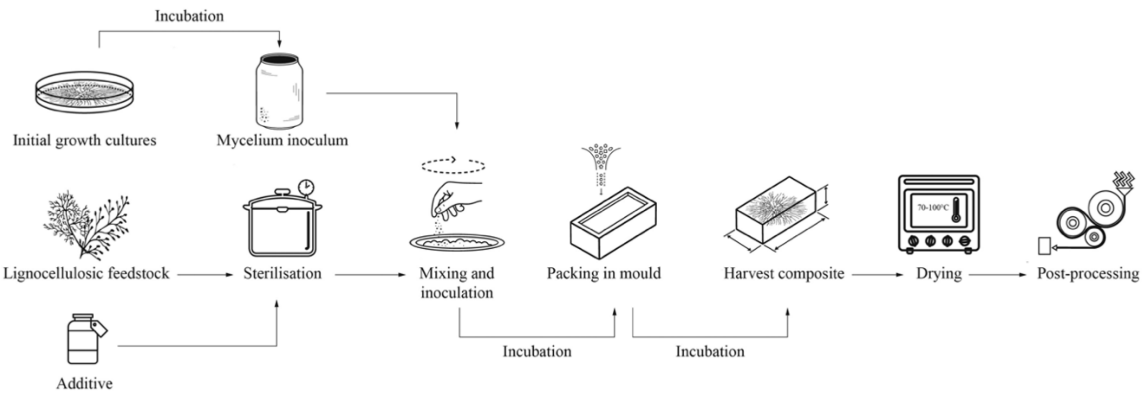

Figure A4. Schematic representation of the mycelium materials production process (Elsacker et al., 2020).

There are two variables that mainly influence the specific characteristics of both the mycelium composites as well as the pure mycelium: the substrate filler and the fungal species (Appels et al., 2019). Waste streams from agriculture and forestry are often used as substrate fillers and can consist of any type of lignocellulose containing organic material e.g., straw, sawdust, wood chips, rice hulls, flax, cotton seed hulls, etc. (Jiang & Li, 2013). However, these fillers have specific qualities that translate into the technical characteristics of the mycelium composites. Additionally, various species of mushrooms are used to produce mycelium materials that each have their specific qualities and molecular composition e.g., beta-glucans, chitin, and other membrane proteins (Fig. A5; El Knidri et al., 2018; Garcia-Rubio et al., 2020; Gow et al., 2017).

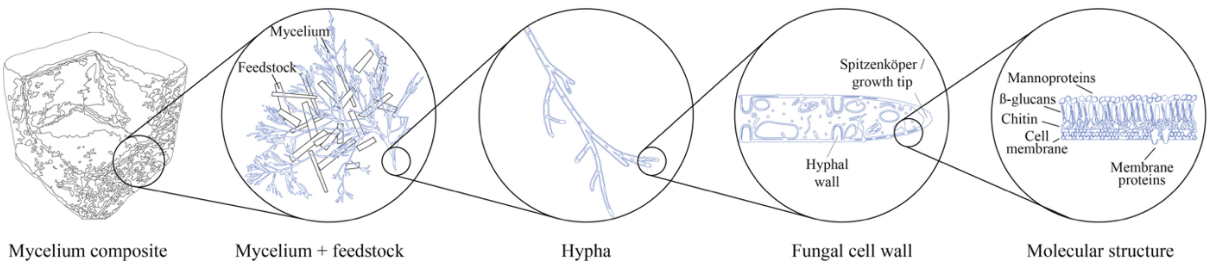

Figure A5. Graphical representation of the interaction between mycelium and its substrate in mycelium composites (left) and the molecular structure of fungal hyphae (right) (Elsacker et al., 2020).

Appendix B – Data and calculations for assessment of environmental impacts

This appendix includes the required data and calculations for the assessment of environmental impacts of the different options for valorisation of one ton SMS and PMW. The following conversion factors were used for the calculations: 1 kg water = 1 L water; 1 kwh = 3.6 MJ.

B.1 ENVIRONMENTAL IMPACTS OF MUSHROOMS PRODUCTION

Only the cultivation process (Fig. A1) was considered for calculation of the environmental impacts of mushrooms production for the valorisation of one ton SMS as the preceding and consecutive processes are not required when directly reutilizing the SMS for mushrooms production (Cunha Zied et al., 2020), or are outside the system boundaries of this thesis. Below are the required calculation steps and used data.

- Conversion of SMS to mushrooms = 48.9% (BE) (Pardo-Giménez et al., 2010)
- Total mushrooms production from one ton SMS = 489 kg mushrooms
- $C_{W:}$ 489 * 2.42 m^3 * 0.69 = 816.5 m^3
- $C_{E:}$ 489 * 149 MJ * 0.60 = 43716.6 MJ
- GWP: 489 * 2.99 * 0.13 = 190. 0 $kgCO_2$-eq.

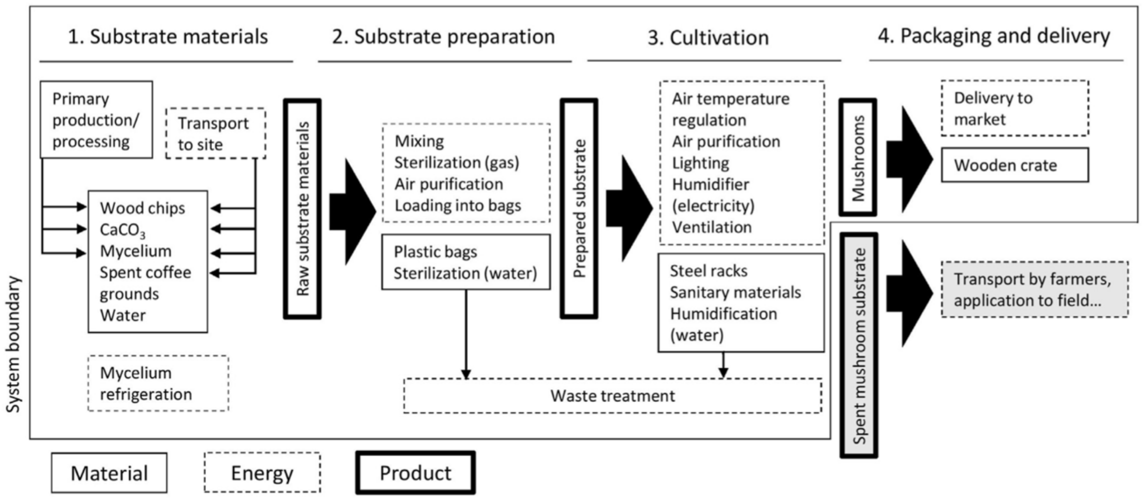

Figure B1. Flowchart and system boundaries of mushrooms production used in the study of Dorr et al. (2021).

Table B1. Values of lifecycle impact categories for environmental impacts of one kg mushrooms production. Data taken from Dorr et al. (2021).

Impact category	Value	Unit	Relative contribution cultivation stage
GWP (with C sequestration)	2.99	$kgCO_2$-eq.	13%
Energy demand	149	MJ	60%
Water scarcity	2.42	m^3	69%

B.2 ENVIRONMENTAL IMPACTS OF CNF PRODUCTION

CNF is produced through the alkaline-lime juice hydrolysis (ALH) method (Fig. A2). Below are the required data and calculations for determination of the environmental impacts from the valorisation of one ton SMS through CNF production.

- Total CNF production from one ton SMS (30% yield*) = 300 kg
- GWP: 300 kg * 3.17 = 951 $kgCO_2$-eq.
- C_E: (300 kg/ 0.006 kg) * 0.07 MJ = 3500 MJ
- C_W: (300 kg/ 0.006 kg) * 60 L = 3000 m^3

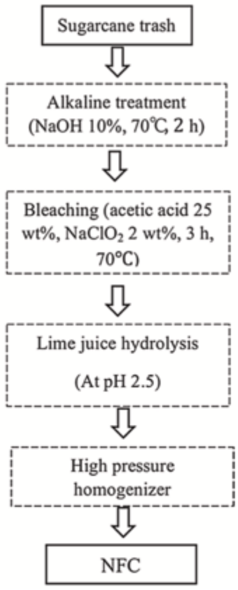

Figure B2. Flowchart of production process of CNF (NFC) used in the study of Haroni et al. (2021).

Table B2.1. Inventory data for the production of CNF. Taken from Haroni et al. (2021).

Inputs	Value	Unit
Sugarcane trash	20	gram
Energy	0.07	MJ
Water	60	L
Outputs		
CNF*	6	gram

*Based on 30% yield (Haroni et al., 2021).

Table B2.2. Values of life cycle impact categories for environmental impacts of one kg CNF production. Data taken from Haroni et al. (2021).

Impact category	Value	Unit
GWP	3.17	$kgCO_2$-eq.

B.3 ENVIRONMENTAL IMPACTS OF CELLULASE PRODUCTION

The environmental impacts of cellulase production concerned the production of cellulase through the submerged aerobic fermentation method (Fig. A3) and pre-treatment of the SMS to produce the carbon source for cellulase production. Below are the required data and calculations for determination of the environmental impacts from the valorisation of one ton SMS through the production of cellulase.

- Total carbon source production from one ton SMS = (1000/441) * 1000 kg
- Total production of cellulase from one ton SMS = ((1000/441) * 1000)/28.3 (80.1kg)
- GWP: 80.1 kg * 7.9 = 632.8 kgCO_2-eq.
- C_E: 80.1 kg * 52.4 MJ = 4197.2 MJ
- C_W: $C_{W\ pre\text{-}treatment} + C_{W\ CE\ production}$
  $C_{W\ pre\text{-}treatment}$ = (1000/441) * 759 kg
  $C_{W\ CE\ production}$ = 80.1 kg * 35.5 kg

  C_W: ((1000/441) * 759 kg) + (80.1 kg * 35.5 kg) = 4564.6 L = 4.6 m^3

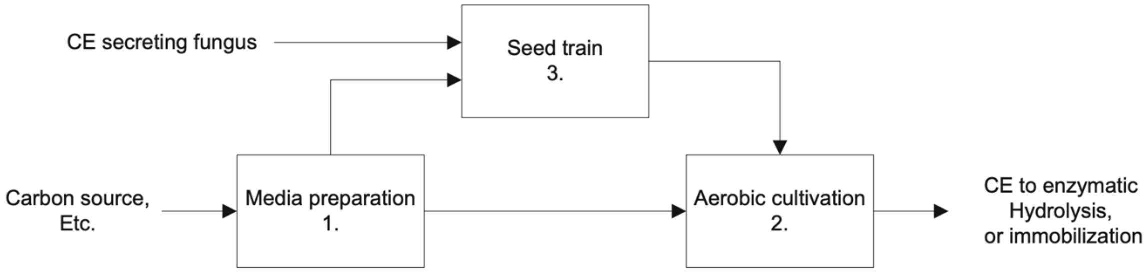

Figure B3. Process overview of CE production through the SmF method. Taken from Gilpin-Andrae et al. (2017).

Table B3.1. Inventory data for the production of 1000 kg pre-treated carbon source for cellulase production. Data taken from Gilpin-Andrae et al. (2017).

Inputs	Value	Unit
Wood chips	441	kg
Water	759	kg

Table B3.2. Inventory data for the production of 1 kg cellulase. Data taken from Gilpin-Andrae et al. (2017).

Inputs	Value	Unit
Carbon source	28.3	kg
Water	35.5	kg

Table B3.3. Values of life cycle impact categories for environmental impacts of one kg cellulase production (including pre-treatment of carbon source). Data taken from Gilpin-Andrae et al. (2017).

Impact category	Value	Unit
GWP	7.9	kgCO_2-eq.
Energy demand	52.4	MJ

B.4 ENVIRONMENTAL IMPACTS OF FUEL PELLETS PRODUCTION

Only process SS2.3 (Fig. A4) was considered for the calculations of the environmental impacts of fuel pellets production for the valorisation of one ton SMS. Below are the calculations steps and used data.

- Total feedstock inputs to produce one kg of fuel pellets = 1.13 kg
- Total fuel pellets production from one ton SMS: (1/1.13) * 1000 = 885 kg
- $C_{W:}$ 1000 * 0 m^3 = 0 m^3
- $C_{E:}$ (1/1.13) * 1000 * 2.45 MJ = 2168.1 MJ
- GWP: (1/1.13) * 1000 * 0.986 * 0.96 * 0.4 = 335.1 $kgCO_2$-eq.

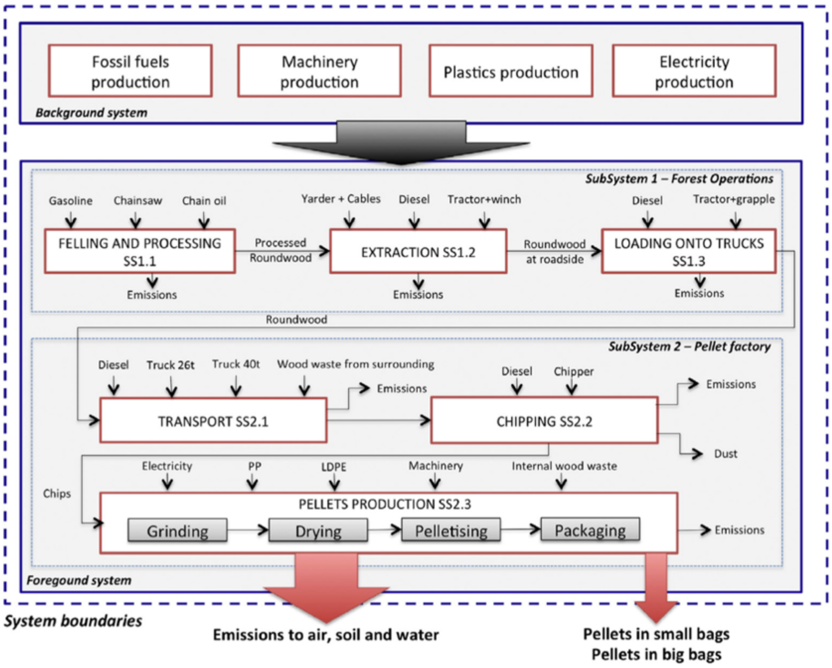

Figure B.4. Flowchart and system boundaries of fuel pellets production used in the study of Laschi et al. (2016).

Table B4.1. Inventory data for the relevant sub-process (SS2.3) of one kg fuel pellets production. Data taken from Laschi et al. (2016).

Inputs	Value	Unit
Wood chips	0.93	kg
Internal wood waste	0.20	kg
Electricity	2.03	MJ
Heat	0.42	MJ

Table B4.1. Global warming potential and relative contributions of sub-processes for the production of one kg fuel pellets. Data taken from Laschi et al. (2016).

	GWP ($kgCO^2$-eq.)
Total	$4.0*10^{-1}$
SS2	98.6%
SS2.3	96%

B.5 ENVIRONMENTAL IMPACTS OF DIETARY FIBRE EXTRACTION

Given below are the required data and calculations for determination of the environmental impacts of valorisation of one ton PMW through dietary fibre extraction. Both dietary fibre and polyphenols are extracted from dry PMW. Therefore, the impacts of drying are left outside the scope of this research.

- Weight loss from drying: 87.5 - 14.9 wt% = 72.6%
- Dry weight PMW: 1000 kg * (1 – 0.726) = 274 kg
- TDF per ton PMW (wet): 274 kg * 0.146 = 40.0 kg
- C_E: 274 kg * (10 *12.2 MJ) = 33,428 MJ
- C_W: 274 kg * (10 * 3.5 m^3) = 9590 m^3

Table B5. Total dietary fibre content (dry wt%) and maximum yield (wt%) through UAE.

	Value	Reference
TDF	52.5*	Table 3
Max. attainable yield (%)	27.81	Bagherian et al. (2011)
Yield (mg/g)	146.0	-

*Average of the TDF content of the concerning mushrooms: (49.9 + 55.0)/ 2.

Table B5.1. Average values for moisture content of dry and wet PMW.

PMW	Moisture (wt%)	Reference
Wet	87.5*	Bano et al. (1988); Sim et al. (2017)
Dry	14.9**	Table 3

*Average moisture content of wet PMW: (85 + 90)/ 2.
**Average moisture content of dry PMW: (11.76 + 18.0)/ 2.

Table B5.2 Values for life cycle impact categories for environmental impacts of the extraction of dietary from 100-gram feedstock. Data taken from Balicki et al. (2020).

Impact category	Value	Unit
Energy demand	12.2	MJ
Water consumption	3.5	m^3
GWP	-	$kgCO^2$-eq.

B.6 ENVIRONMENTAL IMPACTS PHENOLIC COMPOUND EXTRACTION

Given below are the required data and calculations for determination of the environmental impacts of valorisation of one ton PMW through phenolic compound extraction. Similar to dietary fibre extraction, the impacts of drying are left outside the scope of this research.

- Weight loss from drying: 87.5 – 14.9 wt% = 72.6%
- Dry weight PMW: 1000 kg * (1 – 0.726) = 274 kg
- TPC per ton PMW: (274 kg *10^3) * 16.6 mgGAE * 0.351 = 1.6 kg GAE
- C_E: (1.6 * 10^6 g) * 0.01172 kWh * 3.6 MJ = 67,507.2 MJ
- C_W: (1.6 * 10^6 g) * 0.138 m^3 = 220,800 m^3
- GWP: (1.6 * 10^6 g) * 8.0 $kgCO_2$-eq. =12,8*10^6kgCO2-eq

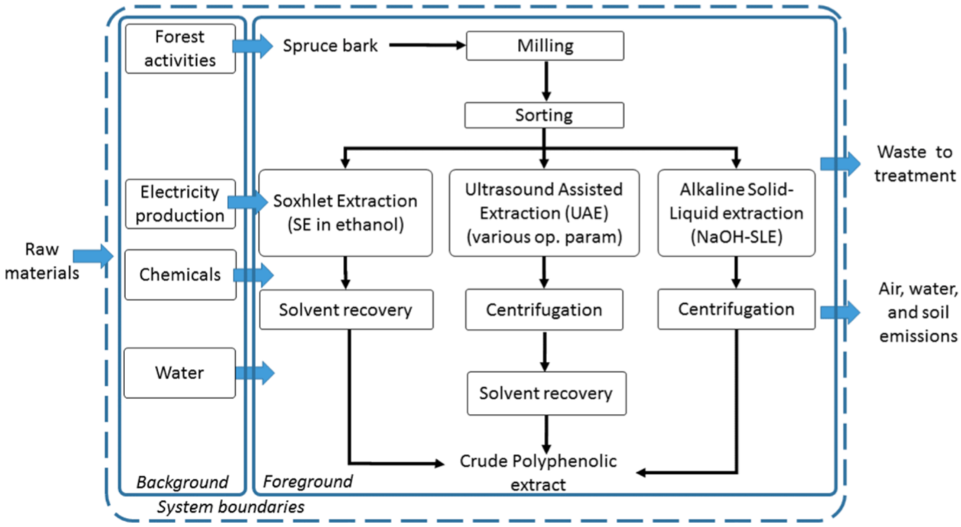

Figure B6. Flowchart and system boundaries of the different methods for extraction of polyphenols used in the study by Barjoveanu et al. (2020).

Table B6.1. Inventory data for the extraction of one mg polyphenols. Taken from Barjoveanu et al. (2020).

Input	Value	Unit
Electricity for heating	0.006	kWh
Electricity for ultrasound generation	0.00314	kWh
Electricity for ethanol recovery	0.0011	kWh
Centrifugation energy	0.00148	kWh

Table B6.2. Values of life cycle impact categories for the extraction of one mg phenolic compounds. Taken from Barjoveanu et al. (2020).

Impact category	Value	Unit
Water consumption	0.138	m^3
GWP	8.0	$kgCO_2$-eq.

Table B6.3. Average values for moisture content of dry and wet PMW.

PMW	Moisture (wt%)	Reference
Wet	87.5*	Bano et al. (1988); Sim et al. (2017)
Dry	14.9**	See Table 3

*Average moisture content of wet PMW: (85 + 90)/ 2.
**Average moisture content of dry PMW: (11.76 + 18.0)/ 2.

Table B6.4. Total phenolic content (dry wt%) and yield for PMW.

	Value	Unit	Reference
TPC	16.6*	mgGAE/g PMW (dry)	See Table 3
Yield	35.1**	%	Priyadarshini et al. (2022)

*Average of the TPC of the concerning mushrooms: (4.2 + 29.0)/ 2.
**Yield under optimal conditions.

Appendix C – Data and calculations for assessment of economic viability

This section includes the data used and calculations made for the assessment of economic viability of the selected valorisation options.

C.1 ASSESSMENT OF ECONOMIC VIABILITY MUSHROOMS PRODUCTION

The data comes from a full-cost analysis of a mushrooms production unit with an annual production capacity of 350 tons (Akritidis, 2018).

Annual production MME	902.5 ton * 489 kg	= 441.3	ton/year
Linear scaling factor	441.3 ton/ 350 ton	= 1.261	
Capital costs	1,350,000 EUR* (441.3/ 350)	= 1,702,350	EUR
Operating costs (incl. raw materials)	800,000 EUR * (441.3/ 350)	= 1,008,800	EUR/year
Revenue	902.5 * 489 kg * 3.33 EUR	= 1,456,290	EUR/year
Annual return	1,456,290 – 1,008,800	= 447,490	EUR/year
ROI	1,702,350/ 447,490	= 3.8	years

Table C1. Used data for assessment of economic viability of mushrooms production.

	Value	Unit	Reference
Capital costs	1.350 million	EUR	Akritidis (2018)
Operating costs (incl. raw material costs)	800,000	EUR/year	Akritidis (2018)
Sales price	3.30	Kg	Akritidis (2018)
Production	489	Kg/ton SMS	Pardo-Giménez et al. (2010)

C.2 ASSESSMENT OF ECONOMIC VIABILITY CNF PRODUCTION

The data comes from a techno-economic analysis of a CNF production unit with an annual production capacity of 1075.2 tons/year. This production output requires an input of 200 kg/h and operation at 24 hours a day for 350 days per year (Bondancia et al., 2020). The process is comparable to the production process described by Haroni et al. (2021) for the assessment of environmental impacts in Section 4.3.2.

Annual material input	350 days * 25h * 200 kg	= 1680 tons	input/year
Annual SMS input MME		= 902.5 tons	input/year
Linear scaling factor	902.5 tons/ 1680 tons	= 0.537	
Capital costs	7,000,000 USD * 0.93 * (902.5/1680)	= 3,497,187.50	EUR
Operating costs	(8,200,000 USD * (902.5/1680)) – (902.5 * 700 USD) * 0.93	= 3,509,177,86	EUR/year
Revenue	9.5 USD * 0.93 * 902.5 tons * 300 kg	= 2,392,076.25	EUR/year
Annual return	2,392,076.25 - 4,096,705.36	= - 1,117,101.61	EUR/year
ROI		= negative	

Table C2. Used data for assessment of economic viability of CNF production.

	Value	Unit	Reference
Capital costs	7 million	USD	Bondancia et al. (2020)
Operating costs (incl. raw materials)	8,2 million	USD/year	Bondancia et al. (2020)
Biomass feedstock	700	USD/ton	Bondancia et al. (2020)
Sales price	9.50	USD/kg	Nechyporchuk et al. (2016)
Production	300	Kg/ ton SMS	Haroni et al. (2021)

C.3 ASSESSMENT OF ECONOMIC VIABILITY CELLULASE PRODUCTION

The data comes from various studies concerned with cost-analyses of cellulase production from lignocellulosic biomass sources. The capital costs are based on an anaerobic cellulase production unit with an annual production of 40 tons (Hong et al., 2013). The operational costs are based on a production unit using *T. reesei* bacteria for production of cellulase (Elilä et al., 2017).

Annual production MME	902.5 tons * 80.1 kg	= 72,290.25	kg/year
Linear scaling factor	72.29 tons/ 40 tons	= 1.807	
Capital costs	4,600,000 EUR * (72.29/40)	= 8,313,350	EUR
Operating costs	72,290.25 kg * 2.12 EUR	= 153,255.33	EUR/year
Revenue	72,290.25 kg * 16.72 EUR	= 1,208,692.98	EUR/year
Annual return	1,208,692.98 –153,255.33	= 1,055,437.65	EUR/year
ROI	8,313,350/ 1,055,437.65	= 7.88	years

Table C3. Used data for assessment of economic viability of cellulase production.

	Value	Unit	Reference
Capital costs	4.6 million	EUR	Hong et al. (2013)
Operating costs (incl. raw materials/ excl. feedstock) *	2.12	EUR/kg	Elilä et al. (2017)
Sales price	16.72	EUR/kg	Liu et al. (2016)
Production	80.1	Kg/ ton SMS	Gilpin-Andrae et al. (2017)

*Assuming the feedstock contributes to 50% of the operating costs including raw materials (Elilä et al. 2017).

C.4ASSESSMENT OF ECONOMIC VIABILITY OF FUEL PELLETS PRODUCTION

The used data comes from a cost-analysis of a fuel pellets production facility with a production capacity of 46,929 tons (Wang et al., 2020). The facility is similar to that in the study by Laschi et al. (2016), which was used for the assessment of the environmental impacts of fuel pellets production. No additional raw materials are required for the production of fuel pellets (Laschi et al., 2016; Wang et al., 2020).

Annual production MME	902.5 tons * 885 kg	= 798.7	tons/year
Linear scaling factor	902.5 tons/ 46,929 tons	= 0.019	
Capital costs	4,403,744 USD * (902.5/46,929) * 0.93	= 84,689.19	EUR
Operating costs	4,046,745 USD * (902.5/46,929) * 0.93	= 77,823.68	EUR/year
Revenue	185 USD * 0.93 * 902.5 tons * 0.885	= 137,418.48	EUR/year
Annual return	137,418.48 – 77,823.68	= 59,594.8	EUR/year
ROI	84,689.19/ 59,594.8	= 1.42	years

Table C4. Used data for assessment of economic viability of fuel pellets production.

	Value	Unit	Reference
Capital costs	4,403,744	USD	Wang et al. (2020)
Operating costs	4,046,745	USD/year	Wang et al. (2020)
Sales price	185	USD/ton	Wang et al. (2020)
Production	885	Kg/ton SMS	Laschi et al. (2016)

C.5 ASSESSMENT OF ECONOMIC VIABILITY OF DIETARY FIBRE EXTRACTION

Based on the results of Table A5.1, the dry weight of the 47.5 tons PMW annually produced by MME is 13,015 kg. Assuming 350 annual 8 hour working days (Priyadarshini et al., 2022) and a full extraction cycle of one hour (Balicki et al., 2020), allows 2800 UAE runs annually and requires a 5L UAE unit. The capital costs of such a production unit are €10,000 (Prado et al., 2017). The ethanol used for extraction of dietary fibres can be recovered for 95% (Barjoveanu et al., 2020). Therefore, every production run requires an ethanol supplementation of 5% of the total volume. The operating costs for a 125L UAE unit are 0.53€/h (Prado et al., 2017).

Linear scaling factor	5L/125L	= 0.04	
Capital costs		= 10,000	EUR
Raw materials costs	5L + (5L * 0.05) $^{(13015/6.25)}$ * 0.79 EUR	= 415.22	EUR/year
Operating costs	(13015kg/ 6.25kg) * 0.53 EUR * 0.04	= 44.15	EUR/year
Revenue	47.5 tons * 40 kg * 6 EUR	= 11,400	EUR/year
Annual return	11,400 EUR – (8,225.48 + 44.15 EUR)	= 10,940.33	EUR/year
ROI	10,000/ 10,940.33	= 0.91	years

Table C5. Used data for assessment of economic viability of dietary fibre extraction.

	Value	Unit	Reference
Capital costs	10,000	EUR	Prado et al. (2017)
Raw material costs *Ethanol*	0.79	EUR/L	Vieira et al. (2013)
Operating costs	0.53	€/h	Prado et al. (2017)
Sales price	6	EUR/kg	Wenger et al. (2018)
Production	40	Kg/ton PMW	Balicki et al. (2020)

C.6 ASSESSMENT OF ECONOMIC VIABILITY OF PHENOLIC COMPOUND EXTRACTION

Based on the results of Table A5.1, the dry weight of the 47.5 tons PMW annually produced by MME is 13,015 kg. Assuming 350 annual 8 hour working days (Priyadarshini et al., 2022) and a full extraction cycle of one hour (Balicki et al., 2020), allows 2800 UAE runs annually and requires a 5L UAE unit. The capital costs of such a production unit are €10,000 (Prado et al., 2017). The ethanol used for extraction of dietary fibres can be recovered for 95% (Barjoveanu et al., 2020). Therefore, every production run requires an ethanol supplementation of 5% of the total volume. The operating costs for a 125L UAE unit are 0.53€/h (Prado et al., 2017).

Annual production MME	47.5 tons * 1.6 kg	= 76	kg/year
Linear scaling factor	5L/125L	= 0.04	
Capital costs		= 10,000	EUR
Raw material costs	5L + (5L * 0.05) $^{(13015/6.25)}$ * 0.79 EUR	= 415.22	EUR/year
Operating costs	(13015kg/ 6.25kg) * 0.53 EUR * 0.04	= 44.15	EUR/year
Revenue	47.5 tons * 1.6 kg * 28 EUR	= 2,128	EUR/year
Annual return	2,128 EUR - (415.22 + 44.15 EUR)	= 1,668.62	EUR/year
ROI	10,000/1,668.62	= 6.0	years

Table C6. Used data for assessment of economic viability of polyphenol extraction.

	Value	Unit	Reference
Capital costs	10,000	EUR	Prado et al. (2017)
Raw material costs	0.79	EUR/L	Vieira et al. (2013)
Operating costs	0.53	€/h	Prado et al. (2017)
Sales price	28	€/kg	Uyttebroek et al. (2018)
Production	1.6	Kg/ ton PMW	Priyadarshini et al. (2022)

Appendix D – Calculations for eco-efficiency indicator values

This section includes the calculations for the eco-efficiency indicator values following the formula mentioned in Section 4.5.

D.1 CELLULASE PRODUCTION OPTION

- GWP: 632.8/ (1,055,437.44/ 902.5) = 0.54 kgCO_2-eq/€
- Ce: 4197.2/ (1,055,437.44/ 902.5) = 3.59 MJ/€
- Cw: 4.6/ (1,055,437.44/ 902.5) = 1.30 m^3/€

D.2 CNF PRODUCTION OPTION

Calculation of the eco-efficiency of CNF production is not possible due to the negative net value.

D.3 FUEL PELLETS PRODUCTION OPTION

- GWP: 335.1/ (59,594.8/ 902.5) = 5.07 kgCO_2-eq/€
- Ce: 2168.1/ (59,594.8/ 902.5) = 32.83 MJ/€
- Cw: = 0 m^3/€

D.4 MUSHROOMS PRODUCTION OPTION

- GWP: 190/ (447,490/ 902.5) = 0.38 kgCO_2-eq/€
- Ce: 43,716.6/ (447,490/ 902.5) = 88.17 MJ/€
- Cw: 816.5/ (447,490/ 902.5) = 1.65 m^3/€

D.5 DIETARY FIBRE EXTRACTION OPTION

- GWP: 12,800,000/ (10,940.33/ 47.5) = 55,574.19 kgCO_2-eq/€
- Ce: 33,428/ (10,940.33/ 47.5) = 145.14 MJ/€
- Cw: 9590/ (10,940.33/ 47.5) = 41.64 m^3/€

D.6 PHENOLIC COMPOUND EXTRACTION OPTION

- GWP: 12,800,000/ (1,668.2/ 47.5) = 3,644,646.92 kgCO_2-eq/€
- Ce: 67,507.2/ (1,668.2/ 47.5) = 1922.19 MJ/€
- Cw: 220,800/ (1,668.2/ 47.5) = 6287.02 m^3/€