Developing novel applications of mycelium based bio-composite materials for design and architecture

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ABSTRACT

The growing need of the industry for alternative materials and products that are biodegradable and derived from renewable resources has recently led researchers from varied fields to search for more sustainable alternatives, and develop natural bio-composites to replace varied petroleum-based products in order to reduce the intolerable stress on the planet environment. In this research, the natural ability of saprophytic fungi to bind and digest lingo-cellulose, is utilized to develop natural bio-composite materials for novel applications in design and architecture.

Previous research has shown a potential to develop products such as packaging, building and insulation materials, leather-like, textile and transparent edible films. However, no research has been found in which all the significant variables were systematically tested. In this work, several fungi species were grown on varied local agricultural-growth wastes to evaluate which pair of fungi-plant material features the most suitable combination for future applications. The fungi; *Pleurotus pulmonarius, Pleurotus ostreatus, Pleurotus salmoneostramineus and Aaegerita agrocibe* where grown on woodchips of Eucalyptus, Oak, Pine, Apple and vine. The samples were tested for selected properties, including chemical changes in organic matter (pH, electric conductivity, water, carbon and nitrogen contents), mycelium growth rate, density and quality impression. By examining these fundamental materials characteristics, we aim to achieve a thorough understanding of the structural and aesthetic opportunities that this novel bio-material should offer. The current stage of the research shows that the most efficient integrations where the samples of *P. ostreatus* grown on Apple or Vine woodchips. Future work will focus on using suitable analytical methods for further understanding of the changes in mycelium and plant structures during the digestion process, and locating essential variable parameters of previous and post processing, to achieve desired material properties and introduce innovative characteristics and functions over existing industrial products and applications.

INTRODUCTION

Utilizing fungi ability to digest varied lingo-cellulose wastes

Recent advances in technological, biological and digital computation abilities, alongside the need for more sustainable manufacturing methods, assist to develop alternative materials and fabrication processes, using multidisciplinary tools. In a world characterized by rapid population growth, intensified agriculture and industrialization, where fast, low-cost manufacturing process encourages a constant growth of production and consumption, leading to an accumulation of waste, environmental pollution, and depletion of natural resources, there is a growing need for alternative materials and products, particularly in the mass industry, that are biodegradable and derived from renewable resources.

A complex enzymatic process enables the white rot fungi with a unique ability to digest highly stable molecules such as the structural polysaccharides of plants (Danai et al. 2012). This process plays a significant role in natural ecosystems and is already widely used for varied agricultural and food applications (Levanon et al. 1988). However, the fungal ability to bind and digest lignin and cellulose fibres of plants can also provide an inherent bonding, forming a natural, light weight bio-composite, in which the fungal mycelium functions as the matrix and holds the plant substrate pieces together, without the use of any synthetic adhesives (Figure 7). The resulting material can therefore be applied as a biodegradable alternative for a wide range of industrial materials, products and applications.

In this paper, the novel concept of utilizing this integration of fungal mycelium with the plant fibers, will be introduced. The paper starts with a general review of current design works done with mycelium. It then presents a preliminary experiment we conducted, to scan for a suitable fungi-plant set, as well as understand whether some analytical methods can assist with the characterization of the final bio-composite material. It concludes that there is a clear connection between the quantitative and qualitative parameters we have tested so far. In future work, we wish to test more analytical methods to better characterize the structural changes during mycelium development and its effect on the final material.

Using mycelium-plant material as a bio-composite in design and architecture

A bio-composite consisting of mycelium-plant material can be applied as a biodegradable alternative for a wide range of industrial materials. At the end of its use, the mycelium product can decompose to be available as a recourse for the development of other organisms in the environment. Previous research has shown a potential to develop products such as packaging (Holt et al. 2012 (Ziegler et al. 2016)), building (Jiang et al. 2016, 2013; Jiang et al. 2017; Travaglini et al. 2014), thermal and acoustic insulation materials (Yang et al. 2014; M. G. G. Pelletier et al. 2013; M. G. Pelletier et al. 2017), decorative house products (Mycoplast), leather (Mycoworks), textile (Hoitink) and transparent edible films (Kumar 2000). In recent years, some workshops and exhibitions (such as Fungal Futures 2016) of international artists and designers who investigate and develop materials using mycelium are taking place. However, most of the current research focus on evaluating the mechanical properties of the raw material on a macro level, using a set of physical tests, such as compressive and tensile strength, stiffness, elasticity, density, dimensional stability, aging, water absorption, thermal and acoustic insulation (Mendez, Autónoma, and Antonio 2016; Lelivelt 2015). Yet, in order to detect and control the final material properties, a bottom up approach is required, in which structural changes within the plant and fungal components should first be identified. Using such approach could contribute and advance the possible applications using mycelium based bio-composites as novel materials.

Characterization of mycelium-plant bio-composite materials properties

The objective of this experiment is to locate the most suitable fungi-substrate combination for further exploration and development of potential materials, products and applications in the field of architecture and industrial design. In this work, four fungi species; *Pleurotus salmoneo-stramineus (P. salmoneo), Pleurotus ostreatus (P. ostreatus), Pleurotus pulmonarius (P. pulmunarius), Aaegerita agrocibe (A. agrocibe)*; where grown on five types of woodchips substrates; *Eucalyptus ecamaldulensis* (Eucalyptus); *Quercus calliprinos*; (Oak), *Pinus halepensis* (Pine); *Vitis vinifera* (Vine- Cabernet Sauvignon);

Malus domestica (Apple- Golden Delicious). Each substrate was tested for selected properties before and after the mycelium growth, including chemical changes in organic matter (water, pH, electrical conductivity, ash, nitrogen content and organic matter digestion); mycelium development was evaluated by rate, density and quality factors.

The water content was measured at the beginning of the experiment to test whether the substrate contains a sufficient amount of water (about 65% is essential for fungal growth and development); And at the end of the experiment, where it is expected to rise or remain stable, due to the release of metabolic water during enzymatic digestion process.

The pH level of the substrate is expected to drop where fungal mycelium have developed. A relatively high initial pH level (around 8) can donate to the selectivity of a substrate, since Pleurotus mushrooms can manage to grow on higher pH levels than other, unwanted fungi types. Furthermore, the digestion process of the fungi lowers the pH level of the substrate to about 5), thus it might assist to indicate the amount of mycelium development on the substrate.

The optimum nitrogen content range for Pleurotus mushroom growth is about 0.6-1%. The organic matter composing wood contains about 50% carbon and around 1% nitrogen. During its growth process, the mushroom digests mostly carbon from the substrate, without consuming nitrogen. Thus, the relative amount of nitrogen in the final material (substrate with mycelium) is expected to be higher compared with the control (only substrate). The change in nitrogen content during mycelium development is therefore another parameter to consider while evaluating mycelium development.

The amount of organic matter is determined by calculating the relative part of ash (non-organic components) from the total mass of the initial substrate material, compared with its amount on the final material (substrate with mycelium). Since the fungi digest organic matter, the relative part of the ash will therefore increase, thus indicate the amount of organic matter digested by the fungi. Hence, the loss of organic matter (ΔOM) indicates the amount of mycelium growth.

By accomplishing this experiment, we aim to understand which fungi-plant set is most suitable to work with for design and architecture applications. Further experiments will include a systematic testing for variations of substrate composition and characteristics, while exploring advanced production methods to fully utilize the possibilities and properties of this material. In future work, mycelium development could be evaluated using quantitative visual inspections such as fluorescence chitin assay (Ayliffe et al. 2013). The unique fungal digestion mechanism could be utilized to manipulate and enhance the mechanical properties of the plant fibres (Dresbøll and Magid 2006) to feature integrated performances of the fungal mycelium that is bound within the plant matter. As demonstrated in previous research (Avni et al. 2017), substrate composition can also affect the structural components of fungal cell wall, therefore assist to manipulate the final material properties.

EXPERIMENTAL

Mycelium cultivation

The mycelium cultivation process was done in the mushroom research lab of MIGAL, northern R&D, Israel. The woodchips substrate for this experiment is pruning sourced from local forest trees and agricultural crops in the Galilee area, Israel. The average particle size is 5X15 mm. The substrate was thoroughly mixed with (50% w/v) water, then placed in a ⌀14cm glass petri dish (150gr wet substrate in each plate) and sterilized in an autoclave (1hr, 121°C). Each plate was inoculated with a disk of ⌀7mm mycelium grown on agar, then incubated at 25°C for 4-5 weeks. Five replicas for each variation of substrate type and fungi specie were used. Two plates of each substrate type (not inoculated with mycelium) where used as control. Mycelium growth rate was calculated by measuring its diameter every 2-3 days. Visual inspection was used to determine mycelium quality and density, ranged from 1-Thin, almost transparent; to 5- thick and firm, white (Figure 1). Mushroom growth parameters were multiply (growth rate mm/day X mycelium density 1-5) and compared with organic matter loss to evaluate their relation.

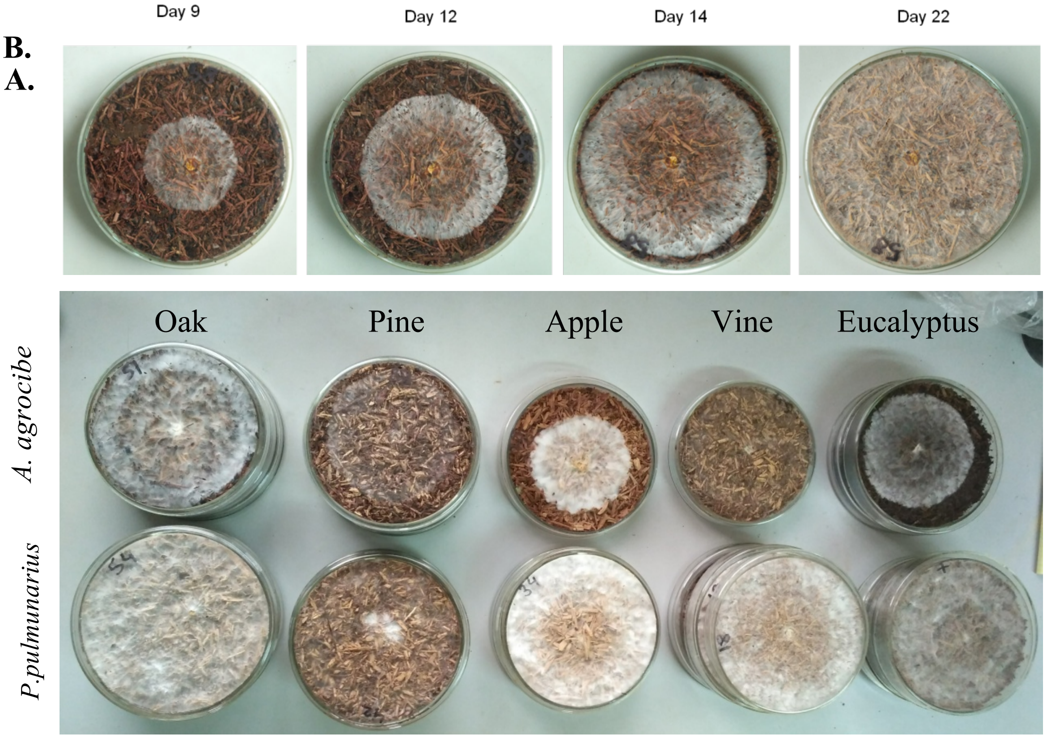

Figure 1: [A] Development of P. pulmonarius mycelium on oak for 16 days (from left to right). [B] Day 28, growth of A. agrocibe (top row) and P.pulmunarius (bottom row) on oak, pine, apple, vine and eucalyptus substrates. mycelium density and growth rate were measured during the experiment.

Chemical parameters:

All chemical properties where tested before and after mycelium growth. Two repeats where measured for each test. pH and conductivity where measured using a sample of substrate soaked in distilled water (1:10 w/w). Water content was determined by oven-drying (48 hours at 105°C). Samples where then milled using a 2mm sieve for carbon and nitrogen tests. Nitrogen content determined according to Kjeldahl, using a Buchi K-435 digestion and B-324 distillation units. For ash content measurement, milled samples were placed in crucible and burned in A Bifatherm multistage MS8 kiln (5 hours at 600°C).

Figure 2: Chemical parameters tests (from left to right); pH and electric conductivity, water, nitrogen and ash contents.

RESULTS AND DISCUSSION

Water capacity

As shown in Figure 3, the initial water content of all selected substrates was 52-54%. During the experiment, the control samples (without fungi) have lost significant amount of water compared with mycelium containing samples. The *P. pulmunarius* samples retained the highest water content during the experiment, followed by the *P. ostreatus, P. salmoneo* and *A. agrocibe (in this order)*. The growth of *P. salmoneo and A. agrocibe* on vine and apple substrates did not develop or had a little growth and their water content dropped similarly to the control. All types of fungi that were grown on the pine substrate contained less water content compared with its control.

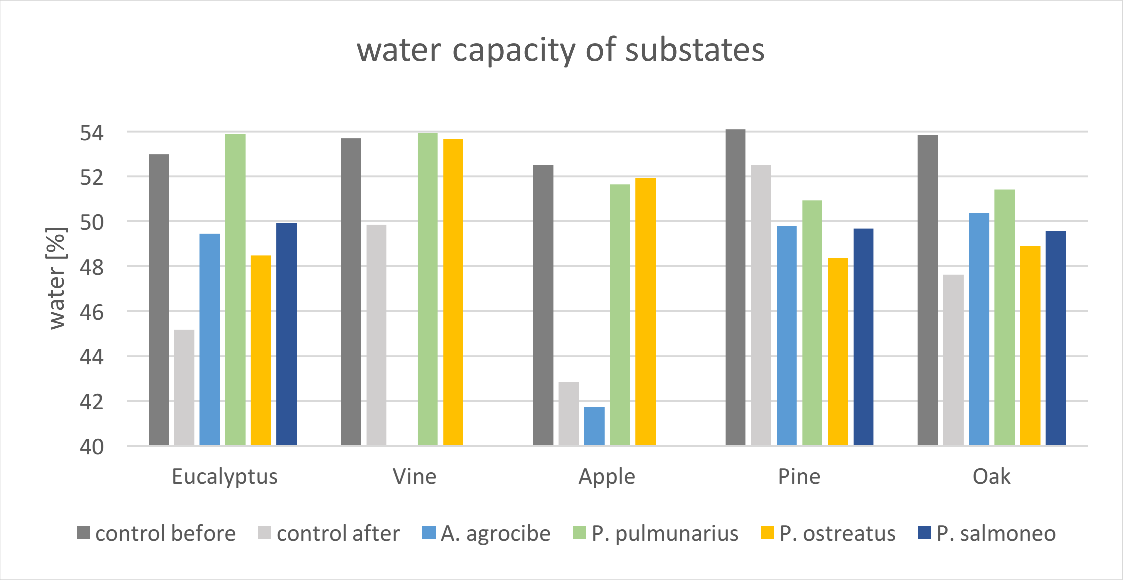

Figure 3: The changes in water content of selected lignocellulosic substrates during growth of several mushroom types. The mycelium retains higher water content compared with the control (before and after the experiment).

The lack of current substrates to hold enough water seems to challenge the growth of some fungi. **Error! Reference source not found.** demonstrates the effect of mycelium development on retaining the water content in the substrate. The control was drier in comparison to samples where mycelium has developed, except for the samples containing pine substrate, which were all drier than its control. This might be related to the very low density of mycelium on these samples (Figure 1). Furthermore, though the initial water content of the apple substrate was the lowest, its combination with *P. ostreatus* and *P. pulmunarius* retained its initial humidity and produced thick, dense mycelium. Considering these results, it seems that changes in water content can point on the level of mycelium development but does not necessarily indicate mycelium density.

pH level

As indicated in Figure 4, the *P.ostreatus* and *P.salmoneo* samples show a significant decrease in pH level, yet all other results do not show a coherent correlation between pH change and mycelium development. The initial pH level of all selected substrates was around 5-5.5. Such pH level is not optimal for woodchips based substrates. This might have influenced mycelium development.

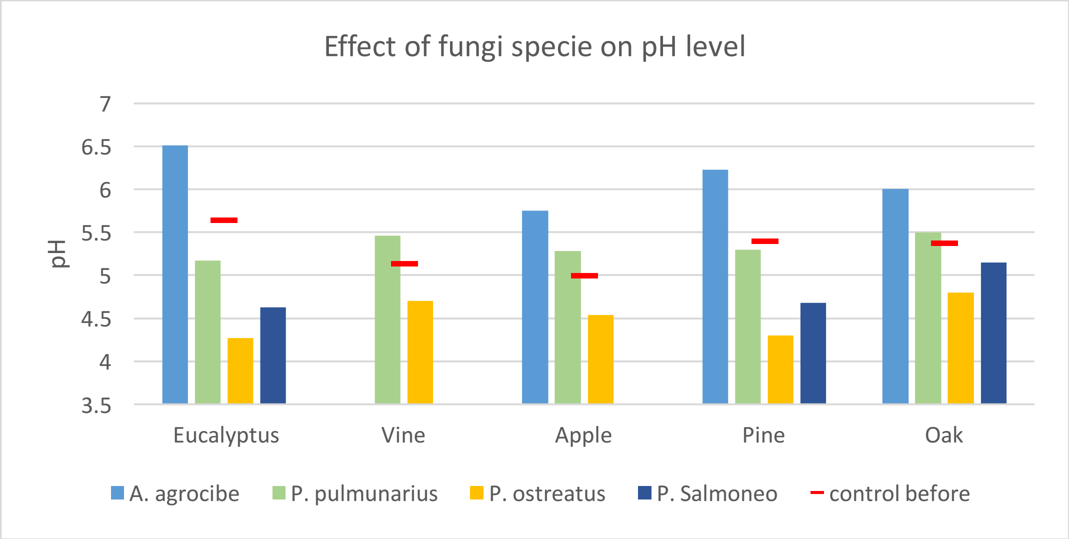

Figure 4: Changes in pH level of selected lignocellulosic substrates during growth of several mushroom types. Due to enzymatic digestion process, the pH level is expected to drop in comparison with the control where fungal mycelium has developed.

Nitrogen content

Figure 5 indicates that in all sets, accept the *A. agrocibe* on oak, the nitrogen level increased during mycelium development. The most significant increase is seen on the eucalyptus substrate, that had a lower initial nitrogen content (0.6%) and increased during the growth of *P. ostreatus* by 0.5%. On all other substrates, the change is smaller (about 2%). The oak substrate had a relatively high initial nitrogen content of (about 0.7%), yet, the final values after the growth of varied fungi did not rise significantly (about 0.1%).

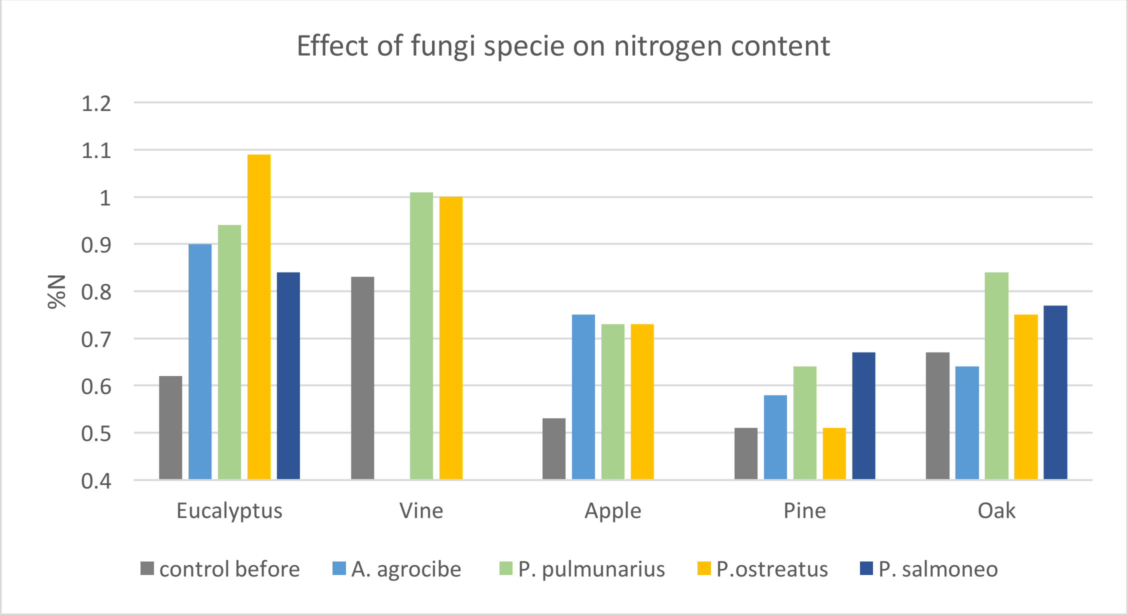

Figure 5: Changes in nitrogen content of selected lignocellulosic substrates during growth of several mushroom types. Due to enzymatic digestion process, the relative nitrogen content is expected to increase in comparison with the control.

Organic matter digestion

As can be seen in Figure 6, the samples of *P.ostreatus* grown on Vine and Apple substrates and the *P.pulmunarius* on oak, have shown the highest loss of organic matter during mycelium development (about 21gr). The smallest change is seen on the pine substrate. The high correlation between the change in organic matter content and mycelium development (Figure 7) indicate that this test is a reliable quantitative index to evaluate mycelium quality.

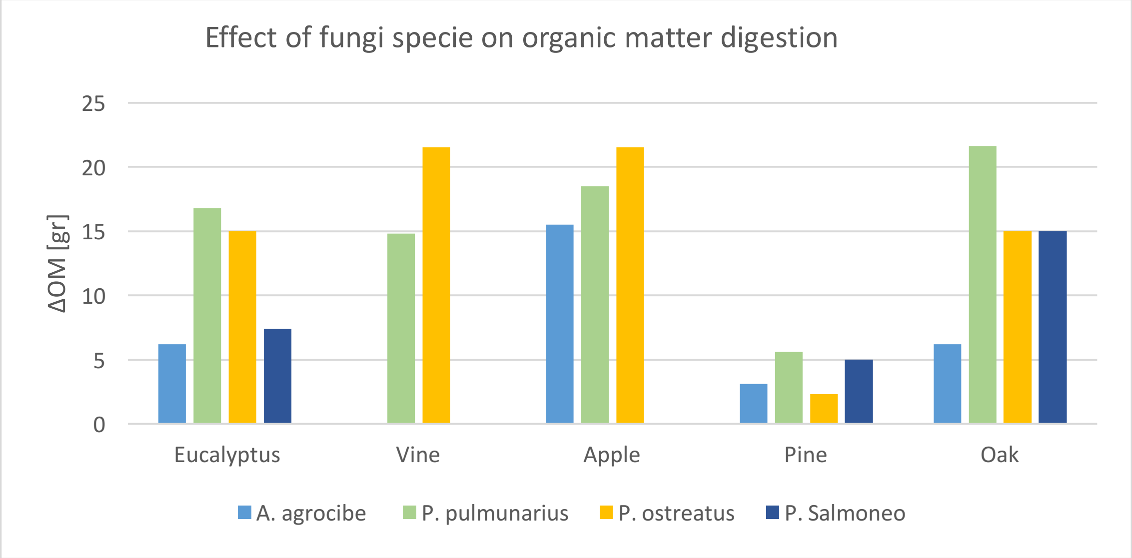

Figure 6: Changes in organic matter content of selected lignocellulosic substrates during growth of several mushroom types. Due to enzymatic digestion process, the relative organic matter content is expected to decrease in comparison with the control. the largest change in organic matter is expected where fungal growth is most developed.

Figure 7: P. ostreatus grown in ⌀ 14cm petri dishes with Apple (left) or Vine (right) woodchips.

Mushroom growth

To evaluate the mycelium quality and suitability for further work, mushroom growth parameters were multiply (growth rate mm/day X mycelium density 1-5) and compared with the amount of organic matter loss (Figure 8). This comparison shows high correlation between the selected parameters (R squared value is close or same to 1). According to this comparison, the mycelium growth on apple and vine substrates (Figure 7), showed the most suitable qualitative and quantitative characteristics so far. According to this comparison, there is a clear correlation between the quantitative change in organic matter content during mushroom growth with the qualitative parameters of mycelium density and thickness tested (Figure 1). Therefore, this comparison is the most reliable index to evaluate mycelium development and suitability for further exploration so far.

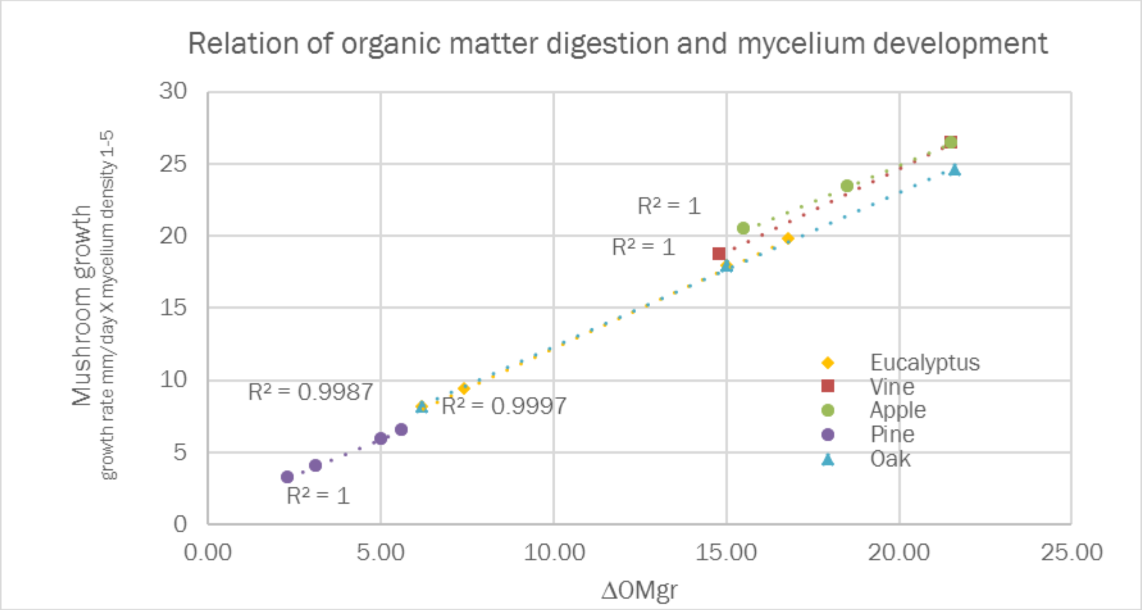

Figure 8: A comparison between the amount of organic matter loss to mushroom growth parameters (growth rate mm/day X mycelium density 1-5) shows high correlation, indicating it as reliable calculation for mycelium development.

Substrate parameters

The results generally indicate a lack of few parameters that are essential for mushroom growth and proper mycelium development. The water absorbance ability of desired substrates should be increased to around 65%; the initial pH level of the substrate should be higher, around 7-8; nitrogen content of the substrate should also start at higher levels, closer to 1%. This could be achieved by a few actions:

a. Increase the surface area of the substrate components by reducing the particle size to around Ø2mm as well as add varied amounts of sawdust (Danay et al. 2012), while maintaining aerobic conditions in the substrate.
b. A prior heat treatment to open and expand air cavities between the wood fibers, thus increase the porosity of the desired substrate.
c. Test additional materials with higher water absorbance ability, such as hemp fibers (Lelivelt 2015), coconut, soy bean meal, wheat bran, gypsum etc. (Danay et al. 2012) should be added to the selected substrate to increase its water capacity to the desired amount. The addition of such substrates can also contribute to its initial nitrogen content and to the mechanical properties (Lelivelt 2015).

CONCLUSIONS

The objective of this experiment is to locate fungi-substrate combinations that are most suitable for further exploration and development of potential materials in the field of architecture and industrial design. A set of 4 fungi species grown on 5 different agricultural plant waste substrates. The samples were tested for selected properties, including chemical changes in organic matter and qualitative evaluation of mycelium development. The high correlation between organic matter loss and mushroom growth parameters assisted to evaluate the efficiency of each substrate and fungi set. Best results are *P.ostreatus* mycelium grown on vine and on apple substrates. Therefore, variations of this fungi-plant sets could be used in further work. Yet, to better evaluate the suitability of each substrate-fungi set for more particular applications, additional analytical methods should be used. there is a need to develop a further set of parameters (physical, chemical and visual) of the varied substrate components to understand their relations with one another and its impact on final material properties.

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