Cellulose-Mycelia Foam: Novel Bio-Composite Material

by

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B.Sc. Chemical Engineering, Amirkabir University of Technology, 2012

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF APPLIED SCIENCE

in

The Faculty of Graduate and Postdoctoral Studies

(Mechanical Engineering)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

December 2016

Abstract

Demand for sustainable products is growing faster than ever before. Because of this, the development of novel sustainable materials is crucial to leverage our environmental resources and to ensure future growth of Canada's economy. In this study, we propose a technology to develop the use of fungal mycelium, the vegetative part of a fungus, through a porous scaffold of cellulose-based foam. A methodology for producing cellulose-mycelia foam (CMF) has been developed by mixing a surfactant with pulp suspension of 1% consistency and *Pleurotus djamor* spawn, mixing at high velocity to entrain air, filtering the suspension, and then holding at incubation conditions suitable for mycelium growth. During the incubation period, temperature (20-25 °C), pH (5-8), humidity (80-100%), ventilation and exposure to light were controlled. Simplicity of production, biodegradability, and 3-D porous structure of the product position this biocomposite as a green alternative to polymeric foams. The structure of the CMF was characterized through fluorescent microscopy during the incubation period. The effect of mycelial growth on the mechanical behaviour of the CMF including compressibility, thermal decomposition, dry and wet strength was investigated during 25 days of mycelial growth. The results indicated that all tested mechanical properties improved after 25 days of mycelial growth. The second set of experiments was run to specify the application of the CMF in a hydraulic filtration system. The pressure drop, permeability, and filtration efficiency of the product were studied. The experimental results showed that the permeability of the CMF decreases by an increase in mycelial growth. The hydraulic filtration efficiency of the product improved from 74% for cellulosic foam to 99.9% for 25 days CMF for removing 20 μm and larger particles. Bioremediation tests also were performed to evaluate the detoxification capability of mycelia in the CMF. Detoxification tests demonstrated that the living mycelia are able to detoxify potassium hydroxide from waste alkaline batteries.

Preface

The work in this dissertation, including all experiments, analysis and writing was conducted by the author, H. Ahmadi. All work was performed under supervision of Dr. James A. Olson.

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Acknowledgements

I would like to express my deepest appreciation to my supervisor, Professor James A. Olson, and my co-supervisor, Professor D. Mark Martinez who continually and convincingly conveyed a sprit of adventure in regard to research. Without their guidance and persistent help this dissertation would have not been possible.

I would specially like to thank Pouyan Jahangiri, Amir Farzad Forughi, Amin Engarnevis, David Sommer, Amirhossein Salimian, and Mohammad Shanbghazani for their invaluable discussions and for their patience with my endless stream of questions.

I would like to acknowledge the invaluable assistance of Dr. Reza Korehei, George Soong, Anna Jamroz, Meaghan Miller, and Emilia Jahangir at the UBC Pulp and Paper Centre for assisting me with performing the experiments.

I would also like to thank the Natural Science and Engineering Research Council of Canada (NSERC) and all members of Green Fibre Networks society for their financial support.

Most of all, I would like to express my heartfelt gratitude to my wonderful parents Mahin and Rostam whom this dissertation is dedicated to, and my lovely brother, Avesta. I have always felt them beside me in Canada, even though they live far away.

Dedication

To my beloved parents

for their unconditional love, support, and encouragement.

Chapter 1

Introduction

1.1 Motivation and advantages of biodegradable composites

Composite materials are engineered materials made from two or more constituents with significantly different mechanical properties. There are two categories of constituent materials: matrix and reinforcement. The matrix surrounds and supports the reinforcements by maintaining their relative positions, while the reinforcement material provides the desired physical properties. An enormous breadth of materials are used for matrices and reinforcements, depending on the application [1].

The rapid growth in the economy, the extraction of natural resources for manufacturing and production, and the patterns of consumers consumption are the main cause of environmental deterioration [2]. Concern over the environment has evolved from the 1960s ecology movement. As the environment continues to worsen, it has become a persistent public concern in developed countries and has recently awakened developing countries to the green movement. Also, the high rate of depletion of petroleum resources has decreased pressures for the dependence on petroleum products while increasing interest in maximizing the use of renewable alternatives. Another primary limitation of using synthetic polymer composites is that their recycling is quite difficult, and it is often preferred to produce new materials rather than recycling. Consequently, time resistant polymeric wastes are becoming highly unacceptable, necessitating the search for alternative materials to existing polymers. The concept of green composites from natural resources emerged to help society achieve sustainable consumption [3]. The word green refers to those materials which are biodegradable, renewable, and sustainable with lower environmental impact. Green composites can be disposed of or composted without harm-

ing the environment [4]. Polymers from natural resources such as cellulose, starch, lignin, cellulose acetate, poly-lactic acid (PLA), polyhydroxylalkanoates (PHA) are degradable and are classified as biopolymers [5]. The development of biocomposites from sustainably sourced biodegradable polymers and natural fibres have attracted great interest in the composite material sciences as a viable alternative to petroleum based composites [6–10]. While many biocomposites boast a natural reinforcement, the matrix is often based on petrochemicals. A renewable alternative for these matrices is the vegetative state of a fungus, the mycelium [11]. In this study, the cellulose fibres were used as a natural reinforcement, and the mycelium acted as a bio-based matrix to develop a novel green biocomposite.

1.2 Background

To better understand the concept of cellulose-mycelia foam, it seems appropriate to have a brief review of cellulose foam and mycelium. A brief background of foam forming technology and its application in the papermaking industry is presented in this section, as well as an introduction to mycelium and its application in filtration and bioremediation. This section also gives a brief review of nonwoven hydraulic filter media to help to understand filtration mechanisms and the applications of fibrous filters.

1.2.1 Foam-paper

Ultralight 3D porous materials have many important applications in the fields of sound and energy absorption, thermal insulation, radiation shielding, and filtration [12–17]. Most of these ultralight materials such as metal foams and aerogels, have traditionally been developed using either expensive materials or complicated procedures which has limited their commercial feasibility and widespread adoption. Another class of materials which are used in foam forming technology are cellulose containing materials. Jahangiri *et al.* [18, 19] recently developed a lightweight, highly porous and 3D shaped cellulose-based material, referred to as a foam-paper, as shown in Figure 1.1. The production of foam-paper shares many similarities with the papermaking process. Foam-paper offers several

key advantages over papermaking technology, including prevention of fibre flocculation, a 3D porous structure and less water and energy consumed in the manufacturing process. Foam-paper can be used in a wide range of applications including insulation, packaging, filtration and acoustics [18, 20]. A brief review of the foam forming process and its application in the papermaking industry is presented in the next section.

Figure 1.1: NBSK foam-paper at 50% air content. (Photographed by Anna Jamroz, UBC Pulp and Paper Centre)

Foam forming: state of the art

The idea of applying foam in the papermaking process was first proposed by Radvan and Gatward [21] in 1972 and Smith and Punton [22] in 1974 to improve the uniformity of the paper. Radvan and Gatward [21] used foam as a means of preventing fibre flocculation in the papermaking process due to the presence of very long fibres or high consistency of pulp suspension. They also developed a foam forming process, referred to as the Radfoam process which employs a discontinuous foam forming unit attached to a small paper machine. Also, another study on the Radfoam process [23] showed that the specific volume (bulk) of a Radfoam-made sheet is 20 to 30 percent higher than a standard handsheet. Smith *et al.* [22] and Tringham [23] also confirmed that paper has a higher uniformity, porosity and strength by using the Radfoam process. Another study by Smith *et al.* [24] on the structure and characteristics of the Radfoam-made sheets showed that

surface tension and bubble spacing in the foam affect the properties of the final product, while chemical effects were found to be insignificant. Recently, Al-Qararah *et al.* [25] at VTT Technical Research Centre of Finland studied the effect of various parameters on bubble size distribution in foam-formed cellulose fibres. They used a CCD camera and image processing to characterize the bubble size distribution as a function of various parameters, such as air content, type and concentration of surfactant, and rotational speed. They found that the average bubble size decreases by increasing the rotational speed of mixing; also, by decreasing air content, the average bubble radius increases. The results showed that the pore size distribution of paper made with foam forming process depends on the properties of the foam.

Recently, with an increasing demand for green and environmentally friendly products, interest in re-applying foaming process for developing novel cellulose-based material has increased [18, 26–29]. Many studies have been done on forming lightweight porous material from nanofibrillated cellulose (NFC) by applying several different methods [26–29]. Deng *et al.* [27] developed a procedure to produce nanoporous cellulose foams by dissolving cellulose in ionic liquid and then freeze drying the solution. The results showed that the foam has a 3D open fibrillar network structure with high porosity and high specific surface area. They also found that cellulose concentration and drying techniques affect the structure and pore size of the foam. Sehaqui *et al.* [28] and Mohammad Ali *et al.* [29] studied characteristics, morphological structure and mechanical properties of NFC foams. They found that the foams have a unique cellular structure and the mechanical properties of the foams can be improve by controlling the cell structure. In 2012, Cervin *et al.* [26] proposed a new foam forming process by using the Pickering emulsion technique to prepare low density cellulose material. NFC was used to stabilize the air bubbles in an aqueous suspension. In this process, NFC stabilized foam was prepared by adding octylamine to NFC, then the mixture was rapidly agitated for 20 min. The resulting mixture was foamed with a stainless steel milk beater for 10 min. The NFC stabilized foam was then filtered by Buchner funnel to drain any excess water. Finally, the foam was dried at ambient conditions. The results showed that the porosity of NFC stabilized foam is higher than other cellulose foams made by freeze-drying, while its mechanical properties are the same as cellulose aerogels and other cellulose foams made

by freeze-drying. Recently, Madani [19] and Jahangiri [18] developed a manufacturing process which has many similarities with the Radfoam process to produce a lightweight porous cellulose-base foam, referred to as a foam-paper. In this process, surfactant is added to the pulp/water suspension at pH of 7. Microbubbles are created by agitating the mixture and the suspension is filtered by a Buchner funnel to drain any excess water. Two techniques of drying were applied on the wet foam: air drying without pressing and freeze drying. They studied the morphology, mechanical behaviour and prospective applications of the foam-paper for different pulp types and different drying methods.

Foam-paper characteristics

When first developed, foam-paper was a novel cellulose based product which required more specific studies to characterize its properties. Madani [19] and Jahangiri [18] put a great effort into studying the properties and behaviour of foam-paper. They found that density of the foam depends on two parameters: air content and cellulose fibre length. The results showed that by increasing fibre length, the values of bulk increases which leads to lower density and lower tensile index (TI). They also found that foam air content has a similar effect on bulk, density and TI. To improve the strength of the final product, various ratio of refined fibres were added to the suspension. The results showed that regardless of the ratio of refined fibres in the foam-paper samples, all samples exhibited improved mechanical properties as compared to hardwood samples. They found that longer fibres contribute to achieving low density while fine or highly fibrillated fibres contribute to the strength by diminishing the values of bulk. The effect of pulp consistency on the properties of foam-paper has been studied [30]. The results showed that the density of the foam increases by increasing the pulp consistency which leads to the lower values of bulk. The effect of air content on the porosity [1] of the product has been studied [18, 19]. The results showed that by increasing air content of the foam, the porosity of final product increases up to 99.7%. Another significant mechanical property is permeability which is the ability of the foam to allow fluid to pass through it. The results showed that increased bulk, indicative of a more open structure

[1] The ratio of volume of pores to the total volume of sample.

which results in higher permeability to air flow [19].

Korehei *et al.* [30] studied three different drying techniques to compare the effect of drying method on foam-paper characteristics. Drying methods which were applied on the foam-paper were vacuum dewatering followed by air drying (VAD), vacuum dewatering followed by freeze drying (VFD), and direct freeze drying (FD). The morphological study by SEM and stress-strain curve of the foam-paper by Dynamic Mechanical Analysis (DMA) revealed that the microstructure of foam-paper was affected by applying different drying techniques. The VAD samples had the most compact structure due to shrinking during the vacuuming and dewatering process; while the FD samples had the highest porosity and the lowest density compare to VAD and VFD samples.

Foam-paper applications

The 3D structure, high porosity, and low pressure drop of foam-papers make these material appropriate for a wide range of applications including filtration, packaging, thermal insulation and acoustic dampening [18, 20, 30].

With increasing public demand for clean air, the capture of harmful aerosol particles by filtration has become the most common method for air cleaning [31]. Fibrous filters with mean pore size ranging between 100 nm to 100 μm are effective for capturing airborne particles and can obtain high separation efficiencies with a relatively low pressure drop [31, 32]. A great deal of research has been done towards making efficient filters from pulp in order to find a low-cost and green alternative for polymeric filters [33–35]. Jahangiri *et al.* [18, 20] studied the effect of fibre morphology and air content of foam-paper on the filtration efficiency and permeability of the product. They generated aerosol particles by a 1% aqueous solution of Sodium Chloride (NaCl). The aerodynamic diameter of the particles that were used for filtration tests were between 14 nm to 670 nm. The results showed that permeability of all samples increases by increasing the air content of the foam. However, by increasing the ratio of refined fibres, permeability of samples decreases to a great extent. This happens due to shorter length and higher specific surface fibres which can block the fluid flow. The results on filtration efficiency of foam-papers showed that by increasing the air content, the filtration efficiency of foam-paper decreases

until it loses its filtration properties at 45% air content. However, they found that by adding 10% to 30% of refined fibres in the structure of foam-paper, the filtration efficiency of the product dramatically increases and the results are comparable with commercial filters. The effect of adding nanofibrillated fibres (NLF) on the filtration efficiency of the product has been studied [18, 20]. The foam made of NLF by freeze drying showed a very high filtration efficiency at high air contents, however it displayed poor mechanical properties.

Another potential application of foam-papers is using them as thermal insulation materials. The effect of porosity and air content on the thermal conductivity of the foam-papers has been studied by Jahangiri *et al.* [18, 20]. The results showed that a slight change in porosity has a great impact on the values of thermal conductivity. By increasing the porosity of the samples, the volume fraction of air increases and consequently the thermal conductivity decreases. Comparing the results with commercial heat insulators showed that highly porous foam-papers could provide a competitive alternative.

Porous materials are generally used as sound absorbing materials due to their efficiency in attenuating acoustic energy. Many studies have been done on the sound absorption by the porous materials [36–39]. Jahangiri *et al.* [18] studied the effect of various parameters such as air content, thickness, porosity and consistency of the foam-paper. The results showed that the absorption coefficient decreases by increasing the air content of the foam-paper. Increasing the thickness and consistency of the product results in increasing the acoustic dampening of the foam-paper. The comparison between foam-papers and commercial acoustic products showed that these materials can be applied as sound absorbing materials. The advantages of using foam-papers instead of some other commercial products are that they are degradable, lower cost, and have a reduced environmental footprint.

1.2.2 Mycelium and sustainability

Since the industrial revolution, humans have had a widespread impact on the planet through industrial wastes and pollutants. In addition to the grave environmental impact, this has also led to serious health concerns of people exposed to these pollutants. The

problems are serious, but fortunately nature has provided us with a potential sustainable biological solution. Fungi are the recyclers of our planet. Their ability to disassemble complex organic molecules into simpler forms helps ecosystems to regenerate. Fungi absorb nutrients and break down biological polymers through their mycelium. Mycelium, as shown in Figure 1.2, is the subterranean mass of string-like hyphae that functions as fungal roots by absorbing nutrients. Through the mycelium, a fungus absorbs nutrients from its environment by a two stage process: first, hyphae secret enzymes into the food source which break down large organic molecules to smaller units such as monomers; then, these monomers are absorbed into the mycelium [40, 41]. That is why it is believed mycelium is the neurological network of nature which has a unique ability to break down and detoxify a great deal of toxic industrial waste and pollution.

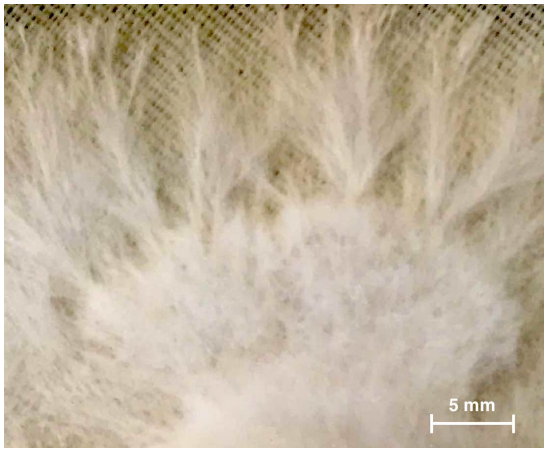


Figure 1.2: Image of mycelial strands.

Mycoremediation

Clean technologies focus on the use of biological methods for the remediation of waste. The process of using fungi to degrade or eliminate contaminants present in water, soils, or air is known as mycoremediation which is a form of bioremediation. This natural degradation process is superior to physical or chemical processes because it is less expensive and causes minimal site disruption. Fungi use three different methods to degrade

a great deal of environmental pollutants: biodegradation, biosorption, and bioconversion [42]. Biodegradation is the process which leads to complete mineralization of the starting compound to simpler ones by living organisms. Many studies have been done on the degradation abilities of mycelium as represented in Table 1.1.

Table 1.1: Role of mycelium in degradation of pollutants.

Mushroom spp.	Waste/Pollutants	Remark
Pleurotus ostreatus	DDT (dichlorodiphenyl-trichloroethane)	DDT was degraded by 48% during a 28 d incubation and 5.1% of the DDT was mineralized during a 56 d incubation[43].
Pleurotus ostreatus	PAHs (polycyclic aromatic hydrocarbons)	Suitable for degradation of PAH-contaminated oil-based drill cuttings[44].
Pleurotus sajor-caju	PAHs	Fungal mycelia of *Pleurotus sajor-caju* degraded 99.99% of PAH-contaminated soil[45].
Pleurotus ostreatus	Oxytetracycline (OTC)	The fungus completely degrades the drug in few days[46].
Pleurotus ostreatus	Crude oil	Total hydrocarbon concentration were reduced by 85% [47].
Pleurotus ostreatus	Oxo-Biodegradable Plastic	The plastic was degraded without prior physical treatment[48].

Biosorption is another important process of removal of heavy metals from the environment. The passive uptake of toxicants/metallic ions/xenobiotics by live or dried biomass can be defined as biosorption. The removal of heavy metal from wastewater is now shifting from the use of conventional methods to the use of biosorption [49]. Many studies have been done on the biosorption capacity of mycelium which are summarized in Table 1.2.

Table 1.2: Role of mycelium in the removal of pollutants by using biosorption process.

Mushroom spp.	Waste/Pollutants	Remark
Pleurotus ostreatus	Cadmium	Mechanism of biosorption was observed[50].
Pleurotus mutilus	Metribuzin pesticide	Adsorption rate of Metribuzin of 70% were obtained at optimum conditions[51].
Trichoderma	Nickel, Cadmium, and Chromium ions	The dried biomass of *Trichoderma* can be used for the treatment of toxic heavy metal ions from the industrial effluents [52].
Pleurotus ostreatus	Zinc ions	*Pleurotus ostreatus* is a potential adsorbent in wastewater treatment due to its great sorption capacity and low cost[53].
Pleurotus sapidus	Uranium(VI) ions	*Pleurotus sapidus* is a potential adsorbent for Uranium(VI) ions removal[54].

Bioconversion is a process of conversion of industrial sludges into some other useful forms. Mushroom is the most important bioconversion product. Any lignocellulosic waste, generated from industries, can be used for cultivation of mushroom. Kulshreshtha *et al.* [55, 56] successfully cultivated *Pleurotus citrinopileatus* and *Pleurotus florida* on the sludge of handmade paper and cardboard industrial waste. Other studies on the cultivation of mushrooms from industrial wastes or residues (including cotton waste, rice straw, sugar beet pulp, and etc.) reported the greatest degradation of the components of the substrates as a result of successful cultivation of different mushrooms [57, 58].

Mycofiltration

Another application of mycelium is to use it as a membrane for filtering out microorganisms, pollutants, and silt. The process of using fungal mycelia to filter toxic waste and microorganisms from the environment is called mycofiltration. Mycofiltration membranes can be applied in farms, urban areas, factories, roads, and harmed habitats [59]. The supports mostly used in biofilters are organic materials such as soil, compost, peat, bark, due to their large availability and low cost [60]. One of the problems associated with fungal growth is heavy mycelial growth occupy the void space in filters which results in increased pressure drop and clogging [61, 62]. This problem causes a significant decrease in the elimination capacity of membrane. Aizpuru *et al.* [63] used porous ceramic rings to control pressure drop. The study also showed that fungal biofilters have a greater potential to remove toluene vapor as compared to bacterial systems. Another application of mycofilters is pathogen management. Taylor *et al.* [64] developed a mycofilter which is capable of filtering *E. coli* from stormwater.

1.2.3 Filter media

A filter medium is any material that, under the operating conditions of the filter, is permeable to one or more components of a mixture, solution, or suspension and is impermeable to the remaining components [65]. In the filtration process, the surface straining is when the particle is too big to enter the entrance of the pore, but the particles may fit through the entrance and may be trapped by one of the four mechanisms of particle capture after they penetrate the surface of the medium [66], as listed below:

- Inertial impaction − occurs when the particle inertia is so high that it has sufficient momentum to break away from the streamlines and adhere to the wall of the pore.
- Interception − occurs when small particles do not have enough inertia to break down the streamlines, however the streamlines may carry them too close to the obstructions where surface forces cause the particles to adhere to the fibres.
- Diffusion − occurs when very small particles (<0.5 μm) exhibit Brownian motion

and move randomly due to collisions with other particles. They may randomly touch the wall surface of the pore.

- Electrostatic attraction − occurs due to electrostatic charge on the particle and/or fibre that will force the particle to be attracted to the fibre with an opposite charge.

Both inertial impaction and Brownian diffusion are much less effective with liquids than with gases [66]. Since the density of a particle is closer to the liquid than a gas, deviation of a suspended particle from the liquid is much less, thus the adherence of a particle by inertial impaction is less likely. Brownian diffusion in liquids occurs to a limited extent because Brownian motion in liquid suspensions is not as noticeable as in gaseous suspensions.

Fibrous materials are a low cost filter media, the fibres can be derived from natural or synthetic sources [65]. Fibrous filters are widely used in many industries, such as pharmaceutical, biotechnology, microelectronics, and semiconductor manufacturing. Fibrous filter media can be classified as woven and nonwoven filters, where nonwoven filters are more commonly used in filtration technology. The properties of fibrous filters is related to the properties of the fibres. Finer fibres are able to trap smaller particles and improve the filtration performance. However, finer fibres produce a weaker filter media. To find the best filter for a specific application, filtration efficiency, pressure drop and mechanical strength must be optimized by selecting the appropriate fibres. A weak material with good filtration performance can be strengthened by supporting it on a stronger substrate but, this often carries an associated manufacturing cost increase which can limit the commercial viability of the product.

Throughout the history of filtration and separation processes, fibrous media in the form of nonwoven filters have been used extensively in water treatment, water desalination, and water discharge treatment plants [65]. Compared to asymmetric membrane filters, nonwoven filters have a high internal surface area which leads to an increase the dirt loading capacity of the filters [67]. Despite having advantages of low cost, high dirt holding capacity, and high filtration efficiency, nonwoven media is limited to removing particles with diameter between 10 and 200 microns [68].

1.3 Cellulose-Mycelia Foam as a filtration media

In pursuit of sustainable products that leverage Canada's available natural resources, this research is conducted to produce a novel eco-friendly biocomposite material, which is called cellulose-mycelia foam (CMF). In this product, cellulose fibres are used as a natural reinforcement, and mycelium act as a bio-based matrix to surround and support the cellulose fibres.

The procedure of making CMF is quite similar to making foam-paper, with the addition of specific fungi grain spawn. In the foam making stage, the air content and consistency of foam are kept constant to evaluate the effect of mycelial growth on mechanical properties of the CMF biocomposite. The samples are kept at cultivation conditions up to four weeks. After observing the desired mycelial growth, they are dried in a low temperature oven to prevent any further mycelial growth. The final product has 3-D structure, high porosity, high strength and is extremely light. This characteristics of the CMF position this biocomposite as a green alternative to polymeric foams. The CMF also can be used in a wide range of applications including insulation, packaging, filtration and acoustic damping.

In the first experimental study, the morphology, thermal stability, compressibility, energy absorbing, wet and dry tensile strength of the CMF are assessed to characterize the mechanical properties of the product, presented in Chapter 2. In all experiments, the time of mycelial growth is considered as an input variable, while other factors including consistency, fibre length, diameter, and properties of fibres are constant.

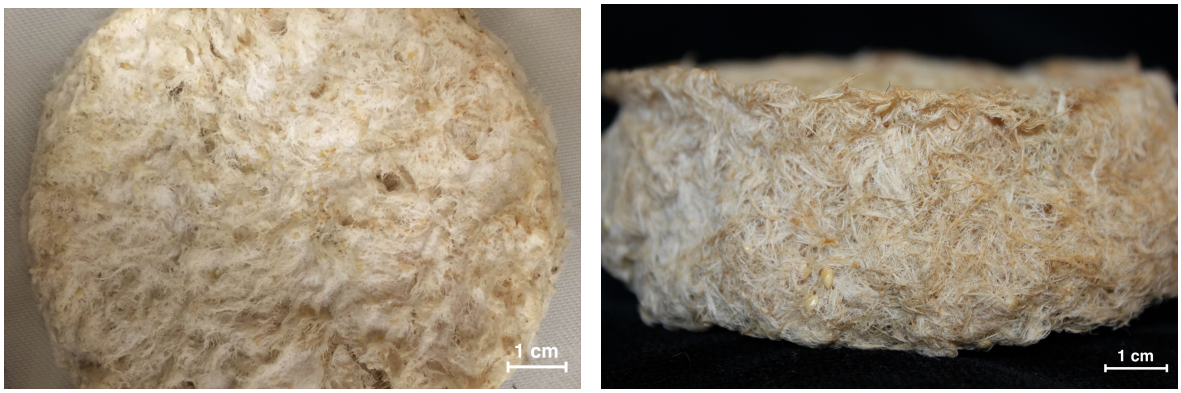
In the second experimental study (Chapter 3), an application of the CMF as a hydraulic filter is studied. Pressure drop, permeability, hydraulic filtration efficiency, and filter quality factor are measured in a small flow circuit. As mycelia can remediate contaminate present in water, soils, or air, the effect of mycelia on the mycoremediation abilities of the CMF to degrade potassium hydroxide are investigated in final part of the study.

Chapter 2

Cellulose-Mycelia Foam: production and characteristics

2.1 Production

Often the limiting factor in adopting foam-paper for an application is strength. To achieve a highly porous foam-paper, low consistency and high air content is required; although these lead to desirable increases in porosity, it also adds weakness in the final product. One way of circumventing this problem is to increase the weight percentage of refined fibres in the structure of foam-papers. Another approach to solve this problem is to add mycelium to the final product. Since mycelium spreads out through the samples, effectively reinforcing the material, it is a good candidate to make a high porous, biodegradable, and sustainable biocomposite with improved mechanical properties. Figure 2.1 shows a sample of the cellulose-mycelia foam.



(a) Top view (b) Side view

Figure 2.1: Image of dried cellulose-mycelia foam after 25 days.

In this study, the methodology for production of cellulose-mycelia foam (CMF) is quite similar to making foam-paper [18], with the addition of specific fungi grain spawn. A standard Northern Bleached Softwood Kraft (NBSK) pulp sheets were used as the fibre source for making 1% consistency pulp/water suspension. In this process, a Bioterge AS 40 surfactant was added to NBSK pulp/water suspension in 10 wt% at pH of 7, as a foaming agent. Second, the suspension of foam and fibres was rapidly agitated to reach 50% air content. Third, the mushroom grain spawn and nutrients (sawdust and cornmeal) were added to the foam suspension while the mixture was being agitated. The suspension was filtered in a 10 cm diameter Buchner funnel under 9.8 kPa of vacuum. Then, the filtered foam was kept at cultivation conditions for 5 to 25 days. Finally, after the desired growth of mycelium was observed, the foam was dried in a low temperature oven at 55 °C for 2 hours, to inhibit any further mycelium growth. The characteristics of the samples that were used for testings are briefly reported in Table 2.1.

Table 2.1: Characteristics of the cellulose-mycelia foam samples.

Mushroom ssp.	*Pleurotus Djamor*
Pulp type	NBSK
Consistency	1 %
Air Content	50 %
Growth time	5 - 25 days
Drying method	Oven-dried
Drying temperature	55 °C
Drying time	2 hours

Fungi grain spawn is used to introduce the fungi into the paper media. Commercially available prepared fungi grain spawn was used. A certified organic rye grain spawn was used which is ideal for inoculating the sterilized substrate, or for use in unsterilized straw, paper or bed-style inoculations. In this study, *Pleurotus Djamor* (Pink Oyster Mushroom) was cultivated because it grows faster than other species. Each species of the fungi requires specific cultivation conditions. In general, fungi do not have chlorophyll and do not perform photosynthesis, therefore exposure to sunlight is not mandatory. However, it does not mean fungi necessarily require a dark environment to grow. The

advantage of growing in the darkness is that dark areas often provide the moisture that the spores need to reproduce. Because fungi are not able to retain the moisture, an environment that has a high humidity is mandatory to avoid water loss. Based on different studies [69–74] on mycelium growth especially on the *Pleurotus* species, the best cultivation conditions are listed below:

- Temperature: 20 - 25 °C [69–71].
- pH: 5 - 8 [69].
- Humidity: 80 - 100%.
- Light: darkness.
- Ventilation: mushrooms breathe and exchange gases, so air circulation and gas exchange is required.
- Time: generally, between 2 to 4 weeks are ideal for mycelium running [70–72].
- Nutrients: cellulose, lignin, fibre content of substrate, husk rice, straw, and corn.

Based on a study on the performance of *Pleurotus ostreatus* [72], total average of mycelium growth time for *Pleurotus* species is 15 to 25 days. Another study on cultivation of *Pleurotus* species [71] showed that the complete spawn run is 2, 3, and 4 weeks for paper, fibre and sawdust respectively. Baysal *et al.* [70] found that the addition of husk rice to waste paper increases the mycelial growth. Another study on the growth of *Pleurotus* species [73] showed that the yield of mushroom is positively correlated to cellulose, lignin, and fibre content of substrates. In this study, straw and cornmeal were used as excess nutrients to improve mycelial growth. Park *et al.* [69] found that the optimal corn steep powder concentration is 10 g/L. Therefore, 1 wt% of nutrients were added to the pulp suspension.

2.2 Morphology and thermo-mechanical properties

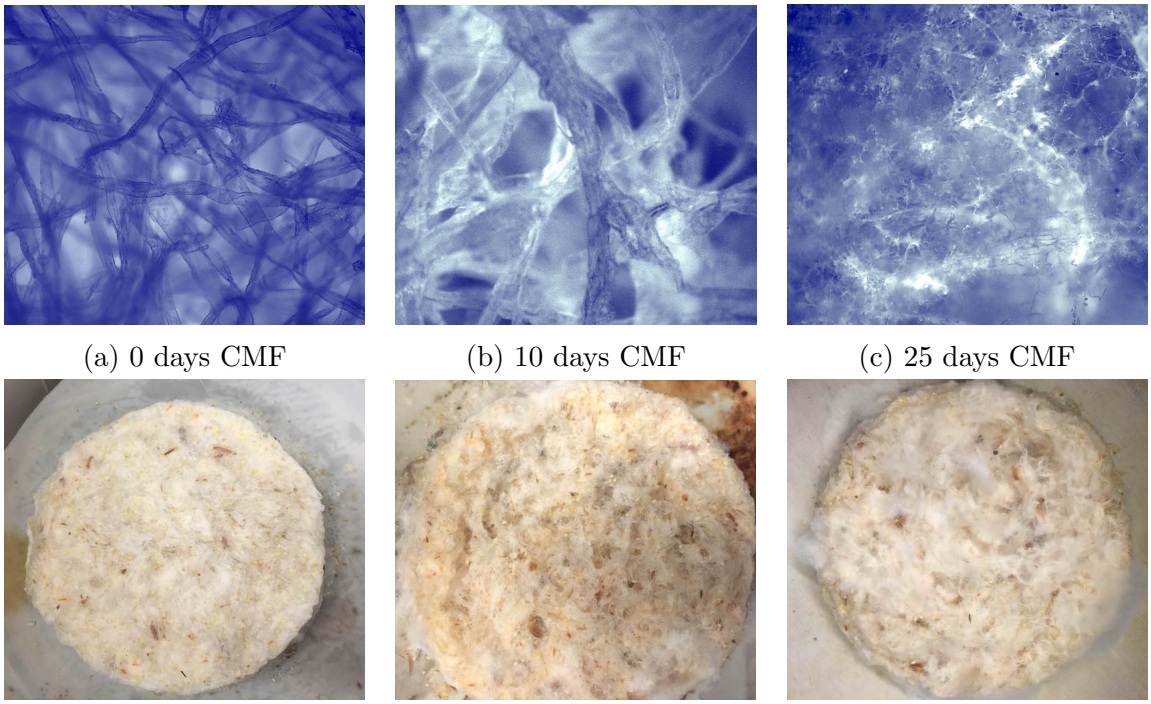
One of the major problems associated with mycelial growth is that mycelia tend to occupy the void space between fibres. It is necessary to study the morphology of the product to find the optimum growth time based on the intended use of the final product. In this section, the morphological characteristics of the CMF during mycelial running were studied. In this research, the time of mycelial growth is considered as an input variable, while metrics such as mass loss, compressibility, and tensile index (TI) are used to evaluate the effect of growth time on the final product.

2.2.1 Morphology

Fungi are morphologically complex microorganisms, exhibiting different structural forms throughout their life cycles [75]. The basic vegetative structure of growth consists of a tubular filament known as hypha that originates from the germination of a single reproductive spore. As the hypha continues to grow, it frequently branches repeatedly to form a mass of hyphal filaments referred to as mycelium. Mycelia secrete a wide variety of enzymes into the environment [76]. Most enzymes are secreted at the edge and in the centre of mycelium to aid in digestion of surrounding organic material. In proteins, and therefore enzymes, the indole group of tryptophan display intrinsic fluorescence [77]. Intrinsic fluorescence has become an important evaluation tool in various biologically relevant studies including morphological studies of mycelium [78–80].

The morphology of the CMF during the growth time of mycelia was studied by using a Nikon Eclipse TE200 fluorescent microscope. A filter cube with 520 - 540 nm excitation and 560 - 620 nm emission was used to attempt to excite and measure the fluorescence of the indole group present in the mycelial enzymes. Figure 2.2 shows the images of the cross-section of CMF.

Fluorescent microscopy revealed that mycelia grow and cover the cellulose fibres. Figure 2.2a showed that the cellulose fibres in the structure of foam-paper (0 days CMF) did not display intrinsic fluorescence, and the fibres were dark in the absence of mycelia. After 10 days of mycelial growth, the fibres became brighter because of mycelial running which leads to the secretion of enzymes (Figure 2.2b). The results showed the cellulose



(a) 0 days CMF (b) 10 days CMF (c) 25 days CMF

(d) 0 days CMF (e) 10 days CMF (f) 25 days CMF

Figure 2.2: Fluorescent micrographs (top) and conventional photographs (bottom) showing the effect of mycelial growth on the microstructure of cellulose-mycelia foam. Note: field of view for all micrographs is 1.4 mm x 1.6 mm.

fibres were covered by mycelial strands after 25 days, as shown in Figure 2.2c. Also, assuming that the excreted enzymes are the primary source of fluorescence, it can be concluded that secretion occurred more in central zones of the colonies compared to the periphery of the mycelia.

As mycelia grows, the volume fraction of cellulose decreases due to the degradation of the cellulose fibres by mycelia as a nutrient and mycelial strands replace the cellulose fibres and occupy the void spaces between them. However, decreased volume fraction of cellulose is negligible compare to increased volume fraction of mycelia in the first 25 days, as extra nutrients are provided in the substrate, and the growth rate is fast. Thus the void spaces between fibres are occupied by mycelial strands which can lead to decreased

porosity of the biocomposite, as can be expressed by the following equation:

$$f_f + f_m + f_v = 1 \tag{2.1}$$

As mycelia grows, the summation of the volume fraction of cellulose fibres f_f and volume fraction of mycelial strands f_m increases, and the void fraction f_v decreases, which leads to decreased porosity and a densely packed structure of the CMF.

2.2.2 Thermal Gravimetric Analysis

To study the effect of mycelial growth on the thermal stability of the CMF, the amount of weight loss as a function of increasing temperature was obtained by thermal gravimetric analysis (TGA), as shown in Figure 2.3. Samples were run in a nitrogen atmosphere on a Q500 TGA manufactured by TA Instruments. The samples were heated from 30 °C to 600 °C, with a constant purge gas rate of 40 ml/min and a scanning rate of 10 °C/min.

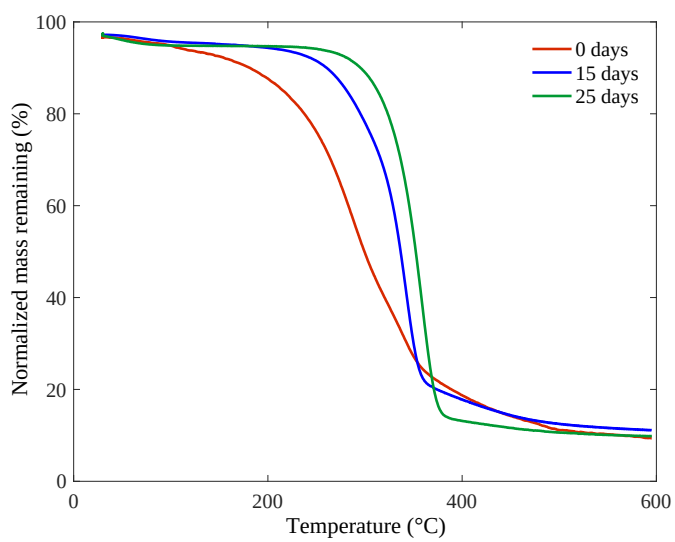


Figure 2.3: The effect of mycelial growth on the thermal stability of the cellulose-mycelia foam.

There are three stages of degradation in the TGA curves of all samples.The initial mass loss below 120 °C can be attributed to the evaporation of moisture. This loss depends on the initial moisture content of the fibres. The second, severe weight loss (250 − 350 °C) and third stage are due to decomposition of the major components of the fibres. The TGA curve of the NBSK cellulose foam, labelled as 0 days in Figure 2.3, showed the thermal decomposition of cellulose begins around 227.5 °C (extrapolated onset temperature); while, the temperature at which the weight loss begins increases with the presence of mycelia. The extrapolated onset temperatures are 312.7 °C and 325.5 °C for 15 days and 25 days respectively. After 600 °C, only ash and char were left,

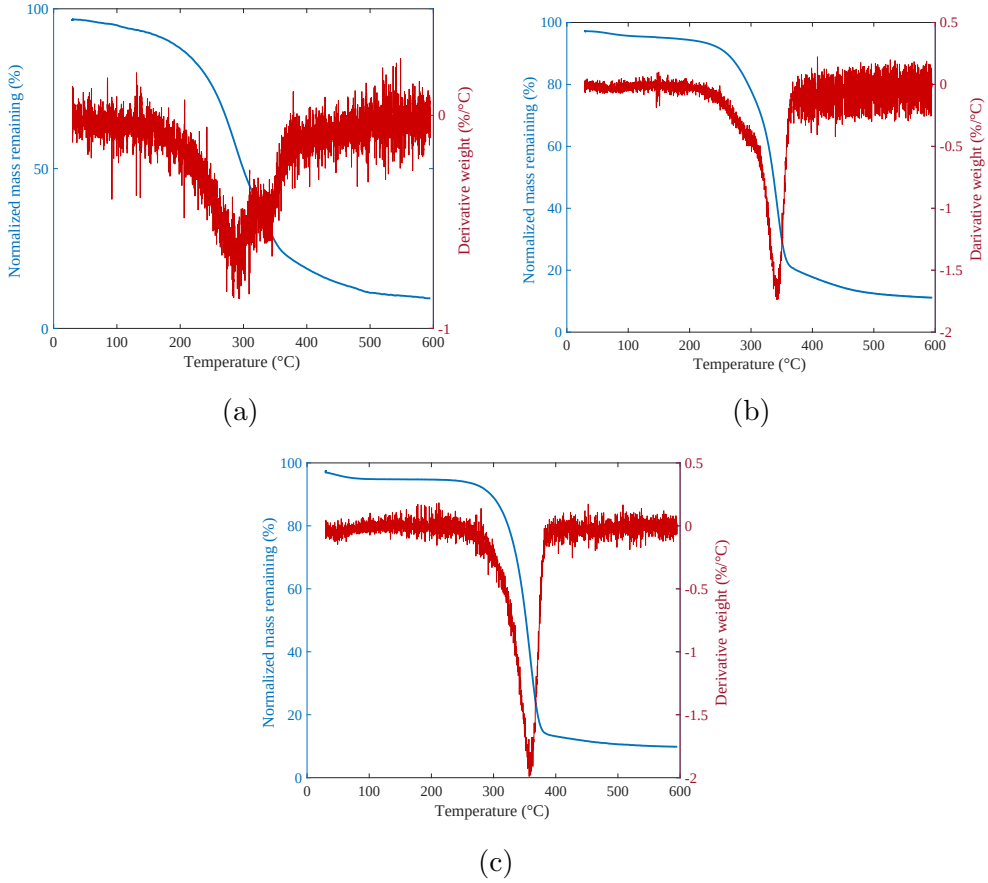


Figure 2.4: TGA curve and first derivative of the weight loss for (a) 0 days (b) 15 days and (c) 25 days CMF.

and the remaining mass was 10% for all samples. It can be concluded that mycelial growth improves the thermal stability of the final product. The best result was obtained after 25 days of mycelial growth which delayed the onset of thermal decomposition to higher temperatures.

Another significant characteristic is the point of greatest rate of change on the weight loss curve. This is also known as the inflection point. The peak calculation of the 1st derivative of the weight loss curve indicates the inflection point of the sample, as shown in Figure 2.4. The noise in the derivative plots is due to random noise in the original data. The inflection point temperature was calculated to be 286.9 °C, 342.4 °C, and 360.3 °C for 0 days, 15 days, and 25 days of mycelial growth time respectively. The results indicated the same trend as extrapolated onset temperature, with the best results obtained after 25 days of mycelial growth. It showed the greatest rate of change occurs around 360 °C which is much greater than the temperature for the pure foam-paper.

2.2.3 Dynamic Mechanical Analysis

To study the effect of mycelial growth on the compressibility of the CMF, the stress-strain curve was obtained by dynamic mechanical analysis (DMA). The test was performed using a DMA Q800 manufactured by TA Instruments operating in controlled force mode. The samples were cut into cylinders of diameter $D = 15$ mm by thickness $L_0 = 10$ mm. The samples were placed in compression clamps under isothermal conditions at 22 °C, and the compressive force was ramped at a rate of 0.5 N/min up to a maximum of 2.0 N. Stress σ and strain ε are recorded throughout the test, and are defined as follows:

$$\sigma = \frac{4F}{\pi D^2} \tag{2.2}$$

$$\varepsilon = \frac{x}{L_0} \tag{2.3}$$

where F is measured compressive load, x is displacement which is measured from the initial dimension of the sample.

The compressive stress-strain curve of samples with different growth days are pre-

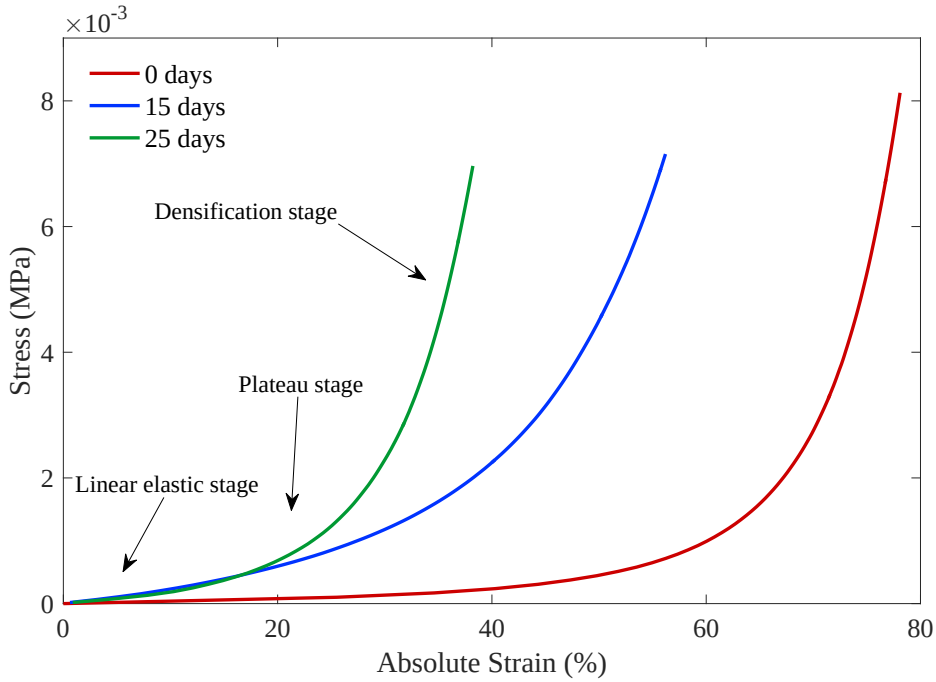


Figure 2.5: The effect of mycelial growth on the compressibility of the cellulose-mycelia foam.

sented in Figure 2.5. Typical of other porous materials, the compressive stress-strain curve indicates three distinct stages: the linear elastic stage, the plateau stage, and the densification stage. At low strains (<5%), the stress-strain behaviour is linear elastic in which the stress elevates linearly with increasing strain. The compressive test results show that in the linear elastic region, the compressive modulus of elasticity is higher for both CMF samples as compared to the foam-paper sample.

In the plateau stage, the stress increases slowly in a wide strain range, which means the structure begins to collapse at an approximately constant stress. The foam-paper showed higher deformation at the constant stress which is due to its high porosity compare to the CMF samples, as shown in Figure 2.5. The 15 days and 25 days CMFs which have more compact structure, resulting in an increase in the plateau stress and a significant reduction in the densification initial strain.

In the third region of the compressive stress-strain curve, referred to as the densification region, the complete collapse occurs with the characteristics of a rapid increase of stress with increasing strain. The densification initial strain was calculated to be 55%, 30%, and 20% for foam-paper, 15 days and 25 days CMF respectively.

The area under stress-strain curve can be related to the energy absorbtion of the material [81]. To compare the effect of mycelial growth on the energy absorption characteristics, the work of compression W_{comp} was calculated for each sample. The compressive work is related to stress σ and displacement x as follows:

$$W_{\text{comp}} = \int F \mathrm{d}x = \frac{\pi}{4} D^2 \int \sigma \mathrm{d}x \tag{2.4}$$

Test results were recorded at discrete intervals, so the compressive work is approximated by numerically integrating Equation 2.4 as follows:

$$W_{\text{comp}} \approx \frac{\pi}{4} D^2 L_0 \sum_{i}^{N} \sigma_i (\varepsilon_{i+1} - \varepsilon_i) \tag{2.5}$$

The compressive work is 6.16 mJ, 171 mJ, and 90 mJ for 0, 15, and 25 days respectively. Compared with foam-paper, the both CMF samples revealed at least 10 times larger compressive work which indicates better energy absorbing properties. It can be concluded from the aforementioned results that growing mycelium in the structure of the foam-paper can improve the compressive strength and energy absorbing characteristics of the biocomposite. It is hypothesized that mycelia grow through the foam paper providing additional structure to the material – acting as a matrix. This leads to decreased porosity of the biocomposite and improved compressive strength.

2.2.4 Tensile and wet strength

Many factors should be taken into consideration when designing a biocomposite, including the length, diameter, orientation, consistency, and properties of fibres; the properties of matrix; and the bonding between the fibres and the matrix [81]. The effect of length, diameter, and consistency of fibres on the structure of foam-paper have been discussed in Section 1.2.1. To study the effect of mycelial growth on the strength of the CMF as

a matrix, the tensile strength test was performed during mycelial running by Thwing-Albert's QC Electronic Materials Tensile Tester. The gauge length and the speed were fixed at 1 cm and 2.0 cm/min respectively. The samples were cut into 15 mm wide strips with sufficient length to be clamped in the jaws as described in TAPPI T 494. The samples were tested in accordance with the standards outlined in TAPPI T 494 and T 456. The dry and wet tensile index were calculated by using the following equations:

$$F = \frac{\mathrm{v}}{L} \tag{2.6}$$

$$TI = \frac{F}{wR} \tag{2.7}$$

where F is the breaking force, v is the separation speed of the jaws, L is the reading load, TI is the tensile index in Nm/g, w is the specimen width in meters, and R is the grammage in mass per unit area (g/m^2).

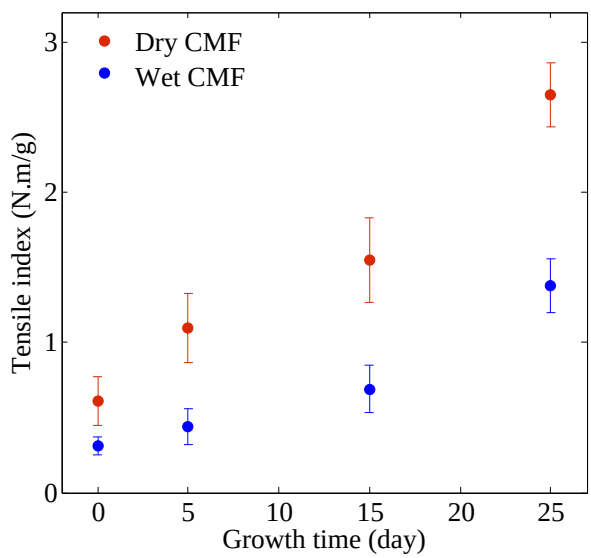


Figure 2.6: The effect of mycelial growth on the tensile index and wet strength of the cellulose-mycelia foam.

Figure 2.6 shows the effect of mycelial growth on the dry and wet strength of the CMF. The results revealed that by increasing the growth time of mycelia in the foam-paper structure, wet and dry tensile index increases at a constant bulk. The dry tensile index of the CMF improved from 0.61 Nm/g for foam-paper to 2.65 Nm/g for 25 days CMF. The wet strength also showed the same trend. The wet tensile index increased from 0.31 Nm/g for foam-paper to 1.38 Nm/g for 25 days CMF.

As we discussed in Section 2.2.1, the mycelial strands occupy the spaces between fibres during incubation period and decrease the porosity of the CMF, which can lead to higher tensile strength at the constant bulk. Another explanation for increased strength can be expressed by the relation of the stress taken by the fibres, the stress taken by the matrix and the strength of the composite [81]. The strength of the composite σ_{c} can be estimated from

$$\sigma_{\mathrm{c}} = f_f \mathrm{TS}_f (1 - \frac{l_{\mathrm{c}}}{2l}) + f_m \sigma_m \tag{2.8}$$

where f_f and f_m are the volume fraction of fibres and matrix respectively, TS_f is the tensile strength of the fibre, l_{c} is the critical fibre length, and σ_m is the stress on the matrix at failure. A critical fibre length for any given fibre diameter can be determined:

$$l_{\mathrm{c}} = \frac{\mathrm{TS}_f d}{2\tau_i} \tag{2.9}$$

where d is the diameter of the fibre, τ_i is related to the strength of the bond between the fibre and the matrix [81]. Substituting Equation 2.9 into Equation 2.8 yields an expression for the strength of composite in terms of the contribution from the fibres, matrix and the bond strength between them:

$$\underbrace{\sigma_{\mathrm{c}}}_{\substack{\textit{Strength of}\\ \textit{composite}}} = \underbrace{f_f \mathrm{TS}_f}_{\substack{\textit{Stress taken}\\ \textit{by fibre}}} + \underbrace{f_m \sigma_m}_{\substack{\textit{Stress taken}\\ \textit{by matrix}}} - \underbrace{\frac{f_f \mathrm{TS}_f^2 d}{4\tau_i l}}_{\substack{\textit{Bond between}\\ \textit{fibre \& matrix}}} \tag{2.10}$$

Equation 2.10 shows that the strength of composite is proportional to the stress taken by fibre, the stress taken by matrix, and the bond between them. Usually the stress that

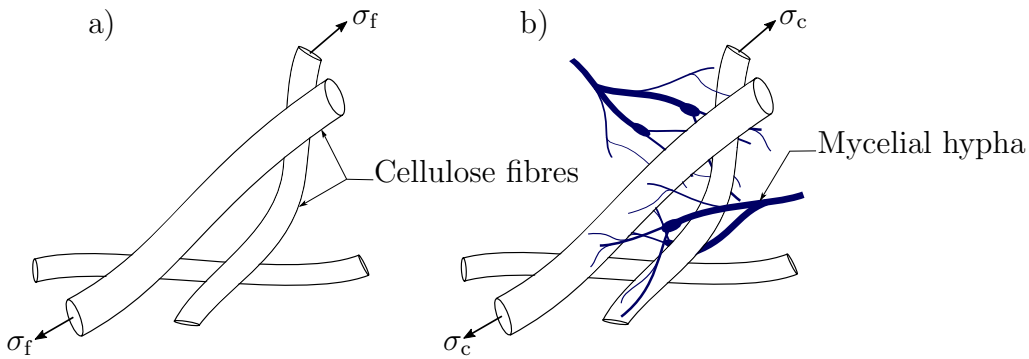


Figure 2.7: a) Interaction between cellulose fibres in foam-paper, b) interaction between cellulose fibres and mycelia in the cellulose-mycelia foam composite.

the matrix can hold until it fails σ_m, is much smaller than tensile strength of the fibre TS_f. Hence, the larger the fraction of matrix f_m is, the smaller the strength of the composite would be if the other factors are constant. The only term left in Equation 2.10 which effects the strength of the composite is the bond between the fibres and matrix. With mycelial growth, the strength of the bond between cellulose fibres and the mycelial strands τ_i increases. As shown in Figure 2.7, the mycelial filament network attach to multiple cellulose fibres improving the connection between individual fibres. This results in reduced critical fibre length l_c. It can be concluded that the tensile strength of the CMF can be optimized by controlling mycelial growth.

Chapter 3

Applications

3.1 Water filtration

Fibrous media in the form of nonwoven filters are widely used in processing water and wastewater as pre-filters [82]. We consider the suitability of the CMF as a biocomposite filter media to eliminate undesirable contaminants from a water system. In this section, the filtration properties of the CMF are studied. The effect of mycelial growth on the pressure drop, permeability, filtration efficiency, and filter quality factor of the CMF are evaluated.

3.1.1 Background

Foam-papers have a very high porosity, high dry filtration efficiency, and a low resistance to flow [18]. If the foam-paper wets, the fibres will absorb water which leads to two significant consequences: the fibres swell, so the void spaces between them reduce which can improve the filtration efficiency, however the mechanical strength drops sharply. To be used as a wet filter, the foam must be fully supported by a strong substrate. One of the original purposes of introducing cellulose-mycelia biocomposite was to provide mechanical support for the foam for liquid filtration. The effect of mycelial growth on the wet strength of the CMF is shown in Figure 2.6, which shows a significant increase in the wet strength with increasing mycelial growth.

The CMF is a wet laid nonwoven filter media with its porous fabric composed of a random array of mycelial fibres within the cellulose fibres. Liquid filtration of nonwoven filter media can not be the only method of purification, because particles of 1 μm or less size can pass through, and further separation is required [66]. It's generally assumed that the flow through nonwoven filter media, which is dominated by viscous effects at

low Reynolds numbers, can be described by Stoke's law. In the creeping flow regime, the effect of gravity and inertia become negligible compared to viscous forces, and Darcy's law can be applied to nonwoven filter medium used in liquid filtration. Darcy's law gives a general description of fluid velocity as a function of pressure gradient, fluid viscosity, and hydraulic permeability. Darcy's law and calculating hydraulic permeability will be discussed further in Section 3.1.3.

3.1.2 Methods

Investigations on the performance of the CMF were conducted for the foam-paper (0 days), 5, 15, and 25 days CMF. The filter pressure drop of samples were measured in a small circuit as shown in Figure 3.1. Each CMF sample was cut into 45 mm diameter disks to fit into the sample-holder in the circuit. Water was pumped into the circuit, and the flow rate was controlled by a low-flow flowmeter with a range of 2 to 22 gph. The pressure drop of the samples were measured by a differential pressure sensor measuring across the inlet and outlet of the sample holder.

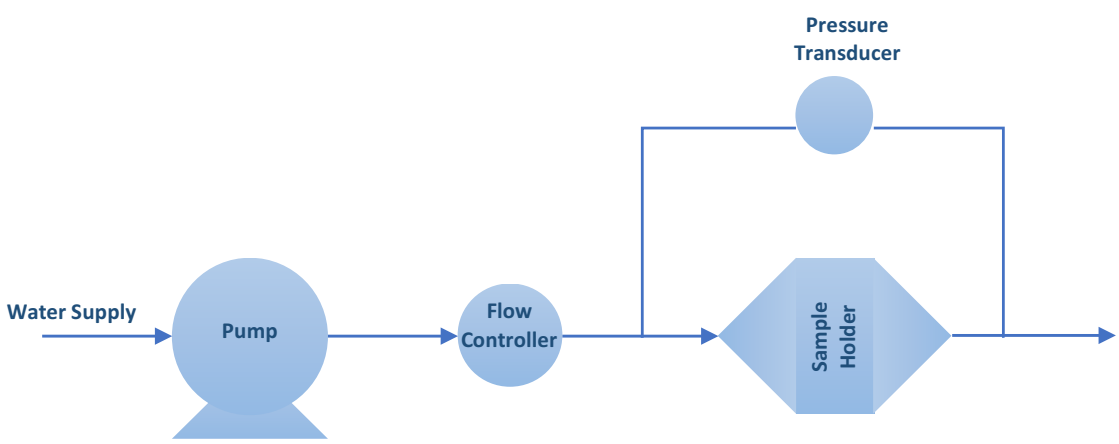


Figure 3.1: The hydraulic circuit for measuring the pressure drop of the CMF filters.

To evaluate the filter performance of the CMF, testing was conducted in accordance

with ISO 12103-1, A2 FINE TEST DUST [83]. Particles varying in diameter from 1 μm to 45 μm were added to the fluid until it reached a specified concentration in grams of dust per litre of water. This solution was circulated continuously at 5 gph ($Q = 5.26 \times 10^{-6}$ m^3/s). The performance of the filters were determined using the Mastersizer 2000 (Malvern, UK) by measuring the particle size distribution of the upstream and downstream flows. The measurements were conducted using a Hydro MU sample dispersion unit, and applying 20 s of ultrasound treatment. In this study, the Mie theory was applied, assuming the following values for the indices: particle refractive index 1.572, particle absorption index 0.1, and refractive index 1.33 for water as a dispersant.

3.1.3 Results and discussion

Figure 3.2 shows the experimental values of the pressure drop against flow rate for various mycelial growth days under continuous loading of water. The results revealed that increasing the growth days of mycelia in the structure of the foam leads to an increased pressure drop(ΔP) at a constant flow rate. The resistance to fluid flow through the CMF is related to mycelial growth that occupies the void spaces between the cellulose fibres which leads to a decrease in the porosity of the final product.

The permeability is a constant intrinsic to the porous medium. The permeability of the filters were calculated by using Darcy's law which describes the flow of a fluid through a porous medium. For a finite 1-D flow as shown in Figure 3.3, Darcy's law is stated as

$$q = \frac{Q}{A} \tag{3.1}$$

$$q = k\frac{\Delta P}{\mu L} \tag{3.2}$$

where Q is the volumetric flow rate (m^3/s), A is cross-sectional area (m^2), q is the volumetric flux (m/s), ΔP is the pressure gradient (Pa), L is the flow path length (m), μ is the fluid viscosity (Pa $\cdot$ s), and k is the hydraulic permeability (m^2). In Figure 3.4 the normalized permeability is shown as a function of mycelial growth days. The results showed that the permeability of the CMF decreases by increasing the mycelial growth

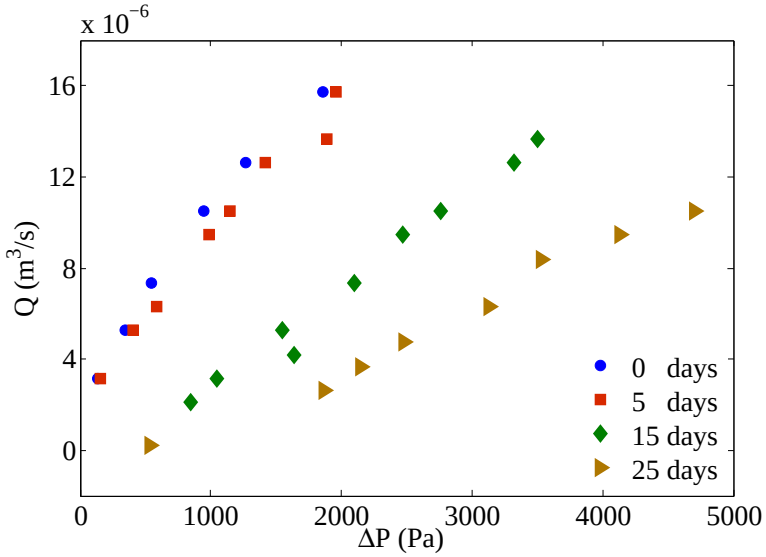


Figure 3.2: The effect of mycelial growth on the pressure drop of the cellulose-mycelia foam under continuous loading of water.

days. An increase in the growth days of mycelia leads to a reduction in the porosity of the final products which diminishes the hydraulic permeability.

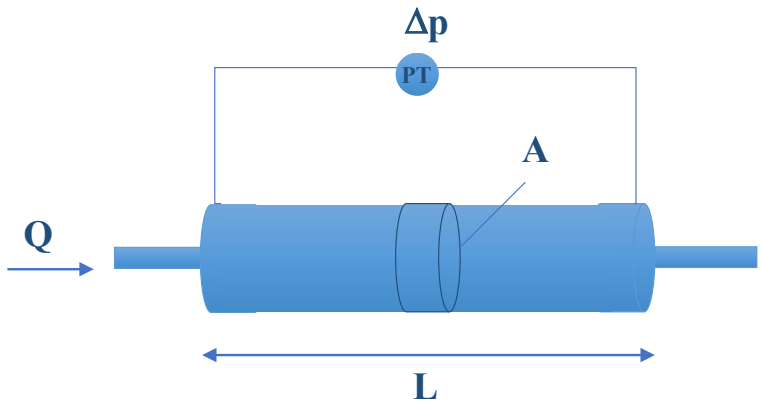


Figure 3.3: Schematic view of a 1-D model of the filtration apparatus used to calculate the permeability of the CMF samples.

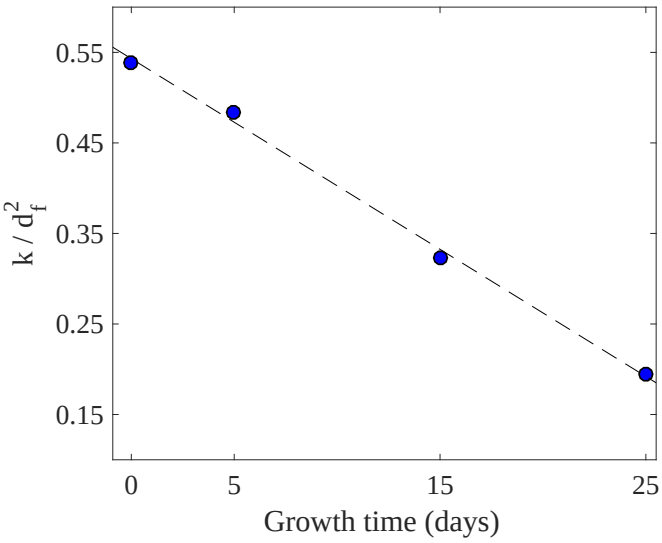


Figure 3.4: The effect of mycelial growth on the normalized hydraulic permeability of the cellulose-mycelia foam.

Filter performance is often expressed in terms of percent efficiency, defined as the ratio of upstream particle concentration compared to the downstream concentration that has passed through. Figure 3.5 shows the hydraulic filtration efficiency of the CMF as a function of particle diameter for different mycelial growth time. An increase in mycelial growth time leads to an increase in filtration efficiency of the product for all particle diameters. The filtration efficiency for foam-paper (0 days) was the lowest and that for 25 days CMF was the highest. This is because the 25 days CMF is lower in porosity compare to the others and interception is the dominant mechanism in hydraulic filtration. It is more likely that the particles touch the surface of the pore wall in the 25 days CMF.

The most commonly used rating in industry to evaluate the hydraulic filter performance is the Beta ratio. Beta ratio is defined as the ratio of the number of particles upstream versus the number of downstream, greater than a given size. Table 3.1 shows the values of Beta ratio and capture efficiency of the CMF filters for 5 μm, 10 μm, and 20 μm. The results showed that the foam-paper (0 days) is 17% efficient at removing

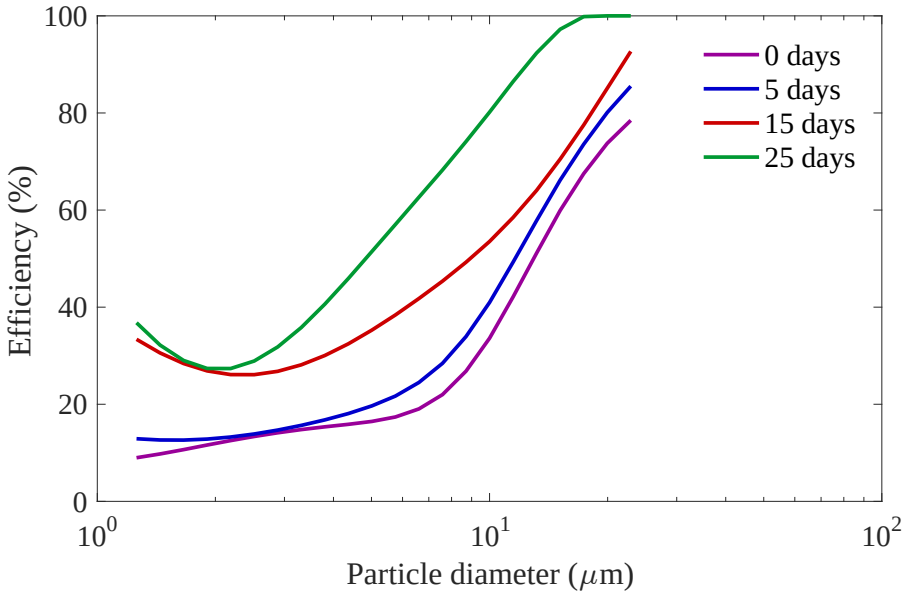


Figure 3.5: The effect of mycelial growth on the hydraulic filtration efficiency of the cellulose-mycelia foam.

5 μm and larger particles, and 74% efficient at removing 20 μm and larger particles, while the 25 days CMF is 50% and 99.9% efficient respectively. The experimental results clearly demonstrated that mycelial growth improves the hydraulic filtration efficiency of the CMF.

Table 3.1: Selected Beta ratio and the corresponding efficiencies of the CMF filters for 5 μm, 10 μm, and 20 μm.

Filter type	$\beta(5)$	Capture efficiency (%)	$\beta(10)$	Capture efficiency (%)	$\beta(20)$	Capture efficiency (%)
0 days	1.2	17	1.5	34	3.8	74
5 days	1.25	20	1.7	41	5	80
15 days	1.5	35	2.1	54	6.7	85
25 days	2	50	5	80	1000	99.9

Filter quality factor is another useful parameter to characterize filter performance which takes both the effect of efficiency and pressure drop into account. Filter quality factor is calculated as

$$QF = \frac{-\ln(1-\eta)}{\Delta P} \tag{3.3}$$

where η is the filtration efficiency, and ΔP is the pressure drop across the filter (Pa). Figure 3.6 shows the calculated quality factor of the CMF for various particle diameters at a constant flow rate ($Q = 5.26 \times 10^{-6}$ m^3/s). The results shows that 15 days of CMF is more efficient than other samples for smaller particles, while 25 days CMF is a better option for larger particles.

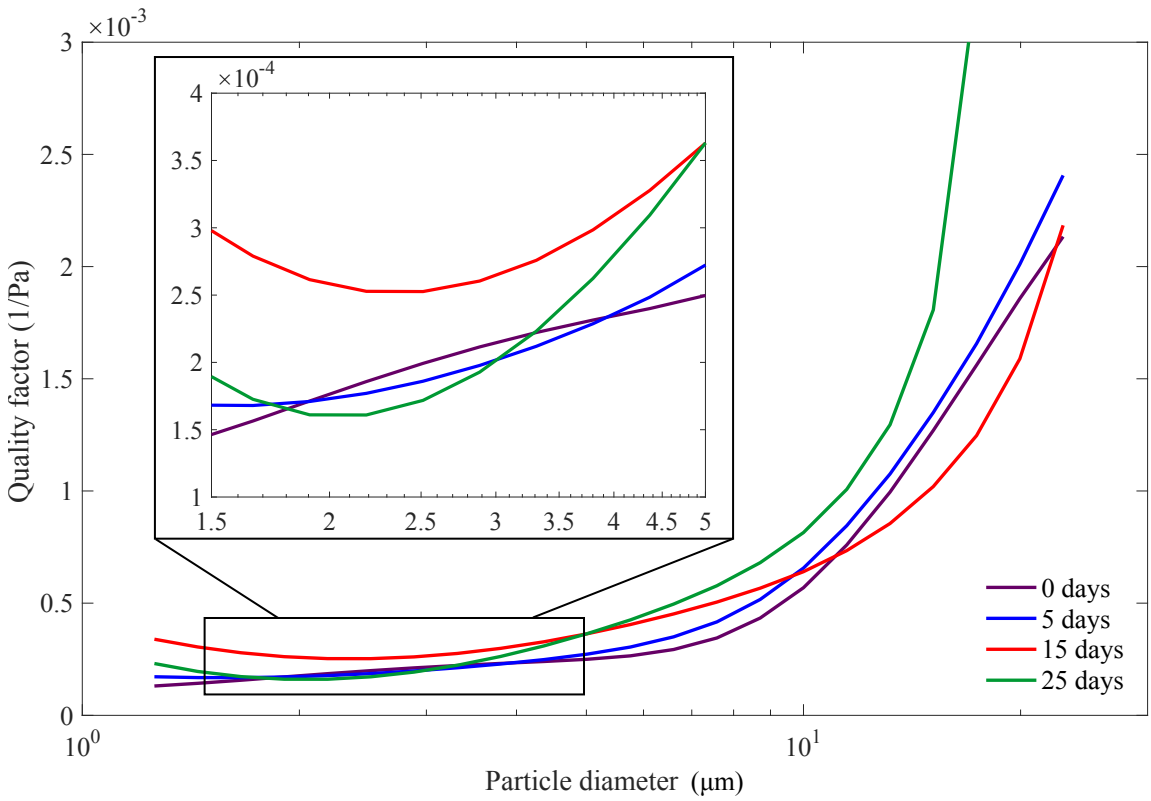


Figure 3.6: Quality factor of the cellulose-mycelia foam as a hydraulic filter media.

3.2 Mycoremediation

The use of fungi to reduce or eliminate contaminate present in water, soils, or air is known as mycoremediation. *Pleurotus* species are a widely cultivated mushroom due to their strong ability to degrade a wide variety of materials and compounds [84]. In this section, the effect of dead and living mycelia on the mycoremediation abilities of *P. djamor* mycelia in the cellulose-mycelia foam to degrade KOH were investigated.

3.2.1 Background

A battery is an electrochemical device which consists of an anode, a cathode, an electrolyte, separators, and the external case. The materials used as electrodes and electrolyte are the main differences between different battery systems. Separators are made of polymeric materials, paper, or paperboard. The external case is composed of steel, polymeric materials or paperboard. The consumption of batteries has increased because of low maintenance, reduced cost, and its requirement by the electronic industry [85]. Based on statistics for Canadian battery sales, primary batteries make up approximately 78% of total sales, by weight, in the country [86]. Alkaline battery is a type of primary battery which is the most common battery chemistry in Canada. It makes up 58% of the total battery market in Canada [86], as shown in Figure 3.7. Today, the majority of these batteries go to landfills at the end of their life cycle. An interest in environmental issues related to battery disposal has been growing [85, 87].

In alkaline batteries, the negative electrode is zinc and the positive electrode is manganese dioxide, and the electrolyte is potassium hydroxide (KOH). The cell is totally enclosed in a high density steel, and a separator is made from non-woven fabric to separate the anode and cathode from the electrolyte solution. A typical initial composition of an alkaline battery is presented in Table 3.2 [88].

The zinc and manganese dioxide are consumed during discharge, while the alkaline potassium hydroxide is not part of the reaction and remains in the batteries. Potassium hydroxide is colourless, odourless, and corrosive. It is soluble in water up to 1 M at 20 °C. This hazardous alkaline may leak by increased duration of residence in landfills. One of the most feasible solutions to potassium hydroxide contamination is to use a

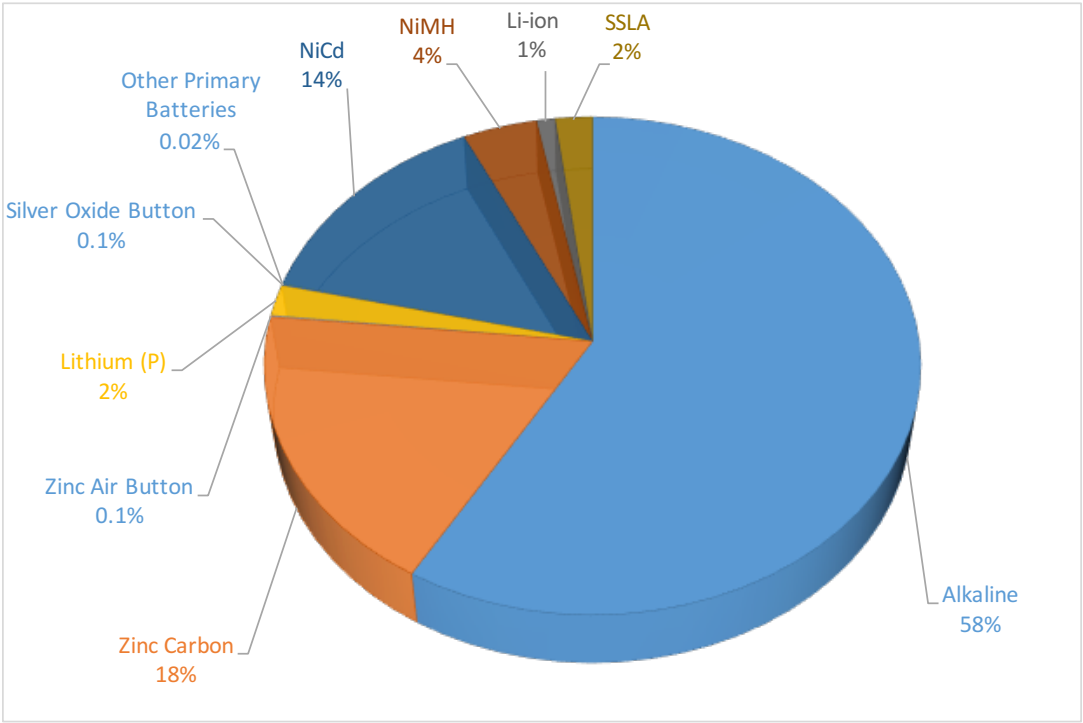


Figure 3.7: Market share for batteries, based on tonnes sold in Canada by Kelleher Environmental [86].

bioremediation process. This study evaluates the ability of the CMF samples to detoxify potassium hydroxide from discharged alkaline batteries.

Table 3.2: An average composition of Alkaline battery [88].

Battery component	Typical composition (%)
Manganese dioxide	32 – 38
Zinc	11 – 16
Potassium hydroxide	5 – 9
Graphite	3 – 5
Steel	19 – 23
Barium sulfate	<5

3.2.2 Methods

To investigate the bioremediation capabilities of the CMF samples, four different samples were used: foam-paper, live mycelia after 5 days of growth, live mycelia after 25 days of growth, and dried CMF after 25 days of growth. Before adding live and dead samples, leaking AA alkaline batteries were placed in the water to obtain strong base toxic solution. The equal amount of solution were added to different containers. The pH of all containers were 12 at the first day of experiment. Then, all different samples were add to the container. A pH meter was used to measure the pH of solutions every 5 days for 60 days. A change in pH indicates the amount of remaining potassium hydroxide in the solution.

3.2.3 Results and discussion

Figure 3.8 shows the pH of solutions as a function of time. The pH of control samples (foam-paper) showed insignificant changes which can likely be attributed to experimental uncertainty in the pH measurement. The pH of the solution with dead samples after 25 days of incubation decreased slightly for 20 days and then remained the same for another 40 days. The pH of the solution for both living mycelia added after 5 days and 25 days of incubation dropped during the 60 day test period. The results for living mycelia after 25 days of incubation showed a sudden decrease in the amount of pH after 20 days.

A decrease in the amount of pH shows a reduction in the hydroxide ion concentration which shows potassium hydroxide was used as a nutrient for mycelia by biosorption mechanism. The following equations define the relation between the pH and the hydroxide ion concentration.

$$\mathrm{pOH} = 14 - \mathrm{pH} \tag{3.4}$$

$$[\mathrm{OH}^-] = 10^{-\mathrm{pOH}} \tag{3.5}$$

It can be concluded the living mycelia have the ability to detoxify the potassium hydroxide solution by biosorption. Figure 3.9 shows the various biosorption mechanisms according to the dependence on the cells metabolism [89]. Veglio *et al.* [89] demonstrated

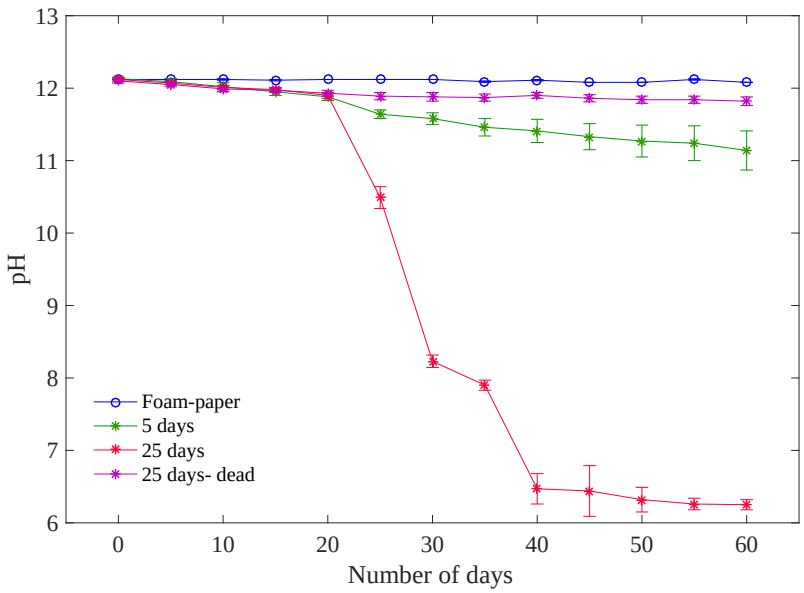


Figure 3.8: Detoxification of KOH by dead and live mycelia of *P. djamor* in contaminated water during 60 days.

that transport across the cell membrane is associated with cell metabolism and may take place only with viable cells. This method of biosorption is not immediate, since it requires the time for the reaction of the microorganism. It is hypothesized that mycelia transport the ions across mycelial cell membranes and convey potassium as an essential mineral nutrient. This is why more mycelial growth leads to faster detoxification as living samples after 25 days incubation obtained the best results during the 60 day test period. However, the slight decrease in the pH for dead samples can be attributed to physicochemical interaction between the ions and functional groups of the cell surface, based on physical adsorption, ion exchange, and complexation which are not dependent on the metabolism. Also, precipitation is another possible theory, which is either dependent on the cellular metabolism or independent of it [89]. Mycelia may excrete compounds which favour the precipitation process.

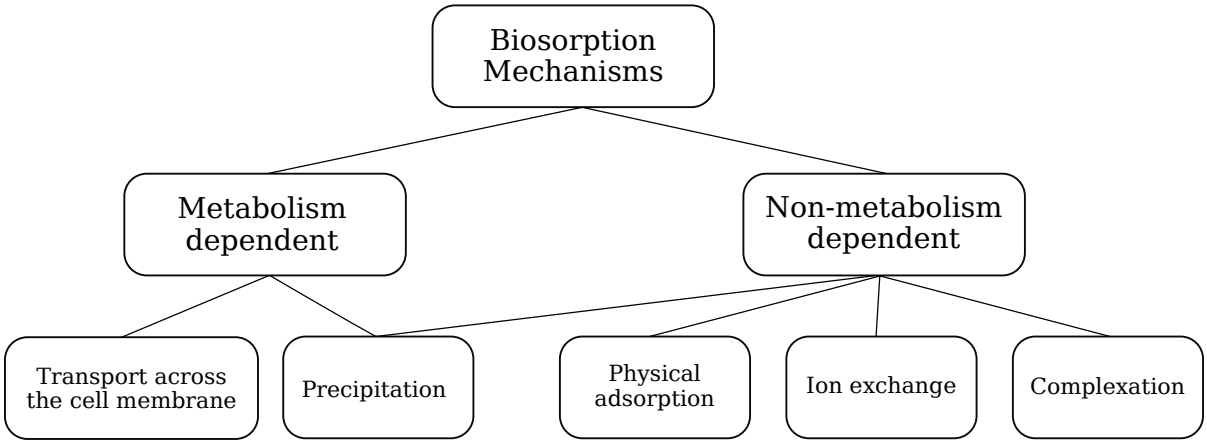


Figure 3.9: Biosorption mechanism according to the dependence on the cell metabolism.

Chapter 4

Conclusions and future work

Since the industrial evolution, humans have had a widespread impact on the planet through industrial wastes and pollutants, and the demand for sustainable products continues to grow. The development of green and biodegradable products is crucial to help ecosystem to regenerate. Cellulose mycelia foams were developed by using foam-forming process in paper industry. These green biocomposite consist of mycelial strands within the cellulose fibres which have a 3D porous structure. The experimental tests show reasonable results which suggest these biocomposite can be useful in a wide range of applications.

The morphological study of the CMF showed that mycelial strands replace the cellulose fibres by decomposition and occupy the void spaces between fibres which lead to a reduction in the porosity of the product. The effect of mycelial growth on the mechanical properties of the CMF were studied. The thermal gravimetric analysis showed increasing mycelial growth days increases the onset temperature of thermal decomposition. The mycelial growth improved the thermal stability of the CMF. The 25 days of mycelial growth improved the onset temperature by 40% compared to the foam-paper. The strain-stress curve of the CMF was obtained by dynamic mechanical analysis. An increase in mycelial growth caused an increase in the plateau stress and a significant reduction in the densification initial strain which leads to better energy absorbing properties. The effect of mycelial growth on the dry and wet strength of the CMF also were studied. The wet and dry tensile index increases by increasing the growth time of mycelia. The dry tensile index improved from 0.61 Nm/g for foam-paper to 2.65 Nm/g for 25 days CMF. The wet tensile index also increased from 0.31 Nm/g for foam-paper to 1.38 Nm/g for 25 days CMF.

The hydraulic filtration properties of the CMF were evaluated by using a small flow

circuit to measure the pressure drop and the particle size distribution of the upstream and downstream flows. The experimental results showed that the resistance to fluid flow through the CMF is related to the mycelial growth time. The results also showed that an increase in mycelial growth leads to a decrease in the permeability, and an increase in filtration efficiency of the product. The experimental results showed that 25 days CMF is 50%, 80%, and 99.9% efficient at removing particles larger than 5 μm, 10 μm, and 20 μm respectively.

To investigate the detoxification capabilities of the CMF samples, the pH of leaking alkaline battery in water were measured every 5 days for 60 days. The results showed that living mycelia is effective to detoxify potassium hydroxide, but the dead mycelia is not, and the CMF samples are not able to detoxify inorganic compounds.

The 3-D, lightweight, highly porous CMF can be applied in a wide range of applications and provide a green alternative to polymeric foams. Due to the time limitation of this project, some characteristics of the product were studied. To have a better understanding of the CMF and its suitability for different applications, other characteristics of the product can be evaluated in future projects such as sound absorption coefficient, air permeability, air filtration efficiency, and thermal conductivity of the CMF. Another area of study which can be significant to increase its commercial viability is working on reducing the incubation time of mycelia. In other words, finding the optimum incubation conditions to obtain the same amount of mycelia in shorter time is crucial to reduce production costs.

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