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# Polyethylene Based Materials for Biofilm Carriers Used in Wastewater Treatment

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**Abstract.** The moving bed biofilm technology is based on biofilm carriers on which consortia of microorganisms attach, develop and grow. Around the world are known many biofilm carrier variants made of varied materials. The most common materials are based on polyethylene since this material has a close to water density. The authors propose a novel biofilm carrier to be used in tertiary treatment for tannery and paper-mill wastewaters. The biological treatment is based on fungal activity. The selected fungal strains will be grown on innovative polyethylene carriers containing cellulose. The carrier will be designed to be exploited in a moving bed bioreactor and to favour fungal growth in the presence of competing bacteria.

## 1. Introduction

Industrial wastewaters can often be recalcitrant to conventional biological treatment methods, due to their complex constitutive matrix (extreme chemical-physical parameters, bio-burden, presence of complex aromatic compounds etc.) [1]. Tannins and Absorbable Organic Halogen compounds (AOXs) are keen to damages aquatic environments. Moreover, they are produced by industrial activities that are highly water demanding. At present, treatment of effluents with high cellulosic content is mostly carried out by aerobic processes (activated sludge), which are not fully effective due to high organic matter concentration (high content of cellulose) and the presence of cellulose derived compounds, among others organochlorine compounds [2]. Reactors used in the treatment of high content cellulosic unsegregated wastewaters, do not respond to final emissary quality demands in terms of chemical oxygen demand (COD) and in terms of content of chlorinated compounds generally defined as absorbable organic halides (AOX). Current techniques of removal of chlorinated phenolic compounds and AOX, are based on membrane filtration and ozonation, energy demanding processes.

Approximately 85% of the water of the pulp and paper-mill industry consumes is only used for processing, leading to generation of massive amounts of contaminated wastewater. The Food and Agriculture Organization (FAO) of the United Nations Secretariat estimated that total effluent discharged annually from pulp and paper-mills is about 40,000 million cubic meters (~200 m<sup>3</sup>/t of pulp and paper). Generated from a variety of manufacturing processes, these discharges often serve as a primary source of pollution for aquatic life, public health and the environment, by generation of considerable amounts of toxins contained in the effluents. Pulp and paper-mill effluents have also regularly undergone secondary biological treatment to remove organic matter such as residual total suspended solids (TSS) and biological oxygen demand (BOD) and address COD and AOX.



Filamentous fungi strains have been highlighted as possessing great applicative potential in the field of wastewater treatment [3]. In recent light, White-Rot-Fungi (WRF) and Ascomycota (ASC) representatives have been successfully used in bio-remediation technologies of polluted effluents [4]. WRF strains possess several adaptive mechanisms to the environment that involve the battery of oxidative enzymes, which are also involved in bioremediation of wastewater effluents processes [5]. This aspect can lead to a greater efficiency of fungal strains, when compared to bacteria, in regards of oxidative mechanisms of environment adaptation [6]. In this context WRF are more efficient than bacteria in expressing oxidative mechanisms of adaptation to the environment.

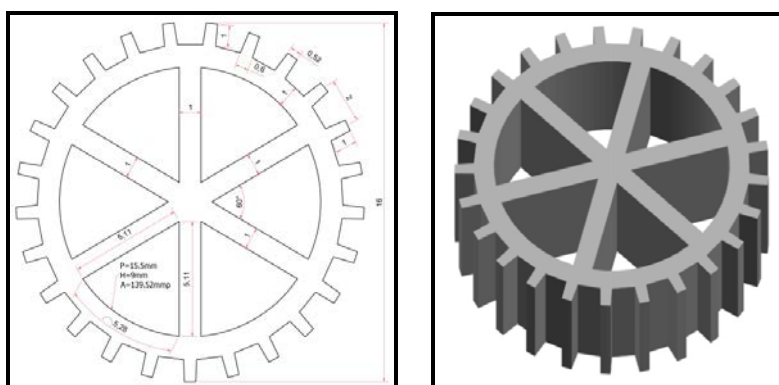
However, these technologies still face great applicative challenges, as fungal mycelium is prone to mechanical breakage, inside the treatment plant, during process. Immobilization of these microbial structures in rigid structures may prove as a viable solution for both mechanical integrity of the microorganism, and maintenance of treatment efficiency. This complex problem will be tackled in FunCell project (COFUND-MANUNET III-FUNCELL, MNET17/ENER-1143, entitled “Exploiting fungi potential for recalcitrant compounds removal from cellulosic wastewaters”), which consists is the development of an innovative myco-based tertiary treatment for tannery and paper-mill wastewaters efficient in removing tannins and AOX, not depleted by consolidated bacterial-based processes. Previous literature work has already explored denitrification properties of some fungal strains in MBBR systems, with higher performance yields that bacteria-driven system [7].

The FunCell technology is dedicated to the depletion of chemical structures recalcitrant to the bacterial oxidative machinery: tannins, AOX and cellulose.

## 2. Moving bed biofilm reactors (MBBRs)

During the last years, the MBBR technology for wastewater treatment [8] has been intensively studied and developed [9], but not already applied in combination with WRF/ASC for wastewater treatment.

New MBBRs structures will be designed, for fungal biofilm attachment, grafted with cellulose that will be tested at different concentration to stabilize growth conditions, in order to improve the fungal competitiveness in non-sterile condition where usually bacteria are overwhelming [10]. The project proposes a new biomedica design, starting from existent polyethylene biofilm carrier (Figure 1), which will be tested to support fungal growth in a not sterile environment typical of wastewater treatment.



**Figure 1.** Proprietary Moving Bed Biofilm Reactor design.

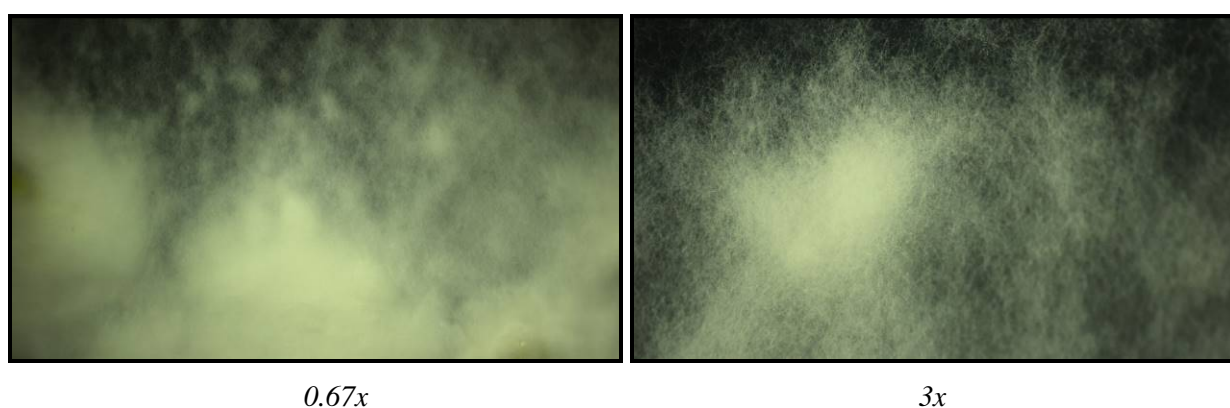
The new biofilm carrier (based on a mixture between high density polyethylene and other materials) is aimed at finding a tradeoff between packed bed and moving bed biofilm reactors and is aimed at differentiating and fine-tuning the gradients of substrate and the shear stress across the reactor bed. Such type of product is relatively new in the field of wastewater treatment, where usually gradients are modulated either in time or in space only at micro-scale level and can be eventually exploited also for bacterial ecosystems.

### 3. Microbial component

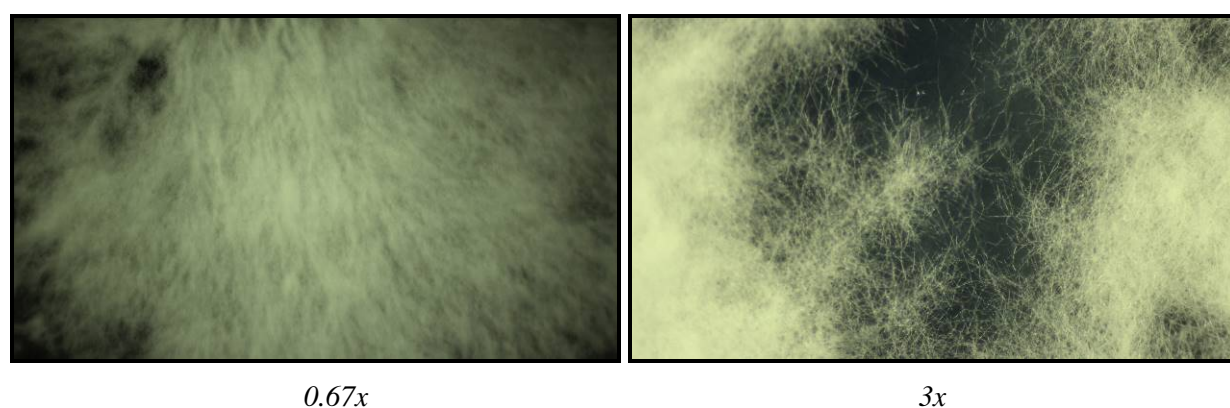
For in-situ testing a pilot MBBR for fungal biomass will be realized for exploiting the capacity of the WRF to produce the battery of extracellular enzymes that are involved in the depletion of recalcitrant compounds. FunCell project will exploit the intracellular enzymes machinery that is at the base of ASC's metabolic capacity towards contaminants of the environment that generally ASC colonize all aspects extremely innovative in the actual scenario of the wastewater treatment.

Preliminary work has already been carried out in the direction of the FunCell project research, and several Ascomycota and Basidiomycota representatives have been successfully grown at fermenter level (Biotec FE 007). Strains were grown in Potato-Dextrose nutritive broth, for 7 days, at 28°C, in 400 mL broth volume, with continuous oxygenation and agitation set to approximately 300 rpm.

The following strains have been grown at both fermenter level, and then passed on PDA plates, for microscopically morphology assessment: of Basidiomycota division: *Ganoderma lucidum* (Figure 2), *Pleurotus ostreatus* (Figure 3), *Polyporus squamosus* (Figure 4), *Agaricus bisporus* (Figure 5), and of Ascomycota division: *Fusarium oxysporum* (Figure 6).



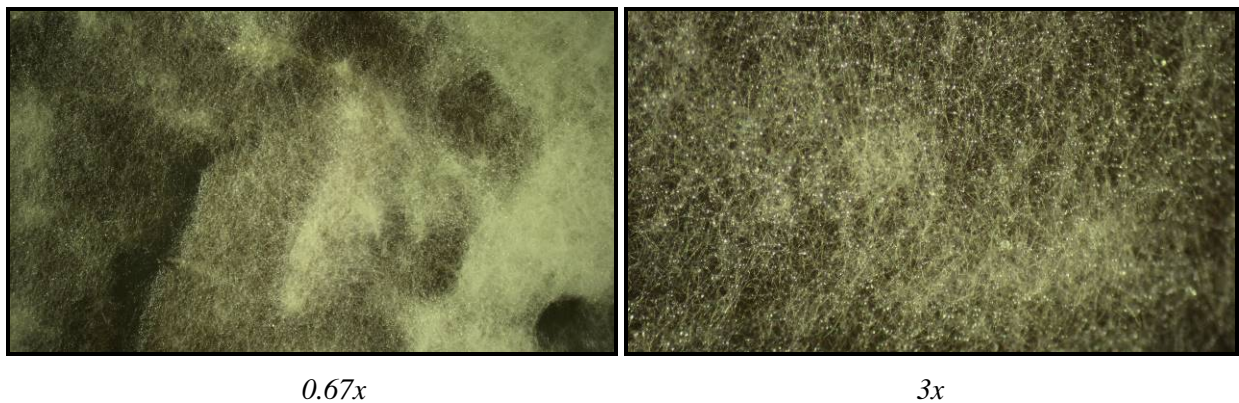
**Figure 2.** *Ganoderma lucidum* morphological analysis.



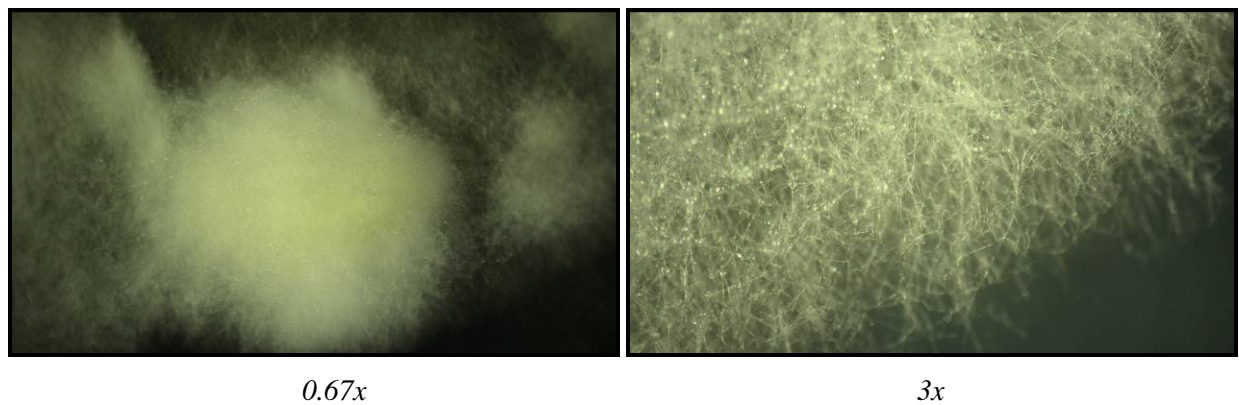
**Figure 3.** *Pleurotus ostreatus* morphological analysis.

Furthermore, for future scaling up of the experimentation [11], the selected fungal strain will be grown on innovative polyethylene carriers containing cellulose, which will allow rapid metabolic development, for greater biomass yield and mechanical fixation inside the MBBRs structures (Figure 7). The new treatment technology will be developed and designed as self-sustaining by the exploitation of an innovative fungal carrier containing cellulose [12]. The growth conditions will allow the exploitation of cellulose as selective carbon source for fungi in environments where bacteria are competitive and can inhibit fungal development.

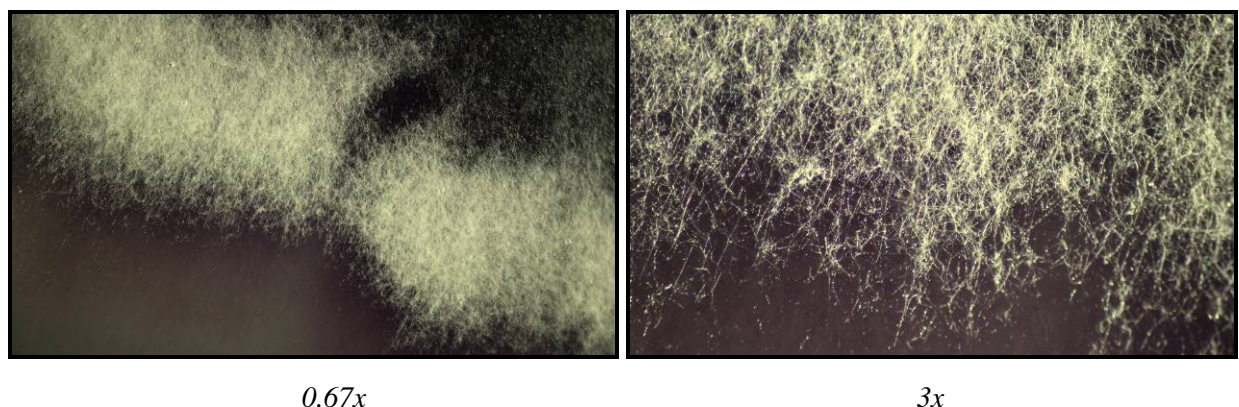




**Figure 4.** *Polyporus squamosus* morphological analysis.



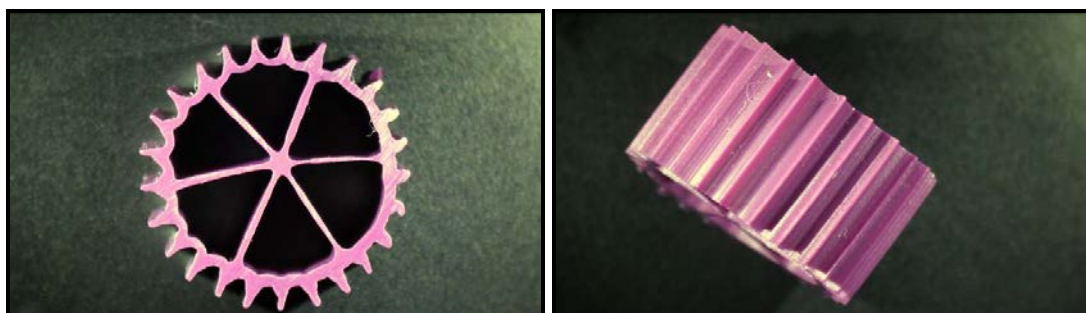
**Figure 5.** *Agaricus bisporus* morphological analysis.



**Figure 6.** *Fusarium oxysporum* morphological analysis.

FunCell project proposes a novel carrier design, grafted with cellulose fibers for the self-sustainability of the fungal biomass.

This will facilitate substrate adhesion, due to biochemical capabilities of the selected strains, which involve secretion of extracellular enzymes, which can break down substrate by combined action of several degradative processes, such as dimethylation, oxidative cleavage of the propane side chain, cleavage of ether bonds between monomers etc.



**Figure 7.** MBBRs microscopically analysis.

#### 4. Conclusions

In the last decades, European regulations on effluents discharge limits became increasingly stringent and several industries found more cost effective to relocate than to adapt to the new limits. This phenomenon impacted several strategic industrial sectors such as textile and tannery industries, pulp and paper, food and beverage and petrochemical industries.

FunCell project is oriented to create non-conventional processes performing better than the “best available technologies”, in the removal of both tannins and AOXs from the effluents and to promote the re-use of treated water and the adoption of a common policy in the frame of the Communitarian and National regulations. The obtainment of a high-quality effluents and effluents with a water quality suitable for reuse will be the two main needs addressed by the project. Also, the goals will be achieved by developing an MBBR treatment specifically designed for fungal metabolism, producing lower amount of sludge accompanied by a higher removal efficiency of pollutant agents. The re-utilisation of the treated wastewaters inside the services of the industrial plants will determine a decrease in the consumption of water in the same sectors.

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