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MBBRs FUNCTIONALIZATION WITH *CERIOPORUS SQUAMOSUS*

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Abstract

Most widely applied technologies wastewater treatment biological technologies are based on the selection of microbial communities dominated by bacteria (activated sludge).Wastewater treatment through Moving Bed Biofilm Bioreactors (MBBRs) has been widely used during the last years. The wastewater treatment with moving bio media consists in adding biofilm carriers (small cylindrical/ round/ plate shaped polyethylene/polypropylene/ polyurethane foam carrier elements) in aerated or anaerobic basins to support biofilm growth. The paper explored the functionalization of MBBRs (polyethylene support) structures in a Biotec FE 007 fermentor, with Cerioporus squamosus strain (a basidiomycete bracket fungus). Scanning Electron Microscopy and optical microscopy analysis were carried out pre and post microbial functionalization on the MBBRs in order to assess the colonization of the MBBRs structures and internalization of microbial biomass. The functionalized MBBRs will be further used in experiments of removal of recalcitrant compounds from cellulosic wastewaters.

Key words: *MBBRs, wastewater, Basidiomycota, fungi.*

INTRODUCTION

Worldwide there are several wastewater treatment processes available. Most widely applied technologies are based on the selection of microbial communities dominated by bacteria (activated sludge). Even though bacteria are effective (Calderón et al., 2012) in the removal of most of the organic compounds from municipal wastewaters, industrial wastewaters contain a significant concentration of several compounds that are not biodegraded by bacteria.

These compounds need to be removed by further treatments grafted on the main pipe of the primary treatment, usually consisting in chemical-physical technologies, demanding for reagents and energy and generating sludge.

Biofilm reactor configurations applied in wastewater treatment include trickling filters, high rate plastic media filters, rotating biological contactors, fluidized bed biofilm reactors, airlift reactors, granular filters, and membrane immobilized cell reactors. A general division between fixed and moving bed processes based on the state of the support material is usually done. Fixed bed systems include all systems where the biofilm is formed on static media such as rocks, plastic profiles, sponges, granular carriers or membranes.

The wastewater treatment with moving bio-media consists in adding biofilm carriers (small cylindrical/round/plate shaped polyethylene/ polypropylene/polyurethane foam carrier elements) in aerated or anaerobic basins to support biofilm growth.

A rotating biological contactor is a biological treatment process used in the treatment of wastewater following primary treatment.

It consists of a series of closely spaced, parallel discs mounted on a rotating shaft which is supported just above the surface of the wastewater.

Microorganisms grow on the surface of the discs where biological degradation of the wastewater pollutants takes place. The rotating packs of disks are contained in a tank or trough and rotate at between 2 and 5 revolutions per minute. The shaft is aligned with the flow of wastewater so that the discs rotate at right angles to the flow with several packs usually combined to make up a treatment train (Lazarova and Manem, 2000).

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Moving bed systems comprise all biofilm processes with continuously moving media, maintained by high air or water velocity or mechanical stirring. Biofilm carrier material (media or bio media) is selected based on size, porosity, density and resistance to erosion. By using a material with a large specific surface area (m^2/m^3) high biological activity can be maintained using a relatively small reactor volume. Small parts made of special materials with close-to-water-density, are immersed in bioreactors. The biofilm carriers are kept in suspension and even mixed with the help of air bubbles generated by the aeration system (aerobic bioreactors) or with the help of a mixer (anoxic bioreactors). This type of support is most effective because it is not clogged. Worldwide there are several models of biofilm carriers.

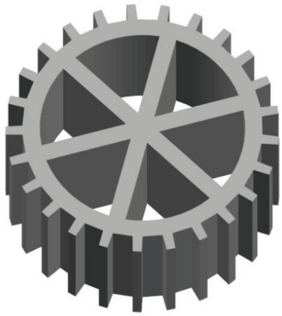
The MBBRs process is based on the aerobic/anoxic biofilm principle and utilizes the advantages of activated sludge and other biofilm systems without being restrained by their disadvantages. The biofilm carriers provide a large protected surface area for the biofilm and optimal conditions for the bacteria culture to grow and thrive. The biofilm that is created around each carrier element protects the bacterial cultures from operating excursions to yield a very robust system for those industrial facilities loaded with process fluctuations.

In recent years, fungi have been largely investigated in wastewater treatment and their potential in the removal of recalcitrant compounds was demonstrated. Thanks to their extracellular enzymes, such as laccases (Spina et al., 2015), peroxidases (Chen et al., 2015) and tannases (Salgado et al., 2016), active on a broad range of substrates, fungi are able to degrade several recalcitrant compounds (pharmaceuticals, endocrine-disrupting compounds, PCBs, herbicides, dyes, tannins etc.). Moreover, the fungal initial oxidative attack increases the biodegradability of the recalcitrant compounds, improving the effectiveness of bacteria in activated sludge.

MATERIALS AND METHODS

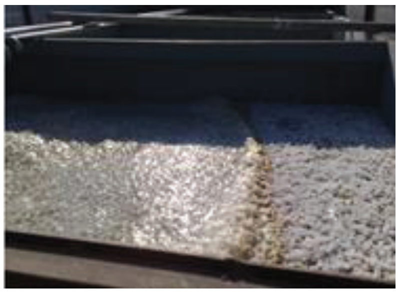
MBBRs

DFR Systems has obtained results in using MBBRs technology and holds a patent for a model of biofilm carrier (Patent no. RO 123174/28.01.2011), (Figure 1).



a) vectorized MBBRs

b) carrier with biofilm



c) MBBRs in treatment tank

Figure 1. Biofilm carrier

In MBBRs systems the biofilm grows protected within small plastic carriers, which are carefully designed with high internal surface area.

These biofilm carriers are suspended and mixed throughout the water phase. The biological wastewater treatment process consists of adding biofilm carriers in aerated or anoxic basins to support biofilm growth. When the microorganisms in the attached biological film die, the film breaks up and peels off from the solid support being carried away by the liquid current. The destroyed cellular material is removed as sludge.

Fungal strain

Cerioporus squamosus is a basidiomycete bracket fungus (also known as dryad's saddle or pheasant's back mushroom) (Spahr, 2009), and has special importance in natural ecosystems, being able to degrade a wide range of cellulosic substrates. The strain develops Dimitic hyphae, which are composed of skeletal hyphae (axial or inflated) (Zmitrovich, 2016).

Fresh culture strain was started in Potato-Dextrose Nutrient Broth (Scharlau), for 4 days at 28^{o}C, at fermenter level (Biotec FE 007), in a final volume of 500 mL, environmental volume for 72 hours at 28°C under aerobic conditions (continuous oxygenation) and agitation set to 250 rpm (Figure. 2).

Figure 2. *Cerioporus squamosus* growing at fermenter level

After strain growing, for 4 days at 28^{o}C, MBBRs pieces (previously sterilized at 121^{o}C for 15') were put in the fermenter, and the process was further continued for 7 days at 28^{o}C.

SEM analysis

Microscopic analysis and morphological characterization was performed by scanning electron microscopy (SEM) using a Quanta 200, Fei (Netherlands) electron microscope (Figure 3). SEM analysis was also conducted on *Cerioporus squamosus strain* grown on Sabouraud-Agar plates, incubated at 28^{o}C for 14 days, for hyphae development, and compared to liquid media growth, which was used for MBBRs functionalization.

Scanning Electron Microscope

SEM specimen chamber

Figure 3. Scanning Electron Microscope, Quanta 200, FEI

Morphological analyses were performed on the GSED detector, ESEM mode, spot beam size of 4.0, 10kV filament voltage, with image acquisition at 27.2 seconds.

Optical microscopy analysis was carried out on an Olympus SZX7 stereomicroscope, with 7:1 zoom ratio, built-in electrostatic discharge protection, and advanced Galilean optical system for highly resolved images. Analyses were carried out at a magnification level of 0.8X, on both treated and untreated MBBRs.

RESULTS AND DISCUSSIONS

Indigenous Ascomycota are the dominant fungal phylum in polluted environments, where they are able to transform or remove also significantly stable and toxic recalcitrant (Marco-Urrea et al., 2015). Polluted wastewaters represent a source of Ascomycota,

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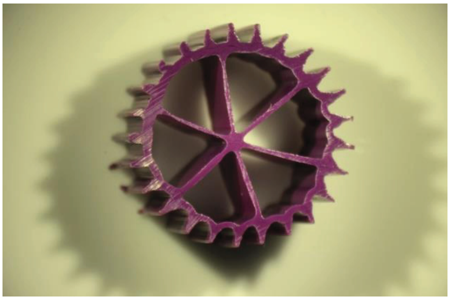
putative degraders for which studies are surprisingly scarce.

In this context, many unstudied fungal species need to be explored, to understand their specific interactions in engineered ecosystems, because they appear to play a primary role in actual polluted scenarios. Some Ascomycota undoubtedly possess great potential for bioremediation purposes (Mariner et al., 2008), and the key seems to be the intracellular enzymatic machinery coded in their genome, rather than the extracellular battery of oxidative enzymes already described for White Rot Fungi.

Optical microscopy analyses revealed *Cerioporus squamosus* biomass deposition inside the MBBR structure (b) when compared to untreated MBBRs (a) (Figure 4).



a) control MBBRs

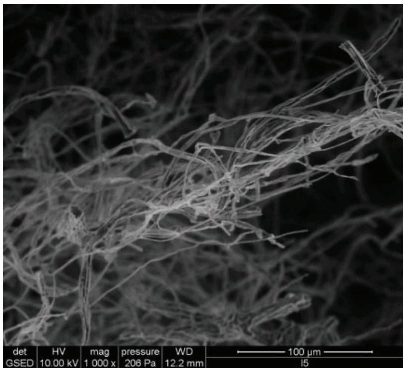
b) functionalized MBBRs

Figure 4. Internalization of biomass inside the MBBRs

Analysis highlighted attachment of microbial biomass inside the MBBRs, but not on the external spaces of the polymeric structures. It can be highlighted that a longer process (more than 7 days) could translate into a higher quantity of biomass that will be internalized between the MBBRs empty spaces (inside the structure).

Another aspect that was noted is that the biomass was not firmly attached by the polymer surface, and could be easily washed.

SEM analysis on *Cerioporus squamosus* standalone strain, grown on Sabouraud-Agar Petri plates, carried out at pressure levels between 200Pa and 208Pa highlighted a fibrillated, branched structure with a high degree of coverage on the carbon band and hyphae size ranging from 1.60 µm to 2.72 µm, for 4000x magnification analysis. The strain has a multi branches hyphae organization, these having dimensional width variations, and pyramidal structure in section (Figure 5).



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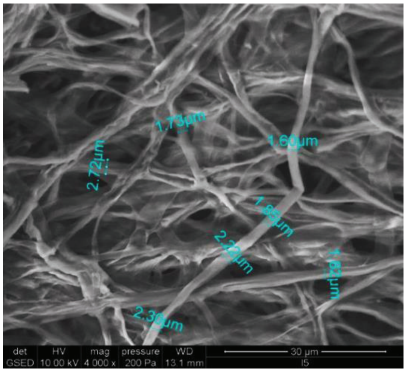
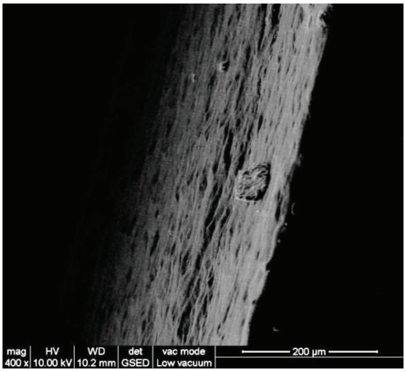


4000x

Figure 5. SEM analysis of morphological characters for *Cerioporus squamosum* strain

Scanning Electron Microscope analysis was carried out on the control MBBRs, but due to high magnification levels allowed by the equipment, combined with the dimensions of the MBBRs, only details of the polymeric walls could be assessed (Figure 6).



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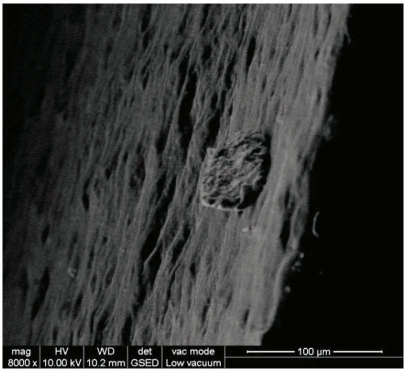

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Figure 6. SEM analysis of control MBBRs

SEM analysis allowed highlighting of a rough surface, at microscopically level, of the MBBRs, which can lead to a greater degree of mechanical attachment of microbial biomass, when compared, for example, with a smooth surface structure.

The internal structure of the MBBRs allows for high quantities of microbial biomass fixation, having a total internal contact area of 837.12 mm^2 (Figure 7).

Furthermore, SEM analysis was conducted in order to assess the morphological characterization of *Cerioporus squamosus* MBBR internalized biomass (Figure 8), grown in submersed environment (fermenter level).

The strain biomass grown in submerged conditions, under aeration presented the same morphology as that grown on solid media plates, highlighting high biomass yield even in only seven days of growth.

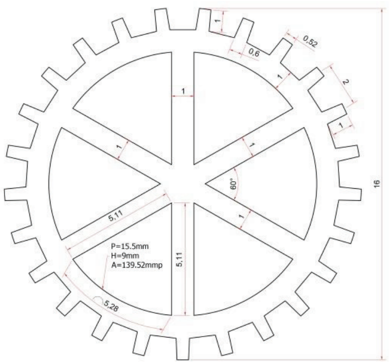
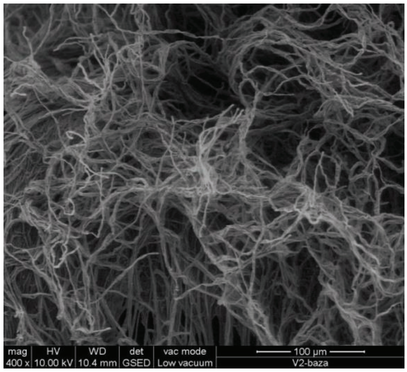


Figure 7. Artificial mobile support (scale 10:1)



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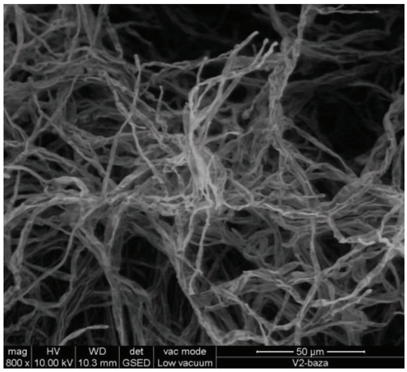

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Figure 8. SEM analysis of morphological characters for *Cerioporus squamosum* strain

CONCLUSIONS

Despite the massive work already done by the scientific community, biotechnologies based on fungi are not mature for full scale application in wastewater treatment.

The present research work successfully explored the functionalization of moving bed biofilm reactor structures with *Cerioporus squamosus* fungal strain, in a fermenter control conditions, and allowed microscopically characterization of the internalized biomass. Future research work will be centred on development of new composite materials with cellulose (cellulose functionalized MBBRs) which will allow the fixation and growth of certain cellulolytic microbial species, and further used in treatment of wastewaters originated from paper-mill and cellulose industry.

The successful implementation of biological processes based on fungi, and their integration with activated sludge, will deeply innovate the state-of-the-art towards more sustainable standards from both an economic and environmental point of view.

The EU industry will benefit from the application of new biological treatment technologies, thanks to more efficient and cost-effective solutions to mitigate their energetic impact and improving effluents quality. Moreover, the proposed technologies must be directly related to greenhouse gas emissions reduction.

The application of biological processes for various compounds removal from sources of wastewater is addressed to save energy since contrarily to other alternative technologies; it does require a lower consumption of energy and chemicals.

This is particularly important in a scenario where climate change has to be considered. Finally, the potential reduction of pollution both at global (reduced GHG emissions) and at local level (improved effluent quality) will improve the quality of the environment with further social and economic benefits.

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