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BIO-AUGMENTATION OF POLYETHILENE BIOFILM CARRIERS BY *Cerioporus squamosus* WHITE ROT FUNGI

Ovidiu IORDACHE[1], Ioana Corina MOGA[2], Cornelia MITRAN[1, 3], Dana CIUTARU[1], Irina SANDULACHE[1], Lucia SECAREANU[1], Gabriel PETRESCU[2], Elena PERDUM[1]

[1]National R&D Institute for Textile and Leather (INCDTP), 16 Lucretiu Patrascanu Street, District 3, Bucharest, Romania
[2]DFR Systems L.L.C., 46 Drumul Taberei Street, District 6, Bucharest
[3]Politehnica University of Bucharest, 313 Splaiul Independentei, District 6, Bucharest

Corresponding author email: iordacheovidiu.g@gmail.com

Abstract

*Fungi have been widely used in industrial wastewater treatment, as real alternative to conventional treatment methods. Specific filamentous fungi (FF) have been used for sludge treatment, bioflocculation, SS concentration reduction, degradation and removal of certain toxic compounds etc. Very few research works exploited the use of fungi augmented carriers inside MBBR systems. Present work explored the bio-augmentation experiments of four polyethylene based carriers with strain of Cerioporus squamosus (*syn. *Polyporus squamosus*), *a basidiomycete bracket fungus. Bio-augmentation was carried out in various conditions (varying certain process parameters) in order to facilitate growth of strain inside the carriers' structure, which were composed of a mix of polyethylene with inorganic and organic compounds, especially designed in order to allow microbial growth inside the carriers' internal space.*

***Key words**: fungi, MBBR, reactors, wastewater treatment.*

INTRODUCTION

HDPE carriers are the heart of a Moving Bed Biofilm Reactor (MBBR) system for treating of industrial wastewater. This system offers a real efficient alternative to traditional methods of wastewater treatment (with activated sludge) (Kruszelnicka et al., 2018). MBBR processes can be successfully used in industrial application, being characterized by quality improvement in BOD and nitrogen removal, limited footprint of the installation, modularity (for future scaling of the plant), minimization of process complexity in terms of operator input, fast recovery from process upsets. Currently, several compositions carriers are being used in the modern MBBR systems: polypropylene (PP), low-density polyethylene-polypropylene (LDPE-PP), and polyurethane foam-polypropylene (PUF-PP) (Sonwani et al., 2019).

The biological wastewater treatment process consists of a transfer of materials from the water to the living cells and vice versa (Adrados et al., 2014). Pollutants from the wastewater pass to the mass of microorganisms, following interfacial contact or adsorption-desorption processes. The adsorbed compounds are trained in enzymatic reactions that take place in multiple stages (Wesenberg et al., 2003; Nilsson et al., 2006). Between the enzyme molecules and the nutrient substrate, reactive complexes are formed which in a later stage decompose, releasing the product or reaction products and the regenerated enzyme that can resume the reactions (Zottia et al., 2014).

The present paper explored the bio-augmentation of four newly developed prototypes of carriers (made of a mix of HDPE, organic and inorganic compounds) with *Cerioporus squamosus* strain which was already successfully tested by the team in residual concentration reduction of some pollutants in samples of textile industry originated wastewater (Iordache et al., 2015; Iordache et al., 2016). *Cerioporus squamosum* is a basidiomycete, with a particular importance in natural ecosystems, being able to degrade a wide range of cellulosic substrates. No studies have been highlighted so far with forced bio-augmentation of wastewater treatment carriers with *Cerioporus squamosus* strain. More than that, the study aimed the treatment of the carriers using two methods: dynamic treatment method and static treatment method.

MATERIALS AND METHODS

Carriers bio-augmentation

In the present study, *Cerioporus squamosus* (of Basidiomycota Phylum) strain was used for bioaugmentation of newly developed HDPE carriers used in MBBR systems for wastewater treatment. The carriers are made of a mix consisting of HDPE and inorganic and organic compounds, in various ratios (patent pending). *Cerioporus squamosus* is a basidiomycete bracket fungus, belonging to a group of fungi that form fruiting bodies with pores or tubes on the underside. Fresh starter strain was grown in Czapek nutritive broth, for 14 days at 28°C.
The bioaugmentation experiments were run by two methods, one in dynamic conditions, and one in static conditions. For dynamic conditions treatment, the HDPE carriers were put in 250 ml Erlenmeyer flasks, counting 20 carriers/flask (Figure 1), and autoclaved for 15' at 121°C.

Figure 1. Pre-autoclaving carriers

Furthermore, Czapek nutritive broth was autoclaved at 121°C for 15 minutes, and poured, in sterile conditions, over the carriers, and brought to sign at 200 ml. From starter culture, 1mL was inoculated in each flask (Figure 2).

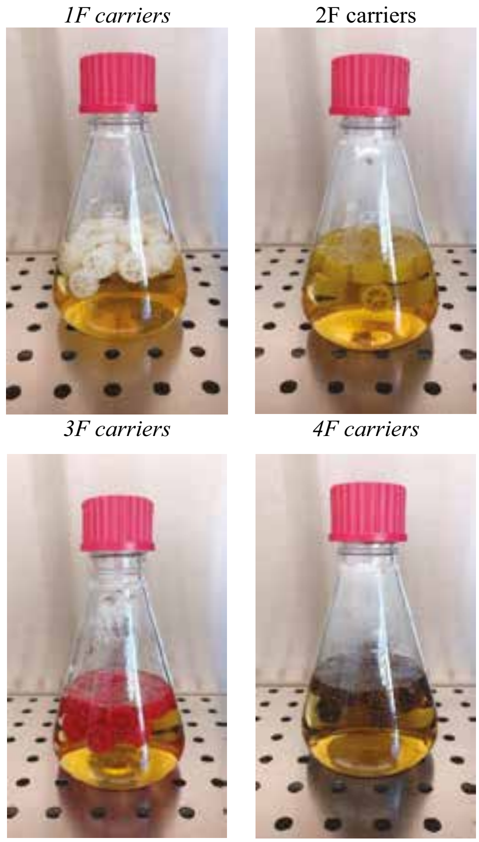
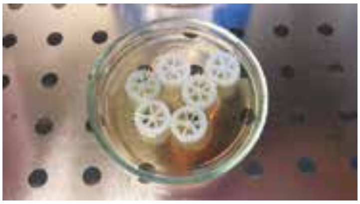


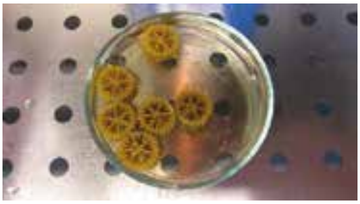
Figure 2. Inoculated flasks containing carriers and Czapek broth

After inoculation, flasks were incubated for 7 days, at 28°C, at 140 rpm, in an SIF6000R incubated shaker, from Medline Scientific.
For bioaugmentation of the carriers in static conditions, six carriers were placed in sterile Petri dishes, over which pre-inoculated Czapek broth was poured in a volume of 20 ml (Figure 3).
The samples were incubated for the same period of time as the samples treated in dynamic conditions, for 7 days, at 28°C.

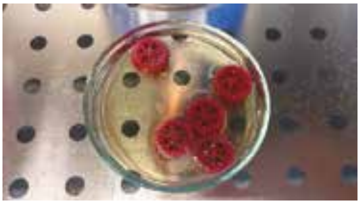
1F carriers



2F carriers



3F carriers



4F carriers

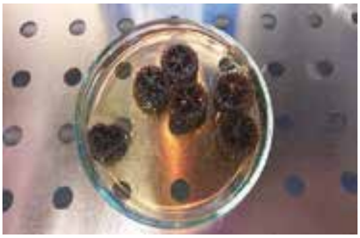


Figure 3. Inoculated Petri dishes containing carriers and Czapek broth

Optical microscopy analysis

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 0.67x, on both sets of the carriers (treated in dynamic and static conditions) in order to highlight the presence of the strain on the surface of the carrier, and in the internal spacers.

RESULTS AND DISCUSSIONS

Present research work explored to bio-augmentation activity of a new generation of HDPE based carriers (patent pending) with *Cerioporus squamosus* strain. The work represents a novelty in this field, as not only *Cerioporus squamosus* gained little attention in treatment of industrial wastewater, but no studies have been found about bio-augmentation with this specific strain of carriers used in MBBR systems.

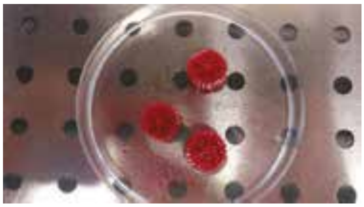
Bio-augmentation experiments were run on four types of carriers in both dynamic and static conditions. Results varied greatly not only from treatment method to treatment method, but also from carrier to carrier.

In dynamic conditions, after incubation at 28^{o}C for 7 days (140 rpm), no microbial biomass could be observed neither in the external spacers or the internal ones (Figure 4).

1F carriers

2F carriers

3F carriers



4F carriers

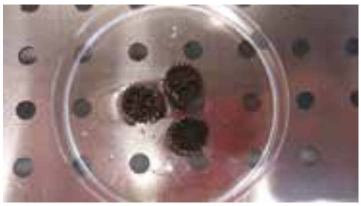


Figure 4. Photographs of post-incubation in dynamic conditions carriers

For exact assessment of presence of biomass in the external and internal spacers, the carriers were analysed under a stereomicroscope (magnification level 0.67x – Figure 5).

1F carriers

2F carriers

3F carriers

4F carriers

Figure 5. Stereomicroscopy of post-incubation in dynamic conditions carriers

Images show no growth of microbial biomass in dynamic conditions. White zones can be observed on 3F (red carriers) and 4F (brown carriers), belonging to the mix compounds. Lack of microbial biomass growth can be caused by several factors, such as: short timespan of the incubation process – usual bacterial biofilm formation in MBBR systems take around 40 days, which is a longer period of time than 7 days used in this experiment; agitation rate – a higher agitation rate can work against mechanical attachment of biomass on the carriers, leading to both short contact time and easy wash of the already attached biomass; nutrients depletion – in order for new biomass to grow in the external and external spacers, the nutrients depletion rate must be high enough so it can "force" the strain to access the carriers as nutritive substrate. This parameter is tightly influenced by process timespan, as a short-lived process won't allow both proper microbial development and nutrients depletion. Future experiments will be concentrated on longer processes timespan and lower agitation rate.

The carriers bio-augmented in static conditions yielded far better results, the method allowing the strain to colonize both external and internal spacers (Figure 6).

1F carriers

2F carriers

3F carriers

4F carriers

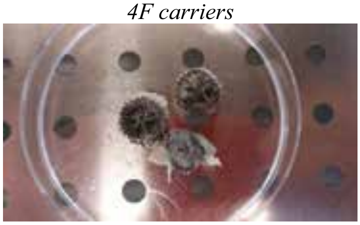
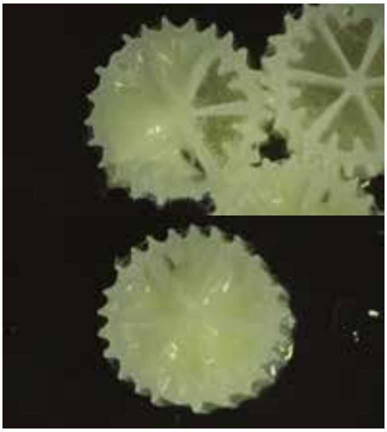


Figure 6. Photographs of post-incubation in static conditions carriers

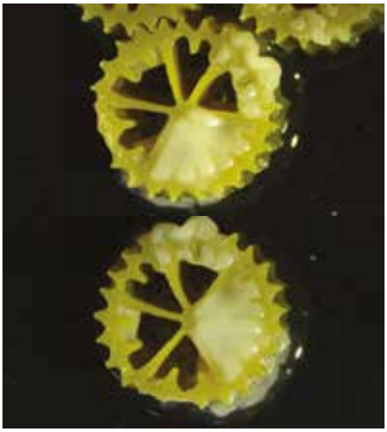
Carriers' treatment in static conditions revealed very good colonization efficiency of *Cerioporus squamosus* strain, with good surface coverage for carriers 1F and 2F and very good for 3F and 4F.

Furthermore, microscopy analysis was carried out on the bio-augmented carriers, for assessment of developed biomass (Figure 7).

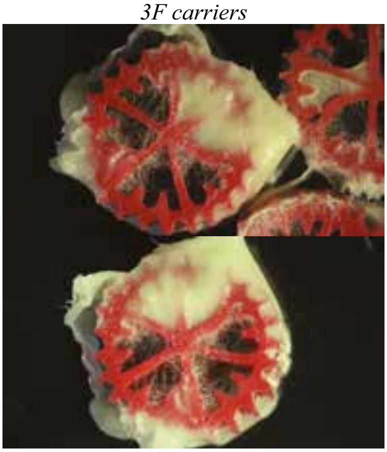
1F carriers



2F carriers



3F carriers



4F carriers

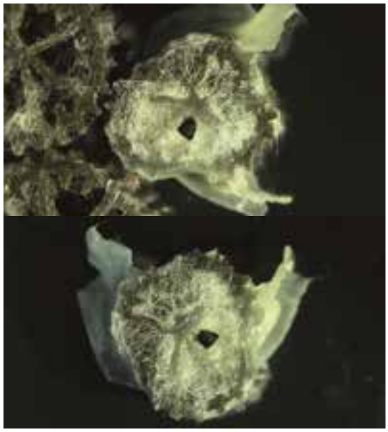


Figure 7. Stereomicroscopy of post-incubation in static conditions carriers

Analyses showed good development on all carriers, with very good development on 3F and 4F carriers. Colonization on 4F carriers was very good, with significant biomass quantity developed on both internal spacers, but also on the exterior of the carriers.

Even though the two methods differ by nature, key differentiators are: significantly lower nutritive broth volume - this allowed faster nutrients depletion, and therefore, faster access of the carriers by the strain, thus leading to good colonization; static contact conditions – this allowed uninterrupted contact between the strain and the carriers, without possibility of being “washed”, like was the case in dynamic conditions treatment.

CONCLUSIONS

The experiments carried out within current research paper explored the potential of bio-augmentation of four new generation carriers (for use in MBBR systems) with Basidiomycota strain, *Cerioporus squamosus*. Two treatment methods were tested, one in dynamic conditions and one in static conditions, for a treatment timespan of 7 days. Even though this is a very short time period for biomass formation on the carriers, when compared to regular timespan of approximately 40 days in regular MBBRs (for formation of bacterial biofilm), the results were more than promising, with static treatment conditions rendering far better results when compared to

dynamic ones. The best results were obtained on 3F and 4F carriers, with very good development of *Cerioporus squamosus* strain, on both external and internal carriers' spacers.
Future work will focus on bio-augmentation of the carriers in dynamic conditions, as this will mimic closely the real in-situ implementation (as similar to MBBR systems). A personalized programme will be aimed, in order to allow proper microbial development on the carriers, that will mix both static treatment conditions and dynamic treatment conditions.

ACKNOWLEDGEMENTS

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