Science of the Total Environment 579 (2017) 366–377

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Study of the effect of the bacterial and fungal communities present in real wastewater effluents on the performance of fungal treatments

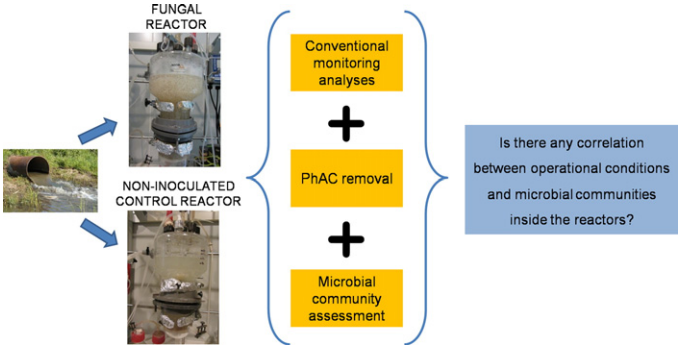
Marina Badia-Fabregat [a], Daniel Lucas [b], Tero Tuomivirta [c], Hannu Fritze [c], Taina Pennanen [c], Sara Rodríguez-Mozaz [b], Damià Barceló [b,d], Glòria Caminal [e,*], Teresa Vicent [a]

[a] Departament d'Enginyeria Química, Universitat Autònoma de Barcelona (UAB), 08193 Bellaterra, Spain
[b] Catalan Institute for Water Research (ICRA), H2O Building, Scientific and Technological Park of the University of Girona, 101-E-17003 Girona, Spain
[c] Natural Resources Institute Finland (Luke), Jokiniemenkuja 1, FI-01370 Vantaa, Finland
[d] Water and Soil Quality Research Group, Department of Environmental Chemistry (IDAEA-CSIC), Jordi Girona 18-26, 08034 Barcelona, Spain
[e] Institut de Química Avançada de (IQAC) CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain

HIGHLIGHTS

- Continuous fungal bioreactors treating real non-sterile wastewaters were performed.
- Interactions between *Trametes versicolor* and indigenous microorganisms were studied.
- DCA and PCA analysis link microbial community and operational conditions.
- Fungi (i.e. *Trichoderma* sp.) and not only bacteria can overtake *Trametes versicolor*.
- Molecular tools should be included as monitoring analysis of non-sterile treatments.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 27 May 2016
Received in revised form 14 November 2016
Accepted 14 November 2016
Available online 23 November 2016

Editor: D. Barcelo

Keywords:
Molecular biology
Microbial communities
White-rot fungi
Bioreactor
Real effluents
Pharmaceutically active compounds (PhAC)

ABSTRACT

The use of the ligninolytic fungi *Trametes versicolor* for the degradation of micropollutants has been widely studied. However, few studies have addressed the treatment of real wastewater containing pharmaceutically active compounds (PhAC) under non-sterile conditions. The main drawback of performing such treatments is the difficulty for the inoculated fungus to successfully compete with the other microorganisms growing in the bioreactor. In the present study, several fungal treatments were performed under non-sterile conditions in continuous operational mode with two types of real wastewater effluent, namely, a reverse osmosis concentrate (ROC) from a wastewater treatment plant and a veterinary hospital wastewater (VHW). In all cases, the setup consisted of two parallel reactors: one inoculated with *T. versicolor* and one non-inoculated, which was used as the control. The main objective of this work was to correlate the operational conditions and traditional monitoring parameters, such as laccase activity, with PhAC removal and the composition of the microbial communities developed inside the bioreactors. For that purpose a variety of biochemical and molecular biology analyses were performed: phospholipid fatty acids analysis (PLFA), quantitative PCR (qPCR) and denaturing gradient gel electrophoresis (DGGE) followed by sequencing. The results show that many indigenous fungi (and not only bacteria, which were the focus of the majority of previously published research) can successfully compete with the inoculated fungi (i.e., *Trichoderma asperellum* overtook *T. versicolor* in the ROC treatment). We also showed that the

* Corresponding author.
E-mail address: gloria.caminal@uab.cat (G. Caminal).

http://dx.doi.org/10.1016/j.scitotenv.2016.11.088

wastewater origin and the operational conditions had a stronger impact on the diversity of microbial communities developed in the bioreactors than the inoculation or not with *T. versicolor*.

1. Introduction

Special concern about the presence of pharmaceutically active compounds (PhACs) in surface waters arose two decades ago (Daughton and Ternes, 1999). PhACs are compounds that are specifically designed to be biologically active even at low concentrations. The main source of PhAC are the effluents of wastewater treatment plants (WWTPs), where conventional activated sludge treatment is not able to properly degrade them. For instance, diclofenac, a common analgesic and anti-inflammatory, has an average removal percentage of only 29% (Verlicchi et al., 2012). Therefore, alternative treatments should be found. The use of ligninolytic fungi has been studied based on their ability to degrade conventional pollutants (Pointing, 2001). The use of ligninolytic fungi immobilised enzymes has recently gained attention for the degradation of PhACs as they can overcome the problem of maintaining active microorganisms (Spina et al., 2015). However, it is common the need for a recurring supply of enzymes in order to maintain the activity of the enzymes for a long period of time.

Successful results were obtained using the whole fungus *Trametes versicolor*, a well-known ligninolytic fungus, with degradation percentages >90% for many PhACs in spiked experiments, as reviewed by Cruz-Morató et al. (2013b). *T. versicolor* has also been demonstrated to degrade PhAC in real wastewater (Badia-Fabregat et al., 2015a; Cruz-Morató et al., 2013a, 2014), even in continuous operation mode (Badia-Fabregat et al., 2015b). Many factors can affect the efficiency of the treatment, such as the configuration of the reactor, the chemical profile of the wastewater, the addition of nutrients and the pH (Anastasi et al., 2010). The two main drawbacks of fungal reactors when working under non-sterile conditions are the overtaking of the inoculated fungus by bacteria and the washing out of extracellular enzymes during continuous operation. However, it was reported that a continuous extracellular enzyme concentration is not crucial to achieve good degradation percentages (Anastasi et al., 2010; Badia-Fabregat et al., 2015b; Blánquez et al., 2004; Yang et al., 2013) and intracellular enzymes have also been reported to play a key role in the degradation of micropollutants (Marco-Urrea et al., 2009). Therefore, in the present study, we focus mainly on the competition between the inoculated fungus and other microorganisms.

Most real wastewater treatment under non-sterile conditions has been conducted for textile wastewater (Blánquez et al., 2008; Hai et al., 2008; Libra et al., 2003; Lu et al., 2009) and, recently, urban and hospital wastewater (Badia-Fabregat et al., 2015a, 2015b; Cruz-Morató et al., 2013a, 2014). Treating urban wastewater with fungi is a greater challenge than treating textile wastewater due to the higher microbial titre of the former, leading to possible competition between the inoculated fungus and the indigenous microorganisms. Therefore, different strategies to avoid or minimise bacterial growth have been implemented in different studies. Some were successful but expensive, such as continuous ozonation of the media (Cheng et al., 2013), whereas others, such as maintaining an acidic pH, did not suppress bacterial growth (Libra et al., 2003). Fungal reinoculation was reported in previous studies for effective control of bacterial growth (Blánquez et al., 2006; Dhouib et al., 2006); thus, it was included in the treatments presented here.

Little is known about fungal and bacterial interactions in liquid media (Weber et al., 2007; Yang et al., 2011) because the vast majority of studies on fungal-bacterial interactions are performed in soil (Mikesková et al., 2012; Rousk and Bååth, 2007). Usually, fungi are not taken into account in the microbiota characterisation of wastewater. However, they represent an important load (e.g., reaching approximately 100 colony forming units (CFU) in a landfill leachate effluent (Tigini et al., 2014)). In the present study, biochemical and molecular tools (phospholipid fatty acids analysis (PLFA), denaturing gradient gel electrophoresis (DGGE) and quantitative PCR (qPCR)) were used to study the microbial communities (both fungal and bacterial) during non-sterile fungal treatment of real effluents (reverse osmosis concentrate (ROC) and veterinary hospital wastewater (VHW)). The present study, thus, focuses on the microbial communities developed in the different treatments and their relationship with the operational parameters with the final aim to identify the optimal conditions for the development of the fungal activity in the near future. All treatments included a fungal-inoculated bioreactor (I) and a non-inoculated bioreactor (NI) in parallel as a control. Therefore, the main aim of the study was to identify the microbial communities that developed in the continuously operating bioreactors and, for the first time, to statistically correlate them with PhAC removal, fungal survival and the operational parameters and data from traditional monitoring methods (mainly laccase activity, glucose consumption and visual aspects).

2. Materials and methods

2.1. Fungal strain and pellet production

Trametes versicolor (ATCC#42530) was obtained from the American Type Culture Collection and was maintained by sub-culturing on Petri dishes in malt extract (2%) and agar (1.5%) medium at 25 °C. Pellet production was performed as previously described by Blánquez et al. (2004). Briefly, a mycelial suspension was obtained by inoculating four 1 cm^2 plugs from the malt agar plate in a 500 mL Erlenmeyer flask containing 150 mL of malt extract medium (2%, adjusted to pH 4.5). Flasks were incubated at 25 °C in an orbital shaker for 4–5 days. The obtained mycelial mass was ground with a homogeniser and the resulting mycelial suspension was stored in a sterile saline solution (8 g L^{-1} NaCl). This suspension was used to obtain pellets by inoculating 1 mL of the suspension in 250 mL malt extract medium (2%, adjusted to pH 4.5) in a 1 L Erlenmeyer flask. Flasks were incubated at 25 °C in an orbital shaker for 5–6 days.

2.2. Wastewater

The ROC,was obtained from a pilot plant located in Castell-Platja d'Aro WWTP, in the north-east of Spain. The pilot plant was described in detail by Dolar et al. (2012); briefly, it consists of a membrane bioreactor (MBR) that treats urban wastewater, followed by a reverse osmosis unit. The volume treated in the pilot plant is 200 L h^{-1}. The obtained permeate is two thirds of it and, the other third, is concentrate. The ROC was sampled on April 2013 and was stored for a month at 4 °C until its use in the bioreactor experiments. VHW was sampled twice from a veterinary hospital located on the Universitat Autònoma de Barcelona campus (Bellaterra, Barcelona, Spain): on the day that the bioreactor was set up and after a week. The pertinent wastewater in the feed storage tank was replaced by fresh water stored at 4 °C every 2–3 days for VHW and 3–5 days for ROC. The characterisation parameters of the ROC and VHW samples are presented in Table S1.