Improving mycoremediation of acetaminophen: Effect of pH, nitrogen limitation, and co-cultivation

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Abstract
Untreated pharmaceutical pollution and their possibly more toxic metabolites, resulting from outdated traditional wastewater treatment processes, end up in aquatic environments and are hazards to the ecosystem homeostasis. Biological wastewater remediation could supplement traditional methods and overcome the dumping of these biologically active compounds in the environment. Mycoremediation is especially promising due to the unspecific nature of fungi to decompose compounds through exoenzymes and the uptake of compounds as nutrients. In the present study, we improved on the previous advances made using the fungus *Mucor hiemalis* to remediate one of the most commonly occurring pharmaceuticals, acetaminophen (APAP), at higher concentration. The adjustment of pH, nitrogen limitation, and comparison with, as well as co-cultivation with the white-rot fungus *Phanerochaete chrysosporium* were tested. Nitrogen limitation did not significantly improve the APAP remediation efficiency of *M. hiemalis*. Maintaining the pH of the media improved the remediation restraint of 24 h previously seen. The APAP remediation efficiency of *P. chrysosporium* was far superior to that of *M. hiemalis* and co-cultivation of the two resulted in a decreased remediation efficiency compared to *P. chrysosporium* in single.
Keywords: Acetaminophen, mycoremediation, micromycetes, Mucor hiemalis, Phanerochaete chrysosporium

1. Introduction

The occurrence of pharmaceuticals in aquatic environments, especially surface waters, are being reported more often worldwide, conceivably due to increased usage combined with the inefficiencies of the wastewater treatment methods currently used (Jones et al., 2007).

Fungi in aquatic environments play a greatly underestimated role in food webs and show significant capabilities to degrade organic material. Due to the unspecific nature of their exoenzymes, fungi could also perform a major role in the biotransformation and biodegradation of organic pollutants in aquatic systems. Macromycetes have been successfully used for the degradation of pharmaceuticals (Marco-Urrea et al., 2009); however, few have explored the use of micromycetes, which could have a broader use in biotechnological applications compared to mushrooms.

Mucor hiemalis f. *irnsingii* (DSM 14200; Zygomycota), a strain isolated from a ground water source in Germany, was previously used for the remediation of diclofenac (Esterhuizen-Londt et al., 2017), and APAP (Esterhuizen-Londt et al., 2016a, b). After 24 h of exposure to environmentally relevant concentrations (up to 20 ng/mL APAP), *M. hiemalis* was able to internalize and bioaccumulate up to 50% of the APAP it was exposed to without experiencing an amplified oxidative stress status. However, after 24 h, remediation halted, possibly due to the environment becoming too acidic. For higher APAP concentrations (100 ng/mL and more), the remediation efficiency dramatically decreased even within the first 24 h.

In the present study, the aim was to investigate if the previous APAP remediation potential of *M. hiemalis* could be improved by nitrogen starvation, pH adjustment, and co-cultivation with the white rot fungus *Phanerochaete chrysosporium* (Basidiomycota), which is considered a model remediation fungus due to its ability to degrade aromatic polymer compounds.

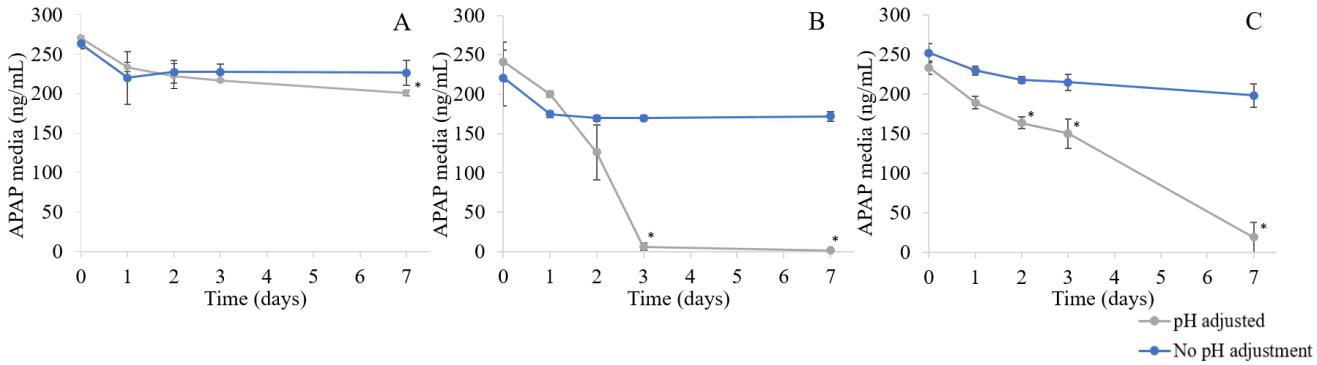

Figure 1. Mycoremediation of APAP by (A) *M. hiemalis* in nitrogen-limited media with and without pH regulation, (B) *P. chrysosporium* in nitrogen-limited media with and without pH regulation, and (C) Mycoremediation of APAP using both fungi in co-cultivation with and without pH regulation. Data represent mean concentrations in ng/mL ± standard error. * denotes statistical significance ($p < 0.05$)

2. Results and Discussion

2.1. *Nitrogen limitation and pH*

Theoretically, when limiting the medium nitrogen, the fungus would be forced to use APAP as a nitrogen source; however, this did not improve the elimination of APAP from the media ($p = 0.189$). By maintaining the pH at 8, the APAP remediation by *M. hiemalis* was improved by 12% compared to when unadjusted ($p = 0.410$) (Figure 1 A).

2.2. *Phanerochaete chrysosporium*

The APAP remediation ability of *P. chrysosporium* (Figure 1B) was far superior to that of *M. hiemalis* (Figure 1A). *P. chrysosporium* was able to remove 97% and 99% of the APAP (250 ng/mL) added to its environment with pH maintenance, after 3 and 7 days respectively ($p < 0.001$) (Figure 1B). Without pH control, the APAP elimination percentage was reduced to only 23% (Figure 1B), again emphasizing the need for a buffered system.

2.3. *Co-cultivation*

It was expected that co-cultivation would cause competition for nutrients and thus result in increased remediation. Co-cultivation of *M. hiemalis* and *P. chrysosporium* however resulted in a reduced APAP remediation efficiency (Figure 1C) compared to that achieved with *P. chrysosporium* in single (Figure 1B). With co-cultivation, 35% of the APAP was removed after 3 days, and 97% after 7 days (Figure 1C).

M. hiemalis was previously proven as an excellent candidate for diclofenac remediation (Esterhuizen-Londt et al., 2017) and APAP at concentrations below 20 ng/mL (Esterhuizen-Londt et al., 2016). In the present study, the APAP remediation efficiency of *M. hiemalis* could be improved through pH maintenance and thereby bridge the 24 h remediation limitation previously seen (Esterhuizen-Londt 2016a). However, the APAP remediation ability of *P. chrysosporium* far exceded that of *M. hiemalis*, deeming it a better option for the remediation of APAP. Interestingly, co-cultivation did not improve the elimination of APAP. This indicates that an ideal strain for the remediaion of specific contaminant could be paired; and can be compared to a lock and key.

3. Conclusion

Mycoremediation has the potential to serve as a sustainable, effective, and environmentally friendly remediation tool against a vast range of environmental pollutants. However, screening of strains against pollutants and parameters optimization, as well as finding the most effective combination of fungi to treat an environmental pollution issue is needed to improve mycoremediation as a practical tool for water purification. The practicality of mycoremediation in the environment and biotechnological applications at a larger scale needs to be investigated.

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