

ACTIVATING BIOCATALYST PRODUCTION TO ENHANCE THE REMOVAL OF VETERINARIAN ANTIBIOTICS FROM WASTEWATER

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Abstract: Data around the world shows that antibiotics in farm and municipal wastewater are a serious ecotoxic threat to receiving water bodies. Here, preliminary results from a new treatment process to remove veterinary antibiotics (i.e. trimethoprim, sulfamethoxazole and tylosin) from wastewaters are presented. The treatment process focuses on degrading pharmaceuticals by controlling the production and activation of microbial peroxidases and glucosidases in dairy farm and municipal wastewater. Enzymes production induction is done by maintaining dissolved oxygen concentration low (at 0.5 0.1 mgDO/L) and adding hard to degrade organics (lignin) to wastewater sludge cultivated in laboratory sequential batch reactors. Dairy farm runoff is seen to contain microbial populations capable of consistently reducing antibiotics influent concentration by 60 to 90%. This is likely due to adaptation from prior exposure to the antibiotics in the farm run off environment.

Keywords: Emerging contaminants, antibiotics, biological degradation

Introduction

The world's fresh water resources are exposed to thousands of modern, man-made organic chemicals including pharmaceutical compounds and agrochemicals (Figure 1a).^{1,2} At very low concentrations (e.g. nano-gram per liter) these "Organic Micro-Pollutants" (OMPs) can reduce the quality of receiving waters and their associated ecosystems.^{3,4} Figure 1b shows an experiment to trace the diffusion of micropollutants through fresh water streams.

Current wastewater treatment technologies (ozonation, advanced oxidation, UV treatment and enzymatic inactivation) are prohibitively expensive and often ineffective in removing the majority of OMPs.² For instance, Figure 1c shows the efficiency percentages to remove 25 common micropollutants by wastewater treatment plants in 12 countries. Micropollutants like atrazine, carbamazepine, diclofenac and sulfamethoxazole are poorly removed from wastewater and, therefore reach fresh water bodies. In farms, antibiotics (e.g. trimethoprim, sulfamethoxazole and tylosin) are the common OMP present in runoff water from stables and milking areas.

The objective of this study is to develop a wastewater treatment technology to degrade OMPs by activating microbial biocatalysts. Figure 2 depicts the proposed water treatment approach. Many wastewater microbes have the capacity to synthesize OMP-degrading oxidoreductase enzymes. Good examples of such oxidoreductases are peroxidases, cytochromes and laccases. Our focus is to investigate how the synthesis of such enzymes can be induced by manipulating microbes culturing conditions. Given that oxidoreductases are generally involved in electron equivalent generation/transfer processes,⁵ we hypothesize that changing cultures' electron donor and acceptor sources will stimulate the synthesis of oxidoreductases and therefore enhance the degradation of OMPs in water.

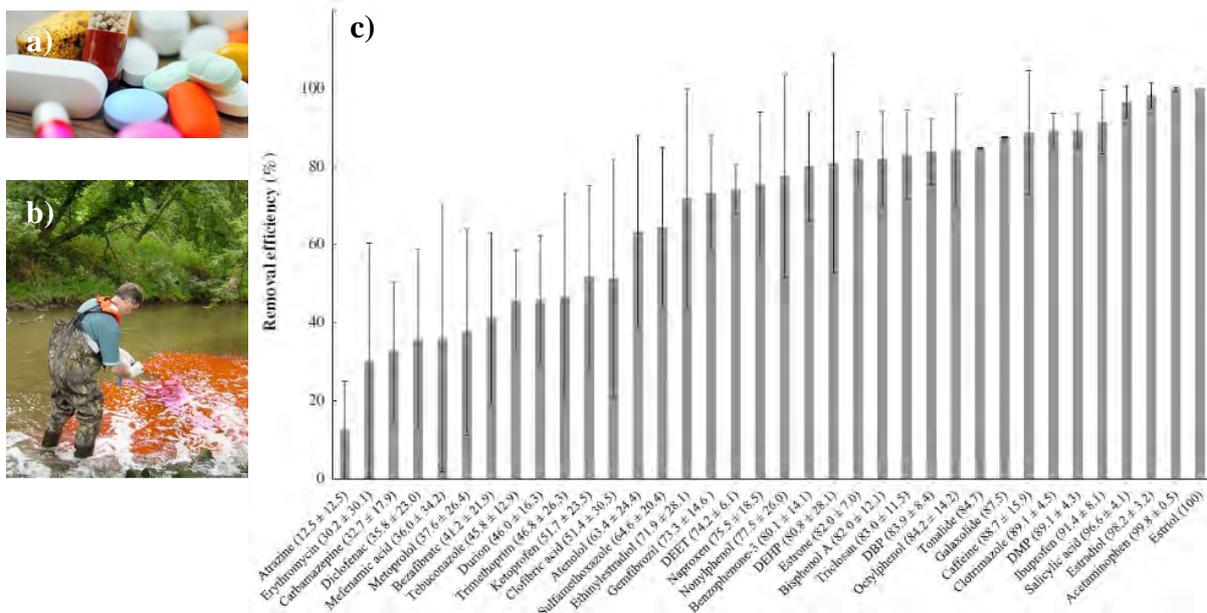


Figure 1: a) New contaminants such as antibiotics and pharmaceuticals are reaching our water resources; b) A tracing experiment of OMPs diffusion in fresh water streams; c) Removals of the selected micropollutants in 25 wastewater treatment plants in 12 countries¹

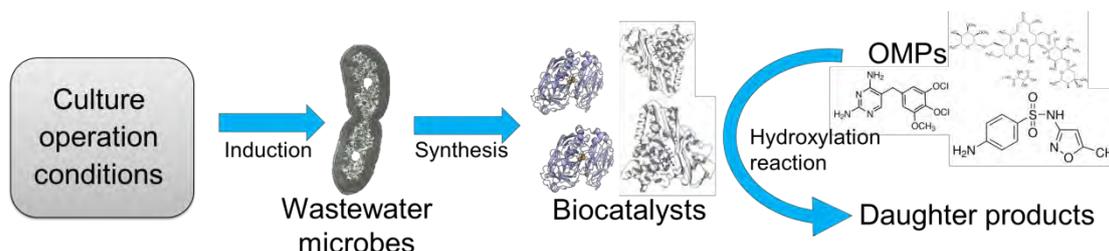


Figure 2: Proposed OMP degradation mechanism based on biocatalyst activation.

Material and Methods

Mixed microbial populations from municipal and dairy farm wastewater sludge were cultivated to investigate the synthesis of biocatalysts (i.e.: laccases, peroxidases, cytochromes) and their effect on degrading antibiotic OMPs (i.e. trimethoprim (TMP), sulfamethoxazole (SMX) and tylosin (TYL)). Figure 3 depicts the overall experimental approach.

Biocatalysts synthesis was induced by exposing activated sludge to conditions resulting in microbial stress responses. Municipal and dairy farm microbial sludge was cultivated in one litre sequential batch reactors (SBR) with either acetate or lignin as carbon sources under microaerobic conditions. In total we tested four experimental conditions. 0.2 mg/L of OMPs dissolved in methanol were added each SBR cycle. COD and NH_4^+ measurements were performed according to standard methods. TMP, SMX and TYL (and other OMPs) were analysed using LC-MS. Biocatalyst activity was measured using colorimetric assays implemented in micro-plate format (Figure 3). Chemical oxygen demand and ammonium were measured in the effluent of generated each SBR cycle; while biocatalyst activity in biomass and OMPs concentration in effluent were measured on cycles 1, 5 and 10.

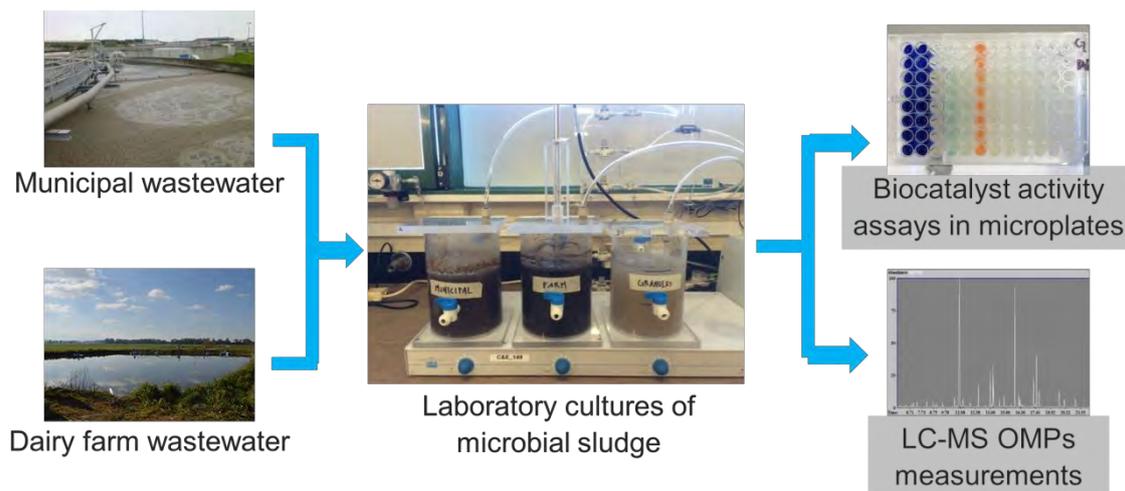


Figure 3: Overview scheme of experimental setup. Wastewater sludge from a municipal wastewater treatment plant and dairy farm was sampled and then acclimatized to experimental conditions in the lab. Sludge cultures had a work volume of 1L and were run in sequential batch mode for 10 cycles of 24 hours.

Results and Conclusions

Figure 4 shows the effluent NH_4^+ concentration in culture effluent in the four tested cultures. Prior OMP addition, cultures on acetate removed NH_4^+ efficiently but those on lignin did not. After 10 SBR cultivation cycles, organic micro-pollutants (OMPs) significantly disrupted the nitrogen removal capacity of municipal sludge but had no effect on dairy farm sludge. This result suggests the presence of OMPs resistant microbes in dairy farm wastewater.

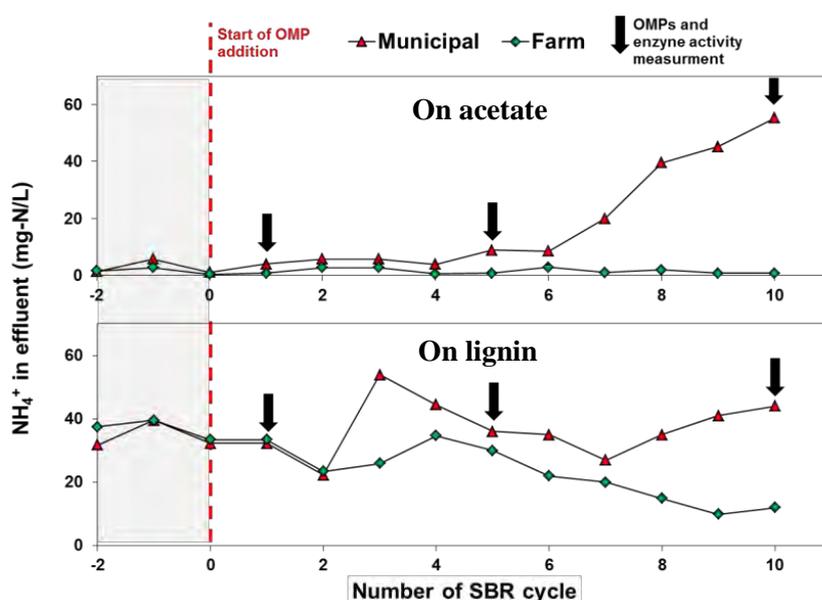


Figure 4: Nitrogen removal of experimental cultures. Arrows indicate the points of OMPs and enzyme activity measurement.

Sludge cultured on acetate removed OMPs only during the first SBR cycle and lost their capacity to do so after 10 SBR cycles. Farm sludge cultured with lignin continuously removed OMPs through various SBR cycles.

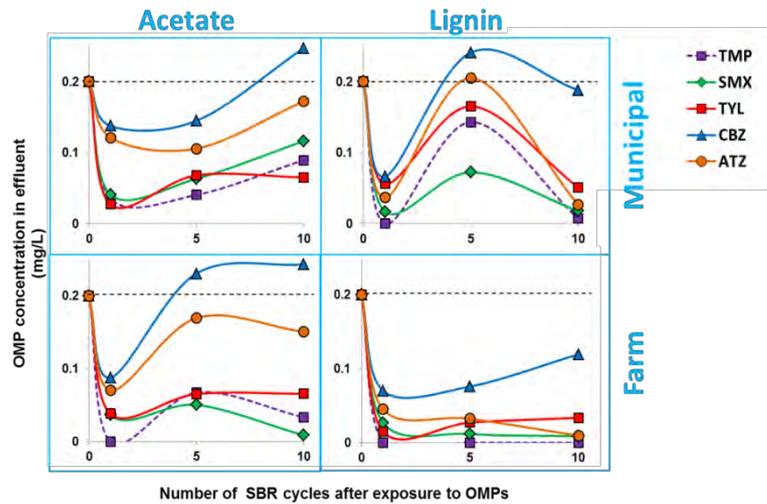


Figure 5: OMPs measurements in effluent. OMPs were added at 0.2 mg/L in each SBR cycle

Regarding the biocatalysts, lignin peroxidase activity was detected in all conditions however at low values. Horse radish peroxidase was also detected in all the conditions reaching the highest activity in municipal on acetate cultures. No consistent laccase, β -glucosidase and cytochrome P450 activities were detected

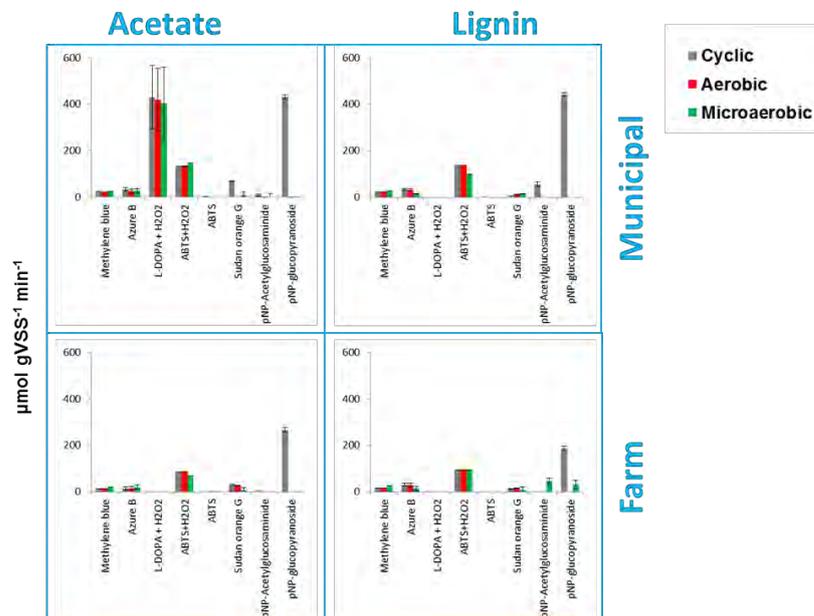


Figure 6: Enzyme activity measurements. Lignin peroxidase = methylene blue and azure B; Horse radish peroxidase = L-DOPA and ABTS; Laccase = ABTS and sudan orange G; β -glucosidase = pNP substrates.

Research contribution

- This study provides „proof of concept“ for the removal of OPMs via microbial activity and offers a solution for controlling emerging pollutants.
- Generation of fundamental knowledge of how microbial populations interactively adapt and regulate their metabolism and enzyme synthesis in response to controlled environmental perturbations

References

- (1) Luo et al. 2014, Science of Total Environment 473:619-641;
- (2) Oulton et al. 2010. Journal of Environmental Monitoring 12:1956-1978;
- (3) Vos et al. 2000, Critical Reviews in Toxicology 30:71-133;
- (4) Roig et al. 2013, Crit Rev Env Sci & Technol 42:2297-2351;
- (5) Chubukov et al. 2014, Nature Review 12:327-340.