



# Degrading Organic Micropollutants: The Next Challenge in the Evolution of Biological Wastewater Treatment Processes

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Global water scarcity is driving the need for identifying new water source. Wastewater could be a potential water resource if appropriate treatment technologies could be developed. One of the barriers to obtaining high quality water from wastewater arises from the presence of organic micropollutants, which are biologically active at trace levels. Removal of these compounds from wastewater by current physico-chemical technologies is prohibitively expensive. While biological treatment processes are comparatively cheap, current systems are not capable of degrading the wide range of organic micropollutants present in wastewater. As current wastewater treatment processes were developed for treating conventional pollutants present at mg/L levels, degrading the ng/L levels of micropollutants will require a different approach to system design and operation. In this paper we discuss strategies that could be employed to develop biological wastewater treatment systems capable of degrading organic micropollutants.

**Keywords:** emerging contaminants, organic micropollutants, biocatalysis, metabolic diversity, biodegradation, oxidoreductases

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## WASTEWATER, A WASTED RESOURCE

The Oxford English Dictionary defines wastewater as superfluous water, or water that has served its purpose. This definition of wastewater is ingrained in our perception and attitude. Yet, this definition would appear to be outdated in light of the emerging view that wastewater represents an unutilized water resource and not a waste product to be discarded. Furthermore, promoting such use of wastewater is neither new nor radical. Virtually all urban societies already make use of water sources laden with wastewater, albeit in varying amounts. For example, consider the Mississippi, the longest river in North America, which serves as both a water source and wastewater receptor for the numerous towns and cities located along its path. As the river meanders downstream, water increasingly enriched by upstream discharges of wastewater serves as a source of potable water for downstream communities. Such observations support the view that wastewater is better defined as a potential water resource that is currently wasted. A third of the world's population lives in water-stressed countries and this proportion is expected to rise to two thirds by 2025 (Service, 2006). Finding alternate water sources is critical to the survival of these water stressed societies. Although wastewater has been overlooked as a potential water resource, it could become an option if appropriate treatment technologies can be developed.

While biological treatment processes are significantly cheaper to build and operate, current designs are not capable of delivering high quality water suitable for a variety of purposes. Despite several advances since the inception of biological wastewater treatment processes, with an initial focus on removing organic carbon through to subsequent extension to include nitrogen and phosphorous removal, these systems continue to only be able to effectively degrade substances present at mg/L concentrations.

Over the past decade the rapid increase in instrument sensitivity has led to wide detection of ng/L levels of biologically active contaminants, referred to as emerging pollutants or organic micropollutants, in the environment. These contaminants include pharmaceuticals, personal care products, steroid hormones, industrial chemicals, and pesticides (Galloway et al., 2010; Oulton et al., 2010; Vandenberg et al., 2013; Richardson and Ternes, 2014; Petrie et al., 2015; Richardson and Kimura, 2016). Discharges from wastewater treatment plants are acknowledged as a major source of these contaminants in receiving environments (Spongberg and Witter, 2008; Bartelt-Hunt et al., 2009; Loganathan et al., 2009). Various studies have highlighted the need to remove the ng/L levels of these contaminants from the treated wastewater discharge due to their negative effect on human and animal health and ecotoxicology effects (Escher et al., 2011)—for example, a recent critical review concluded that doses of bisphenol A up to 4 orders of magnitude lower than the currently prescribed lowest observed adverse effect level of 50 mg/kg/day reliably produced effects in animals (Vandenberg et al., 2013) and another study reported changes in sex hormones associated with exposure to bisphenol A in men (Galloway et al., 2010). The cost of treating impacted environments can be large. London's drinking water is reported to be tainted with synthetic estrogens from excreted contraceptive pill residues in the Thames that have resulted from the water having gone through the equivalent of six people before reaching London. The cost of cleaning up Britain's contaminated waterways is estimated to exceed £30bn (McKie, 2012). Development of appropriate technologies is needed to convert wastewater into a water resource, potentially even for meeting potable needs. This paper aims to stimulate a discussion on ways to enhance the performance of biological treatment systems to achieve this vision.

## CURRENT WASTEWATER TREATMENT APPROACHES

The biological treatment approach, specifically the activated sludge process, is the most commonly used wastewater treatment technology. While it was originally designed to only remove organic carbon, it was subsequently extended to remove nitrogen and phosphorous. Transitioning between aerobic, anoxic, and anaerobic conditions is key to achieving biological nutrient removal. Doing so triggers the utilization of different electron acceptors and donors, therefore promoting the transformation of C, N, and P compounds. This strategy has continued to be

exploited with the development of new system configurations, such as the Anammox (Lackner et al., 2014), SHARON (Hellings et al., 1998; van Dongen et al., 2001), and Nareda (van der Roest et al., 2011) processes. Different redox environments influence organic micropollutant removal in different ways. Various studies have reported that organic micropollutant removal occurs to varying extent in biological treatment systems via a combination of biosorption and biodegradation. Maximum achievable removal for micropollutant such estrogens, nonylphenolics and metals was recorded at the highest sludge retention times (SRT) and hydraulic retention time (HRT) studied (Petrie et al., 2014). This micropollutant removal is related to the concomitant reduction in food: microorganism ratio. Nevertheless, studies have concluded that micropollutant degradation is insensitive to different SRTs (Falås et al., 2016). Other aspects influencing micropollutant removal in biological treatment processes are heterotrophic activity (Majewsky et al., 2010), pH (Gulde et al., 2014), and suspended/attached growth configuration (Falås et al., 2013). Although the heterogeneity of micropollutants in wastewaters makes removal difficult to predict since their chemistry is so diverse. For instance micropollutants can be broadly classified as easily, moderate and poorly degradable. The reality is that the biological processes are not designed to remove these pollutants, resulting in their incomplete removal and detection in final effluents and receiving surface waters (Joss et al., 2006; Racz and Goel, 2010). No specific strategies appear to have been successfully developed to enhance micropollutant removal by biological systems. Instead, organic micropollutant removal is typically achieved using physicochemical processes. These processes include adsorption in to organic matrices, passive effluent treatment in wetlands and aquifers, advanced oxidation processes (e.g., ozonation, UV treatment, photocatalysis, and Fenton oxidation), membrane filtration (nanofiltration and reverse osmosis membranes), and membrane biological reactor (Bolong et al., 2009; Rossner et al., 2009; Oulton et al., 2010; Luo et al., 2014). The shortcomings of these treatment systems are high investment and maintenance costs, generation of toxic residuals, and complex treatment procedures (Grassi et al., 2012). Operational difficulties of these physicochemical processes also need to be considered. For instance, wetlands and aquifers require large surface areas and large HRTs, which significantly complicates its implementation in urban areas. Similarly, advanced oxidation processes require chemicals not easily available (e.g.,  $O_3$ ,  $F_2^+$ ,  $H_2O_2$ ) and the non-selective nature of these reactions can result in formation of daughter products that are more toxic than the parent micropollutant (Rosal et al., 2009). Other concerns may also need consideration: energy and chemical inputs of ozonation processes are substantial (Cañizares et al., 2009); the Fenton process requires rigorous pH control (Chong et al., 2012); specially configured reactors are needed to maximize light exposure by UV and photocatalysis based water treatment, or water turbidity could significantly influence treatment efficiency (Chong et al., 2012); and membrane filtration and membrane biological reactors treatments routinely suffer from membrane fouling and biofilm formation (Guo et al., 2012).

## ENHANCING BIOLOGICAL WASTEWATER TREATMENT

### Exploiting Microbial Diversity

Complex microbial consortia composed by bacteria-bacteria, bacteria-archaea and bacteria-fungi can be developed to enhance biological degradation of micropollutants. Oxidase enzymes have great potential as biocatalysts for micropollutant and organic waste breakdown. Two of such class of enzymes are the oxygenase Cytochromes P450 (CYPs or Cyt P450), a highly efficient group of monooxygenases responsible for the destruction of drugs and toxins in organisms, and the laccases (EC 1.10.3.2), a class of copper-containing oxidase enzymes used by microorganisms to break down lignin (Riva, 2006; Kumar, 2010). Both enzymes have been shown to efficiently degrade a vast array of organic micropollutants in pure enzyme assays (Harms et al., 2011; Lah et al., 2011). Despite the demonstrated effectiveness of these enzymes, their use in wastewater treatment is just starting to be investigated and has not been implemented in pilot or full scale (Lah et al., 2011). Current biological systems are reliant on using prokaryotic bacteria, which can rapidly oxidize organic carbon but generally do not express Cyt P450. Additionally, no applications of bacterial laccases have yet been realized due to their limited characterization (Werck-Reichhart and Feyereisen, 2000). In comparison, many fungi produce Cyt P450 (Lah et al., 2008; Kelly and Kelly, 2013) and only fungal laccases are used currently in biotechnological applications (Sharma et al., 2007; Harms et al., 2011). A system integrating suspended phase bacteria with activated fungi to transform conventional and emerging categories of pollutants can potentially take advantage of both organisms. Fungi can be activated by inducing the production of high levels of oxidase enzymes and at the same time stimulating enzyme activity (e.g., by providing  $H_2O_2$  as cofactor for bacterial Cyt P450). By controlling the carbon source and electron acceptor regimens, it is possible to induct biocatalyst expression and activation (Price et al., 2013; Chubukov et al., 2014). As whole-cell catalyzed reactions are reportedly 10- to 100-fold slower than reactions catalyzed by free enzymes (Sotirova et al., 2008), cell membranes can be permeabilized to enhance extracellular laccase secretion and promote cellular influx of pollutants to membrane bound Cyt P450. In addition, gel encapsulated of fungi or bacterial cells can be implemented for improved performance. Alternatively, iterating between different redox conditions can promote the growth of different microbial populations to improve micropollutant biodegradation (Falás et al., 2016).

### Exploiting Metabolic Diversity

New bioprocesses to treat wastewater can be developed by exploiting the diverse metabolic capabilities of microbes. Traditionally, only metabolic capabilities in relation to aerobic heterotrophy, aerobic nitrification and anoxic denitrification, and phosphate accumulation have been used in biological wastewater treatment systems. Exploiting alternative metabolic capabilities such as heterotrophic sulfate-reduction (Zhang and Wang, 2014), autotrophic sulfur-oxidizing denitrification (Hao

et al., 2014; Pokorna and Zabranska, 2015), anaerobic methane-oxidation denitrification (Raghoebarsing et al., 2006), partial nitrite reduction to nitrous oxide for energy generation (Scherson et al., 2013) and electron shuttle redox biotransformation (Van der Zee and Cervantes, 2009) could improve micropollutant removal from wastewaters. These metabolic capabilities can also be exploited to remove inorganic micropollutants (e.g., heavy metals and radioactive elements) in which redox transformations can alter the solubility and precipitate the contaminants (Groudev et al., 1999; Gadd, 2010).

### Exploiting Biocatalyst Diversity

Many enzymes are “promiscuous” biocatalysts capable of transforming a variety of substrates that share structural similarity with their primary substrate. These promiscuous or generalized enzymes: (i) are frequently not essential, (ii) maintain low metabolic fluxes, and (iii) require less regulation of enzyme activity to control metabolic flux in dynamic environments than do specialized enzymes (Nam et al., 2012). This non-specificity makes them promising biocatalysts for organic micropollutant degradation. Bacteria are constantly developing new catabolic pathways in order to either access sources of carbon, energy and nutrients or simply to detoxify new compounds. However, unraveling these degradation processes in nature is made difficult by the large number of chemicals, their occurrence at mostly low concentrations, and the number of unknown chemicals resulting from bacterial transformation and biodegradation. Genomics has revealed that many microorganisms have far greater potential to produce specialized enzymes and metabolites than was thought from classic bioactivity screens; however, realizing their degradation potential has been hampered by the fact that many specialized metabolite biosynthetic gene clusters are not expressed in laboratory cultures (Keller et al., 2005; Miller et al., 2010; Mora-Pale et al., 2014; Rutledge and Challis, 2015). Theoretically bacteria can utilize the full space of catabolic biochemical reaction types and initiate several pathways to degrade micropollutants (Kolvenbach et al., 2014). For example, multiple biodegradation mechanisms have been discovered for bisphenol A (BPA), an industrial chemical found in a variety of plastics and epoxies and a putative endocrine disrupting compound. Similarly, enzymatic biodegradation pathways may be discoverable for other compounds using biocatalyst and daughter products screening techniques such as metagenomics (Fernández-Arrojo et al., 2010), metaproteomics, and metabolomics (Villas-Bôas and Bruheim, 2007; Helbling et al., 2010, 2012).

## IMPLEMENTATION

Current designs focus on removing conventional pollutants (organic and inorganic substances present) that are present at much larger concentrations than micropollutants to be metabolized as a source of carbon and energy. The low concentrations of organic micropollutants suggests that these substances are more likely to be cometabolized. Hence, system design will need to focus on ways of stimulating the production of enzymes that could degrade the micropollutants.

Current wastewater treatment system designs contain the basic ingredients required to formulate new processes for efficiently degrading micropollutants using the strategies discussed in the sections above. Biological treatment systems contain a wide variety of active microorganisms, and employ different redox environments are used to stimulate a wider range of chemical transformations. Rapid degradation of micropollutants could be achieved by stimulating the production of enzymes (e.g., oxidoreductases and laccases) by using hard-to-degrade substrates or by employing other environmental stresses. Liquids from anaerobic digestate could serve as carbon source that promotes such activity. The performance of such systems could be further enhanced by employing operational strategies that avert a lowering of enzyme production resulting from adaptation of microbial consortium to the imposed stress. Two plant configurations could potentially be developed using these approaches: (i) main stream micropollutant removal (i.e., modification of existing bioprocesses) or (ii) add-on reactors for micropollutant removal (i.e., dedicated tanks to remove micropollutants in the effluent of treatment plant).

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## CONCLUSIONS

Numerous organic micropollutants are present in wastewater. To convert wastewater into high quality water will require their removal. Current wastewater treatment systems are not designed to degrade organic micropollutants. Physicochemical treatment systems are effective but expensive while biological approaches cheap but ineffective and inconsistent. The next stage in the evolution of biological wastewater treatment processes could be based on discovering and employing novel metabolic traits of unconventional microbes (e.g., sulfur or iron oxidizing bacteria), or inducing the synthesis of enzymes capable of degrading micropollutants. Implementation of these new bioprocesses will involve the use of different waste streams used as substrate and new process operation strategies such as dynamic substrates control.

## AUTHOR CONTRIBUTIONS

NS developed the overall concept. NS and OP equally contributed to the writing of the article.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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