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Ionic liquid biodegradability depends on specific wastewater microbial consortia

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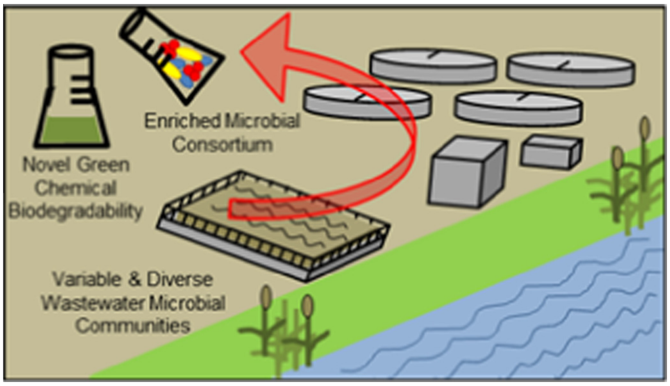
HIGHLIGHTS

- Variation in wastewater microbial communities impacts standard biodegradability assay results.
- Proactively assessing biodegradability of new chemicals is influenced by microbial variability.
- Ionic liquid biodegradation is influenced by microbial community composition in a standard assay.
- Microbial consortia capable of fully degrading three common ionic liquids were enriched.
- Enriched microbial consortia that degrade novel chemicals can prevent pollutant release.

GRAPHICAL ABSTRACT



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ABSTRACT

Complete biodegradation of a newly-synthesized chemical in a wastewater treatment plant (WWTP) eliminates the potential for novel environmental pollutants. However, differences within- and between-WWTP microbial communities may alter expectations for biodegradation. WWTP communities can also serve as a source of unique consortia that, when enriched, can metabolize chemicals that tend to resist degradation, but are otherwise promising green alternatives. We tested the biodegradability of three ionic liquids (ILs): 1-octyl-3-methylpyridinium bromide (OMP), 1-butyl-3-methylpyridinium bromide (BMP) and 1-butyl-3-methylimidazolium chloride (BMIM). We performed tests using communities from two WWTPs at three time points. Site-specific and temporal variation both influenced community composition, which impacted the success of OMP biodegradability. Neither BMP nor BMIM degraded in any test, suggesting that these ILs are unlikely to be removed by traditional treatment. Following standard biodegradation assays, we enriched for three consortia that were capable of quickly degrading OMP, BMP and BMIM. Our results indicate WWTPs are not functionally redundant with regard to biodegradation of specific ionic liquids. However, consortia can be enriched to degrade chemicals that fail biodegradability assays. This information can be used to prepare pre-treatment procedures and prevent environmental release of novel pollutants.

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1. Introduction

Ecosystems are constantly exposed to anthropogenic stressors, particularly in the form of chemical pollutants. One way to proactively reduce the impact of chemicals on human and environmental health is to develop greener replacements for hazardous chemicals that are used at a large scale. An overarching theme in the field of green chemistry is that it is better to prevent pollution at its source by proactively testing chemicals and processes, than to clean up hazardous waste after it has been released. Specific goals include designing synthesis methods for chemicals that are less hazardous and more energy efficient, as well as creating final chemical products that are recyclable and biodegradable (Anastas and Warner, 1998). In particular, complete microbial metabolism of a chemical in a wastewater treatment plant (WWTP) aeration tank results in permanent removal of the pollutant before it can enter into the aquatic environment. Therefore, it is critical to understand whether a novel chemical will completely degrade within a standard WWTP, or whether specific microbial consortia are required to carry out catabolic processes.

Biodegradability of a novel chemical is typically assessed using a standardized protocol. A diverse set of established protocols can be used to classify a chemical as “readily biodegradable” (e.g. OECD, 2006; OECD 309; ASTM 5988; ISO 14593; reviewed in Coleman and Gathergood, 2010; Stolte et al., 2011). Chemicals that achieve this standard are assumed to biodegrade during their residence time in a WWTP and are categorized as being low risk aquatic pollutants (OECD, 2006). Typically, these tests rely upon collecting a sample from a WWTP aeration tank and then inoculating the microbial community into liquid mineral media that contains the chemical of interest as the sole carbon source. Over the course of 28 days, a variety of metrics can be measured including dissolved organic carbon (DOC) concentration, biochemical oxygen demand (BOD) or CO_2 production to determine whether the microbial community actively utilizes the chemical as a carbon source for growth, or adapts to the presence of the chemical. The test chemical is classified as “readily biodegradable” when a specified threshold is reached during a 10-day window of the 28-day test (OECD, 2006).

The WWTP microbial communities used in these assays are highly diverse (e.g. Wagner et al., 2002; Ye and Zhang, 2013), and so are assumed to be functionally redundant. Several recent studies have shown that the community structure of WWTP microbial communities varies spatially within the plant (e.g. Ye and Zhang, 2013; Wells et al., 2014), temporally within the plant (e.g. Kim et al., 2013; Ju et al., 2014), by geographical location of the plant (e.g. Zhang et al., 2012), by the type of treatment system implemented (e.g. Boon et al., 2006) and due to local adaptation of microbial communities within the plant to specific concentration waste streams (e.g. van der Meer, 2006; Kraigher et al., 2008). However, it is unknown whether functional redundancy in these communities is sufficient to provide consistent results in biodegradability assays and accurate predictions of biodegradability for newly developed green chemicals. Conversely, variation in aeration tank communities can serve as an important source of catabolic potential for promising new green chemicals that may not meet biodegradability standards. Microbial isolates have provided novel remediation strategies for numerous chemicals that have been released into the environment. For example, methyl tertiary butyl ether (MTBE), once thought to be a green fuel additive, was successfully biodegraded by *Methylibium petroleiphilum* PM1 when it became a troubling groundwater contaminant following unpredicted release into a California aquifer (Hicks et al., 2014).

We tested whether functional redundancy in WWTP communities allows for consistent biodegradability of a well-studied class of green chemicals called room temperature ionic liquids (ILs). ILs typically contain a bulky cation (e.g. imidazolium, pyridinium, phosphonium, ammonium) and an inorganic anion (e.g., Br^-, Cl^-, $[NTf2]^-$, etc.) (e.g. Brennecke and Maginn, 2001). The potential for numerous varieties of cation–anion combinations allows for endless engineering opportunities to meet many application requirements. To date, diverse ILs have been engineered to fulfill many functions, including gas separation agents, lubricants, reaction media, solvents in electrochemical and dye-sensitized solar cells, and batteries, refrigeration cycles, slow-release active drug candidates, cellulose degradation and an industrial-scale acid scavenging technique (reviewed in Freemantle, 2010; Pernak et al., 2011). Many studies have proactively examined the eco-toxicological and biodegradability potential of ILs. In general, ILs that are engineered to have lower octanol-water partition coefficients (K_{ow}) are more likely to dissolve in water, but are less likely to cross cellular membranes and bioaccumulate within organisms (e.g. Deng et al., 2012) and dicationic ILs generally have lower toxicity and higher biodegradability than monocationic ILs (Steudte et al., 2014). In this study, we examined the biodegradability of a few representative ILs: 1-octyl-3-methylpyridinium bromide (OMP), 1-butyl-3-methylpyridinium bromide (BMP) and 1-butyl-3-methylimidazolium chloride (BMIM) (Fig. S1). We chose these ILs because they embody a known spectrum of IL structures from readily biodegradable to highly recalcitrant. Previous studies indicate that OMP is readily biodegradable and that the pyridinium ring can be completely metabolized within the standard assay test period (Docherty et al., 2007, 2010; Harjani et al., 2008, 2009). Conversely, assays testing the biodegradability of BMP have produced varying results (Pham et al., 2009; Docherty et al., 2010), and BMIM typically resists biodegradation (Gathergood et al., 2004; Docherty et al., 2007). Some studies have shown that supplementing the BMIM cation with a readily degradable anions or anions that promote oxidation aids in the biodegradation process (Garcia et al., 2005; Fabiańska et al., 2012). However, the imidazole ring itself is typically recalcitrant and remains a problematic obstacle to achieving full biodegradation of imidazolium-based ILs (Gathergood et al., 2004; Docherty et al., 2007; Coleman and Gathergood, 2010; Fabiańska et al., 2012; Zgajnar et al., 2014).

The goals of our current study are two-fold: (1) Assess whether temporal and location-specific consistency of WWTP microbial communities influences biodegradability of OMP, BMP and BMIM; (2) Enrich for microbial consortia originating from the WWTP aeration tank that are capable of biodegrading ILs that are shown to be recalcitrant using standard biodegradability assays. Since ILs have such high potential for wide-spread use, providing information about functionally-degrading microbial consortia is crucial for emerging pollution prevention and potential pre-treatment options.

2. Materials and methods

2.1. Sample collection

We collected 5 L grab samples from the aeration tanks at the Kalamazoo Water Reclamation Plant (KZ, Kalamazoo, Michigan, USA) and the South Bend Wastewater Treatment Plant (SB, South Bend, Indiana, USA) on August 22, 2012, December 19, 2012, and May 28, 2013. No precipitation was recorded on the collection dates. We transported and aerated samples and measured total suspended solids as described previously in Docherty et al. (2007). We also removed triplicate 45 mL samples and froze them immediately at −80 °C for DNA extraction and microbial community analyses. Metadata for all samples collected were provided by WWTP technicians, including average settled solids (percent), suspended solids (SS, mg L^{-1}), dissolved oxygen (DO, mg L^{-1}) and temperature (°C).