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

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 catalytic 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 CO₂ 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 catalytic 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 unexpected 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 (Kow) 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. 1). 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 (Garica 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).
2.2. Standard biodegradability assays

We set up all biodegradability assays according to the OECD 301A: Dissolved Organic Carbon (DOC) Die-Away Test (OECD, 2006). This protocol states that if an organic test chemical is mixed with mineral media and an aerated sample from a WWTP aeration tank, it can be considered readily biodegradable if it degrades by 70% within a 10-day window of a 28-day period. Details of the experimental set-up are described in the Supplementary Materials.

To quantify biodegradation potential, we filtered 300 μL of sample from each bottle through a 0.22 μm syringe filter (Millex) throughout the 28-day period for each experiment. We measured the absorbance of triplicate filtrates at 265 nm for pyridinium treatments and controls and 210 nm for imidazolium treatments and controls. We used a UV 96-well plate (Corning Life Sciences) and an Epoch microplate spectrophotometer (BioTek, Winooski, VT) for absorbance measurements. We corrected absorbance by subtracting out the absorbance of triplicate blanks of UltraPure water. Changes in absorbance indicate possible breakdown in IL ring structure and biodegradation of the ring. We assessed for significant differences by comparing triplicate treatment measurements to triplicate abiotic control measurements using repeated measures analysis of variance (ANOVA) followed by a Tukey’s post hoc test. Significance was assessed at $\alpha = 0.05$ using SYSTAT V.10 statistical software. A comparison of absorbance and DOC measurement techniques is discussed in the Supplementary Materials.

2.3. Wastewater microbial community composition

We thawed each of the triplicate 40 mL samples that were frozen from the initial inocula and performed total DNA extraction and amplification of the 16S rRNA gene as described in the Supplemental Materials. We conducted restriction digests using HhaI (New England Biolabs) to prepare terminal restriction fragment (TRF) as described in the Supplemental Materials. We analyzed TRFs using Applied Biosystems Peak Scanner Software v1.0 and organized the TRFs by sizes and peak heights for all base pair sizes that were within 2 base pairs above and below an average size. We removed all TRFs that were below 25 bp and above 900 bp in size. We then summed all peak heights for all TRF sizes to calculate a total peak height per sample. We used this value to calculate the proportional peak height of each of the grouped TRFs to the total peak height for the sample.

2.4. Enrichment culture preparation

Following the 28-day standard test for biodegradability conducted in August 2012, 200 mL of sample from each treatment bottle was transferred into 800 mL of new sterile mineral media + the respective IL. Concentrations of ILs in this first transfer were 40 mg C L$^{-1}$. The bottles were shaken at 150 rpm in the dark at room temperature for three weeks and transferred again. We continued to transfer microbial inocula into new media every three weeks, increasing concentrations of ILs to 60, 80 and 100 mg C L$^{-1}$. Throughout these experiments, we continued to measure the absorbance of filtered samples once per week at 265 nm for pyridinium and 210 nm for imidazolium treatments. After several enrichment transfers, we noted decreases in absorbance of BMP in one of the replicates originating from the KZ WWTP and decreases in absorbance in OMP and BMIM in two of the replicates originating from the SB WWTP. We interpreted a decrease in absorbance as indicative of successful enrichment of a microbial community capable of biodegrading each IL. We also visually noted increases in biomass throughout the 3 week incubation time. We continued to transfer only those successful enrichments into sterile mineral media containing 60–200 mg C L$^{-1}$ of each IL.

2.5. Enrichment culture biodegradation of ILs

In March 2012, we began an experiment to measure biodegradability of the three ILs using the enriched microbial communities. We set up the experiments as described for the standard biodegradability assays above, but with double the volume in 2 L Pyrex bottles. We inoculated experimental treatment bottles and biotic controls with 20 mg SS enrichment culture corresponding to each IL. We measured absorbance of the cation ring as described above every two days until levels reached a minimum. Additionally, we measured DOC, microbial respiration and used $^1$H NMR spectroscopy to examine biodegradation products as described in Supplementary Materials. Triplicate 50 mL samples were removed at the final time point of each experiment and frozen at $-80$ °C for microbial community characterization. Absorbance and DOC measurements were compared to abiotic controls over time and respiration measurements were compared to biotic controls over time using repeated measures ANOVA and Tukey’s post hoc tests as described above. We extracted genomic DNA, amplified the 16S rRNA gene and performed TRF analysis on the samples taken from the final time point of the enrichment cultures as described above.

2.6. Microbial community composition

Standardized TRFs × sample tables were used as input for multivariate statistical analyses to assess the factors driving differences in community composition among WWTP samples and enrichments. We visualized patterns of variation in community composition using principle coordinates analysis (PCoA) implemented in the R Statistics Environment (function = cmdscale) (Cox and Cox, 2001). PCoA of both WWTP samples and enrichment cultures were based upon pairwise Bray-Curtis distances generated from the standardized TRF × sample tables. We used PERMANOVA implemented with the adonis function in the vegan package for the R Statistics Environment to test hypotheses about the major drivers of WWTP and enrichment culture community composition (Oksanen et al., 2013). Additionally, environmental covariates (Table S1) were visualized on our WWTP PCoA to identify likely proximate variables explaining any spatial and temporal differences in community composition. For WWTP samples, date of collection (categorical), WWTP location, and their interaction were considered, and with enrichment community composition we tested for an effect of the IL amended as potential substrate.

3. Results and discussion

3.1. Wastewater treatment plant characterization

The KZ WWTP treats 28.0 million gallons of water per day (MGD. 105,991 m$^3$ per day), serves a population of 75,000 people and discharges to the Kalamazoo River in southwest Michigan. The SB WWTP treats 48.0 MGD (181,700 m$^3$ per day), serves a population of ~101,000 people and discharges to the St. Joseph River in northwest Indiana. We collected samples to characterize aeration tanks on dates when no precipitation was recorded (Table S1). We examined the microbial community structure from triplicate samples collected from each WWTP at each time point. Community profiles differed by WWTP location and collection time in all cases (Fig. S2). Location (SB vs. KZ) and sample date had significantly interacting effects on WWTP community composition (PERMANOVA, $p < 0.005$; Fig. 1). The average Bray–Curtis distance between replicate samples was 0.16. The addition of environmental covariate vectors to our PCoA of WWTP community composition suggests that differences in SS correlates with differences in...
community composition across sites while differences in DO and temperature partially correlate with temporal changes in WWTP community composition.

Differences in community structure between WWTPs have been observed previously (Zhang et al., 2012). There are clear geographical differences in activated sludge collected from North America vs. Asia, and community composition is best classified by the type of waste the treatment plant receives (Zhang et al., 2012). For example, saline or slaughterhouse sewage results in different activated sludge microbial communities than from urban and suburban water use (Zhang et al., 2012). However, the KZ and SB WWTPs serve similarly-sized cities, but housed significantly different microbial communities at each time point in our study. Technicians at both facilities did not record any disruption in WWTP function during the weeks that samples were collected for this study, indicating that water treatment was operating under normal conditions. There are many factors that can influence the composition of aeration tank microbial communities. Environmental factors, such as precipitation, temperature and pH can influence cell density, waste concentration and specific nitrification rates. However, operating conditions, including organic loading rate, feed composition, reactor configuration and solids retention time (SRT) can all influence microbial diversity as well (Pholchan et al., 2010; Vuono et al., 2015). For example, a press disturbance experiment investigating reduction in SRT from 30 days to 12 or 3 days resulted in a shift in sludge microbial community composition from K-strategists to r-strategists, with demonstrated losses in bacterial diversity following the disturbances (Vuono et al., 2015). Our observation that significant temporal variation is based on DO and temperature aligns with results of previous studies that have examined activated sludge community composition using high throughput sequencing techniques (Kim et al., 2013). Oxygen-based parameters have been shown to influence the community composition of general taxa that were present at all sample time points, whereas temperature influences community composition of numerous rare taxa that fluctuate seasonally (Kim et al., 2013). As such, temporal variation can drive the abundance of functionally important taxa. For example, nitrifying bacteria (Nitrosospira, Nitrosonomas) can be more abundant in activated sludge in the summer, while phosphate-removing and denitrifying taxa (Tetrasphaera, Paracoccus) are more abundant in the winter (Ju et al., 2014). Despite taxonomic variation exhibited several studies, including ours, metagenomics-based approaches indicate a high level of functional redundancy in activated sludge microbial communities across seasons (Ju et al., 2014). This suggests that WWTPs maintain a stable functional community and equivalent levels of broadly-defined wastewater treatment (i.e. respiration, nutrient removal) over time.

3.2. Standard biodegradability assays

We tested whether taxonomically variable activated sludge microbial communities provide equivalent functionality with respect to biodegradation of specific organic chemicals. We tested for biodegradability of OMP, BMP and BMIM using a modified OECD 301A protocol, measuring absorbance of the cation ring of each IL. Overall, absorbance measurements indicate that the IL ring was metabolized in OMP-based tests only, and BMP and BMIM were not metabolized (Table S2). Our results indicate variation in OMP biodegradability by WWTP location and time. OMP was completely metabolized by the microbial communities collected from the SB WWTP in December 2012 and May 2013, but not August 2012 (Figs. S3–S5). Additionally, microbial communities collected from the KZ WWTP did not completely metabolize OMP at any time point, though they did partially break down the chemical in August 2012 and December 2012 (Figs. S3–S5).

Geographical and temporal variation in OMP biodegradability was surprising, given past observations of consistent OMP biodegradability (Docherty et al., 2007, 2010; Stolte et al., 2008). In general, ILs that have longer substituted alkyl groups and a pyridinium cation tend to be more readily biodegradable than those with shorter alkyl substitutions (Coleman and Gathergood, 2010). Our group originally classified OMP as readily biodegradable using the DOC-Die Away test and a microbial community collected from the SB WWTP in April 2005 (Docherty et al., 2007). This result was replicated using a microbial community collected from the SB WWTP in September 2006 (Docherty et al., 2010). Other research groups have shown OMP to consistently pass biodegradability tests as well (e.g. Stolte et al., 2008; Harjani et al., 2009).

In contrast, we observed uniform results for BMP and BMIM using samples from both WWTP locations and across time (Table S2). In all tests, neither of these ILs was metabolized, indicating that they would not be adequately treated in a WWTP aeration tank and could pose a risk as a recalcitrant aquatic pollutant when used in significant quantities (Figs. S3–S5). Recalcitrance has been a common observation in standard assays performed previously using BMIM (Boethling and Voutschka, 2012; Coleman and Gathergood, 2010; Zgajnar et al., 2014). However, published biodegradability results for BMP are more variable. For example, using standard biodegradability assays, BMP has been shown to: completely resist biodegradation (Docherty et al., 2007), be completely metabolized in 40 days, but not be classified as readily biodegradable (Docherty et al., 2010), and be classified as readily biodegradable (Pham et al., 2009).

Biodegradability assays attempt to equilibrate all other variation in WWTP abiotic characteristics through the initial nutrient removal by aeration and the equal amounts of SS added to all assay bottles. Many factors can influence the results of biodegradability assays. For example, the necessary microorganisms for biodegradation of the chemical may not be present in the environment, nutrient levels or other abiotic conditions (e.g. temperature, salinity, pH, O2) are not adequate to support the appropriate microbial population or community (Stolte et al., 2011). Similarly, the chemical concentration of the substrate may be too high, causing a toxic effect to microorganisms before they can metabolize it, or the substrate may covalently bind to physical surfaces, making it inaccessible (Stolte et al., 2011). Additionally, biodegradability assays preclude the
possibility of co-metabolism with other chemicals in the waste stream, which may underestimate biodegradation of low-concentration pollutants (Fischer and Majewsky, 2014). Our results demonstrate another consideration for standard biodegradability assays: while the microbial community inoculated into each assay is treated as a constant by the protocol, clearly there is demonstrated variation in community composition within and between WWTPs. Thus, another critical factor that can explain differences in biodegradability results for test chemicals is variation in WWTP aeration tank microbial communities, which may be due to a number of environmental and operational differences at water treatment facilities. While this variation has been shown to contain sufficient functional redundancy to maintain broad WWTP functions, (e.g. Ju et al., 2014) it is not sufficient to provide consistent biodegradative function for ILs. An additional consideration is that the test chemicals themselves may exhibit selective toxicity to important, but rare, members of a successful microbial consortium. A recent study indicates that ILs inhibit microbial respiration of activated sludge inocula used in standard OECD tests (Markiewicz et al., 2013). By extension, as novel chemicals enter the waste stream: (1) WWTP aeration tanks may not harbor a microbial consortium with the ability to remove them and/or (2) they will exhibit toxic effects on the entire microbial community, but effectively remove rarer taxa that are required for full biodegradation. In both cases, this could result in unintentional release of the chemical into the aquatic environment.

3.3. Enrichment culture biodegradability assays

A solution to the issue of inconsistent biodegradability results lies in the WWTP microbial communities themselves. It is possible that microbial consortia which require the activity of rare taxa in the WWTP communities are capable of efficiently degrading test chemicals, but they are out-competed by more dominant taxa within the constraints of the standard biodegradability assay. To test this hypothesis, we began enrichment procedures to select for microbial consortia capable of biodegrading OMP, BMP and BMIM. Following enrichment procedures, we performed biodegradability assay measurements for each IL using enrichment cultures as microbial inocula. For each test, we measured absorbance of the cation ring (Fig. 2), changes in DOC concentrations, microbial respiration and $^1$H NMR spectra (Figs. S6–S8) over a test period following repeated rounds of enrichment. Results of these measurements clearly indicate that subsets of our enrichment cultures were capable of rapidly biodegrading each IL. Full degradation of each IL by their respective enrichment cultures was achieved by day 10 for OMP, day 8 for BMP and day 38 for BMIM (Fig. 2). This observation was corroborated by decreases in DOC on the same dates (Figs. S6–S8, A), indicating that cleavage of the cation ring is the rate limiting step in complete metabolism of these ILs. We observed increases in microbial respiration preceding rapid biodegradation for OMP and BMP (Figs. S6 and S7, B), indicating heterotrophic activity on the IL as a carbon substrate. $^1$H NMR data verifies that both the alkyl chains and heterocycle components of OMP and BMP were metabolized during this time period (Figs. S6 and S7, C). Interestingly, respiration rates did not increase in the BMIM test until after catabolism of the imidazolium ring began (Fig. S8, B). This may be due to the formation of a transient biodegradation product in the BMIM. $^1$H NMR data indicates that two metabolic products of BMIM were generated and excreted during degradation (Fig. S8, C). The major metabolite appears at day 7 and is degraded by day 31. The minor metabolite appears at day 10 and persists in solution until the end of the study. Kinetic analysis and identification of the major and minor metabolites were beyond the scope of the current study, but will be examined in future work.

3.4. Enrichment culture characterization

We examined the microbial community structure of the enrichment cultures that successfully biodegraded each IL. Fragment analysis results indicated that enrichment communities capable of IL degradation were significantly different in their composition (PERMANOVA, $p < 0.01$) (Fig. 3, Fig. S9). Enrichments capable of degrading BMIM and BMP were much more similar to each other than either was to the OMP enrichments (Fig. 3). Interestingly, the two enrichments capable of degrading OMP were quite
different from each other, but were still more different from the communities enriched for degradation of BMIM and BMP (Fig. 3). Our results indicate that distinct microbial communities were enriched to biodegrade each of the three ILs, all of which resisted biodegradability in at least one of the standard biodegradability assays. It is important to note that TRF results must be interpreted with caution, because several bacterial taxa may have the same restriction site in their 16s rRNA gene.

The results of this work, combined with recent efforts from other groups, are a promising step toward proactively coupling novel chemical design with microbial biodegradation solutions. For example, Zhang et al. (2010) observed that a pure culture of a soil Corynebacteria spp. was able to degrade N-ethylpyridinium tetrafluoroborate [BF₄⁻] and N-ethylpyridinium trifluoroacetate [CF₃COO]⁻, but could not metabolize BMIM hexafluorophosphate [PF₆⁻]. While this bacterium was not able to metabolize BMIM, another promising study indicated that the bacterium Sphingomonas paucimobilis was capable of degrading ILs with the BMIM cation, as well as a suite of pyridinium-based ILs (Abrusci et al., 2011). Fungal degradation of ILs may also be a promising option. One study indicated that 80% biodegradation of BMIM [PF₆⁻] was achieved in a bioreactor using an isolated filamentous fungal culture (Esquivel-Viveros et al., 2009).

In contrast, the results of our work clearly indicate that distinctive communities degrade BMP and BMIM vs. OMP. This pattern likely results from selection for certain enzymatic capabilities to degrade long vs. short alkyl-substituted cations coupled with microbial resilience to the differing toxic effects these ILs. By implementing a multi-organism approach to biodegradation of novel chemicals, it may be possible to provide functionally-redundant microbial consortia that are specifically crafted to quickly degrade an individual chemical and remain metabolically active at high concentrations and in complex environments.

The results of this study demonstrate that biodegradability of a chemical can vary temporally and by WWTP location because of differences in WWTP microbial communities. While high level functions of the WWTP may be preserved, consistency of IL biodegradability, and the organisms mediating biodegradation, is not maintained. As such, chemicals that are thought to be “readily biodegradable” based on standard assays performed at a particular location and time may resist traditional wastewater treatment at other locations and time points, and may pose a risk as aquatic pollutants. Conversely, promising novel chemicals that could serve as greener replacements for more hazardous counterparts might be passed over because they do not meet biodegradability standards.

While we have described a proactive solution to this issue by enriching for specific microbial consortia that are capable of rapidly biodegrading chemicals of interest, further work is necessary to determine whether this approach is feasible in more complex systems. The tests conducted here relied upon long selection times and high concentrations of test chemicals in highly stable enrichment cultures that are unavailable in an aeration tank. While they are a useful starting point, standard biodegradability assays preclude many industrial and environmental conditions that will determine chemical degradability beyond microbial community composition. For example, IL stability can become altered through wear at high temperatures when used as lubricants (Pisarova et al., 2012; Stolte et al., 2012), or through hydrolysis as a result of altered pH and temperature (Steudte et al., 2012), which may alter, or possibly promote, subsequent biodegradability by microorganisms and their potential toxicity. Once in the aeration tank, presence or absence of nutrients and cofactors (Markiewicz et al., 2011), adaptation of aeration tank microbial communities to operating conditions (e.g. Vuono et al., 2015), co-metabolism with other waste streams (Fischer and Majewsky, 2014) and physicochemical environmental parameters (e.g. Pholchan et al., 2010) can all substantially influence biodegradation rates of ILs in sewage sludge. Further studies that test the effectiveness of these enriched microbial consortia under non-standard environmental conditions and in a high-throughput system are required. We suggest that this information may be used to enhance pre-treatment of chemicals (such as ILs) in bioreactors at the industrial source, since supplementing existing WWTP communities with the appropriate microbial consortium for specific chemical degradation can be complicated by multiple waste streams, operating and environmental conditions. Pre-treatment of a single waste stream could be used to successfully prevent release of non-biodegradable chemicals into the environment, when they are not completely metabolized in a wastewater treatment facility.

Acknowledgements

Thanks to the Center for Environmental Science and Technology and Magnetic Resonance Research Center (University of Notre Dame), Jim Coloso, Kristen Bergh, Dr. Christopher Pearl, Dr. Carla Koretsky, Ron Janssen, staff at the Kalamazoo, MI and South Bend, IN WWTPs. Funding was provided by WMU Biological Sciences and Chemistry Departments, Faculty Research and Creative Activities Award (WMU-OVPR), Interdisciplinary Award (WMU-CAS) and Lee Honors College. REU Supplement support for BKB was provided by NSF CBET-1134238.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2015.05.016.

References


