Microplastic is a Means for Transporting Naproxen-Transforming Microbes in the Environment

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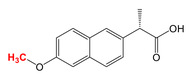
Abstract

One route for microplastic deposition in the environment is in treated wastewater effluent. As microplastic travels through the wastewater treatment facility it will interact with and may bind pharmaceuticals, as well as the microorganisms that are part of this engineered system. We hypothesize that there will be differential biotransformation activity between the microorganisms associated with microplastic particles and the planktonic cells present in the bulk liquid. Using naproxen (Aleve), a common nonsteroidal anti-inflammatory drug, as a model compound we examined these differences. Anaerobic digester sludge was used to establish enrichment cultures under methanogenic conditions. Naproxen was provided as the sole carbon source and was delivered to the cultures using naproxen-coated microplastic particles. Naproxen loss and metabolite formation were monitored by HPLC. Primary enrichment cultures showed loss of naproxen and accumulated the metabolite 6-O-desmethyl naproxen. Once naproxen transformation was complete, colonized microplastic and planktonic cells were separately used to inoculate fresh medium containing neat naproxen. Naproxen was again completely transformed in cultures inoculated with colonized microplastic, but no loss was observed with transfers of planktonic cells. DNA fingerprints of the 16S rRNA gene by Illumina sequencing showed differences between the microbial community on the beads and the bulk liquid. Furthermore, PCR analysis of the *mcrA* gene, a biomarker for methanogenesis, indicated the presence of the gene on colonized microplastics and not in planktonic cultures. This suggests that methanogens that benefit from naproxen transformation are associated with the microplastic. These results demonstrate that the presence of microplastic affects the ability of the microbial community to transform naproxen. We propose that microplastics can provide a colonization surface for naproxen-utilizing microorganisms and can serve as a mechanism for transporting these organisms from wastewater treatment facilities into the environment.

Background

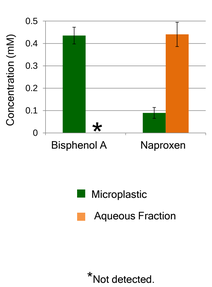
- Microplastic pollution has been recognized as a concern in marine environments, but only recently has been identified as a concern in freshwater systems as well[1].
- Wastewater treatment effluents were identified as one source of environmental microplastic deposition[2].
- One study estimated that the majority of the plastic particles were removed during the skimming process, but 1 particle per 20 mL remained in the return activated sludge[3]. The United States disposes about 783 million liters of sludge per day, containing an estimate of 39 billion plastic particles, per day, every day[4].
- Microplastic is a surface that can be colonized by environmental microorganisms[5].
- Microplastic and persistent organic pollutant interactions have been studied to evaluate the potential for sorption and subsequent environmental mobilization[6]. Less is known, however, about the interaction between microplastic and pharmaceuticals, both of which may be only partially removed during the wastewater treatment process[7].
- Our work uses naproxen as a model compound. Naproxen is a widely used over-the-counter non-steroidal anti-inflammatory medication. It is also a frequent wastewater contaminant that is found at detectable levels in the environment[8].



- We have previously shown that naproxen is transformed through O-demethylation by a highly enriched consortium from anaerobic digester sludge. The consortium is dependent upon the interactions of demethylating acetogens, syntrophic acetate oxidizers, and methanogens[9].
- We hypothesize that accumulated contaminants on the microplastic surface will serve as a carbon source for microorganisms, creating a microhabitat in which microorganisms capable of degrading contaminants are transported from wastewater treatment into the environment via microplastic.

Microplastics Sorb Pharmaceuticals

- Substrate-coated microplastic was incubated in sterile artificial wastewater under anaerobic conditions.
- Sample periodically and quantify the amount of bisphenol A or naproxen in the liquid fraction and microplastic fraction by HPLC.
- At all time points, bisphenol A remained associated with the microplastic fraction.
- Approximately 80% of the naproxen was soluble, but the remaining 20% was associated with the microplastic. **This demonstrates that even reasonably soluble compounds may associate with microplastic.**



*Not detected.

Methods Overview

Establish enrichment cultures with anaerobic digester sludge (20% v/v) under methanogenic conditions.

Purify polyethylene microplastic beads from consumer products.
Mix solvent-washed microplastic with naproxen dissolved in methanol.
Evaporate methanol under N_2.

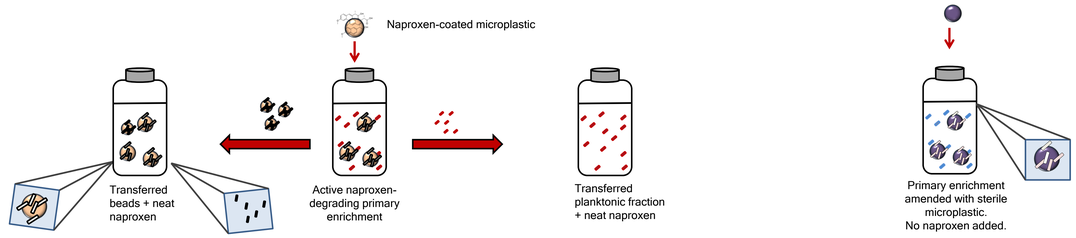
Amend enrichment cultures with naproxen-coated microplastic. Include cultures amended with sterile microplastic as a background control.

Monitor naproxen loss and metabolite formation by HPLC.
Reamend with additional naproxen-coated microplastic as necessary.

Remove 0.3 ml microplastic from original culture and transfer to fresh medium. Transfer remaining liquid culture to an equal volume fresh medium.
Amend resulting cultures with neat naproxen.

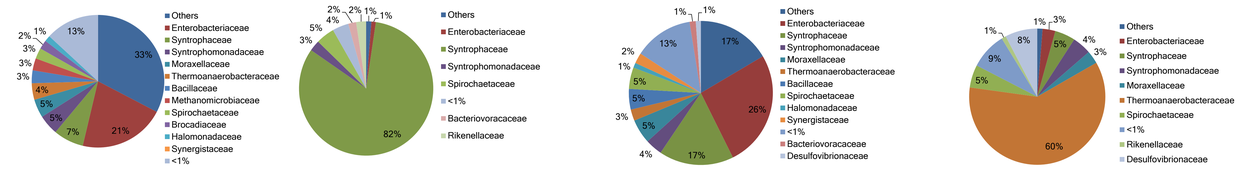
Monitor naproxen loss and metabolite formation by HPLC. Recover microplastic and planktonic fractions separately. Extract DNA for next generation 16S rRNA gene sequencing.

Primary Enrichment and Culture Transfer

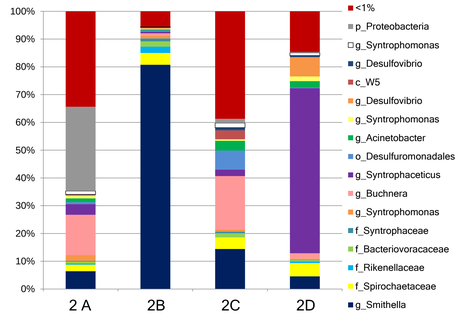


Microbial Community Analysis

1. Relative Abundance - Family



2. Relative Abundance - OTU

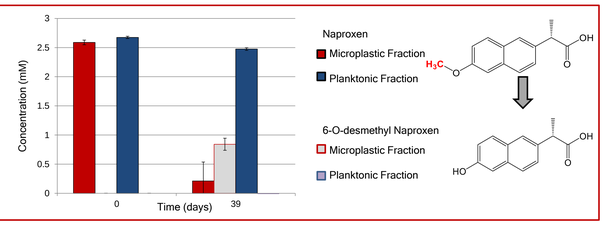


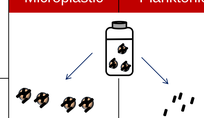
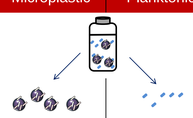
16S rDNA Analysis

- Microplastic from naproxen-amended enrichments (1A, 2A) had a very different microbial community than unamended microplastic control (1D, 2D). Naproxen transformation has changed the microbial community composition, which is reflected in the community colonizing the microplastic.
- There was similarity at the family level between the colonized microplastic (1A) and planktonic (1C) cultures, which is to be expected given that they originated from the same bottles. Microplastic (1A) consisted of more unidentified sequences than Planktonic (1C), and there was significantly more *Syntrophaceae* in the latter.
- Interestingly, the planktonic fraction (1B) associated with the microplastic culture was highly enriched in *Syntrophaceae*. Specifically, this corresponded to a single OTU in the *Smithella* genus (2B), which is a known syntroph[15].
- The microplastic community (2A) also consisted of a group of unidentified proteobacteria that comprised 30% of the OTUs, whereas this group was only 2% of the total sequences in the planktonic culture (2C).
- *Enterobacteriaceae* was a significant fraction of the sequences found in the microplastic (1A) and planktonic (1C) cultures. Members of this family have been reported to O-demethylate aromatic compounds[16].

Naproxen Transformation is Associated with Microplastic

- Naproxen disappeared with concomitant formation of 6-O-desmethyl naproxen in the cultures inoculated with colonized microplastic.
- Naproxen was not transformed by the planktonic fraction, which indicates that the microbes involved in naproxen demethylation were not active.
- **These results show that naproxen transformation activity is transferred with colonized microplastic.**



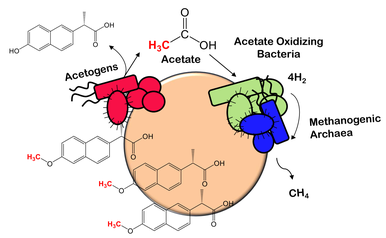
Biomarker Description	Microplastic Transfer, Microplastic	Microplastic Transfer, Planktonic	Planktonic Transfer, No Microplastic	No Naproxen Control, Microplastic	No Naproxen Control, Planktonic
					
Acetogenesis *acsB*[10]	+	+	+	-	-
Acetogenesis *fhs*[11]	+	+	+	+	+
Methanogenesis *mcrA*[12]	+	+	-	+	+
Benzoyl-CoA Pathway *bamA*[13]	+	-	-	-	-
Fumarate Addition *bssA*[14]	+	-	-	+	-

Colonized Microplastic has a Unique Functional Gene Profile

- Based on previous findings, we expect acetogens, acetate-oxiders, and methanogens in the naproxen degrading community.
- Biomarkers for acetogenesis, including acetyl-CoA synthase (*acsB*) and formyltetrahydrofolate synthetase (*fhs*) were present in all cultures with naproxen, but only the latter was detected in the control cultures without naproxen.
- The colonized microplastic from naproxen transforming cultures was positive for both aromatic compound degradation (benzoyl-CoA pathway) and the fumarate addition biodegradation pathway. The microplastic contained in the control without naproxen was also positive for the fumarate addition gene. Neither gene was amplified from any of the planktonic cultures, and highlights differences in functional capabilities in the microbial communities that are colonizing microplastic.
- **Significantly, the planktonic transfer did not have a biomarker for methanogenesis, but the transferred microplastic cultures did. This strongly suggests that methanogens are not present in this treatment. This complements the HPLC data above, which shows little naproxen loss in this culture and is consistent with our previous findings of slowed naproxen transformation when methanogenesis is inhibited[9].**

Discussion

- We have previously shown that naproxen is transformed through O-demethylation by a highly enriched consortia from anaerobic digester sludge. The consortium was dependent upon the interactions of demethylating acetogens, syntrophic acetate oxidizers, and methanogens[9].



- Community sequence analysis revealed abundant representation of microbial families that contain members known for syntrophy. This is important, because we predict syntrophic interactions between acetate-oxidizing bacteria and methanogenic archaea to be key to naproxen transformation. Our results further support this interaction, as the planktonic cultures that were negative for a methanogenesis biomarker were also unable to transform naproxen. Therefore, without the methanogens present, the cascade of events required for naproxen transformation cannot occur.
- Our results demonstrate that microorganisms involved in naproxen transformation colonize microplastic, thus allowing this function to be transferred with microplastic. Therefore it is reasonable to anticipate that pharmaceuticals and microorganisms will interact with microplastic during the wastewater treatment, resulting in environmental deposition of both microbes and contaminants.

Conclusions

A microbial food web was established on microplastic, which provided a physical location for naproxen metabolism and allowed biodegradation to be transferred. This work shows the impact microplastic has on biodegradation processes as it travels from wastewater treatment and into the environment.

Future work

- We are undertaking archaeal 16S rDNA sequence analysis to understand the distribution of methanogens under different treatment conditions.

Acknowledgements

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