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Assessment of the anaerobic degradation of six active pharmaceutical ingredients

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ABSTRACT

Research examined the anaerobic degradation of 17α-ethynylestradiol, acetaminophen, acetylsalicylic acid, ibuprofen, metoprolol tartrate, and progesterone by methanogenic bacteria. Using direct sample analysis and respirometric testing, anaerobic degradation was examined with (a) each compound as the sole organic carbon source and (b) each compound at a lower concentration (250 μg/L) and cellulose serving as the primary organic carbon source. The change in pharmaceutical concentration was determined following 7, 28, 56, and 112 days of anaerobic incubation at 37 °C. Only acetylsalicylic acid demonstrated significant degradation; the remaining compounds showed a mixture of degradation and abiotic removal mechanisms. Experimental results were compared with BIOWIN, an anaerobic degradation prediction model of the US Environmental Protection Agency. The BIOWIN model predicted anaerobic biodegradability of the compounds in the order: acetylsalicylic acid > metoprolol tartrate > ibuprofen > acetaminophen > 17α-ethinylestradiol > progesterone. This corresponded well with the experimental findings which found degradability in the order: acetylsalicylic acid > metoprolol tartrate > acetaminophen > ibuprofen.

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1. Introduction

Over the past decade, studies have measured the presence of pharmaceutical and personal care products (PPCPs) in the aquatic environment and traced the primary source of these emerging pollutants to wastewater treatment plant effluent (Hirsch et al., 1999; Kolpin et al., 2002; Servos et al., 2005; Stumpf et al., 1999; Weigel et al., 2004; Zuccato et al., 2005). In response to these findings, government agencies now recommend the disposal of unused medications in municipal solid waste (MSW) as opposed to sewage disposal (FDEP, 2006; MDEQ, 2007; MOEA, 2006; ONDCP, 2007). Whether disposed in wastewater treatment systems or MSW landfills, pharmaceutical compounds will encounter anaerobic conditions. Therefore, the examination of the anaerobic degradability of pharmaceutical compounds is necessary to assess their fate in these systems. Respirometric techniques have been used successfully for assessing anaerobic biodegradability (Kelly et al., 2006; Shelton and Tiedje, 1984) and as a result standard test methods have been developed (ASTM, 2008; OECD, 2006; USEPA, 1998).

Researchers have examined the anaerobic degradation of a small number of pharmaceutical compounds within wastewater treatment plants. Carballa et al. (2007b) examined the fate of 13 PPCPs during anaerobic digestion of sewage sludge. In a follow up study, the researchers then examined the fate of several PPCPs in sewage sludge subjected to ozone pretreatment (Carballa et al., 2007a). Others have examined the fate of estrogens in a municipal sewage treatment plant with nitrification and denitrification (Andersen et al., 2003). However, research examining long-term pharmaceutical degradation in landfill environments has not been completed.

The objective of this study was to assess the potential for anaerobic degradation of six pharmaceutical compounds by methanogenic bacteria using batch respirometric procedures while incorporating direct analytical measurement. The six pharmaceuticals were selected based upon prescription frequency in the United States from 2002 to 2004, defined daily dose, over-the-counter usage, measurement in waste medication collection programs or environmental samples, and detection in municipal solid waste (Musson and Townsend, 2009). Using two differing initial concentrations, the biodegradation of each pharmaceutical was examined over an extended period due to the extended retention period of landfills in comparison to wastewater treatment plants. The test results were then compared to the degradability predicted for each compound by BIOWIN, a United States Environmental Protection Agency (US EPA) anaerobic degradation model.

2. Experimental

2.1. Selection of target pharmaceutical compounds

Various methods have been used to identify pharmaceutical compounds with the potential for release to the environment and previous researchers have commonly utilized prescription rate data

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(Christensen, 1998; Khan and Ongerth, 2004; Sedlak and Pinkston, 2001). Using a similar method, six pharmaceutical compounds were selected based upon prescriptions in the United States from 2002 to 2004, defined daily dose, over-the-counter usage, measurement in waste medication collection programs or environmental samples, and detection in municipal solid waste (Musson and Townsend, 2009). The six pharmaceuticals were acetaminophen, acetylsalicylic acid (measured as salicylic acid), 17α-ethinylestradiol (EE2), ibuprofen, metoprolol tartrate, and progesterone. The complete method is described by Musson (2008).

2.2. High concentration sample preparation

Sample preparation and initial test concentrations were based upon US EPA test method OPPTS 835.3400 (USEPA, 1998). Triplicate samples of each compound were prepared in sterile, 500 mL glass sample bottles. The pure, powdered compound was weighed into a sample bottle. Two milliliters of ultrapure water were added to each bottle to prevent airborne loss and flushing of the bottle headspace was commenced using nitrogen (80%) and carbon dioxide (20%) through a glass tube inserted into the bottle.

A microbial nutrient solution was prepared as described in US EPA test method OPPTS 835.3400 (USEPA, 1998). The nutrient solution was heated and sparged with the nitrogen/carbon dioxide gas mixture. Upon boiling, the solution was cooled to 37 °C and additional nutrients, 10.56 g of sodium bicarbonate, and 400 mL of anaerobic digester sludge were added to reach a sludge content of 10% (v/v) and a final volume of approximately 4 L. The anaerobic sludge was obtained from a laboratory digester with a retention time of 20 days. The digester was maintained on a hormone and additive free dog food for 3 months prior to the experiment (Pet Promise dry adult dog food). Each sample bottle was then filled with 300 mL of the inoculated nutrient media to result in a final compound concentration equivalent to 50 mg/L of organic carbon. This resulted in individual pharmaceutical concentrations ranging from 61 mg/L to 83 mg/L as shown in Table 1.

The samples were sealed by a butyl rubber two-leg lyophilization stopper and capped while the glass tube used to deliver the flushing gas was removed. The sample headspace was then flushed for an additional 5 min through a supply and vent needle inserted through the stopper septum. In addition to the test samples, triplicate control samples without any pharmaceutical compound were prepared to measure the baseline gas production of the inoculation solution. Furthermore, to verify sufficient bacterial activity and to provide blank samples for quality control, triplicate samples containing readily biodegradable cellulose instead of the pharmaceutical were also prepared.

To measure potential compound loss to abiotic mechanisms such as adsorption or chemical degradation, triplicate samples of each compound were prepared using autoclaved inoculation media. Prior to dispensing to the samples, the inoculation media was autoclaved for 17 min at 121 °C, held at room temperature for 24 h, and the autoclaving repeated. Following autoclaving, the solution was sparged with nitrogen/carbon dioxide, cooled to 37 °C, and then dispensed to the samples as previously described.

All samples were wrapped in aluminum foil to prevent possible photodegradation and stored in an incubator at 37 ± 3 °C for 56 days. This time frame was chosen to match the standard anaerobic degradation test methods (ASTM, 2008; OECD, 2006; USEPA, 1998). Approximately 3 to 5 h after creation, the sample bottles were vented by inserting a gas-tight glass syringe through the septum. This permitted the increased pressure from heating of the flushing gases to equilibrate with atmospheric pressure through expansion within the syringe.

Table 1
Pharmaceutical compound high concentration test results.

Compound	Concentration (mg/L)			Reduction (%)
	Initial	Abiotic	Active	Overall
Acetaminophen	78.73	79 ± 3	70 ± 4	11
Acetylsalicylic acid	83.41	88 ± 6	70 ± 8	16
17α-ethinylestradiol	61.75	4 ± 1	0.1 ± 0	>99
Ibuprofen	66.12	53 ± 4	49 ± 3	28
Metoprolol	71.49	54 ± 1	41 ± 3	42
Progesterone	62.39	0.4 ± 0.3	<0.008	>99

Note: samples incubated at 37 °C for 56 days.

2.3. Low concentration sample preparation

PPCPs are typically detected at the μg/L or ng/L level in the environment (Daughton and Ternes, 1999). The high test concentrations prescribed by standard test methods in the literature may inhibit biological activity and result in erroneous conclusions of nondegradability. To examine the possibility of anaerobic degradability at lower concentrations, samples of each compound were prepared at 250 μg/L instead of the higher concentration (mg/L) used in the initial experiment. A total of twelve samples were created for each compound. This permitted triplicate samples to be analyzed at four differing time periods of incubation to observe degradation trends.

Aqueous stock solutions of 75 mg/L were prepared for acetaminophen, acetylsalicylic acid, and metoprolol tartrate. Due to their low aqueous solubility, 75 mg/L stock solutions of 17α-ethinylestradiol and progesterone were prepared in ethanol and ibuprofen was prepared as an aqueous solution at 20 mg/L. Stock solutions were pipetted into empty sample bottles and the ethanol was allowed to evaporate for the 17α-ethinylestradiol and progesterone samples to prevent any impact on available organic carbon or bacterial function. Continuous flushing of the bottle headspace through a glass tube inserted into the bottle was commenced using oxygen-free nitrogen vice the nitrogen/carbon dioxide mixture used previously. This permitted the measurement of carbon dioxide generated from anaerobic degradation.

Nutrient solution was prepared as previously described; however the addition of sodium bicarbonate was reduced from 10.56 g to 5.28 g due to the removal of carbon dioxide from the sparging gas. After preparation, control samples used to measure baseline gas production of the nutrient media were created. Following control sample creation, cellulose was added to the remaining nutrient solution to provide the same 50 mg/L of organic carbon used previously. This was necessary due to the large reduction in pharmaceutical concentration requiring an additional organic carbon source for microbial growth. Each sample was then filled with 300 mL of the inoculation media, sealed, flushed, and vented as previously described. To verify sufficient bacterial activity of the media and to act as quality control blank samples, one sample without any pharmaceutical addition was prepared for each compound for a total of 6 blank controls.

As in the prior experiment, samples were also prepared using autoclaved media to measure abiotic loss. A total of five abiotic samples were created for each compound to permit the analysis of one sample at 7 days of incubation, one at 28 days of incubation, and triplicate samples following 112 days of incubation. Cellulose was not added to the solution until after the completion of autoclaving to prevent any alteration of the cellulose. Following autoclaving, the solution was cooled in an ice bath to 37 °C with nitrogen sparging, the required cellulose was added, and 300 mL of solution were placed into each sample bottle. All sample bottles were wrapped in aluminum foil to prevent possible photodegradation and stored in an incubator at 37 ± 3 °C for 0, 7, 28, or 112 days. All samples for a specific compound (biologically active, abiotic, and controls) were created on the same day to reduce variability in biological activity between samples due to potential changes over time in activity of the source anaerobic sludge digester used to create the samples.