Report for 2003IA37B: Fate of Veterinary Antibiotics in Manure Lagoons

There are no reported publications resulting from this project.

Report Follows

Fate of Veterinary Antibiotics in Manure Lagoons

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Problem and Research Objectives

Much of the current research on manure management is focused on the reduction of odor and the proper disposal and treatment of manure. An area that needs attention is the fate of veterinary antibiotics in manure management systems. Antibiotics are commonly and heavily used in livestock production to prevent and cure sick animals and to improve the life expectancy and weight gain of the animals.

The fate of these chemicals is of environmental importance as it has been shown that highly resistant pathogens may develop within the manure management facilities and that these chemicals may interfere with the endocrine system of various aquatic species. Currently, research on the fate of these compounds in the manure management system and in the environment is very limited. The objectives of this study are to investigate the fate of two common antibiotics, tylosin and sulfamethazine, used in the swine industry. The focus will be on the sorption and degradation of these antibiotics in manure lagoons under anaerobic and aerobic conditions.

Methodology

The proposed research consists of analytical methods development, batch sorption studies and batch degradation studies. The antibiotic to be tested for this period is tylosin, a major antibiotic used in the swine industry. Manure will be obtained from two lagoons identified as slurry LR and slurry WTC. The manure will be characterized by measuring the slurry pH, total organic carbon, total dissolved solids, potassium, sodium, and ammonia.

A key aspect of studying antibiotics in the environment is the ability to analyze the antibiotics in various media and in low concentrations. Extraction of antibiotics from both liquid and sludge from waste manure using suitable solvents will be tested. To assess the extraction of the solvents, sludge or liquid manure will be spiked with a known amount of antibiotic and the recovery of the antibiotic determined. Triplicate samples will be used to assess reproducibility. The antibiotics will be analyzed using liquid chromatograph and liquid chromatograph-mass spectroscopy (LC-MS).

Batch sorption experiments will be conducted according to the American Society of Testing and Materials E1195-01 (ASTM, 2002). In a typical test, seven to eight sets of vials will be prepared with a given mass of manure in each vial along with liquid manure. The vials will be spiked with tylosin. Sodium azide will be added to each vial to inhibit microbial degradation. Each set of vials consists of either duplicate or triplicate vials to check for reproducibility of the sorption experiments. The amount sorbed will be estimated from the difference between the initial and final concentration of antibiotic in solution.

Anaerobic degradation studies will be conducted using a series of 120 mL serum bottles containing sludge from manure lagoons. The serum bottles will be spiked with a given amount of antibiotic, and the vials will be purged with nitrogen to ensure dissolved oxygen is removed. The vials will then be sealed using crimp-typed aluminum caps and Teflon-coated rubber septa. At different times, vials will be sacrificed and the concentrations of the antibiotics in both liquid and solid phases will be analyzed. The parent compound remaining and metabolites, if any, will be determined using LC-MS. Aerobic degradation experiments will be similarly conducted.

Principal Findings and Significance

Analytical Methods Development. Three different solvents' compositions were tested: methanol, methanol/acetontrile/0.1 M ascorbic acid (45:45:10, v:v:v), and acetonitrile/isopropyl alcohol. The average recoveries of tylosin from slurry LR and WTC as a percentage of the tylosin added using acetonitrile/isopropyl alcohol were 99% and 93%, respectively for vials sacrificed within 0.5 hours of spiking. For slurry LR, recoveries averaged about 41% after 24 hours for acetonitrile/isopropyl alcohol extraction. Methanol and acidified methanol each gave an average 38% recovery after 24 hours. Adding KOH to acetonitrile/isopropyl alcohol produced a higher proportion of tylosin B to tylosin A in the extracts. Of the three extraction solvents tested, acetonitrile/isopropyl alcohol was found to be the better extractant.

Anaerobic Studies. For both slurry LR and WTC, there was a rapid loss of tylosin (60% to 85%) within the initial 24 hours, after which the loss of tylosin slowed down. The rapid loss of tylosin within the first 24 hours may be due to sorption of tylosin to the solids. Substantial sorption of tylosin (90%) has been reported within 1–6 hours after spiking in soil and manure mixtures (Ingerslev and Halling-Sørensen, 2000). Half-lives may be used to compare the differences in the various treatments, but since the loss of tylosin was very rapid, the time for 90% disappearance of tylosin was chosen as a comparison. The estimated time necessary for 90% tylosin loss was 40 and 310 hours for slurry LR and WTC, respectively. The 90% disappearance times for slurry LR and WTC with azide were 90 hours and 500 hours, respectively indicating that faster degradation occurred in the unamended slurries.

Aerobic Studies. As in the anaerobic studies, there was a rapid loss of tylosin but with a lower amount of residual tylosin remaining at the end of the 72 hours. Less than 1% of the tylosin added remained after 12 days of aeration in slurry LR. The 90% disappearance time for aerated slurry LR was 12 hours as compared to 40 hours for the anaerobic slurry LR. For slurry WTC, the 90% disappearance times were 26 hours and 310 for the aerobic and anaerobic slurries, respectively.

Loss of tylosin in manure slurries can be attributed to biotic and abiotic degradation and to sorption (i.e. the formation of non-extractable bound residues). Biodegradation and abiotic degradation may occur but strong sorption to slurry solids was likely the primary mechanism of tylosin loss. Residual tylosin continued to persist in the slurry after 8 months of incubation indicating that tylosin degradation in lagoons is incomplete and that tylosin residues will be carried over to agricultural fields if the manure is land applied.

Tylosin Degradates. A degradate eluting at 20.6 minutes appeared within 12 hours after spiking in all unamended and azide-amended anaerobic and aerobically incubated assays. Neither aeration nor sodium azide affected the amount of degradate production. The degradate compound was not detected in slurry source materials before tylosin addition or in tylosin tartrate standards at pH 7.0 but did appear in sterile lagoon liquids and water at pH 9.2. The degradate's peak mass response was 934.5 mass units (mu) and its major fragmentation ion was 772.5 mu when analyzed with LC-MS-MS under positive ionization mode.

Publications

A paper was submitted to the Journal of Water Environment Federation for review and publication. Part of this work will be presented as a poster at the WEFTEC Conference in October 2004 and platform presentation at the American Association of Microbiology in September 2004.

References

Ingerslev, F.; Halling-Sørensen, B. (2001). Biodegradabillity of Metronidazole, Olaquindox, and Tylosin and Formation of Tylosin Degradation Products in Aerobic Soil-Manure Slurries. *Ecotoxicol. Environ. Saf*. 48: 311-320.