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THE RELATIVE ROLES OF AMMONIA OXIDIZING BACTERIA VERSUS HETEROTROPHIC BACTERIA IN BIOTRANSFORMING 17<ALPHA>-ETHINYLESTRADIOL UNDER LOW GROWTH RATE CONDITIONS

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ABSTRACT: Biotransformation of synthetic estrogen 17<alpha>-ethinylestradiol (EE2) within wastewater treatment plants (WWTPs), particularly nitrifying activated sludges (NAS), has been well documented. Cometabolic action on EE2 by bacterial communities within NAS has been speculated to be governed by ammonia monooxygenase (AMO) activity. This paper will present initial findings regarding the fate and transport of EE2 by the ammonia oxidizing bacterium (AOB) Nitrosomonas europaea versus mixed heterotrophic cultures enriched for oxygenase enzyme activity or low oxygenase activity under physiological conditions typical of WWTPs. Sorption experiments are complete and indicate that sorption of 14C-EE2 onto all biomass types is best described using the Freundlich isotherm. For heterotrophic cultures, isotherms indicate that approximately 5% of EE2 would be removed via biomass sorption, and the results correlated reasonably well with KOC. Although Freundlich was the best predictor of the small amount of sorption that occurred on chemostat-grown N. europaea, the amount of EE2 sorbed was insignificant for this culture. These results highlight the need for conducting biomass-specific sorption evaluations. Batch or chemostat grown N. europaea followed by high versus low oxygenase activity-enriched heterotrophic cultures are being used to investigate biotransformation of radiolabeled 14C-EE2 and non-radiolabeled EE2. Batch biotransformation of EE2 (- 295 m/z) by N. europaea was observed with 98% of the parent compound being transformed over 28.5 days of incubation. LC-MS analyses have revealed the generation of 3 daughter compounds corresponding to -385.2 m/z, -223.1 m/z and -313.1 m/z. Characterization of these compounds through LC-MS/MS is underway. Radiolabeled chemostat experiments performed in series (AOB followed by heterotrophic cultures) are underway and will be reported at the conference. These experiments will indicate transformation results and intermediate fate. Finally, this work will also offer insights into the relationships between cometabolic flux and cellular indicators of bioenergetic activity, such as ATP budget and proton motive force generation/dissipation, through cometabolic flux analysis (CoMFA). Similar AOB-heterotroph serial experiments will also be conducted with radiolabeled carbamazepine and iopromide, and non-radiolabeled trimethoprim. Results for EE2 will certainly be ready for the conference and presented, and any additional results available from the other micropollutants will be presented as well.

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