

# Optimizing Removal of Estrogenic Compounds in Biosolids: A Comparison of Anaerobic, Post-Aerobic, and Cambi Digestion Processes

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## ABSTRACT

Anaerobic digestion is a key treatment step during wastewater treatment because it is often the final significant solids treatment process prior to land application. Land application of biosolids is a vital practice for sustainable waste management; therefore, the presence of estrogenic compounds in biosolids is one concern to this practice. Three digestion processes were studied to determine the optimal method for removing these estrogenic micropollutants: mesophilic anaerobic digestion (MAD), MAD with a post-aerobic digestion phase, and thermal hydrolysis pretreatment followed by MAD. Specific estrogenic compounds were quantified via liquid chromatography/mass spectrometry and total estrogenicity was quantified using the yeast estrogen screen (YES) assay. MAD followed by post-aerobic digestion resulted in a significant reduction in estrogenicity.

**KEYWORDS:** Anaerobic digestion, microconstituents, biosolids, thermal-hydrolysis, YES assay

## INTRODUCTION

More than half of the biosolids produced in the United States are used for land application (USEPA, 2002). The continued and some would argue, expanded, practice of land applying biosolids is essential for minimizing the carbon footprint of wastewater treatment. One evolving concern to the practice of land application is the presence of microconstituents such as estrogenic compounds in biosolids. Once land applied, the potential exists for these compounds to leach into groundwater, runoff into surface water, or be taken up by organisms from the biosolids themselves (Kinney et al., 2008). Indeed, in one study demasculinization was observed in sheep that grazed on fields fertilized with digested sludge (Erhard and Rhind, 2004). Although it is likely that many microconstituents present in land-applied biosolids are degraded in the soil (Hesselsoe et al., 2001), it is still preferential to minimize the amount of potentially harmful compounds in biosolids prior to land application. Further driving the need for better removal of these micropollutants from biosolids is the EU Working Document on Sludge (2000), which

would prohibit land application of biosolids that have additive concentrations of nonylphenol and nonylphenol mono- and diethoxylates greater than 50 mg/kg of dry solids. Thus, the removal of microconstituents from biosolids is an important global issue.

Sludge stabilization is frequently achieved through mesophilic anaerobic digestion (MAD), but MAD does not sufficiently remove estrogenicity from biosolids (Holbrook *et al.*, 2007). Furthermore, MAD converts nonylphenol ethoxylates (NPEO) into nonylphenol (NP) (Giger *et al.*, 1984), and NP has even greater estrogenic activity than the parent nonylphenol ethoxylate compounds (Routledge and Sumpter, 1996). One option for increased removal of microconstituents following anaerobic digestion is subsequent aerobic digestion. An aerobic digestion phase following MAD has been shown to significantly decrease NPEO and NP concentrations (Knudsen *et al.*, 2000; Hernandez-Raquet *et al.*, 2007, thereby decreasing estrogenicity as well.

The Cambi digestion process, which incorporates thermal hydrolysis pretreatment to MAD (TH-MAD) is another type of enhanced digestion that is being used at full-scale operations (Pickworth *et al.*, 2006; Kepp *et al.*, 2001). At a Cambi plant in Norway, sludge is pretreated at 170°C for 30 minutes. This hydrolysis process allows the total solids concentration in the feed to the digesters to be in the range of 10%-12% TS, thus reducing digester volume requirements (Kepp *et al.*, 2001). Less foaming during digestion also results after thermal hydrolysis as a result of the destruction of filamentous flocs (Muller, J. 2000). Furthermore, Class A biosolids are produced, as the heat treatment kills pathogens. While research has been done on the operational parameters of the Cambi process, an evaluation of its ability to enhance estrogenic removal has not been performed.

Municipalities that land apply biosolids would benefit from the knowledge of how the removal of estrogenicity is enhanced through various digestion processes. This research therefore analyzed the removal of specific microconstituents and estrogenicity from three systems: MAD, MAD followed by post-aerobic digestion (MAD-AER), and the Cambi process (TH-MAD). Individual estrogenic compounds were quantified using liquid chromatography/mass spectrometry (LC/MS) and total estrogenicity was determined using the yeast estrogen screen (YES) assay.

## **METHODOLOGY**

### **Reactor Operation**

The MAD and MAD-AER bench-scale reactors were operated at the Virginia Polytechnic Institute and State University. The MAD reactor (Minibrew 6.5 gal fermenter, Hobby Beverage Equipment Co., Temecula, CA, shown in Figure 1) was operated at an HRT of 15 days and a sludge volume of 15 L (4 gal). The reactor in which post aerobic digestion occurred had an HRT of 5 days and a volume of 6 L (1.6 gal). Measurements such as volatile solids reduction, total solids reduction, COD reduction, alkalinity, and pH were made in accordance with *Standard Methods for the Examination of Water and Wastewater* (AWWA, 1998). The reactors were fed with influent municipal wastewater sludge containing 50% primary and 50% biological solids.



**Figure 1. Bench scale mesophilic anaerobic digesters**

The TH-MAD reactors were operated on a pilot-scale system by RDP Technologies, Inc. in Norristown, PA, US. Details on the process have previously been described by Wilson et al. (2008). During the TH-MAD process, sludge was treated with high pressure and temperature (150°C) prior to being digested under mesophilic anaerobic conditions for 15 days.

Five influent and effluent samples were collected from the reactors: (1) raw influent sludge to the reactors, (2) post thermal hydrolysis before MAD occurred in the pilot plant, (3) effluent from MAD, (4) effluent from MAD-AER, and (5) effluent from TH-MAD reactors. These samples were packed in a cooler and sent to the University of Minnesota for analysis.

### **Sample cleanup and analysis**

Sludge and biosolids samples were freeze dried at the National Lacustrine Core Repository (LacCore) located at the University of Minnesota. Dried solids samples were then weighed and extracted overnight using a Soxhlet extraction apparatus with methanol and dichloromethane. The solvent extracts were combined and reduced in a heating bath under vacuum (Rotovapor R-3000, BUCHI Laboratory Equipment, Postfach, Switzerland). Known aliquots of each extract

were removed for YES assay analysis. The YES assay used has been previously described by Routledge and Sumpter (1996). YES assay results yielded total estradiol-equivalent values, such that total estrogenicity could be compared between samples.

The remaining extract was further processed for analysis by LC-MS. First, normal-phase chromatography was used to further purify the extracts in clean-up columns made with alumina, silica, and sodium sulfate. Extracts were solvent exchanged into cyclohexane, loaded onto the cleanup columns and subsequently eluted with respective volumes of cyclohexane, dichloromethane, and acetone. The acetone and dichloromethane fractions were collected, volume reduced, and solvent exchanged into cyclohexane where all fractions were pooled. As a final cleanup step, extracts were run through a gel permeation column (GPC) that was packed with Bio-Beads S-X3 (Bio Rad Co., Australia). Relevant GPC fractions were volume reduced and exchanged into methanol. Final volume reduction occurred by blowing down the extracts under a stream of nitrogen. The final extracts were analyzed by LC/MS (Hewlett Packard, 1100 LC-MSD). Chromatographic separation was achieved on a Polar RP column (4 micron, 150 mm x 2.0 mm, Phenomenex, Torrance, California, USA). Compounds analyzed included nonylphenol, triclosan, bisphenol A, and estradiol.

## **RESULTS**

### **YES Results – Total Estrogenicity**

Semi-quantitative comparisons between the biosolids samples were made to determine which process reduced the most estrogenicity. Using the YES assay results, the MAD-AER process significantly reduced estrogenicity as compared to the MAD sample. These results are in agreement with previous work that showed the reduction of estrogenic compounds during post-aerobic treatment of anaerobically digested biosolids (Knudsen et al., 2000; Hernandez-Raquet et al., 2007). The YES results did not provide a direct quantification of the total estrogenicity of raw sludge because the raw sludge samples were toxic to the yeast. Thus, the total reduction of estrogenicity could not be assessed. The TH-MAD sample was also toxic to the yeast. One possible reason for this was that the TH-MAD sample contained higher ammonia concentrations.

### **Liquid Chromatography-Mass Spectrometry Results**

Similar to the YES results, concentrations of specific estrogenic compounds decreased after MAD-AER digestion. These results further suggest that a post-aerobic digestion phase offers value with respect to the removal of estrogenic compounds. One interesting result was that NP increased from the raw sludge sample to the MAD effluent sample. This phenomenon of NP increasing during anaerobic digestion was originally shown by Giger et al. (1984), and is a result of NPEOs breaking down to produce NP under anaerobic conditions. The TH-MAD process yielded a greater concentration of NP in the digested biosolids when compared to MAD alone. Perhaps the thermal hydrolysis phase enables the effective breakdown of NPEOs, allowing more NP formation during MAD. More detailed research is needed to help clarify the mechanisms by which TH-MAD can enhance NPEO conversion to NP.

## **DISCUSSION/CONCLUSIONS**

Post aerobic digestion appears to be best method for reducing the estrogenicity of biosolids.

Should a WWTP only have one concern – reducing estrogenicity – a post-aeration system is recommended. However, post-aeration does require additional costs and increases the carbon footprint of a plant so each municipality must decide if the additional cost of aerobic digestion is justified. TH-MAD offers benefits such as digester volume reduction and production of Class A biosolids. Nevertheless, the possibility of more complete conversion of NPEOs to NP could result in higher initial levels of the more estrogenic compound, NP, being land applied. The benefit of the TH-MAD process is that the NPEOs and NP may persist in soil for less time as more total conversion would have occurred in the digester. A toxicity model that incorporates the fate of NPEOs and NP with their exposure hazards would help clarify the effects of more complete NPEO conversion to NP in a reactor (the TH-MAD process) versus less complete conversion (the MAD process), resulting in NP persisting in soil longer but at lower peak concentrations. Future research will determine the mechanism by which the TH-MAD process potentially increases NPEO degradation during MAD.

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## REFERENCES

- American Water Works Association, Water Environment Federation, and American Public Health Association. (1998) *Standard Methods for the Examination of Water and Wastewater*. 20th ed. Washington, D.C.
- Erhard, H. W., & Rhind, S. M. (2004). Prenatal and postnatal exposure to environmental pollutants in sewage sludge alters emotional reactivity and exploratory behaviour in sheep. *Sci. Total Environ.*, **332** (1-3), 101-108.
- EU (2000). Working Document on Sludge 3<sup>rd</sup> Draft. Unpublished, 19p.
- Giger, W., Brunner, P. H., & Schaffner, C. (1984). 4-nonylphenol in sewage-sludge - accumulation of toxic metabolites from nonionic surfactants. *Science* (Washington, DC, U.S.), **225** (4662), 623-625.
- Hernandez-Raquet, G., Soef, A., Delgenes, N., & Balaguer, P. (2007). Removal of the endocrine disrupter nonylphenol and its estrogenic activity in sludge treatment processes. *Water Res.*, **41** (12), 2643-2651.
- Hesselsoe, M., Jensen, D., Skals, K., Olesen, T., Moldrup, P., Roslev, P., et al. (2001). Degradation of 4-nonylphenol in homogeneous and nonhomogeneous mixtures of soil and sewage sludge. *Environ. Sci. Technol.*, **35** (18), 3695-3700.

- Holbrook, R. D., Novak, J. T., Grizzard, T. J., & Love, N. G. (2002). *Environ. Sci. Technol.*, **36** (21), 4533-4539.
- Kepp, U., Panter K., Solheim, O.E. (2001). High Dry Solids Digestion. *CIWEM/Aqua Enviro 6th European Biosolids and Organic Residuals Conference*, UK
- Kinney, C. A., Furlong, E. T., Kolpin, D. W., Burkhardt, M. R., Zaugg, S. D., Werner, S. L., et al. (2008). Bioaccumulation of pharmaceuticals and other anthropogenic waste indicators in earthworms from agricultural soil amended with biosolid or swine manure. *Environ. Sci. Technol.*, **42** (6), 1863-1870.
- Knudsen, L., Kristensen, G. H., Jorgensen, P. E., & Jepsen, S. E. (2000). Reduction of the content of organic micropollutants in digested sludge by a post-aeration process - a full-scale demonstration. *Water Sci. Technol.*, **42** (9) 111-118.
- Muller, J. (2000). Disintegration as a key-step in sewage sludge treatment. *Water Sci. Technol.*, **41** (8) 123-130.
- Pickworth, B., Adams, J., Panter, K., & Solheim, O. E. (2006). Maximising biogas in anaerobic digestion by using engine waste heat for thermal hydrolysis pre-treatment of sludge. *Water Sci. Technol.*, **54** (5), 101-108.
- Routledge, E. J., & Sumpster, J. P. (1996). Estrogenic Activity of Surfactants and Some of Their Degradation Products Assessed Using a Recombinant Yeast Screen. *Environ. Toxicol. Chem.*, **15** (3), 241-248.
- U.S. Environmental Protection Agency (2002) *Biosolids Applied to Land: Advancing Standards and Practices*; EPA-832R02005; Washington D.C.
- Wilson C.A., Murthy S.N., Novak J.T. (2008). Laboratory-Scaled Digestibility Study of Wastewater Sludge Treated by Thermal Hydrolysis. *Residuals and Biosolids Conference 2008*, Water. Env. Fed., Philadelphia, PA.