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Pyrolysis of Wastewater Biosolids Significantly Reduces Estrogenicity

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Abstract: Most wastewater treatment processes are not specifically designed to remove micropollutants. Many micropollutants are hydrophobic so they remain in the biosolids and are discharged to the environment through land-application of biosolids. Micropollutants encompass a broad range of organic chemicals, including estrogenic compounds (natural and synthetic) that reside in the environment, a.k.a. environmental estrogens. Public concern over land

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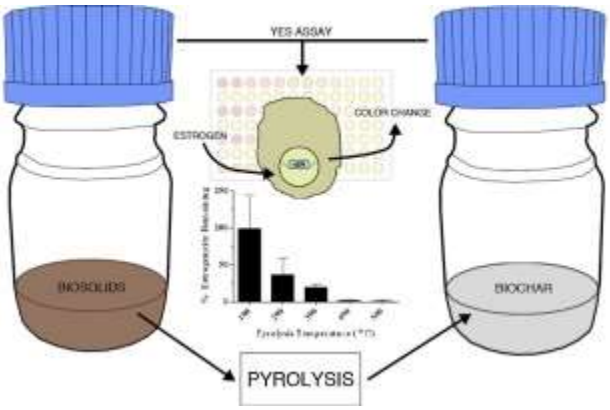
application of biosolids stemming from the occurrence of micropollutants hampers the value of biosolids which are important to wastewater treatment plants as a valuable by-product. This research evaluated pyrolysis, the partial decomposition of organic material in an oxygen-deprived system under high temperatures, as a biosolids treatment process that could remove estrogenic compounds from solids while producing a less hormonally active biochar for soil amendment. The estrogenicity, measured in estradiol equivalents (EEQ) by the yeast estrogen screen (YES) assay, of pyrolyzed biosolids was compared to primary and anaerobically digested biosolids. The estrogenic responses from primary solids and anaerobically digested solids were not statistically significantly different, but pyrolysis of anaerobically digested solids resulted in a significant reduction in EEQ; increasing pyrolysis temperature from 100 °C to 500 °C increased the removal of EEQ with greater than 95% removal occurring at or above 400 °C. This research demonstrates that biosolids treatment with pyrolysis would substantially decrease (removal > 95%) the estrogens associated with this biosolids product. Thus, pyrolysis of biosolids can be used to produce a valuable soil amendment product, biochar, that minimizes discharge of estrogens to the environment.

Graphical abstract



Keywords: Thermal processes, Anaerobic digestion, Estradiol, Biosolids handling, Biochar

1. Introduction

Biosolids are a valued soil amendment with over half of biosolids being land applied in the United States,[25] but there is also concern regarding the estrogenic compounds and other micropollutants associated with biosolids.[18] Estrogenic compounds, including natural estrogens, such as estrone (E1), 17-β-estradiol (E2), and estriol (E3), and xenoestrogens have raised concern due to their wide array of

biological impacts and wide-spread occurrence in the environment. Xenoestrogens, such as bisphenol-A (BPA), are synthetic chemicals that bind to the estrogen receptor and modify endocrine pathways in the same manner as natural estrogens.[33] Many estrogenic compounds are hydrophobic, with log *n*-octanol-water partitioning coefficient (log K_{ow}) values greater than 3, and partition to biosolids that are often treated via anaerobic digestion (AD).[10]

The impacts of estrogens on organisms are highlighted by results from aquatic studies. Estrogenic compounds diffuse into cells and bind with the estrogen receptor to form the hormone-receptor complex. This complex interacts with an estrogen response element of a target gene and increases gene expression for various proteins used in a diverse range of cellular processes. These processes include regulating the expression of certain genes and secretion of specific hormones, and coordinating diverse processes such as cell division, cell differentiation, and tissue organization.[7] The impacts of estrogens have been observed on fish populations. When approximately 5 ng/L of the synthetic estrogen 17-α-ethinylestradiol (EE2) were experimentally added to a previously undisturbed lake (a concentration that represents the total estrogenicity found in wastewater treatment plant (WWTP) effluents), the fathead minnow (*Pimephales promelas*) population declined to near extinction.[14] Vajda et al.[28] reported that the male population of white suckers (*Catostomus commersoni*) was only 20% of the total population downstream of a WWTPs outfall that contained several estrogens. Upstream of the outfall the male population was 46% of the total population. This finding was especially important because it suggests that low level environmental concentrations of estrogens can have impacts on fish in real-world environments. Less work has been done on the impacts of estrogens in the environment following land application of biosolids, but estrogenic compounds are also associated with municipal biosolids.[15] Following land application, estrogens can bioaccumulate in earthworms,[15] or be transported with runoff following rainfall.[32]

Anaerobic digestion is widely used for municipal solids stabilization i.e., reduction of odor, pathogens, and potential for putrefaction, but a consensus on the impact of AD on removal of total estrogenicity (combined estrogenic biological effect measured in E2 equivalents reported as EEQ) has not been reached. Matrix complexity

can make it difficult to quantify estrogens in biosolids. Therefore, few studies describe the impact of sludge stabilization on estrogen fate, and among these studies the conclusions varied.[9] In batch AD experiments, more than 80% removal of human-derived estrogens was measured,[4] but in a study on a full-scale WWTP no significant removal of E1, E2, and E3 was observed.[24] A different full-scale study even reported increased EEQ in mesophilic anaerobic digesters.[10] AD potentially increased normalized EEQ (moles of estrogens per mass of solids) because solids were destroyed, but estrogenic compounds were not; therefore the estrogen concentrations relative to the solids mass increased.[10] Additionally, the estrogenic compound nonylphenol is more hormonally active than its parent compounds, the nonylphenol ethoxylates, and it is readily formed during AD; this transformation could have also contributed to the increased estrogenicity observed after AD.[26,8]

Estrogenic compounds are present in anaerobically digested biosolids, and other treatment options would need to be considered if less hormonally active soil amendments derived from biosolids were desired. Pyrolysis is an abiotic thermal process that decomposes organic material through elevated temperatures in an oxygen-depleted environment[16] and potentially produces a byproduct that is less hormonally active than biosolids. Pyrolysis of biosolids yields a solid fraction (biochar), a gas fraction (py-gas), and a liquid fraction (py-oil), which are all usable byproducts.[31,13,20] The py-gas and py-oil can be combusted for energy[21] with the organic fraction of the py-oil having a heating value comparable to conventional fuels like coal and the py-gas having a value comparable to coke oven gas.[13] Pyrolysis of biosolids has been gaining interest as a biosolids management technology, and a pilot-plant processing 1 ton per year of biosolids has been in operation in the Sanitation Districts of Los Angeles County with full-scale operation expected in 2016.[30]

The specific applications for biochar could be different than those for biosolids because pyrolysis decreases the amount of plant-available nitrogen.[11] Biochar, though, is added to agricultural soil as a beneficial amendment because it can increase soil drainage, plant growth, stress reduction, and carbon sequestration.[1,5,23,22,17] Therefore, it is used as an agricultural soil amendment.[29] Pyrolysis has been shown to remove organic pollutants from the solid phase by

volatilization and decomposition reactions. A pilot-scale pyrolysis reactor operating for 30 min at 450 °C removed 1.3 and 0.32 mg/kg of polychlorinated biphenyls (PCBs) and hexachloronenzene (HCB), respectively, to below detection limits of less than 0.004 and 0.012 mg/kg, respectively.[3] Pyrolysis also removed dioxins and PCBs by greater than 99.9% from sediments at 800 °C.[12] Based on these studies, it was expected that pyrolysis could remove estrogenic compounds through a similar action because pyrolysis temperatures are typically higher than the melting temperatures of estrogenic compounds. While it seems promising that this heat treatment process would reduce estrogenicity in biosolids, the impact of pyrolysis on estrogenic compounds has not yet been quantified.

The objective of this research was to quantify the impact of pyrolysis on the removal of estrogenicity from biosolids. It was hypothesized that pyrolysis would remove estrogenic compounds from the solid phase and produce a biochar that was less-hormonally active than biosolids. Wastewater solids samples were collected from a full-scale WWTP, and pyrolysis was performed in a lab-scale reactor to determine the impact of pyrolysis on the removal of estrogenicity. Solid samples were extracted and analyzed for EEQ via the yeast estrogen screen (YES) assay, and a rapid sample clean-up method was developed to reduce sample toxicity to the yeast.

2. Materials and methods

2.1. Wastewater treatment plant sample collection

Wastewater solids samples were collected at the South Shore WWTP in Oak Creek, WI and analyzed for EEQ to compare to samples that had undergone pyrolysis. The South Shore WWTP has a capacity of 300 MGD and has a flow profile of approximately 52% residential, 33% commercial, and 15% industrial; the treatment plant employs primary sedimentation, activated sludge, and anaerobic digestion. The anaerobic digesters are fed primary solids and the activated sludge solids are conveyed to a facility for heat drying. The anaerobic digester receives primary sludge, is mesophilic, and has an average solids retention time of 21 days. Primary solids (PS) were taken from the settled solids that leave the primary clarifiers (and are eventually fed

to the anaerobic digesters) and anaerobically digested biosolids (ADB) were taken from the effluent of the anaerobic digesters. One PS and one ADB grab sample was collected in May 2014 from sample taps off of PS and ADB pipelines in the WWTP. Samples were collected in 1-L plastic bottles that had been rinsed with methanol and dried and were immediately transported to the lab.

Samples were frozen within one hour of collection and subsequently lyophilized using a freeze dryer (Millrock BT Series, Kingston, NY). Lyophilization was used instead of oven drying to minimize loss of estrogens due to volatilization. Lyophilized samples were stored for approximately 24 h at room temperature in acetone-rinsed aluminum tins until extraction.

2.2. Pyrolysis of anaerobically digested biosolids

Batch pyrolysis experiments were performed to produce biochar at different temperatures. Lyophilized ADB samples were homogenized using a mortar and pestle and approximately 0.2 g were added to Pyrex flasks that were previously heated at 500 °C for 30 min. The flasks were covered with aluminum foil and sparged with argon to remove headspace oxygen. Sparged flasks were placed in a muffle furnace at either 100, 200, 300, 400, or 500 °C for 1 h and then removed and allowed to cool in a desiccator. Initial and final mass values were recorded, solids were transferred to acetone-rinsed aluminum tins, and solid samples were extracted as described below. Removal efficiency of EEQ from pyrolysis at different temperatures was determined on a mass basis as follows:

equation(1)

$$\%EEQ\ Removal = 100 \times \frac{[EEQ_{ADB} * m_{ADB} - EEQ_B * m_B]}{EEQ_{ADB} * m_{ADB}}$$

where *EEQ* is the solids estrogenic equivalents (ng EEQ/g solids), *m* is the mass of solids in the flask (g), ADB denotes anaerobically digested biosolids, and B denotes biochar.

2.3. Sample extraction and processing for YES assay

Lyophilized samples (0.1–0.3 g) were extracted with approximately 25 mL hexane in aluminum-foil-capped 50 mL beakers and ultrasonicated (Branson 5800, Danbury, CT) for 30 min. Liquid extract was transferred to sterilized 100 mL glass bottles with screw-top caps. The variability of solid extraction efficiency was determined by extracting one PS solid sample in triplicate.

Hexane extracts were toxic to yeast and were cleaned using packed columns to remove toxicity prior to YES analysis. Cleanup columns were prepared by dry-packing 1 g of sodium sulfate, 1 g of 5%-activated silica gel, 1 g of 5%-activated alumina, and 1 g of sodium sulfate into sterile 10 mL disposable syringes. To condition columns for nonpolarity, 10 mL of methanol, followed by 10 mL of hexane were passed through the columns and discarded. 2 mL of hexane extract were then added to the column followed by 10 mL of hexane rinse and elution by methanol (20 mL). The combined hexane rinse and methanol eluent were collected in sterilized, 50 mL beakers, evaporated to near-dryness and reconstituted in 2 mL of methanol that were pipetted into sterile amber glass vials and stored at 4 °C until YES analysis. Triplicate aliquots of one hexane extract sample were cleaned up using separate columns, and the eluents were analyzed to determine variability from clean-up columns. One cleaned extract was plated in the 96-well plate in triplicate to determine reproducibility during plating of the assay. Spike and recovery experiments were also performed in which a known mass of the YES assay E2 standard (54 µg/L) was added to a clean-up column to estimate recovery of EEQ during this clean-up step; it is possible that other estrogenic compounds could have different recovery values from E2.

2.4. Recombinant yeast estrogen screen (YES) assay

The YES assay was performed on cleaned samples to quantify total EEQ activity. Using the YES assay as opposed to measuring individual compounds accounts for a cumulative biological response of all estrogenically active chemicals (including human derived estrogens and synthetic estrogens) similar to what occurs in the environment.[10]

The YES assay employs the human estrogen receptor and may not accurately reflect the response of environmental organisms to the array of estrogenic compounds present, but provides an indicator of estrogenicity in samples. Interpretation of the impact of pyrolysis on removal of estrogenicity is based on results of this assay, and not on measurement of individual compounds. The steps and explanation behind the YES assay was described in detail previously.[26] In short, this process involves extracting estrogenic compounds from samples and concentrating them in a solvent (e.g. hexane). The solvent is then added to wells in a 96-well plate and allowed to evaporate so that the estrogens remain in the wells. A yeast culture that contains the human estrogen receptor and chlorophenol red-β-d-galactopyranoside (CPRG) is added to the wells. When estrogens bind with the receptor, an enzyme is produced that converts CPRG from yellow to red and this color change (indicative of estrogenicity) is quantified using a spectrometer. The YES assay was performed according to the method of Routledge and Sumpter[26] with a few additions and modifications. Briefly, (1) the absorbance at 620 nm was measured to determine yeast growth over the incubation period, (2) 20 μL from the dilution plate was added to the assay plate instead of 10 μL, and (3) the stock 17β-estradiol (E2) solution was prepared in methanol instead of ethanol. Absorbance was measured using a plate reader (SpectraMax, Molecular Devices, Sunnyvale, CA) and connected software (SoftMax Pro Data Acquisition and Analysis Software, Molecular Devices, Sunnyvale, CA). Absorbance at 540 nm was corrected as shown in Eq. 2 to correct for background absorbance and turbidity as previously described by McNamara et al.:[19]

equation(2)

$$\begin{aligned}\text{Corrected } A_{540} &= A_{540_{\text{total}}} - A_{540_{\text{initial}}} \\ &\quad - 1.07^{*}\left[A_{620_{\text{total}}} - A_{620_{\text{initial}}}\right]\end{aligned}$$

where corrected A_{540} is the absorbance used for dose-response analysis, $A_{540total}$ and $A_{620total}$ are the absorbance values after 3–5 days at 540 and 620 nm, respectively, $A_{540initial}$ and $A_{620initial}$ are the absorbance values initially after plate preparation.

Dose-response curves were generated from corrected absorbance values using statistical software (GraphPad Prism 6.04, GraphPad Software, La Jolla, CA). Using a nonlinear, variable slope, four parameter regression, the effective-concentration for 50% response (EC_{50}) was determined. EEQ were calculated as follows:

equation(3)

$$EEQ = \left(\frac{E2\,Standard\,EC_{50}}{Solid\,Sample\,EC_{50}} \right)$$

where E2 Standard EC_{50} and Solid Sample EC_{50} are the effective concentrations for a 50% response (ng E2/L and g solids/L, respectively).

2.5. Statistics

GraphPad Prism 6.0 was used for all statistical analysis including *t*-tests for comparing two data sets and analysis of variance (ANOVA) for comparing more than two data sets. All statistics reported for significant differences were analyzed at a 95% confidence interval ($p < 0.05$).

2.6. Chemicals and reagents

All chemicals used for the YES assay are reported elsewhere[26] and were purchased from Sigma-Aldrich (Milwaukee, WI). 17-β Estradiol (E2) (≥98%), silica gel (high-purity grade, 60 Å pore size) and aluminum oxide (activated, neutral) were also purchased from Sigma-Aldrich (Milwaukee, WI). Sodium sulfate anhydrous (granular), and hexanes (98%) were purchased from EMD Millipore. Methanol was HPLC grade and was purchased from Alfa Aesar (Ward Hill, MA).

3. Results and discussion

3.1. QA/QC on YES assay with clean-up method

Clean-up methods are often required to reduce toxicity of sludge samples for the YES assay.[6] The cleanup method of Citulski and Farahbakhsh[6] was modified for rapid throughput to reduce toxicity of

PS and ADB samples to the YES assay (Fig. 1). PS samples that were not processed through clean-up columns did not elicit an estrogenic response on the YES assay. When the same PS sample was processed through a clean-up column, the resulting extract elicited a complete S-curve response. These results demonstrate that this modified cleanup method is one convenient method to reduce sludge toxicity towards yeast in the YES assay. Samples that were processed using this method elicited an expected curve, but an alternative oven drying method has also been employed by others to reduce sludge toxicity to yeast.

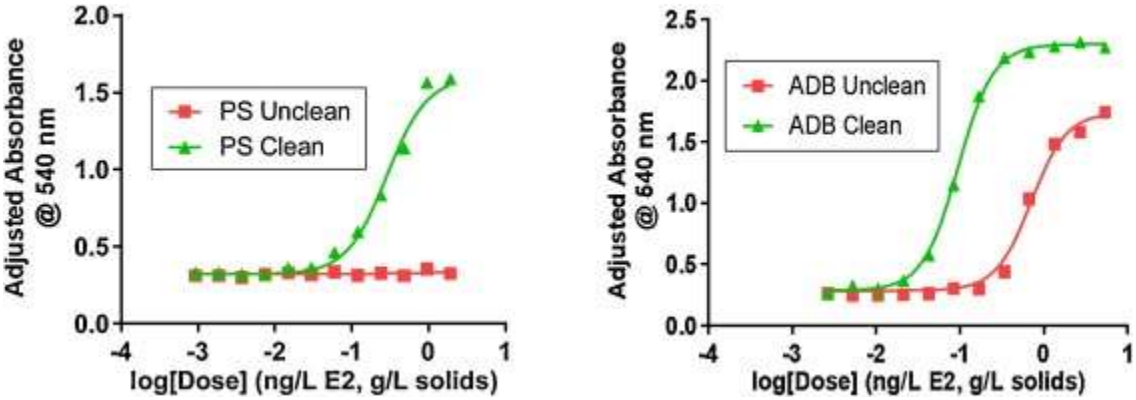


Fig. 1. Cleanup columns reduce toxicity to the yeast. The primary solids (PS) sample (left) inhibited yeast at all tested concentrations and only yielded a response after being processed through the cleanup column. The anaerobically digestion biosolids (ADB) sample (right) was not as toxic as PS, but response was inhibited when compared to the full s-curve of the cleaned ADB sample.

Clean methanol samples spiked with E2, referred to as blank spikes, were also processed in the same manner as actual samples through cleanup columns to determine if samples would lose EEQ after passing through the cleanup columns. The EEQ of the triplicate blank spikes processed through the columns were statistically different from the EEQ of the blank spikes that were not processed through the cleanup columns ($p = 0.028$, *t*-test); the column-processed samples had 28 ± 11% recovery of the original samples' EEQ. This low and variable recovery of estrogenic response suggests that results should be interpreted with caution and only compared with samples processed in the same manner. Furthermore, actual estrogenicity of samples may be greater than reported values because of unrecovered estrogenic fractions.

One PS sample was extracted with hexane in triplicate, processed through clean-up columns in triplicate, and plated in triplicate to determine the variability through each of these steps (Fig. 2). The EEQ values from each of these steps were not significantly different (one-way ANOVA, *p*-value = 0.437), suggesting that the method was reproducible and no particular step substantially increased variability. As seen in Fig. 2 the standard deviation of EEQ for the extraction step was the largest with a coefficient of variation (COV) of 21%, while the clean-up columns and plating steps had lower standard deviation values with COVs of 7% and 4%, respectively.

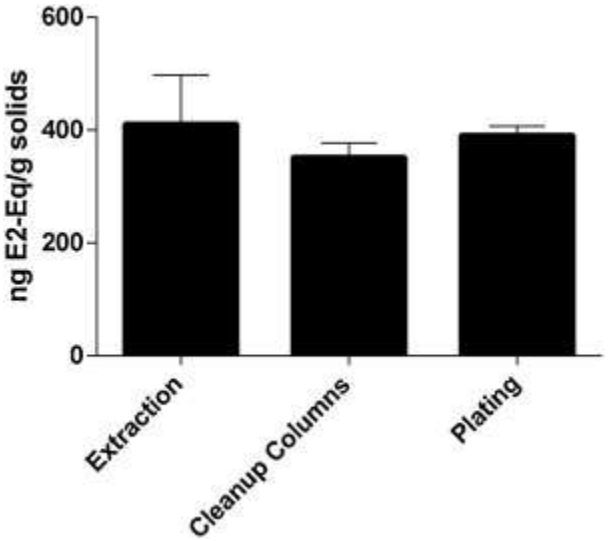


Fig. 2. The impact of sample processing steps on reproducibility of yeast estrogen screen (YES) assay method on a single primary solids (PS) sample analyzed in triplicate. Bars and error bars represent the average and standard deviation of triplicate samples.

3.2. Temperature dependence of estrogenicity removal during pyrolysis

Pyrolysis temperature had a large impact on the removal of estrogenicity from biosolids. EEQ removal increased as pyrolysis temperature increased, with almost complete removal (>95%) occurring at or above 400 °C (Fig. 3). The samples were significantly different from each other (ANOVA, $p < 0.05$). At 200 °C and higher the biochar samples were significantly different from the influent ADB samples (Tukey's multiple comparison's test, *p*-value < 0.05). The melting temperatures of several common estrogenic compounds are

below 300 °C (Table 1), so the effectiveness of pyrolysis on EEQ removal is reasonable. After compounds melt into the liquid phase they will partition to the gas phase (away from the biochar) as liquid-gas phase equilibrium is approached. The boiling points for several of the estrogens listed in Table 1 are less than 400 °C so these compounds will presumably volatilize at pyrolysis temperatures of 400 °C or higher.

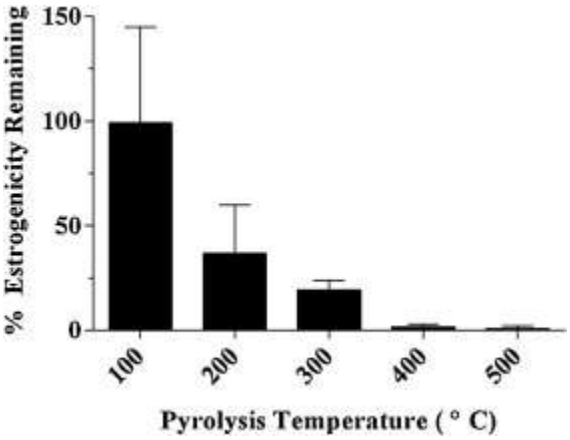


Fig. 3. Increasing pyrolysis temperature improves percent removal of estradiol equivalents (EEQs) from biosolids. Removal was based on mass balance taking mass removal through pyrolysis into account as shown in Eq. (1). Error bars represent standard deviation of triplicate samples.

Table 1. Common estrogenic compounds and chemical properties.

Common estrogenic compounds	**T_M (°C)**	**T_B (°C)**
E1 (estrone)	260[a]	392
E2 (17-β-estradiol)	222[a]	395
17-α-estradiol	222[a]	395
EE2 (ethinyl estradiol)	183[a]	411
E3 (estriol)	290[a]	432
OP (4-octylphenol)	83	311
NP (nonylphenol)	42[a]	295[a]
NP1EO (nonylphenol monoethoxylate)	116	370
NP2EO (nonylphenol diethoxylate)	140	405
Triclosan[b]	137	374
Bisphenol-A	132	364

All data from *EPI Suite* estimations, except ([a]) from *EPI Suite* experimental database. bEstrogenic as shown by.[27]

After initial volatilization from the biochar, the estrogenic compounds could either partition to the py-oil or py-gas, or be transformed through thermal decomposition. More research is needed

to determine if transformation is occurring. Because the YES assay measures the total estrogenic response of a sample as opposed to individual estrogenic compounds, it takes into account any transformation products that might also be estrogenic and residing in the final biochar product. Therefore, biochar produced at 400 °C or higher has substantially less parent estrogenic compounds and residual estrogenic metabolites than biosolids not treated via pyrolysis. The estrogenic compounds and potential transformation products that transfer into the py-gas or py-oil could potentially be oxidized when these high energy byproducts are subsequently combusted in an internal combustion engine or other equipment for energy recovery. Commonly studied pyrolysis temperatures are above 400 °C and sometimes are significantly higher than the temperatures used in this study,[16] suggesting that the pyrolysis process, if used in full-scale, would remove greater than 95% of the estrogenic load in biosolids.

3.3. Time dependence of estrogenicity removal during pyrolysis

Pyrolysis residence time is also an important factor in estrogenic removal. While the average removal increased over the first 30 min, statistically significant removal did not occur until 60 min at 500 °C (*p*-value = 0.0002, see Fig. 4). After 5 min, there was an apparent increase in estrogenicity in one sample which yielded a large standard deviation, but the EEQ of these triplicate samples were not significantly different from the EEQ of the influent triplicate samples (*p*-value = 0.5375).

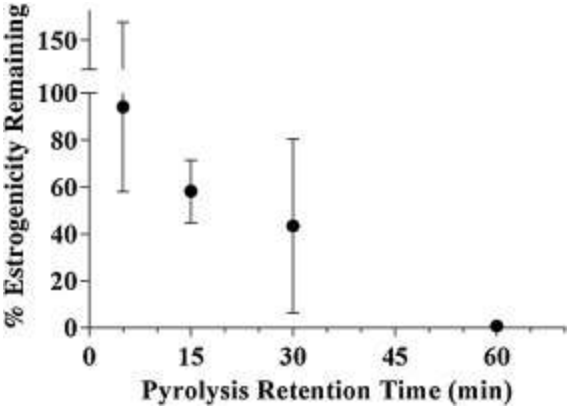


Fig. 4. Estrogenicity remaining after pyrolysis experiments with different reaction times at 500 °C. Data points represent the average value from triplicate experiments,

and error bars represent the standard deviation. No significant removal is seen until a 60 min retention time.

3.4. Estrogenicity of wastewater biosolids samples compared to biochar

Pyrolysis of ADB substantially reduced estrogenicity in the resulting biochar product (Fig. 5). The PS and ADB samples taken from a WWTP, as well as laboratory produced biochar made from pyrolysis of the ADB, were all analyzed for estrogenicity (total EEQs). The average EEQ values of the PS and ADB samples were between 400 and 500 ng EEQ/g solids. The true estrogenicity of these two samples could be different because the extraction efficiency from these two sample matrices could be different. Biochar, however, did have significantly lower EEQs than both the PS and ADB sample sets (*t*-tests *p*-value < 0.05) as the EEQ of each biochar replicate was below 12 ng EEQ/g solids.

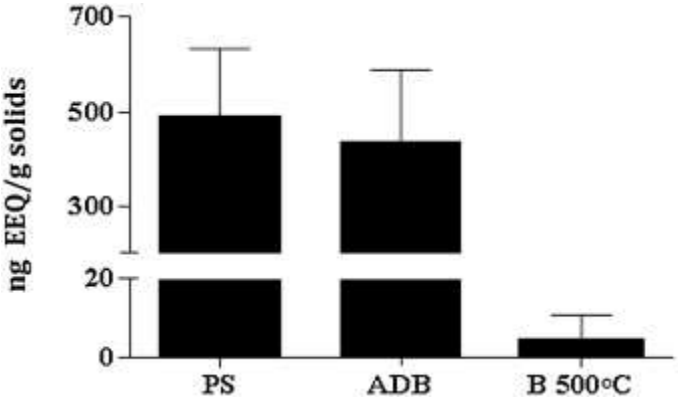


Fig. 5. Pyrolysis reduces estradiol equivalents (EEQs) of wastewater solids; EEQ are quantified as nanograms of estradiol equivalents per gram of solids (ng E2-Eq/g solids). Biochar produced at 500 °C (B 500 °C) has significantly lower EEQ compared to primary solids (PS) and anaerobically digested biosolids (ADB). Values represent the average of triplicate samples, and error bars represent standard deviation.

An accurate assessment of estrogenicity removal through these full-scale digesters would require a more thorough sampling scheme, but nevertheless the biochar samples demonstrated much lower estrogenicity than the ADB samples. Pyrolysis is a re-emerging treatment option that can remove the majority of estrogenicity from biosolids samples. The questions of where the estrogenic compounds go and if they are transformed remain to be answered and are important issues for a complete understanding of how pyrolysis can

contribute to WWTP facilities. If estrogenic compounds are not destroyed then they will be transferred into the py-gas or py-oil that could be destined for combustion. The fate of estrogens during combustion of py-gas needs to be considered along with the potential formation of toxic compounds, such as dioxins.[2,34] With respect to the solids product, estrogens are removed from the product to be land applied (i.e., biochar), making biochar produced under proper conditions of time and temperature a less-hormonally active product than anaerobically digested biosolids.

4. Conclusions

- The clean-up method developed for this research results in only moderate and variable recovery, but effectively reduces wastewater solids toxicity allowing for comparative analysis of estrogenicity of biosolids samples via the YES assay.
- An increase in pyrolysis temperature increases the removal of estrogenicity, and a reaction temperature of 400 °C or higher is required to remove >95% of estrogenicity.
- Estrogenic compounds may be volatilized or transformed out of the solid phase biochar. Further investigation is necessary to determine the fate of estrogenic compounds within pyrolysis while evaluating the formation of toxic compounds during combustion of py-gas.
- Biochar has significantly lower estrogenicity than primary solids and anaerobically digested solids suggesting that treatment by pyrolysis would reduce estrogenicity.

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Appendix A. Supplementary data

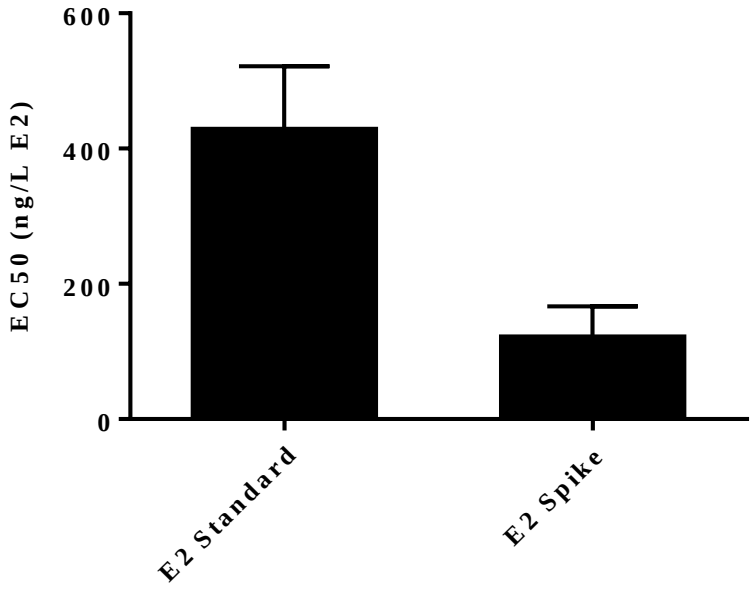

Figure S1. E2 recovery through the cleanup columns. The E2 standard column shows the EC-50 value of the standard solution used for the YES assay, while the E2 spike column shows the standard after being placed through the cleanup columns. Error bars represent standard deviation of triplicate samples.