Effect of microwave pretreatment on fate and removal of steroidal hormones during anaerobic digestion of municipal waste sludge

H. Hamid*, C. Eskicioglu**

* School of Engineering, University of British Columbia, Okanagan Campus, 1137 Alumni Ave., Kelowna (BC) V1V 1V7, Canada (E-mail: *hannahamid@ymail.com).*
** School of Engineering, University of British Columbia, Okanagan Campus, 1137 Alumni Ave., Kelowna (BC) V1V 1V7, Canada. Tel. +1-250-807-8544. Email*: cigdem.eskicioglu@ubc.ca*.

Abstract
Fate and removal of 16 steroidal (estrogenic, androgenic and progestogenic) hormones were studied during advanced anaerobic digestion using microwave (MW) pretreatment. Effects of pretreatment temperature (80, 120, 160^{o}C), digester operating temperature (mesophilic at 35 ± 2^{o}C, thermophilic at 55 ± 2^{o}C) and digester solid retention time (SRT: 20, 10, 5 days) were studied on biogas (methane) production as well organic removal efficiencies from municipal sludge samples. In order to determine the potential effect of MW irradiation, hormones were quantified in total (sludge) and soluble phases of the digester influents and effluents before and after pretreatment and digestion. Seven of the 16 hormones tested were above the reporting limits (RLs) in one or more of the samples. Hormone concentrations in total phase of un-pretreated (control) and pretreated digester feeds ranged in <157 – 2491 ng/L and <157 – 749 ng/L, respectively. Pretreatment with MW resulted in both release from the sludge flocs and attenuation of hormones in the soluble phase. Simultaneous accumulation and removal of 17β-estradiol (E2), and estrone (E1) in soluble phase indicated possible transformation among these estrogenic hormones.

Keywords
Estrogen; androgen; biosolids; microwave pretreatment; anaerobic digestion

INTRODUCTION

Removal of endocrine disrupting compounds (EDCs) is not a design criterion for conventional wastewater treatment plants (WWTPs) and the incomplete removal results in presence of these pollutants in the semisolid residue (biosolids) of WWTPs. In many countries, including Canada, land application of biosolids, which is considered to be a sustainable sludge disposal option, is causing public awareness and concern. Therefore, selection of an optimum biosolids treatment method is very important in order to minimize the concentration of EDCs in biosolids

Production of heat or electricity by recovered methane and reduced greenhouse gas emission has made anaerobic digestion a favourable sludge treatment option in today's world. Recent developments in this field indicate that thermal (microwave or conventional heating), chemical and mechanical disintegration techniques before anaerobic digestion can substantially increase the methane recovery potential and degradation of biosolids. However, the effects of these pretreatments on removal of steroidal hormones in advanced anaerobic digesters are yet to be studied.

As the pretreatments disintegrate the complex polymeric network in waste sludge samples, it is highly likely that some hormones initially encapsulated within the polymeric network may be released into the soluble phase and render themselves more or less biodegradable depending on the changes on their molecular structures at different pretreatment conditions (low or elevated temperatures or mechanical shear). Furthermore, as sludge pretreatments solubilize organics (hormones), it could be postulated that there is a potential to shift the ultimate disposal route of residual hormones in digestate from a landfill or agricultural land to main wastewater stream. Therefore, the objective of this work was to study the effect of MW hydrolysis on fate and removal of 16 steroidal hormones (synthetic and natural displayed in Table 1) during advanced anaerobic

digestion of municipal biosolids. The effects of pretreatment and digestion temperature as well as solid retention time (SRT) were also evaluated at the laboratory scale.

MATERIALS AND METHODS

Substrate and inocula

Sewage sludge cake was collected bi-weekly from Kelowna Pollution Control Centre (British Columbia (BC), Canada). Fermented and gravity thickened primary sludge and waste activated sludge, thickened by a dissolved air flotation unit, are pumped separately and mixed (40:60, v/v) and dewatered by centrifugation. The dewatered sludge cake with a total solids (TS) content of 17.5 ± 1% (w/w) was used for MW pretreatment. Previous studies indicated that heating concentrated sludge samples minimizes the input energy requirement per dry weight by minimizing heat dissipation in the water. MW pretreated and un-pretreated (for controls) sludge cakes were then diluted to attain a typical feed concentration of 3.4% TS (w/w) for anaerobic digesters. The mesophilic and thermophilic inocula were collected from the full-scale anaerobic digesters located at Penticton and Annacis Island WWTPs (BC, Canada), respectively. The inocula were acclimatized to MW pretreated sludge at a temperature of 175^{o}C to avoid any possible acute inhibition at elevated hydrolysis temperatures for 7 months.

Microwave pretreatment

A closed-vessel microwave digestion system (ETHOS-EZ, Milestone Inc., Connecticut, USA) operating at 2.45 GHz with maximum power, temperature and pressure of 1200 watts, 300^{o}C and 35 bars, respectively was used. Sludge cake was irradiated with similar heating profiles (at 7.5^{o}C/min ramping rate) to reach desired temperatures of 80, 120 and 160^{o}C in 12 pressure sealed vessels.

Digester studies

Eight semi-continuous flow digesters (fed once a day) were run for SRTs of 20, 10 and 5 days. Four of the digesters were operated at the mesophilic temperature (35^{o} C) and the remaining four were at the thermophilic condition (55^{o} C) in temperature controlled shakers. At each operational stage, once the steady state (< 10 % variation in biogas production rate) has been reached, the digesters were maintained for over a period of three SRTs. Effluents were collected during this time as 7-day composite samples in LDPE bottles and refrigerated at 4^{o}C for hormone analysis. Chemical oxygen demand (COD), TS, volatile solids (VS), pH, alkalinity, ammonia, volatile fatty acids (VFA) of effluent and feed along with biogas composition were monitored twice a week. Digester pH and biogas volumes were measured daily.

Analytical techniques

Characterization of influent and effluent. Total solids, VS, alkalinity and ammonia were analyzed according to *Standard Methods* procedure 2540 B, 2540 E, 2320B and 4500D, respectively (APHA, 2005). Total volatile fatty acids in the digesters and biogas composition in the headspace were determined using Agilent 7890A and 7820A gas chromatographs, respectively.

Hormone analysis. Digester effluent and feed samples were centrifuged at 8,000 rpm for 20 min and the supernatants were collected and filtered through 1 µm filter papers. The filtrate, which represents the soluble fraction of sample, along with feed and collected effluent were used for hormone analysis. Prior to extraction and clean-up procedures, samples were adjusted to the required pH (2.0) and spiked with surrogates. The sludge samples were extracted by sonication with aqueous buffered acetonitrile and with pure acetonitrile, concentrated by rotary evaporation. Aqueous samples and the diluted extracts of sludge samples were cleaned up by solid phase extraction. After addition of recovery standards, the extract was filtered and analyzed by liquid chromatograph coupled to a triple quadrupole mass spectrometer (LC/ESI-MS/MS) in positive and negative ionization modes to analyze a total of 17 hormones displayed in Table 1.

Table 1. List of hormones analyzed and their estimated method detection limit

Hormone	Category	Soluble phase (ng/L)	Sludge sample (ng/g)
Estrone (E1)	Natural estrogen	11.7	5.9
17β-Estradiol (E2)	Natural estrogen	14.9	7.4
17α-Estradiol	Natural estrogen	6.6	3.3
Estriol (E3)	Natural estrogen	175	88
17α-Ethinylestradiol	Synthetic estrogen	38.5	19.25
Equilin	Natural estrogen	11.7	5.9
17α-dihydroequilin	Natural estrogen	8.3	4.1
Equilenin	Natural estrogen	2.64	1.32
Testosterone(Tr)	Natural androgen	1.57	0.79
Androstenedione (Ad)	Natural androgen	1.76	0.88
Androsterone (An)	Natural androgen	30.3	15.1
Progesterone (Pr)	Natural progestogen	3.02	1.51
Norethindrone	Synthetic progestogen	2.58	1.29
Allyl Trenbolone	Synthetic progestogen	2.64	1.32
Mestranol (Ms)	Synthetic estrogen	100	50
Norgestrel	Synthetic progestogen	6.1	3.1

RESULTS AND DISCUSSION

Effects of microwave pretreatment on steroids in digester feed

Effects of MW pretreatment on hormone release/attenuation are shown in Figure 1. There was no consistent pattern among different steroid concentrations with an increase in MW irradiation temperature in soluble (Figure 1a) or total phases (Figure 1b) of digester feeds.

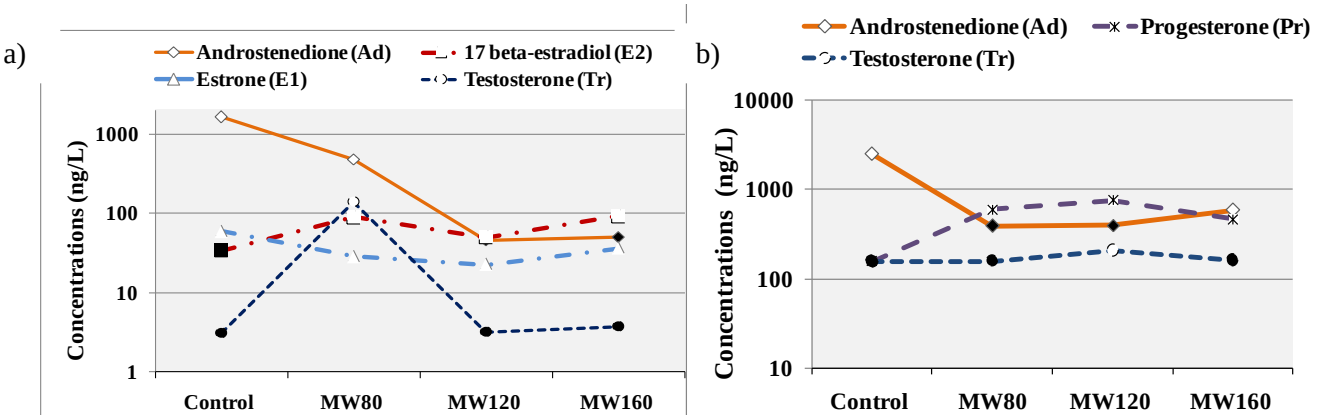

Figure 1. Effect of microwave (MW) on hormone concentrations in the a) soluble (supernatant) phase and b) total (sludge) phase of the influent (Control: un-pretreated; 80, 120 and 160: MW pretreatment temperatures in ^{o}C; single data point concentrations; black filled data points represents concentrations below reporting limit).

Pretreatment led to attenuation of Ad and Tr in both soluble and total phases of the digester influents with an increase in the MW temperature. Similarly, E1 decreased in the soluble phase with an increase in the pretreatment temperature and was below the RL in the total phase. The attenuations could have been caused by abiotic transformation of the hormones. Autoxidation of cholesterol and plant sterols by heating is well documented in the literature (Osada et al., 1993) and could explain the results obtained in the current study involving steroids with similar ring structures to sterols. E2 was the only hormone showing some release with increasing temperature in the soluble phase.

Effects of microwave pretreatment on anaerobic digestion of steroid hormones

The concentrations of E1 and E2 at various SRTs (in soluble phase) are displayed in Figures 2a and 2b, respectively. Despite the known inefficiency of conventional anaerobic digesters to biodegrade

the hormones, below the RL concentrations of estrogenic hormones made it difficult to estimate their removal efficiencies from the total phase. However, the soluble phase concentrations indicated accumulation of E1 and removal of E2 in both controls and pretreated digesters. Previous studies indicated that, E1 is an intermediate compound of E2 degradation (Czajka and Londry, 2006), which may be responsible for the observed accumulation of E1. Despite the lower concentrations of Ad and Tr in the pretreated influents, the effluent concentrations revealed higher accumulation in both phases in the pretreated digesters compared to the controls. Possible explanation of such accumulation could be microbial assisted transformation of hormones. Bioconversion of phytosterols had been reported to cause accumulation of androgens (e.g., androstenedione (Ad)) in anoxic river sediments receiving pulp and paper mill effluent (Jenkins et al., 2004).

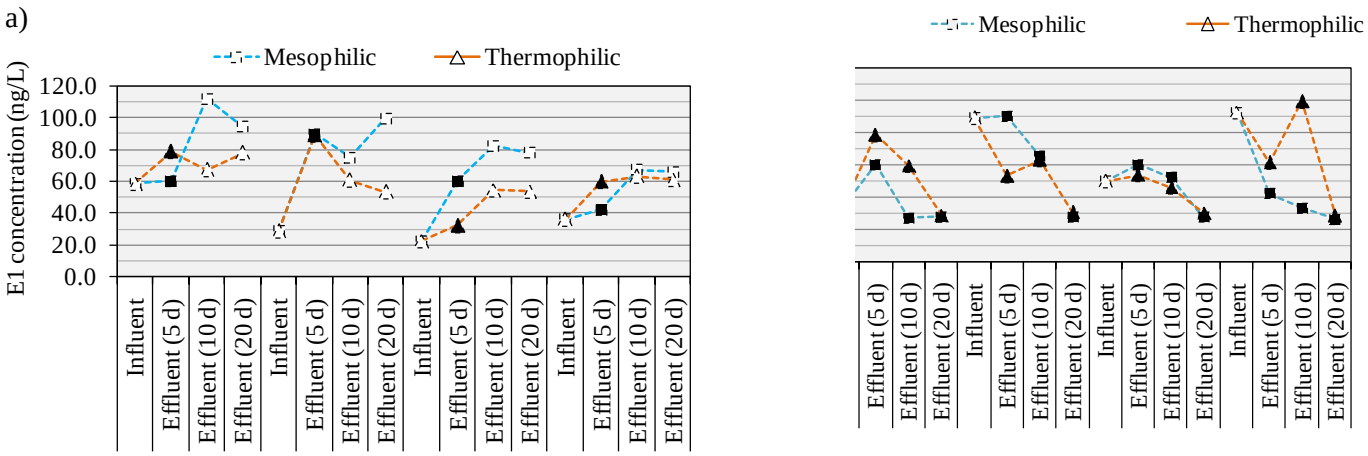

Figure 2. Concentrations of a) estrone (E1) and b) 17β-estradiol (E2) in soluble phases of the influent and effluents at different solid retention times (SRTs) (Control: un-pretreated; 80, 120 and 160 are ultimate microwave pretreatment temperatures in ^{o}C unit; black filled data points were below reporting limit (RL). RL varied as E1: 32 – 90 ng/L, E2: 26.2 – 90 ng/L).

Microwave pretreatment resulted in relative (to control) improvement of volatile solids removal efficiencies for pretreated digester in the range of 124-163% and 98-117%, respectively for mesophilic and thermophilic digesters at SRT of 5 days. Similarly, pretreated digesters produced 101-121% and 57-81% higher methane, respectively for mesophilic and thermophilic digesters, over controls at same SRT.

CONCLUSION

Pretreatment by MW showed release of comparatively more hydrophobic hormones (i.e. E2) in the soluble phase and attenuation of some other hormones (i.e. E1, Ad, Tr). Low biodegradation efficiency of steroidal hormones in anaerobic digesters resulted in accumulation of Ad, E1 and Tr in both soluble and total phase. Possible microbial biotransformations of the steroidal hormones were observed; however, this needs further investigation.

REFERENCES

APHA, 2005 Standard methods for the examination of water and wastewater, Washington DC, USA.

Czajka, C. P. and Londry, K. L., 2006. Anaerobic biotransformation of estrogens. *Science of the Total Environment* **367**(2-3), 932-941.

Jenkins, R. L., Wilson, E. M., Angus, R. A., Howell, W. M., Kirk, M., Moore, R., Nance, M. and Brown, A., 2004. Production of androgens by microbial transformation of progesterone in vitro: A model for androgen production in rivers receiving paper mill effluent. *Environmental Health Perspectives* **112**(15), 1508-1511.

Osada, Kyoichi, Kodama, Takehiro, Yamada, Koji and Sugano, Michihiro, 1993. Oxidation of cholesterol by heating. *Journal of Agricultural and Food Chemistry* **41**(8), 1198-1202.