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Partial characterization of biosolids: Lipophilic organic components

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ABSTRACT

Biosolids from a municipal sewage treatment plant were studied from the standpoint of characterizing the matrix as well as targeting specific substances. Part of this interest stems from the land application of biosolids that may contain pharmaceuticals and personal care products (PPCPs). Targeted analytes were subjected to higher specificity than usual, and this was achieved either through multidimensional chromatography or through high resolution mass spectrometry (accurate mass determination) or both. The bacterial endpoints consisting of fecal sterols and fecal sterones were present as expected and were generally greater than ppm levels meaning that they could pose significant interferences to target analytes at low ppb levels. Thus, one of our goals in characterization was to identify some of these major components as an aid in formulating cleanup strategies for the determination of PPCPs. Target analytes included nonyl phenols, polybrominated diphenyl ethers (PBDEs), and estrogens. These ranged from ppm levels for the first two groups to low or sub ppb levels for the estrogens. The nonyl phenols were further confirmed using high resolution voltage scanning and mass peak profiles at high resolution. The PBDEs were further confirmed using high resolution selected ion recording as were the estrogens.

KEYWORDS

Biosolids;
Lipophilic;
Fecal sterols;
Nonyl phenols;
PBDEs;
Accurate mass.

INTRODUCTION

Biosolids (sewage sludge) represent the end product of bacterial digestion and applied treatments of raw sewage in a municipal sewage treatment facility[1]. The precipitated material from the aqueous solution consists of a relatively intractable material made up of both inorganic and organic substances that have reached an environmental sink. The characterization of this material remains incomplete but more is being revealed by various workers throughout the world[2].

In recent times the interest has shifted from disposal of the biosolids material in landfills to the preferred use of processed material as a soil amendment[3]. The question of course arises as to the potential exposure to biota that would result from such an application in the environment as well as effects on human health that might arise from eating crops exposed to the amended soil or animals eating such crops before going to market. Air transport of dust made up of such material would also be a potential concern. Additionally, migration of substances from biosolids into groundwa-

ter or ultimately into drinking water due to leaching is of concern[4].

In examining biosolids issues, one should maintain the distinction between the case of biosolids arising from treated human waste and applications of animal manure that has been used as fertilizer for hundreds if not thousands of years or to the practice of using raw human waste on crop lands. The biosolids have usually undergone additional treatment to destroy pathogens either by heat or irradiation after the considerable bacterial degradation and additional process treatment that has taken place. Thus, the biosolids are designated Class A or Class B depending on the extent of elimination of pathogens[5]. Some proponents advocate the additional step of composting to remove/degrade the remainder of the objectionable compounds that are currently known to reside in biosolids[6].

A number of papers have now started filling in some of our questions about the types of contaminants in biosolids. The review by Rogers[1] included mention of a variety of compounds including organochlorine pesticides (e.g., aldrin), PCBs, and chlorophenols. A large presence for nonyl phenols and surfactants was mentioned. The large and diverse class of pharmaceuticals and personal care products (PPCPs) was described and most of these compounds (e.g., antibiotics) would be found in the polar fraction of biosolids components[7,8]. The synthetic musks are one group of PPCPs that partition with the lipophilic fraction. Methods were given that included extraction and cleanup procedures.

Oberg et al. described the occurrence of PBDEs in over 100 sludge samples from Sweden where the predominant tetra, penta, and hexa congeners were found as well as the decabromodiphenyl ether[9]. Ying and Kookana pointed out that high levels of triclosan in biosolids could be a concern in soil applications[10]. Synthetic musks were also determined in biosolids.[11] Nonylphenols, pthalates, and PCBs were determined in biosolids and soil in an effort to follow the fate of such contaminants after soil amendment using biosolids[12].

A number of papers have focused on the polar analytes (contaminants) found in biosolids. Giger et al. reported methodology for extraction and determination of antibiotics including the fluoroquinolone antibiotics. Extraction difficulties and relatively low recoveries were noted[13]. Mottaleb and Brumley reviewed the separations used in determining PPCPs in a variety of environmental matrices[14]. The National Research Council has presented an overview of issues concerned with applying biosolids to land[15].

In this work we partially characterize the lipophilic components of biosolids to yield some perspective on the relative contributions of major components and provide definitive confirmations of identity on select contaminants.

EXPERIMENTAL

Method for nonylphenols

GC/MS

A 40 m 0.18μm film 0.18 mm ID column (DB5ms Agilent-J&W) was used with temperature programming: 60^0C for 1 min followed by 60-300@15^0C/min to 300^0C. Scan range 50-500 under EI conditions. Injector temperature was 280EC. Internal standard was d14-terphenyl (quantitation ion m/z 244, 2.5ng/μL in the extract, 1 ^{0}L injection) and quantitation was performed by summing the total ion current between 12.2 and 14.2 min using technical nonyl phenol as a standard. Technical nonyl phenol is a multicomponent mixture of nonyl phenols and octyl phenols and has more than 13 major/minor components.

Extraction/Cleanup

The method uses a SW-846 type extraction of biosolids in methylene chloride/acetone by either Soxhlet or ASE. The ASE conditions were a 10g cell, 80^0C, 2500 psi, 15 min extraction.

An SPE cleanup isolated the nonyl phenols in methylene chloride and methylene chloride/hexane fractions on silica. A 3 mL Si SPE cartridge (Supelco) is washed with hexane; sample is applied in 1 mL of hexane and then the sample is eluted with 2 mL hexane, 2 mL hexane/methylene chloride (50/50 v/v), 2 mL methylene chloride, and 2 mL acetone.

Method for PBDEs

Extraction/Cleanup

The method uses a SW-846 type extraction of BS in methylene chloride/acetone by either Soxhlet or ASE. The extract is concentrated and then fractionated on

silica using SPE to isolate the fraction containing the PBDEs. A 3 mL Si SPE cartridge (Supelco) is washed with hexane; sample is applied in 1 mL of hexane and then the sample is eluted with 2 mL hexane, 2 mL hexane/methylene chloride (50/50 v/v), 2 mL methylene chloride, and 2 mL acetone. The PBDEs are found in the hexane fraction.

GC/MS

A 40 m 0.18μm film 0.18mm ID column (DB5ms Agilent-J&W) was used with temperature programming: 60^{0}C for 1 min followed by 60-300@15^{0}C/min to 300^{0}C.

The method uses quantitation ions from specific PBDE congeners (congeners 181, 28, 183, 47, 99, 154, 66, 85, 153, 100, 155) for quantitation (m/z 403.8, 405.8, 407.8, 483.7, 485.7, 487.7, 561.6, 563.6, 565.6, 641.5, 643.5, 645.5, 719.4, 721.4, 723.4) . The internal standard is PCB#204 (m/z 427.8, RT=15.77, 100 pg/μL in the extract, 1μL injection).

Method for estrogens

Extraction/Cleanup

The method uses a SW-846 type extraction of BS in methylene chloride/acetone by either Soxhlet or ASE. The extract is concentrated and then fractionated on silica SPE into hexane, hexane/methylene chloride, methylene chloride, and acetone fractions. The estrogens (estrone (EST), 17?-estradiol (ESD), and ethynyl estradiol (EED)) are found in the acetone fraction. The method uses quantitation ions of the individual estrogens. The concentrated extract was further concentrated by a gentle nitrogen stream and evaporated to dryness in a 60 mL glass tube. The dry film was redissolved with 1 mL hexane for application to the SPE cartridge cleanup using silica.

SPE cleanup

A 3-mL cartridge of LC-Si (Supelco, Bellafonte, PA, USA) was washed with 6 mL hexanes with a small portion retained above the frit. The 1-mL sample extract was added and pulled through along with 2 mL of hexanes, always retaining a small liquid level above the frit. Next, a solvent gradient was applied in the following sequence: 2 mL (v/v, 50/50) hexanes/methylene chloride, 2 mL methylene chloride, 3.5 mL acetone. The estrogens were recovered in the acetone fraction and concentrated to dryness under a gentle nitrogen stream in a 13-mL test tube. A 1-mL aliquot of hexanes was added and used to wash down the walls. The hexanes solution was then filtered prior to injection on the HPLC porous graphitic carbon (PGC) column.

HPLC fractionation on PGC

A 1-mL hexanes solution of the sample was loaded on a Valco 10-port injector (VICI, Houston, TX, USA) for injection on a 5μm PGC HyperCarb (Hypersil, Keystone, Bellefonte, PA, USA) column 4.6 mm ID x 100 mm. A flow rate of 2.5 mL/min of mobile phase was used. The gradient consisted of: 100% hexane for 2 min, from 2 to 5 min develops 18.7% toluene; from 5 to 10 min develops 50% toluene, 20% acetonitrile; from 10 to 16 min develops 100% acetonitrile; from 16 to 18 maintains 100% acetonitrile; from 18 to 26 develops 100% toluene; from 26 to 30 min develops 100% hexane; from 30 to 36 min maintains 100% hexane to equilibrate before the next run. Fractions were collected by a Foxy Junior Collector (ISCO, Lincoln, NE, USA) with 1 min/13-mL test tube. Tube 9 contained EED and 10 contained EST and ESD. Estriol is not collected in either tube so no native contribution is present to affect quantitation using estriol as an internal standard[16].

GC/MS

This work was performed on an Agilent 5973 GC/MSD with a 6890 gas chromatograph. A 40 m (0.18 mm ID with 0.18 um film) DB5ms (Agilent-J&W) was used with a He flow of 0.5 mL/min. The injection parameters were 1μL pulsed splitless injection (40 psig pulse pressure), 280^{0}C injection temperature, and transfer line temperature of 280^{0}C. The GC initial temperature at 105^{0}C was held for 1.0 minute, then increased to 300^{0}C at a rate of 20^{0}C/min and was held for 20.0 minutes. The transfer line was set at 280^{0}C.

The following ions (m/z) were monitored by compound: 504.4 for estriol (3-TMS derivative) as internal standard (530 pg/μL in the extract, 1μL injection); 342.2, 257.1, 218.1, 327.2 for EST (1-TMS derivative); 416.3, 285.2, 326.2, 232.1 for ESD (2-TMS derivative); 368.2, 285.2, 301.2, 232.1 for EED (1-TMS derivative); 425.3, 440.3, 301.2, 285.2 for EED (2-TMS derivative).

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RESULTS AND DISCUSSION

Overview of results

This work partially characterizes BS in terms of the organic base/neutral extractables (hydrophobic components). The extraction conditions were consistent with published work on the extraction of biosolids for these types of analytes where we have used pressurized and heated solvent extraction. The very polar and ionic compounds such as the fluoroquinolone antibiotics constitute a more complex analytical problem with regard to characterization of their presence in BS and are not treated in this work.

The base/neutral fraction consists of several major components and classes of compounds. The total ion chromatogram in figure 1 under the conditions of full scan EIMS illustrates the relative amounts of some major biosolids components in a single visible context.

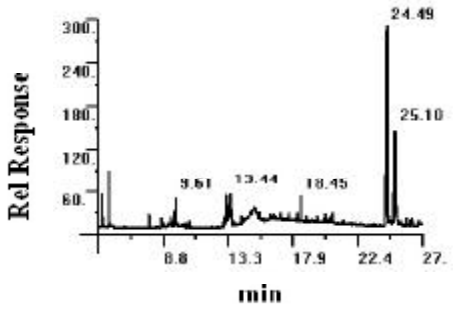

Figure 1: Total ion chromatogram of the organic extract of biosoids, EI full spectrum m/z 50-500. The extract was not derivatized for this comparison of relative responses. Example compounds: hydrocarbon greases such as tridecane at RT=9.61 and tetradecane at RT=10.54; nonyl phenols are found ca. RT=13.44 min (lesser amounts of alkyl benzenes are present in front of and within the same area); galaxolide is at RT=14.28; elemental sulfur is in the broad peak centered ca. RT=15.0; diethylhexyl phthalate occurs at RT=18.45; and the fecal sterols and sterones begin ca RT=24.49 min

The expected presence of fecal sterols and corresponding sterones (ketone forms such as coprostanone relative to coprostanol) are the major components and consist of coprostanol, epicoprostanol, dihydro cholesterol, ethylcholestanol, and coprostanone. The presence of coprostanol has long served as an indicator of the possible contamination of surface water and sediments by raw sewage. Some unmetabolized cholesterol and plant sterols remain present in this matrix. These components were confirmed using standards and the correspondence of retention time and spectra. Recognition of the high levels of these substances is important in developing cleanup procedures for target analytes.

Fecal sterols and sterones represent endpoints of bacterial degradation both in the human intestine and apparently in the wastewater treatment although the occurrence of compounds completely unchanged (e.g., fatty acids) as they reach this sink is also observed. There occur a number of high molecular weight hydrocarbon greases among the smaller peaks along the baseline. Another prominent peak corresponds to galaxolide, a synthetic musk. A major presence of nonyl phenols is observed along with the unsulfonated alkyl benzenes that accompany linear alkyl benzene sulfonate surfactants. A large peak due to bis(ethylhexyl)phthalate is seen as well as a broad hump due to elemental sulfur. These components present the picture of a highly hydrophobic composition with the presence of some moderately polar hydrophobics such as the estrogens more than 1000 times less concentrated than the fecal sterols. We know of course from other work that there is a highly polar and ionic composition as well including inorganic ionic substances accompanying this lipophilic material.

Among major components contributed by various sources outside of the fecal substances are the musks (galaxolide), nonyl phenols, and PBDEs.

The matrix represents an environmental sink resulting from bacterial degradation and application of precipitation agents removing both hydrophilic and hydrophobic components from a water system. Thus, the primary concern may be one of long term degradation and the occurrence of various scenarios that could liberate various components from this reservoir of waste products. In this regard, the advisability of using composting to further break down substances and to provide increased availability of nutrients becomes a consideration.

Nonyl phenols

The extraction conditions were consistent with published work on the extraction of BS for these analytes. Nonyl phenol is a major component of BS with levels found at 684 ppb in a municipal biosolids material. All

components representing nonyl phenols and other related phenols (e.g., octyl phenols) were included in the overall level. This level is consistent with a range of levels reported for BS. Detection limits for nonyl phenols in biosolids were not determined because they constitute a major characteristic component in the material. Figures 2A and 2B show how mass peak profiles lead to accurate and sensitive mass assignment even with capillary GC peaks and reveal whether interferences affect the peak shape[17]. The AF in the figures is the agreement factor produced by using a nonlinear least squares calculation based on the R factor of Hamilton[18].

PBDEs

The extraction conditions were consistent with published work on the extraction of BS for these analytes. The PBDEs were done as a congener-specific analysis with the following results:

PBDE congener#	Amount
181	313 ppb
28	24.2 ppm
183	886 ppb
47	3.74 ppm
99	5.17 ppm
154	424 ppb
66	172 ppb
85	912 ppb
153	959 ppb
100	677 ppb
155	23 ppb

The results range from low ppm levels to tens of ppb levels on a per congener basis. The data confirm the previous reports in the literature of the prominent occurrence of PBDEs in BS and presents the data on a congener specific basis. These levels represent amounts comparable to those of the nonyl phenols and the musks that are major components. Figure 3 illustrates results for pentabromo congeners via the m/z 563.6216 ion using HRSIR monitoring.

Recovery of PBDEs was checked using spiked matrix with congener #155 and averaged 123% confirming our expectation that hydrophobic analytes are extracted efficiently as expected. Based on congener #155, PBDEs are detectable in biosolids at about 10 ppb with a signal to noise ratio of 8:1.

Estrogens

The estrogens were determined using ion monitoring for EST, ESD, and EED with estriol as the internal standard (HPLC fractionation removed any naturally occurring estriol). Figure 4 indicated an interference free response for EST and a representative low resolution (MID) spectrum produced from the monitored ions. ESD and EED were presumptively present at lower levels in comparison and were subject to coelution of additional components. The fact that the ions exhibited by the target analytes are also very common in the steroidal type structures of constituents of biosolids as well as their presence at a thousand fold higher level together raise specificity issues. The low resolution data was judged to have weak presumptive evidence for

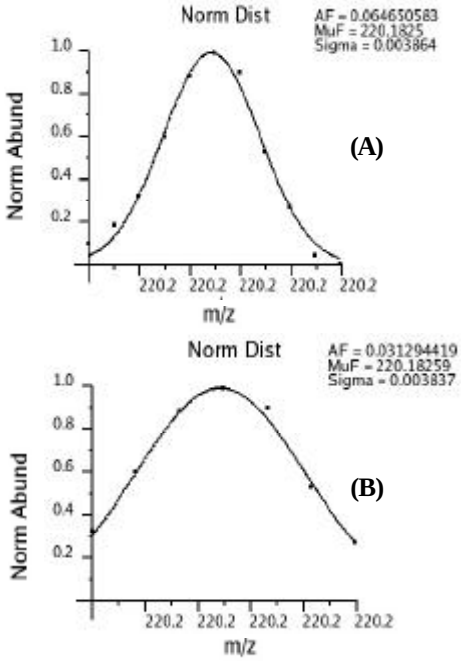

Figure 2: Mass peak profile of the molecular ion of nonyl phenols at 10000 resolution (a): 11 point profile with assignment 0.9 ppm in error and (b): 7 point profile with assignment 0.4 ppm in error

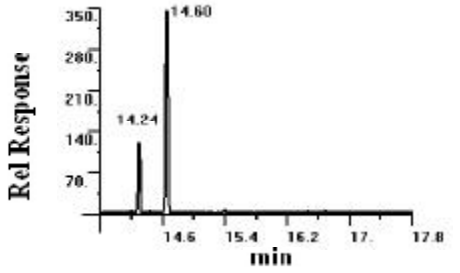

Figure 3: Example of m/z 563.6216 of PBDE #100 and 99 at RT 14.24 and 14.60 min at 10000 resolution

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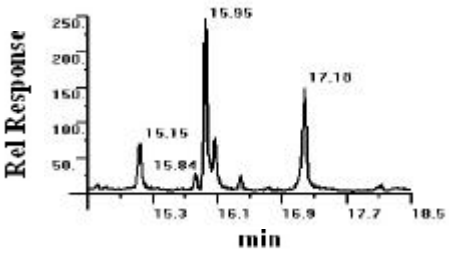

Figure 4: Low resolution EI ion monitoring for the estrogens (EST, ESD, EED) after cleanup and derivatization using BSTFA; EST at RT= 15.84

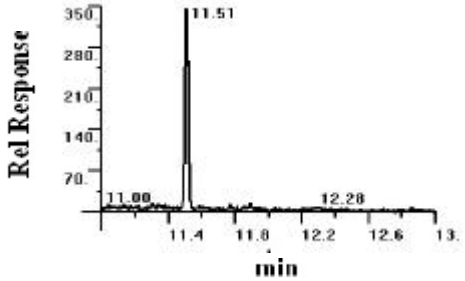

Figure 5: High resolution (10000) SIR of m/z 342.2015 for EST at RT 11.51

ESD and EED which were not confirmed.

Recovery of EST was checked by matrix spikes with relatively high levels with an average of 77% recovery obtained. This determination has a more complex cleanup associated with it, and therefore the recovery has decreased relative to other analytes. The detection limits of EST, ESD, and EED lie in the 1 to 10 ppb range.

High resolution mass spectrometry

Three techniques were used in high resolution experiments and all were carried out at 10000 resolution. Accelerating voltage scans allowed a limited mass range to be acquired on capillary GC peaks. In the case of nonyl phenols the molecular ion (m/z 220) and a fragment ion at m/z 191 were mass measured accurately. The agreement with theory for m/z 220 (calc 220.1827, obs 220.1836, 4 ppm) and for two ions at m/z 191 (calc 191.1436, obs 191.1440 for the loss of C2H5 2 ppm; calc 191.1800, obs 191.1803 for the loss of CHO 1.5 ppm). As a further aid to the elucidation of the elemental composition for the molecular ion, we employed mass peak profiling to seek a lower error in the accurate mass determination[17]. This technique uses small incremental masses in the vicinity of the observed accurate mass and acquires the response using the SIR descriptor. The areas of the ions are plotted and a best fit of the data to a gaussian peak is carried out using the full nonlinear equation of the peak and is assessed through the use of the Hamilton RFactor[18]. The molecular ion at m/z 220 (calc 220.1827, obs 220.1825 0.9 ppm for 11 data points and 220.1826 for the central 7 data points 0.4 ppm) was particularly accurate for a capillary GC peak (Figure 2).

The HRMS SIR technique for PBDEs resulted in the confirmation of the quantitated compounds with the proper relative abundances (Figure 3). Occasionally, PBDE congeners are subject to some interference on one ion in the low resolution results and the HRMS SIR obtains the proper ratio suggested by theory.

In the case of the estrogens, an acceptable low resolution mass spectrum was obtained for EST as stated previously. Nevertheless, recourse to HRMS SIR gave a strong confirmation to the molecular ion at m/z 342 for EST (Figure 5). ESD and EED had yielded presumptive but equivocal evidence and could not be confirmed by low resolution mass spectrometry. Weak responses (about 20 fold less than that of EST) at retention times slightly different than ESD and EED were also obtained for the respective molecular ions. It was concluded that neither ESD nor EED could be confirmed and may be effectively removed in the BS pathogen removal process.

CONCLUSION

This partial characterization has resulted in the identification of major components of biosolids. The fecal sterols and sterones together with high levels of other contaminants such as nonyl phenols present cleanup challenges in determining traditional nonpolar analytes and new compounds targeted from PPCPs that may be 1000 times lower in concentration. Choice of SPE Si is effective for analytes not eluting with the sterols. Application of multidimensional chromatography using PGC with HPLC provided additional separation for the estrogens and use of HRMS afforded increased specificity and selectivity in determining elemental compositions and in SIR monitoring at high resolution.

NOTICE

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REFERENCES

[1] H.R.Rogers; Sci.Total Environ., **185**, 3 **(1996)**.
[2] T.A.Ternes, N.Herrmann, M.Bonerz, T.Knacker, H.Siegrist, A.Joss; Water Res., **38**, 4075 **(2004)**.
[3] G.A.O'Connor; Sci.Total Environ., **185**, 71 **(1996)**.
[4] M.J.La Guardia, R.C.Hale, E.Harvey, T.M.Mainor; Environ.Sci.Technol., **35**, 4798 **(2001)**.
[5] 'Guidance for Controlling Potential Risks to Workers Exposed to Class B Biosolids', Publication Number 2002-149, DHHS, NIOSH, CDC, July, **(2002)**, (assessed at http:/ / www.cdc.gov/ niosh/ docs/ 2002-149/ pdfs/ 2002-149.pdf on 05/16/2007). **(2007)**.
[6] J.V.Fermante J.Meggan; Pollution Engineering, **29**, 40 **(1997)**.
[7] <http://www.epa.gov/nerlesd1/chemistry/org-anal/home.htm> (accessed 10/30/2007) <http://www.epa.gov/ppcp/> (accessed 10/30/2007) **(2007)**.
[8] K.Oberg, K.Warman, T.Oberg; Chemosphere, **48**, 805 **(2002)**.
[9] G.-G.Ying, R.S.Kookana; Environ.Internat., **33**, 1099 **(2007)**.
[10] L.I.Osemwengie; J.Environ.Monitoring, **8**, 897 **(2006)**.
[11] R.Gibson, M-J.Wang, E.Padgett, A.J.Beck; Chemosphere, **61**, 1336 **(2005)**.
[12] W.Giger, A.C.Alder, E.M.Golet, H.-P.E.Kohler, C.S.McArdell, M.A.Mottaleb, W.C.Brumley; Trends Chromatogr., **2**, 11 **(2006)**.
[13] National Research Council, "Biosolids Applied to Land," The National Academy Press, Washington, DC., **(2002)**.
[14] L.Riddick, E.L.Gentry, M.McDaniel, W.C.Brumley; Internat.J.Environ.Anal.Chem., **86**, 299 **(2006)**.
[15] W.C.Brumley, A.H.Grange, V.Kelliher, D.B. Patterson, A.Montcalm, J.Glassman, J.Farley; JAOAC International, **83**, 1059 **(2000)**.
[16] W.C.Hamilton; Acta Cryst., **18**, 502 **(1965)**.