Fate of Zinc Oxide Nanoparticles during Anaerobic Digestion of Wastewater and Post-Treatment Processing of Sewage Sludge

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Ⓢ *Supporting Information*

ABSTRACT: The rapid development and commercialization of nanomaterials will inevitably result in the release of nanoparticles (NPs) to the environment. As NPs often exhibit physical and chemical properties significantly different from those of their molecular or macrosize analogs, concern has been growing regarding their fate and toxicity in environmental compartments. The wastewater—sewage sludge pathway has been identified as a key release pathway leading to environmental exposure to NPs. In this study, we investigated the chemical transformation of two ZnO-NPs and one hydrophobic ZnO-NP commercial formulation (used in personal care products), during anaerobic digestion of wastewater. Changes in Zn speciation as a result of postprocessing of the sewage sludge, mimicking composting/stockpiling, were also assessed. The results indicated that "native" Zn and Zn added either as a soluble salt or as NPs was rapidly converted to sulfides in all treatments. The hydrophobicity of the commercial formulation retarded the conversion of ZnO-NP. However, at the end of the anaerobic digestion process and after postprocessing of the sewage sludge (which caused a significant change in Zn speciation), the speciation of Zn was similar across all treatments. This indicates that, at least for the material tested, the risk assessment of ZnO-NP through this exposure pathway can rely on the significant knowledge already available in regard to other "conventional" forms of Zn present in sewage sludge.
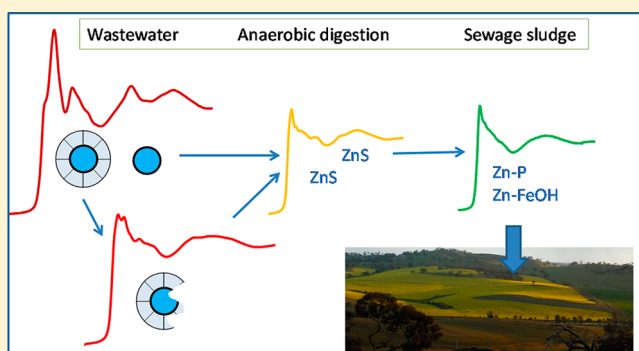
INTRODUCTION

Over the past decade, rapid innovation and commercialization in the field of nanotechnology has ensured continuous growth in this sector (e.g., ref 1). As a result, an increasing number of manufactured nanomaterials (generally defined as engineered particles with at least one dimension <100 nm) have been incorporated into products and manufacturing processes. This growth has prompted an equivalent increase in concern regarding the safety of manufactured nanomaterials with respect to human and environmental health. This is because, as with most products of human activities, their intentional or accidental release to the environment is largely unavoidable (e.g., refs 2,3).

Zinc oxide nanoparticles (ZnO-NPs) are released indirectly to the environment as a result of their increasingly common use in cosmetic products, sunscreen formulations, paints, plastics, and packaging (e.g., ref 4). As is the case for most metal-containing nanoparticles used in everyday consumer products, the main environmental release pathway for ZnO-NP is most likely through wastewater treatment and the utilization of sewage sludge in agriculture.[5,6] Recently, Kaegi et al.[7] investigated the behavior of a silver (Ag) nanomaterials in a pilot wastewater treatment plant. Their results indicate that most of the Ag in both the sludge and the effluent was present in the form of Ag_2S, suggesting that the Ag nanoparticles did not pass through the wastewater treatment process unchanged. Silver sulfide nanoparticles were also found by Kim et al.[8] in a field-sampled sewage sludge. In the case of ZnO-NPs, however, information regarding their fate during wastewater treatment is still lacking. However, several authors have investigated the potential toxicity of ZnO-NPs to the wastewater treatment process. Zheng et al.[9] reported that large concentrations of ZnO-NPs induced a significant reduction in the wastewater

biological nitrogen removal. Mu et al.[10] reported that while SiO_2-, TiO_2-, and Al_2O_3-NPs did not affect methanogenesis during anaerobic digestion of activated sludge, ZnO-NPs significantly inhibited this process at large concentrations. Although the fate of the ZnO-NP during treatment may affect the toxicity if changes in speciation occur (i.e., dissolution to the free ion or precipitation as ZnS), these authors did not investigate potential changes in speciation during the treatment process.

In addition to the lack of information regarding the transformation of ZnO-NP during the wastewater treatment process, there is a paucity of information concerning the effects of product formulation chemistry on the fate of ZnO-NP during wastewater treatment. This is an important knowledge gap given that ZnO-NPs are commonly dispersed in special formulations to facilitate their inclusion and stability in products such as sunscreens and other personal care products. Furthermore, there is presently a dearth of knowledge as to whether the speciation of metals originating from manufactured nanomaterials is the same in the final sewage sludge product as it is for metals released to the sewer as dissolved species. Zinc has been recognized as an important metal in terms of sewage sludge quality for many years as sewage sludge is commonly used as a soil amendment. Zinc is an essential micronutrient for plant growth and its deficiency is a major global challenge (e.g., ref 11), however excessive Zn concentrations can have negative impacts on soil health (e.g., ref 12). As a result, a large amount of information regarding the risk assessment of Zn in sewage sludge destined for reuse in agriculture is available and most countries have set limits on the permissible Zn concentration that can be added via sewage sludge addition to agricultural soils. If the final speciation in sewage sludge of Zn originating from ZnO-NPs is similar to that of Zn originating from other sources commonly present in wastewater (e.g., dissolved Zn), then the ZnO-NP risk assessment for this pathway of exposure would already be covered by the present knowledge.

In the study reported here, we have used bench-scale anaerobic digestors to investigate: (a) the chemical transformation of ZnO-NPs during anaerobic wastewater sludge treatment; (b) whether this transformation is influenced by dispersion of the ZnO-NPs in capric/caprylic triglyceride (commonly used in personal care products); (c) whether the final Zn speciation in sewage sludge spiked with ZnO-NPs prior to anaerobic digestion differs from that of Zn already present in field-collected wastewater or added to it in the form of soluble salt. Furthermore, as we have recently reported that Zn speciation in "fresh" sewage sludge (i.e., collected immediately after anaerobic digestion) is substantially different from that in sewage sludge subjected to stockpiling/composting,[13] we also investigated Zn speciation in the ZnO-NP-sewage sludge materials after a period of simulated stockpiling.

■ EXPERIMENTAL SECTION

Zinc Oxide Nanoparticles. Three ZnO-NP materials were used in this study. A commercial sample (ZnO-NP1), identical to the OECD sample NM-112[14] was made available from Micronisers Pty Ltd., Dandenong, Victoria, Australia. X-ray powder diffraction and transmission electron microscopy (data not shown) indicated a crystallite size of 30−40 nm and dynamic light scattering studies gave a d_{90} of 39 ± 6 nm. The second material tested (ZnO-NP2) is identical to ZnO-NP1 but is formulated as a stable dispersion in capric/caprylic triglyceride (65% w/w). This material is used as a base for various sunscreen and personal care product formulations. The third material (ZnO-NP3) was a cobalt-doped ZnO sample (d_{90} = 35 ± 5 nm), in which the Co has previously been shown to substitute isomorphously for the Zn ions in the ZnO wurtzite lattice by use of channelling-enhanced microanalysis of the X-ray emission spectra from individual nanoparticles.[15] This material has been prepared specifically for risk assessment studies as the Co-doping may facilitate the traceability of the nanoparticles. The molar ratio of Zn:Co of the sample tested in this study was 29:1 (3.8 atom % Co substitution) as determined via ICP-MS.

Experimental Setup. Samples of primary and secondary wastewater sludge were obtained from the Bolivar municipal wastewater treatment plant in Adelaide, South Australia. A feedstock mixture of 60% primary sludge and 40% secondary sludge (i.e., waste activated sludge) was prepared to obtain approximately the same sludge composition commonly used in wastewater treatment plants to feed anaerobic digesters. The basic feedstock mixture was characterized for pH, alkalinity, total and volatile solids.[16] The results are reported in Table S1 of the Supporting Information, SI.

The trials were set up in a 37 °C room using a series of benchtop anaerobic digesters designed to treat 2 L of sludge. Each digester was equipped with both pH and redox potential monitoring probes, and temperature probes. Two three-way stopcock valves were installed in each digester to keep the system isolated from the atmospheric air while allowing gas and digestate sampling. Gas was collected in 0.5 L Tedlar gas sampling bags. The system was continuously stirred using a magnetic stirrer during the digestion process to ensure thorough mixing of the substrate. In addition, for sampling periods of up to 3 days, additional digesters were set up and sampled destructively. The 60:40 primary:secondary sludge feedstock was seeded with a small amount (50 mL per 2 L digester) of anaerobic digester sludge from the Bolivar wastewater treatment plant. As the total alkalinity measured in the feedstock mixture (2600 mg $CaCO_3$ L^{-1}) was in the lower range of values commonly observed for municipal wastewater sludge the alkalinity of the feedstock was amended with sodium bicarbonate prior to start-up of the digesters in order to create sufficient buffering capacity to maintain suitable pH conditions during the digestion process.[17]

The experimental design consisted of a control (no added Zn), a salt control where Zn was added as a solution of $ZnCl_2$, and three separate ZnO-NP treatments. All of the Zn materials were added in the necessary amounts to reach a final concentration in the sewage sludge of approximately 1000 mg kg^{-1}. As the sewage sludge produced at Bolivar typically contains 400 mg kg^{-1} Zn, the Zn addition in the treatments represented approximately 60% of the total. As species representing 5−10% of the total should be identifiable using XANES, this addition was deemed appropriate to assess the fate of ZnO-NPs by XANES while remaining in a realistic scenario concerning total Zn concentration in the sewage sludge. Appropriate amounts of ZnO-NP1 and ZnO-NP3 were dispersed in ultrapure (UP, 18.2 MΩ Milli-Q) water by sonication as specified by Singh et al.[14] As the triglyceride dispersion (ZnO-NP2) is in the form of a thick, water-repellent cream, the appropriate amount of material was dispersed in 5 mL of vegetable oil prior to addition to the bioreactor as vegetable oil is a likely component of domestic wastewater. Immediately after addition (time 0 h), and after 3 h, 24 h, 3d, and 10d, 200 mL of digestate was sampled from each treatment,

Table 1. Zinc Concentrations and Partitioning (K_d) in the Supernatants and Sewage Sludge at the End of the Digestion Process[a]

treatment	supernatant Zn (mg L^{-1})	sewage sludge Zn (mg kg^{-1})	K_d (L kg^{-1})	pH	Eh (mV)	biogas (mL)
control	0.21 (0.01)	421 (16)	1963	7.2 (0.4)	−481 (25)	7875
zinc salt	1.47 (0.01)	939 (18)	637	6.9 (0.4)	−473 (27)	7300
ZnO-NP1	0.71 (0.02)	890 (2)	1258	6.7 (0.5)	−480 (35)	6825
ZnO-NP2	0.54 (0.03)	905 (28)	1667	6.8 (0.2)	−476 (23)	6140
ZnO-NP3	1.01 (0.02)	850 (29)	915	6.8 (0.4)	−483 (33)	7150

[a]Average pH and Eh over the digestion process and cumulative biogas produced by the each reactor are also reported. Standard errors are reported in parentheses.

immediately frozen, and later freeze-dried. After 30 days, the digestors were disassembled and the sewage sludge was separated from the supernatant by centrifugation for 30 min at 3000 g. The sewage sludge was then frozen and freeze-dried. In order to simulate stockpiling/composting of the sewage sludge, 10 g of fresh sewage sludge from each treatment was maintained at 37 °C and cycled through alternating wetting and drying periods. Water was added in the form of UP water (up to 60% maximum water holding capacity) 1−2 times per week, at which time the samples were thoroughly mixed to increase aeration. This intense regime was maintained for 2 months to simulate the longer stockpiling process at the wastewater treatment plant (which usually lasts between 6 months and 3 years).

Throughout the digestion process pH, redox potential and temperature were collected every 15 min using a data-logger. The total gas volumes were measured daily using a syringe method as described by Owen et al.[18] and gas composition was analyzed, at day 10 and 20, by gas chromatography.

The samples collected after 30 d, including both the supernatant and the sludge, were microwave digested in reverse aqua regia (1:3 HCl:HNO$_3$) and analyzed by ICP-MS. Quality control measures included the use of procedural blanks and repeat analysis of a certified sludge reference material (NIST SRM 2781).

Zinc Speciation by X-ray Absorption Spectroscopy (XAS). The investigation of ZnO-NP in complex environmental matrices, such as wastewater and sewage sludge, is complicated by the "native" Zn present in the samples. Techniques such as TEM, which have been successfully employed by Kaegi et al.[7] to investigate Ag-NP in sewage sludge, are difficult to apply for Zn as this element is normally present in relatively large concentrations in wastewaters and sludge. Therefore, in this study we relied on the specific signature of ZnO-NP as assessed by X-ray absorption near-edge structure (XANES) spectroscopy to investigate the behavior and transformation of added NPs. XANES was conducted to observe changes in zinc speciation as a function of time during the anaerobic digestion process (0, 3, and 24 h, 3 and 10 days), at the end of the digestion process (30 d), and after the stockpiling/composting simulation period. XANES data were collected at the Materials Research Collaborative Access Team's (MRCAT) beamline 10-BM, Sector 10 located at the Advanced Photon Source (APS), Argonne National Laboratory (ANL), Argonne, IL.[19] The electron storage ring operated at 7 GeV in top-up mode. A liquid N$_2$ cooled double crystal Si(111) monochromator was used to select incident photon energies and a platinum-coated mirror was used for harmonic rejection. The monochromator was calibrated by assigning the first derivative inflection point of the K-edge of Zn metal (9659 eV) and a Zn metal foil spectrum was collected congruently with each sample scan. Samples were

pressed into pellets and three XANES scans were collected for each sample in fluorescence mode using a silicon drift detector (SDD). Zinc standards included, in addition to the 3 ZnO-NPs, 22 Zn spectra used by Donner et al.[13] in a study of Zn speciation in sewage sludge. These samples were analyzed as finely ground powders (diluted in boron nitride to approximately 1000 mg kg^{-1}). Principal component analysis (PCA) of the normalized sample spectra was used to estimate the likely number of species contained in the samples, while target transformation (TT) was used to identify relevant standards for linear combination fitting (LCF) of the sample spectra.[20] PCA and TT were performed using SixPack[21] while data normalization and LCF were performed using Athena.[22] The fitting range was −30 to +100 eV relative to the Zn K-edge. For each sample, the combination of standards with the lowest residual parameter was chosen as the most likely set of components.[23]

RESULTS AND DISCUSSION

The final Zn concentration in the supernatant and sewage sludge of the control treatment was 0.21 mg L^{-1} and 421 mg kg^{-1}, respectively. In the treatments where Zn was added, total concentrations in the sewage sludge ranged from 890 to 939 mg kg^{-1}, whereas Zn in the solution phase was 0.70−1.40 mg L^{-1} (Table 1). These results indicate that in all cases, the majority of the Zn remains in the sewage sludge. The partition coefficient (K_d) of Zn in the various treatments was largest in the control (1963 L kg^{-1}) and smallest in the salt treatment (637 L kg^{-1}) with K_d values for the Zn-NP treatments having intermediate values (Table 1). This indicates that the Zn derived from the ZnO-NP is not partitioning in larger measure in the solution phase when compared to the salt added treatment. The total concentration in all sewage sludges was within the range recently reported by Donner et al.[13] for six Australian sewage sludges. At these concentrations, toxic effects to the anaerobic digestion process are not expected. For instance, Mu et al.[10] reported no effects on methanogenesis at a loading of ZnO-NP of 6000 mg kg^{-1} of total suspended solids. In the current experiment, parameters indicative of the functioning of the digesters, i.e. pH, redox potential, gas generation, and gas quality, were similar across the various treatments. For instance, the redox potential rapidly decreased within the first day, from approximately −300 to −470 meV and remained constant throughout the digestion process. Methane production occurred in all digestors (ranging from 60 to 80% of the total biogas at the two sampling points) and total gas production was similar across the treatments (Table 1).

Zinc Speciation during Digestion and in Sewage Sludge. The Zn K-edge spectra of the ZnO-NPs are reported in Figure 1. As can be easily evinced, the spectra of the 3 ZnO-NPs tested are practically identical and very closely resemble
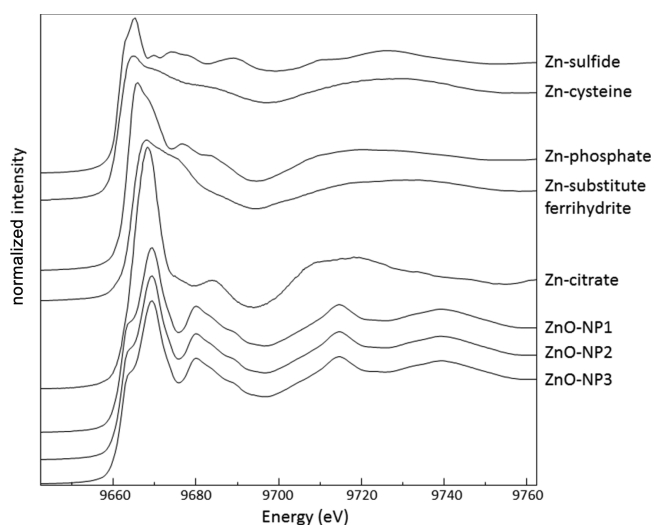
Figure 1. Normalized Zn K-edge XANES spectra of the standards that were represented in the LCFs solutions (Table 2) and of the ZnO-NPs.

the XANES spectra reported for a Sigma Aldrich ZnO-NP material by Scheckel et al.[24]

The PCA analysis conducted using the 35 sample spectra (5 treatments × 7 sampling times) indicated that 4 components were necessary to describe the data set as the IND value reached a minimum at this number of components. Therefore, the LCF analysis was performed using every primary to quaternary combination of the relevant standard spectra and the final results provided good fits to the experimental data (see R-values in Table 2). Target transformation of the data was used to identify the reference spectra to be used in the LCF through selection of standards having a SPOIL <2 (for a discussion of SPOIL value cutoffs see ref 23). Of the 22 reference spectra, 10 had a SPOIL between 1.31 and 1.88 and included Zn sorbed/substituted ferrihydrite, Zn bound to organic ligands, Zn phosphate and Zn sulfide (Table S2 of the SI). Therefore, the LCF was performed using the spectra of these 10 standard compounds and, in the case of the ZnO-NP treatments, the spectrum of the relevant ZnO-NP. As uncertainty in species apportionment is estimated to be approximately 5% of the total amount of the target element, species contributing less than 5% in the LCF solutions were not reported.[25,26] The spectra of the standards that featured in the LCF solutions of the data are reported in Figure 1, and the spectra of the other standard compounds are shown in Figure S1 of the SI.

Upon addition of soluble Zn (Zn salt) and ZnO-NP, differences in speciation can be observed between some of the treatments. The XANES spectra for the samples collected immediately after mixing of the Zn materials are reported in Figure 2a and the solutions of the LCF procedure in Table 2. It is important to point out that even though the samples were immediately stored at −20 °C, changes in Zn speciation may have occurred before the samples were frozen and freeze-dried as the sample volume was considerable (200 mL in volume) and freezing was not instantaneous. Within the period between sample collection and freezing, the added soluble Zn quickly equilibrated with the system as can be seen by the similarity of the spectra between the control and the Zn salt treatment (Figure 2a). In both treatments, approximately 50% of the Zn was present in a form resembling that of ZnS with a lesser

contribution from Zn bound to thiol groups (i.e., cysteine) and Zn phosphate. The Zn edge position of ZnS (e.g., wurtzite and sphalerite) and of Zn complexed by cysteine is considerably lower than that of other Zn species and can be readily identified. However, it must be kept in mind that other S-containing compounds, could also be present as the LCF procedure necessarily relies on the selection of a few representative standards for each class of compounds (e.g., ref 27).

When ZnO-NPs were added, a large proportion of the added Zn was converted to the same species present in the control and salt treatments (Figure 2a, Table 2). In the case of ZnO-NP1 and ZnO-NP3, the LCF revealed that only approximately 10% of the total Zn was in the form of ZnO-NPs. Even taking into account the presence of the "native" Zn in the wastewater (approximately 40% of the total), it is clear that the majority of the added ZnO-NPs quickly undergo a transformation most likely due to their dissolution followed by complexation/precipitation reactions. In the case of ZnO-NP2, the contribution of the ZnO-NP2 standard spectrum to the overall Zn speciation is larger than in the other two treatments (38% of the total Zn). If the contribution of the "native" Zn in the wastewater is discounted, this result indicates that approximately 70% of the ZnO-NPs do not rapidly convert to other species. We suggest that the increased stability of these NPs is due to the hydrophobic nature of the capric/caprylic triglyceride dispersion. This nanoparticle coating is likely to temporarily protect the ZnO-NPs from the external medium and retard their dissolution.

The spectra of the samples collected after 3 h of incubation indicate a further conversion of Zn to sulfide in several treatments (i.e., control, Zn salt, and ZnO-NP3). The proportion of ZnO-NPs in the treatments that received these materials was slightly reduced but the spectra of ZnO-NPs was still part of the LCF solution of the sample spectra (Figure 2b, Table 2).

After 24 h in the anaerobic digestors, the spectra of ZnO-NPs were not part of the LCF solution for the ZnO-NP1 and ZnO-NP3 samples. Over a similar time frame, in a simple system in which ZnO-NPs were equilibrated with kaolin, Scheckel et al.[24] observed the apparent disappearance of the NP signal and the appearance of a signal related to Zn sorption on clay. In contrast, 20% of the total Zn in the sample receiving the ZnO-NP in capric/caprylic triglyceride dispersion was still attributable to this species. This provides further evidence of a protection mechanism of this NP formulation. In all of the other treatments, approximately 70% of the total Zn was present in the form of sulfides (Figure 2c, Table 2).

The spectra of the samples collected at 3 and 10 days of anaerobic digestion indicate a progressive increase of the Zn fraction attributable to sulfides (Figure 2d,e and Table 2). The spectral signature of ZnO-NP was further reduced in the ZnO-NP2 treatment where it accounted for 12% of the total Zn at day 3. At day 10, the spectra of the ZnO-NPs did not contributed to any of the LCF solution indicating that after this period of time the NPs are completely transformed and the ZnO converted to other species, mainly sulfides. Kaegi et al.[7] observed a similar behavior for Ag-NPs spiked into a pilot wastewater treatment plant even though minor amounts of metallic Ag were detected in that case, suggesting formation of a passivating Ag_2S surface layer over the metallic Ag core.

At the end of the anaerobic digestion process, which lasted 30 days, the large majority of Zn was found in the sewage

Table 2. Best Fit Zn Speciation As Identified by Linear Combination Fitting (LCF) of K-Edge XANES Spectra[a]

sample	ZnO-NP (1,2,3)	citrate	cysteine	phosphate	sulfide	substituted ferrihydrate	R-factor
			control				
0 h		9 (0.4)	26 (3)	13 (1)	52 (3)		0.0002
3 h		9 (0.6)	7 (0.4)	14 (2)	70 (4)		0.0002
24 h		7 (0.5)	17 (4)	7 (1)	70 (4)		0.0002
3 days		5 (0.5)	16 (4)		78 (4)		0.0002
10 days		5 (0.5)	16 (3)	5 (1)	74 (4)		0.0002
fresh sludge			15 (3)	5 (3)	80 (6)		0.0002
aged sludge			15 (3)	38 (3)		48 (3)	0.0021
			Zinc salt				
0 h		9 (0.6)	14 (4)	21 (2)	55 (5)		0.0002
3 h		7 (0.6)	24 (3)		69 (5)		0.0002
24 h		5 (0.5)	19 (4)		74 (4)		0.0002
3 days			9 (4)		87 (5)		0.0002
10 days			6 (4)		89 (6)		0.0003
fresh sludge					96 (6)		0.0002
aged sludge			16 (4)	37 (6)		48 (3)	0.0002
			ZnO-NP1				
0 h	12 (1)	6 (0.4)	36 (3)		45 (3)		0.0001
3 h	12 (1)	6 (0.4)	33 (3)		49 (3)		0.0001
24 h		7 (0.5)	18 (4)	5 (1)	70 (4)		0.0001
3 days		7 (0.4)	12 (3)	13 (1)	67 (3)		0.0001
10 days			7 (2)	9 (2)	83 (5)		0.0002
fresh sludge		5 (0.5)	11 (3)		85 (4)		0.0002
aged sludge		5 (1)	18 (3)	41 (4)		35 (2)	0.0012
			ZnO-NP2				
0 h	38 (1)	6 (0.5)	21 (3)		34 (3)		0.0001
3 h	31 (1)	8 (0.5)	26 (3)		33 (3)		0.0001
24 h	21 (1)	5 (0.5)	26 (4)		47 (3)		0.0001
3 days	12 (1)		22 (6)	14 (1)	46 (6)		0.0002
10 days		6 (0.4)	30 (3)	15 (1)	49 (3)		0.0001
fresh sludge		7 (0.5)	10 (3)	14 (1)	67 (4)		0.0002
aged sludge		6 (2)	17 (2)	35(4)		42 (6)	0.0011
			ZnO-NP3				
0 h	9 (0.4)		34 (3)	12 (2)	44 (4)		0.0002
3 h	7 (0.5)		23 (2)	7 (1)	62 (6)		0.0002
24 h			20 (2)	5 (0.4)	74 (4)		0.0002
3 days				5 (0.4)	94 (7)		0.0002
10 days		5 (0.7)		5 (0.4)	90 (6)		0.0003
fresh sludge		7 (0.5)		12 (1)	79 (4)		0.0002
aged sludge		6 (2)	21 (5)	30 (7)		42 (7)	0.0011

[a]Species proportions are presented as percentages and the values in brackets show the percentage variation in the calculated values. Goodness of fit is indicated by the R-factor.

sludge (Table 1) rather than the digestate supernatant following centrifugation, indicating that the digestion process removed the vast majority of the added Zn from the effluent release pathway. The Zn speciation in the "fresh" biosolid was dominated by sulfides which represented between 67 and 96% of the total Zn (Table 2, Figure 2f). The remaining Zn species were identified as phosphate and cysteine-bound. Over the course of the digestion process the Zn apportioned to thiol ligands (modeled by Zn-cysteine in our case) generally decreased with increasing digestion time. Organic thiol-containing ligands have been shown to play an important role in precipitation and growth of ZnS nanoparticles in sulfidic aquatic systems.[28] The role of these organic compounds is that of reducing crystal growth by surface stabilizing ZnS nano-particles, a phenomenon that is also employed to manufacture ZnS nanomaterials.[29] We hypothesize that organic compounds containing thiol groups may play a similar role in wastewater

anaerobic digestion and may play a role in controlling the kinetic reaction leading to ZnS. In any case, the final speciation of Zn in all the treatments is consistent with the speciation of Zn observed in three "fresh" biosolids by Donner et al.[13]

The two months of incubation simulating composting/stockpiling of the "fresh" biosolids significantly affected Zn speciation (Table 2, Figure 3). During this anaerobic phase, ZnS was oxidized and Zn speciation drastically shifted toward association with Fe oxy/hydroxides, modeled by Zn sorbed on ferrihydrite in the LCF procedure. In addition, the proportion of Zn complexed by phosphate increased significantly as a result of this composting process. The results obtained here are similar to those obtained for stockpiled sewage sludge from Bolivar by Donner et al.[13,30] Composting/stockpiling of sewage sludge is often utilized prior to land application of these materials and, even in cases where "fresh" sewage sludge is directly spread on land, the increasingly aerobic conditions
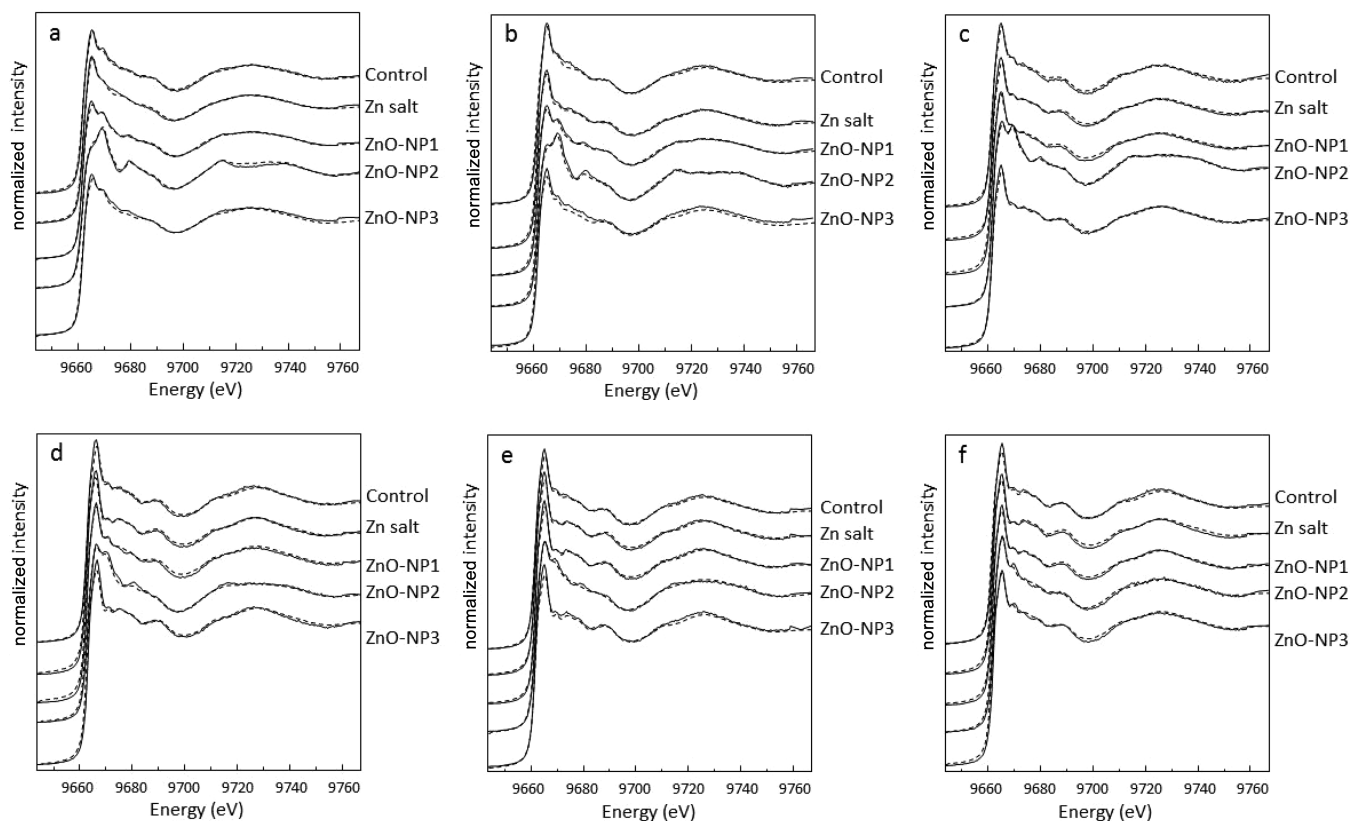
Figure 2. Normalized Zn K-edge XANES spectra of the samples collected from the digestors after 0 h (a), 3 h (b), 24 h (c), 3 days (d), 10 days (e), and at the end of the anaerobic digestion period (f). Dotted lines show the best 4-component linear combination fit of reference spectra as documented in Table 2.
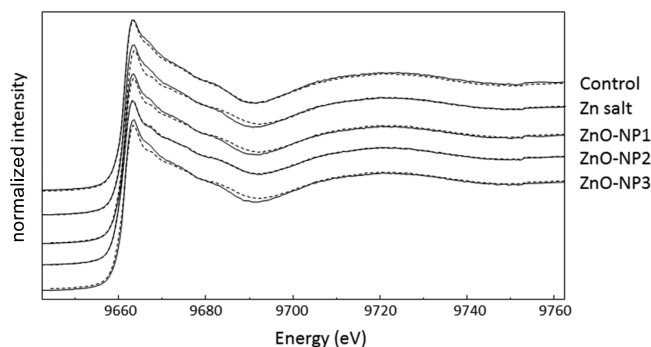

Figure 3. Normalized Zn K-edge XANES spectra of the sewage sludge samples after 2 months of aerobic incubation simulating composting/stockpiling. Dotted lines show the best 4-component linear combination fit of reference spectra as documented in Table 2.

would favor the oxidation of sulfides and organic matter.[31] Interestingly, Donner et al.[13] observed that while ZnS was not present in "aged" sewage sludge, Cu sulfides were more persistent probably because of their higher kinetic stability.[32] As sulfides were identified as the major Ag species in sewage sludge[7,8] it would be important to assess whether these species are also stable upon composting/stockpiling or in the soil environment.

Implications for the Environmental Risk Assessment of Manufactured ZnO-NPs. This study indicates that Zn, either normally present in the wastewater or added in the form of soluble salt or ZnO-NPs, undergoes considerable changes in speciation during the anaerobic phase of wastewater sludge treatment. Zn added as soluble salt and as ZnO-NPs dispersed in water is, within 24 h, transformed to the same species as the "native" Zn, which normally occurs in wastewater. A delay in this transformation process was observed in the case of ZnO-NPs dispersed in an apolar medium commonly used in personal care products. This observation supports the consideration that NP stability may be significantly influenced by surface coating or characteristics of commercial formulations.[33] However, this delay was short-lived and, given the residence time in anaerobic bioreactors, is unlikely to translate into a difference in Zn speciation at the end of the treatment process. This implies that the environmental risk assessment of ZnO-NPs for the wastewater-sewage sludge-agriculture pathway, at least for the formulations employed in this study, can rely on the abundant information already available concerning Zn in sewage sludge.

It is also important to note that the speciation of Zn in "fresh" sewage sludge is significantly different from that which is present after post-treatment biosolids processing. These changes in speciation have been previously observed for both Zn and Cu[13] and may also be relevant in the case of other metals that may be present in wastewater as a consequence of NP dispersion. As Ag_2S has been identified as a major species in sewage sludge,[7,8] further research should be conducted to investigate the stability of metal sulfides under the aerobic conditions found during composting/stockpiling or upon field application.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional information is available in regard to the characterization of the feeding material used in the anaerobic digestors

(Table S1) and the Zn standards used to fit the XANES data (Table S2 and Figure S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Meyer, D. E.; Curran, M. A.; Gonzalez, M. A. An examination of existing data for the industrial manufacture and use of nano-components and their role in the life cycle impact of nanoproducts. *Environ. Sci. Technol.* **2009**, *43* (5), 1256−1263.

(2) Colvin, V. L. The potential environmental impact of engineered nanomaterials. *Nat. Biotechnol.* **2003**, *21* (10), 1166−1170.

(3) Grieger, K. D.; Hansen, S. F.; Baun, A. The known unknowns of nanomaterials: Describing and characterizing uncertainty within environmental, health and safety risks. *Nanotoxicol.* **2009**, *3* (3), 1−U17.

(4) Ju-Nam, Y.; Lead, J. R. Manufactured nanoparticles: An overview of their chemistry, interactions and potential environmental implications. *Sci. Total Environ.* **2008**, *400* (1−3), 396−414.

(5) Mueller, N. C.; Nowack, B. Exposure modeling of engineered nanoparticles in the environment. *Environ. Sci. Technol.* **2008**, *42* (12), 4447−4453.

(6) Gottschalk, F.; Sonderer, T.; Scholz, R. W.; Nowack, B. Possibilities and limitations of modeling environmental exposure to engineered nanomaterials by probabilistic material flow analysis. *Environ. Toxicol. Chem.* **2010**, *29* (5), 1036−1048.

(7) Kaegi, R.; Voegelin, A.; Sinnet, B.; Zuleeg, S.; Hagendorfer, H.; Burkhardt, M.; Siegrist, H. Behavior of Metallic Silver Nanoparticles in a Pilot Wastewater Treatment Plant. *Environ. Sci. Technol.* **2011**, *45* (9), 3902−3908.

(8) Kim, B.; Park, C.-S.; Murayama, M.; Hochella, M. F., Jr. Discovery and characterization of silver sulfide nanoparticles in final sewage sludge products. *Environ. Sci. Technol.* **2010**, *44* (19), 7509−7514.

(9) Zheng, X.; Chen, Y.; Wu, R. Long-term effects of titanium dioxide nanoparticles on nitrogen and phosphorus removal from wastewater and bacterial community shift in activated sludge. *Environ. Sci. Technol.* **2011**, *45* (17), 7284−7290.

(10) Mu, H.; Chen, Y.; Xiao, N. Effects of metal oxide nanoparticles (TiO_2, Al_2O_3, SiO_2 and ZnO) on waste activated sludge anaerobic digestion. *Biores. Technol.* **2011**, *102* (22), 10305−10311.

(11) Cakmak, I. Enrichment of cereal grains with zinc: Agronomic or genetic biofortification? *Plant Soil* **2008**, *302* (1−2), 1−17.

(12) Giller, K. E.; Witter, E.; McGrath, S. P. Heavy metals and soil microbes. *Soil Biol. Biochem.* **2009**, *41* (10), 2031−2037.

(13) Donner, E.; Howard, D. L.; de Jonge, M. D.; Paterson, D.; Cheah, M. H.; Naidu, R.; Lombi, E. X-ray absorption and micro X-ray fluorescence spectroscopy investigation of copper and zinc speciation in biosolids. *Environ. Sci. Technol.* **2011**, *45* (17), 7249−7257.

(14) Singh, C.; Friedrichs, S.; , N.; Gibson, K. A. Jensen, M.; Levin, R.; Birkedal, G.; Pojana, W.; Wohlleben, S.; Schulte, K.; Wiench,; T. Turney, O.; Koulaeva, D.; Marshall, K.; Hund-Rinke, W.; Kördel,; Klein, C.L. *NM-Series of Representative Manufactured Nanomaterials. Zinc Oxide Characterisation and Test Item Preparation*; European Commission, Joint Research Centre, Institute for Health and Consumer Protection: Luxembourg, 2011; p 115, ISBN 978-92-79-22215-3.

(15) Casey, P. S.; Rossouw, C. J.; Boskovic, S.; Lawrence, K. A.; Turney, T. W. Incorporation of dopants into the lattice of ZnO nanoparticles to control photoactivity. *Superlat. Microstruct.* **2006**, *39* (1−4), 97−106.

(16) Eaton, A. D.; Franson, M. A. H., Eds.; *Standard Methods for the Examination of Waterand Wastewater*, 21st ed.; American Public Health Association (APHA), American Water Works Association (AWWA) & Water Environment Federation (WEF): Washington DC (USA), 2005.

(17) Soares Damasceno, L. H.; Rodrigues, J. A. D.; Ratusznei, S. M.; Moraes, E. M.; Zaiat, M.; Foresti, E. Effect of bicarbonate alkalinity on gravimetric solids analysis in anaerobic wastewater treatment. *Interciencia* **2007**, *32* (9), 610−614.

(18) Owen, W. F.; Stuckey, D. C.; Healy, J. B., Jr.; Young, L. Y.; McCarty, P. L. Bioassay for monitoring biochemical methane potential and anaerobic toxicity. *Water Res.* **1979**, *13*, 485−492.

(19) Kropf, A. J.; Katsoudas, J.; Chattopadhyay, S.; Shibata, T.; Lang, E. A.; Zyryanov, V. N.; Ravel, B.; McIvor, K.; Kemner, K. M.; Scheckel, K. G.; Bare, S. R.; Terry, J.; Kelly, S. D.; Bunker, B. A.; Segre, C. U. In *The New MRCAT (Sector 10) Bending Magnet Beamline at the Advanced Photon Source*, 10th International Conference on Synchrotron Radiation Instrumentation, Australian Synchrotron, Melbourne, AUSTRALIA, Sep 27-Oct 02, 2010; Australian Synchrotron: Melbourne, Australia, 2010; pp 299−302.

(20) Malinowski, E. R. *Factor Analysis in Chemistry*; John Wiley: New York, 1991.

(21) Webb, S. M. SIXpack: A graphical user interface for XAS analysis using IFEFFIT. *Phys. Scr.* **2005**, *T115*, 1011−1014.

(22) Ravel, B.; Newville, M. ATHENA, ARTEMIS, HEPHAESTUS: Data analysis for X-ray absorption spectroscopy using IFEFFIT. *J. Synchrotron. Rad.* **2005**, *12*, 537−541.

(23) Gräfe, M.; Donner, E.; Collins, R. N.; Lombi, E. Speciation of metal(loid)s in environmental samples by X-ray absorption spectros-copy. *Anal. Chim. Acta* **2012**, in press.

(24) Scheckel, K. G.; Luxton, T. P.; El Badawy, A. M.; Impellitteri, C. A.; Tolaymat, T. M. Synchrotron speciation of silver and zinc oxide nanoparticles aged in a kaolin suspension. *Environ. Sci. Technol.* **2010**, *44* (4), 1307−1312.

(25) Manceau, A.; Marcus, M. A.; Tamura, N., Quantitative speciation of heavy metals in soils and sediments by synchrotron X-ray techniques. In: Fenter, P., Rivers, M., Sturchio, N. C., Sutton, S., Eds.; *Reviews in Mineralogy and Geochemistry*, Vol. 49: Applications of Synchrotron Radiation in Low-Temperature Geochemistry and Environmental Science, Mineralogical Society of America: Washington, DC, 2002, pp 341−428.

(26) Kirpichtchikova, T. A.; Manceau, A.; Spadini, L.; Panfili, F.; Marcus, M. A.; Jacquet, T. Speciation and solubility of heavy metals in contaminated soil using X-ray microfluorescence, EXAFS spectroscopy, chemical extraction, and thermodynamic modeling. *Geochim. Cosmochim. Acta* **2006**, *70* (9), 2163−2190.

(27) Beauchemin, S.; Hesterberg, D.; Beauchemin, M. Principal component analysis approach for modeling sulfur K-XANES spectra of humic acids. *Soil Sci. Soc. Am. J.* **2002**, *66*, 83−91.

(28) Lau, B. L. T.; Hsu-Kim, H. Precipitation and growth of zinc sulfide nanoparticles in the presence of thiol-containing natural organic ligands. *Environ. Sci. Technol.* **2008**, *42* (19), 7236−7241.

(29) Kho, R.; Nguyen, L.; Torres-Martinez, C. L.; Mehra, R. K. Zinc-histidine as nucleation centers for growth of ZnS nanocrystals. *Biochem. Biophys. Res. Commun.* **2000**, *272* (1), 29−35.

(30) Donner, E.; Ryan, C. G.; Howard, D. L.; Zarcinas, B.; Scheckel, K. G.; McGrath, S. P.; de Jonge, M. D.; Paterson, D.; Naidu, R.; Lombi, E. A multi-technique investigation of copper and zinc distribution, speciation and potential bioavailability in biosolids. *Environ. Pollut.* **2012**, *166*, 57−64.

(31) Martinez, C. E.; Bazilevskaya, K. A.; Lanzirotti, A. Zinc coordination to multiple ligand atoms in organic-rich surface soils. *Environ. Sci. Technol.* **2006**, *40* (18), 5688−5695.

(32) Sato, M. Persistency-field Eh-pH diagrams for sulfides and their application to supergene oxidation and enrichment of sulfide ore bodies. *Geochim. Cosmochim. Acta* **1992**, *56* (8), 3133−3156.

(33) Jarvie, H. P.; King, S. M. Just scratching the surface? New techniques show how surface functionality of nanoparticles influences their environmental fate. *Nano Today* **2010**, *5* (4), 248−250.

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