

Titanium Nanomaterial Removal and Release from Wastewater Treatment Plants

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Titanium (Ti) occurs naturally in soils and as highly purified titanium dioxide (TiO₂) in many commercial products that have been used for decades. We report for the first time the occurrence, characterization, and removal of nano- and larger-sized Ti at wastewater treatment plants (WWTPs). At one WWTP studied in detail, raw sewage contained 100 to nearly 3000 $\mu\text{g Ti/L}$. Ti larger than 0.7 μm accounted for the majority of the Ti in raw sewage, and this fraction was well removed by WWTP processes. Ti concentrations in effluents from this and several other WWTPs ranged from <5 to 15 $\mu\text{g/L}$ and were nearly all present in the <0.7 μm size fraction. As Ti was removed, it accumulated in settled solids at concentrations ranging from 1 to 6 $\mu\text{g Ti/mg}$. Ti-containing solids were imaged in sewage, biosolids, and liquid effluent as well as in commercial products containing engineered TiO₂. Single nanoparticles plus spherical aggregates (50 nm to a few hundred nanometer in size) composed of sub-50 nm spheres of Ti and oxygen only (presumably TiO₂) were observed in all samples. Significantly larger silicate particles containing a mixture of Ti and other metal atoms were also observed in the samples. To support the field work, laboratory adsorption batch and sequencing batch reactor experiments using TiO₂ and activated sludge bacteria verified that adsorption of TiO₂ onto activated sludge biomass occurs. Monitoring for TiO₂ in the environment where WWTP liquid effluent is discharged (rivers, lakes, oceans) or biomass disposed (landfills, agriculture and soil amendments, incinerator off-gas or residuals) will increase our knowledge on the fate and transport of other nanomaterials in the environment.

Introduction

Titanium (Ti) is the ninth most abundant element and the seventh most abundant metal in the Earth's crust (1). With significant worldwide reserves in excess of 600 million tons, the estimated annual production of Ti metal is 90 000 tons, while the annual production of titanium dioxide (TiO₂) is

approximately 4.3 million tons (2). While Ti has numerous industrial applications, from metal alloying to aerospace applications to biomedical devices, approximately 95% of mined Ti is refined into nearly pure TiO₂ through the treatment of Ti-bearing ores with carbon, chlorine, oxygen, or sulfuric acid (3). While currently much of the TiO₂ used today are nanoparticle aggregates or micrometer sized materials (i.e., bulk TiO₂ products), industry trends suggest much higher usage of nano-TiO₂ in the near future (4). Because it is inert, somewhat opaque, and resists fading, TiO₂ is used in an extensive array of consumer products, including paints, paper, plastics, sunscreens, and even food (3, 5–9). TiO₂ is commercially available in the form of dry powder or an aqueous suspension, and in food products, it is used to whiten, increase opacity, or modify texture (10). Typical food products containing TiO₂ include confectioneries, white-colored sauces and dressings, nondairy creamers, and mozzarella and cottage cheeses (11, 12). Food-grade TiO₂ ranges in size from tens to hundreds of nanometers, the common mean diameter being approximately 200 nm (5). The daily human intake of TiO₂ (average size <200 nm) has been estimated to exceed 5.4 mg/day (5). Consumption of TiO₂ leads to a negligible accumulation in people, although Ti is present in human bones (13). Thus, humans should excrete ingested TiO₂, which will then be transported in sewage to wastewater treatment plants (WWTPs). Surprisingly, the only reports of Ti in feces are from rangeland animals and aquaculture fish (14–17). Currently, no publications report on the removal of nanosized Ti or TiO₂ in WWTPs.

TiO₂ is among the most frequently reported engineered materials used in nanotechnology-based consumer products (18). Direct and indirect uses of consumer products containing nanomaterials (e.g., food additives, pharmaceuticals, and clothing) will lead to the release of engineered nanomaterials into domestic sewage (19, 20). A recent study presented evidence of the release of synthetic TiO₂ nanoparticles from paints on building facades and measured a significant amount of TiO₂ nanoparticles in urban runoff after a rainstorm (21). Therefore, in addition to excretion from humans, TiO₂ in sunscreens and paints is likely to be washed or disposed into sewage systems. Serving more than 80% of the U.S. population, municipal WWTPs generally utilize biological treatment, in which dense bacterial communities (activated sludge) sorb and degrade pollutants (22). Recent life cycle predictions of nanomaterial loadings in the environment indicate an important role for WWTPs in nanomaterial removal. The analysis suggested that of the three types of nanoparticles studied, nanosilver, nano-TiO₂, and carbon nanotubes, only the predicted concentrations of nano-TiO₂ in WWTP effluents (0.7–16 $\mu\text{g/L}$) were close to or higher than the predicted no effect concentration level (1 $\mu\text{g/L}$) (23). For that reason, more information is required on the sources, occurrence, and morphology of Ti in wastewater effluents and the factors affecting Ti removal in WWTPs. Furthermore, because TiO₂ has been used for decades, it may serve as a sentinel, or tracer, for the fate of nanomaterials in the environment, especially those that are of similar size and aggregate like TiO₂.

The objectives of this study were to (1) quantify Ti concentrations in a full-scale municipal WWTP; (2) characterize the morphology and composition of Ti-based solids in consumer products plus wastewater effluents and biosolids; (3) quantify Ti concentrations in lab-scale treatment reactors and the sorption capacity of wastewater biomass for TiO₂ nanoparticles. Wastewater biomass is a mixture of

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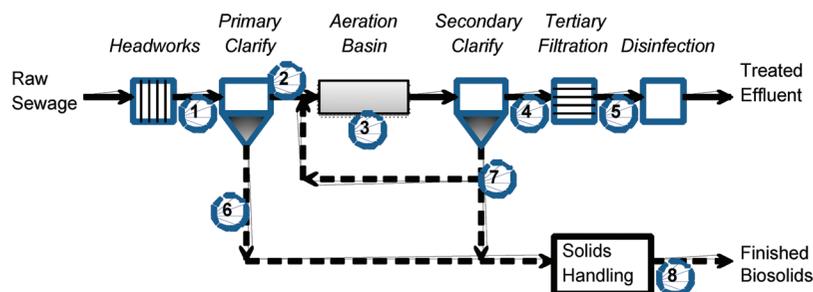


FIGURE 1. Schematic of advanced WWTP including sampling locations (circles): (1) influent, (2) primary effluent, (3) aeration basin, (4) secondary effluent, (5) tertiary effluent, (6) primary solids, (7) secondary solids, and (8) finished biosolids. Solid lines indicate direction of water flow. Dashed lines represent direction of flow of solids.

active bacterial cells, inert or residual biomass, extracellular polymeric substances, protozoa and other higher life forms, mineral precipitates, and influent refractory solids (22), which we hypothesize includes Ti solids. Through the accomplishment of these goals, we develop a more lucid understanding of the potential fate and transport of TiO₂ nanoparticles in WWTPs.

Experimental Methods

Wastewater Treatment Plant Site Description and Sample Collection.

Most of this research was conducted at a wastewater reclamation facility in central Arizona (U.S.A.) that uses an activated sludge process and tertiary filtration treatment (Figure 1). At four different times (7:00, 11:00, 15:00, and 18:00) on each day in June 2008, effluent samples from each unit process, activated sludge from the aeration basins (except at 7:00), wasted solids from the primary and secondary clarifiers, and finished biosolids were collected from the facility. To investigate possible seasonal variations, effluent samples were also collected from each unit process at the same facility once around noon (during peak flow) on a day in January 2009. Additional diurnal, daily, and seasonal monitoring is required to explore the Ti occurrence trends identified in this current research. Approximately 15% of the June samples were collected in replicate, while 100% of the January samples were collected in triplicate. For quality control, field and trip blanks of ultrapure water (<1.5 μS/cm) were included in the sampling. Samples were stored on ice (<4 °C) until return to the laboratory. Portions of each sample were used for suspended solids analysis, nonfiltered analysis, and filtered (GF/F-grade glass microfiber filter, Whatman Inc.) sample analysis. Settled solids from the primary and secondary clarifiers were also collected and analyzed. Activated sludge from aeration basins was collected and used in sorption experiments (described below).

Additional final liquid effluent and biosolid samples were collected from confidential WWTPs in Arizona, California, Colorado, Iowa, Maryland, and three facilities in New York. Samples were shipped to our laboratories by overnight courier.

Laboratory-Scale Experimental Approach. A nanoscale TiO₂ suspension was prepared by adding TiO₂ (Hombikat, Sigma-Aldrich) to ultrapure water, sonicating for 1 h (200 W/L), and centrifuging at $F = 1000$ G for 30 min. Following centrifugation, the supernatant containing suspended TiO₂ was removed and used as stock solution. Hombikat TiO₂ (isoelectric point 5.2) was selected because stable stock solutions could be prepared. Phase analysis light scattering (90 Plus, Brookhaven Instruments Corp., Holtsville, NY) measurement indicated a hydrodynamic diameter of 40 nm (instrument also reports a “real” diameter of 11 nm). Periodic particle size measurements over 1 month indicated a stable TiO₂ suspension (pH 6 and 87 μS/cm conductance). The manufacturer reports the material as a modified anatase with

a purity of ~99% and no information about coatings, so the material was assumed not to be coated.

Batch Adsorption Isotherm Experiment. A TiO₂ test solution (2 mg Ti/L from the TiO₂ stock solution; 1 mM NaHCO₃; pH 7.2) was mixed continuously and equilibrated for 10 h. A wastewater bacteria biomass stock solution was prepared by rinsing the activated sludge three times with a 1 mM NaHCO₃ solution and then resuspending the sludge in the rinsing solution. A series of 60 mL glass vials containing a fixed volume of TiO₂ test solution were spiked with varying amounts of biomass stock solution and then agitated for 3 h. After agitation, the biomass was allowed to settle by gravity (about 30 min), and then 20 mL of supernatant was collected from each sample and analyzed for Ti. Fifteen percent of the samples were conducted in triplicate.

Sequencing Batch Reactor Experiment. Sequencing batch reactors (SBRs) were used to represent the full-scale WWTP operations of aeration and settling. SBRs were constructed using 2 L reactors supplied with compressed air and mechanical mixing units. The SBR contained heterotrophic bacteria acclimated to a feed solution (668 mg/L C₅H₈NO₄Na, 44 mg/L KH₂PO₄, 90 mg/L MgSO₄·7H₂O, 14 mg/L CaCl₂·2H₂O, 10 mg/L yeast extract, and 0.3 mL/L nutrient solution) and was operated to maintain a total volume of 1.6 L, a hydraulic residence time (HRT) of 10 h, and a solids retention time (SRT) of 6 days, which is typical of aerobic WWTPs. The SBR cycle involved 8 h of aeration plus mixing, followed by 2 h of settling time. SRT and HRT were managed by removing a total of 0.25 L of a completely mixed solution plus 0.75 L of settled supernatant once per day. During a separate operating cycle, an additional 1 L of settled supernatant was removed per day. Removed solutions were analyzed for concentrations of Ti and suspended solids and were replaced with the same volumes of feed solution.

Analytical Methods. Because TiO₂ has very low solubility (24), Ti in wastewater is expected to occur solely in solid phases, not in ionic forms. For the quantification method used in this investigation, these solid phases must be transformed into ionic forms by acid digestion. Liquid and solid samples were acid digested using the HNO₃/H₂SO₄ digestion method for Ti as described by Standard Method 3030 G for water and wastewater analysis (25). The digested samples were analyzed by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) (iCAP 6000 Series, Thermo Scientific, Cambridge, U.K.). Suspended solid measurements were made following Standard Method 2540 D (25).

Visual characterization of the Ti-bearing solids was conducted using the scanning electron microscopy/electron dispersive X-ray microanalysis (SEM/EDX) technique (FEI XL-30 equipped with the EDAX system). To minimize potential interferences from organic biomass constituents, we employed a new approach for biosolid examination in which samples were digested with 30% hydrogen peroxide (H₂O₂)

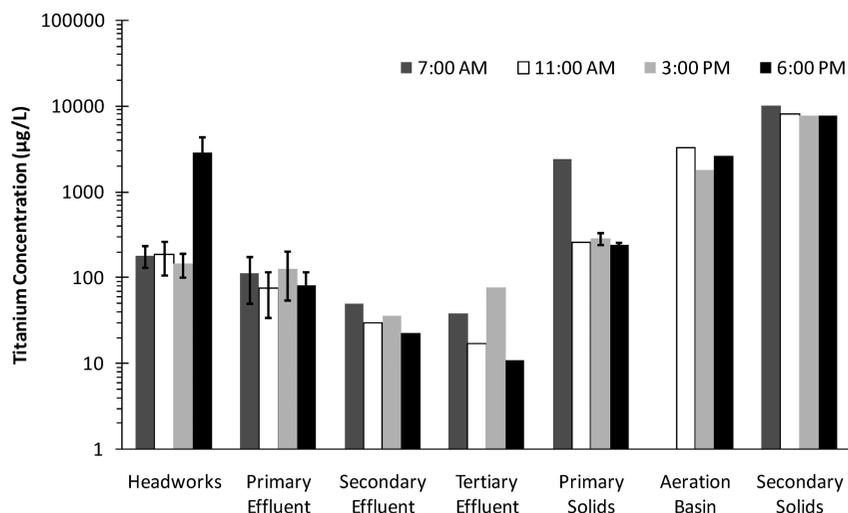


FIGURE 2. June 2008 Ti concentrations for four sampling periods at an Arizona WWTP. Sampling points are identified in Figure 1.

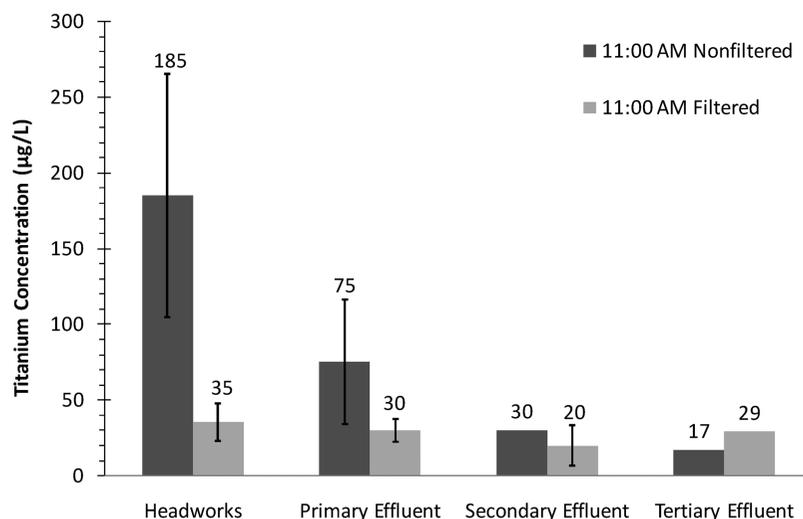


FIGURE 3. June 2008 Ti concentrations of 11:00 a.m. Arizona WWTP filtered and nonfiltered headworks and process effluent samples.

at 90 °C until foaming ceased (26). Hydrogen peroxide oxidized the organic matter and facilitated the solubilization of inorganic constituents. TiO_2 is chemically inert under these conditions and does not dissolve or change shape, as verified in control experiments using commercial TiO_2 . Oxidized samples were centrifuged ($F = 1000$ G) on a SEM stub, air-dried, and then analyzed by SEM/EDX. A backscatter detector was used to differentiate between heavier elements such as Ti, which appeared white, and lighter elements, which appeared darker.

Results and Discussion

Titanium Occurrence and Fate in Wastewater Treatment Plants. Figure 2 presents the results of the June 2008 WWTP sampling campaign. During the 11:00 a.m. sample set, representative of peak plant flow conditions, the Ti concentration at the headworks (plant influent) was 185 $\mu\text{g/L}$; this decreased to 17 $\mu\text{g/L}$ in the tertiary effluent. Thus, approximately 91% of the Ti that entered the treatment plant was removed from wastewater. The removed Ti accumulated in plant solids: primary solids, activated sludge biomass, and secondary solids. Samples collected at other times of the day exhibited similar removal and solid-accumulation trends across the WWTP, with the exception of the 18:00 sample from the headworks. This sample had elevated Ti levels (2800 $\mu\text{g Ti/L}$) that were statistically different from other samples at that location ($p < 0.01$), perhaps due to an industrial source

(e.g., paint, polishers, semiconductor wastes) discharged into the sewers during the day. The average overall removal of Ti for the four different sampling times in June 2008 was 79% \pm 23%.

For the WWTP sampled, the average per capita sewage production is approximately 250 L/day. The daily load to the WWTP from all Ti sources is much higher, on the order of 45–50 (mg/person)/day. Using the effluent Ti concentration of 17 $\mu\text{g/L}$ in the tertiary effluent, the daily load entering the environment equates to approximately 4.2 (mg/person)/day which is very close to the reported internal human consumption rate previously reported (5).

Figure 3 compares Ti concentrations in filtered (nominal filter pore size of 0.7 μm) and nonfiltered samples at 11:00 a.m. in June 2008. In general, Ti concentrations in filtered samples were lower than those in nonfiltered samples. The difference between Ti levels in filtered and nonfiltered samples is particularly obvious for samples containing relatively higher concentrations of total suspended solids, such as plant influent and primary effluent, which contained 221 and 76 mg/L TSS, respectively. For the tertiary effluent, which had low solid levels (4 mg/L TSS), the Ti concentrations in the filtered and nonfiltered samples are approximately the same. The distinction between Ti concentrations in filtered and nonfiltered samples of relatively high TSS suggests that the majority of Ti associated with suspended organic matter is of a size larger than the pore size and/or that the

TABLE 1. June 2008 Average Concentrations for Four Sampling Periods

sample description	filtrate Ti ($\mu\text{g/L}$)	total Ti ($\mu\text{g/L}$)	total suspended solids (mg/L)	total Ti normalized to solids (mg Ti/g TSS)
headworks	34 ± 3.2	843	336	1.6 ± 0.4
primary effluent	66 ± 51	99	97	1.0 ± 0.1
aeration basin	14 ± 9	2572	2220	0.9 ± 0.3
secondary effluent	40 ± 48	35	7	— ^a
tertiary effluent	20 ± 9	36	6	— ^a
primary solids	— ^b	803	1220	0.7 ± 0.2
secondary solids	— ^b	8464	7542	1.1 ± 0.1

^a Indicates samples where TSS was too low to accurately quantify normalized Ti concentrations. ^b Solid samples were not further filtered.

Ti formed aggregates greater than $0.7 \mu\text{m}$ in size. Thus, spectroscopic analysis was carried out to determine the morphology and composition of the Ti in wastewater tertiary effluent (see next section).

Ti concentrations in filtered samples exhibited less variability during the day across the treatment processes compared to the total (nonfiltered) samples collected (Table 1). The average tertiary treated Ti concentration for the four sampling times was $36 \mu\text{g/L}$ ($20 \pm 9 \mu\text{g/L}$ in filtered samples), compared to $843 \mu\text{g/L}$ ($34 \pm 3 \mu\text{g/L}$ in filtered) at the headworks. These effluent concentrations were at the low end of our detection capabilities but were verified through Ti spike-addition and recovery tests. While total Ti removal was $79 \pm 23\%$, removal of filterable Ti was only $42 \pm 22\%$. Thus, large sized Ti which may include larger naturally occurring Ti-silicate minerals or large titanium oxide aggregates (see later discussion of Figure 5) were removed more efficiently than smaller sized Ti that passed through the $0.7 \mu\text{m}$ filter. Other factors may also be associated with this size dependency. For example, some of the smaller Ti particles could be coated, functionalized, or have surfaces that were otherwise organically modified such that their stability/removability was affected. Our reported WWTP effluent Ti concentrations ($10\text{--}50 \mu\text{g/L}$) are in the range predicted by recent models ($0.7\text{--}16 \mu\text{g/L}$) (23).

Compared to other locations along the treatment train, the highest total Ti concentrations ($2572 \mu\text{g/L}$) were found within the activated sludge system (i.e., aeration basin) (Table 1), with the exception of secondary solids, which contain dewatered activated sludge materials. In an activated sludge system, a portion of the settled secondary solids are recirculated back to the head of the aeration basin (Figure 1) to maintain a sludge residence time (SRT) longer than the hydraulic residence time (HRT), resulting in a dense biological community with a relatively stable concentration (2220 mg TSS/L). Because of this recirculation, the biosolids are repeatedly exposed to inorganics, such as Ti-bearing solids, in the WWTP flowstream. Because the concentrations of Ti associated with particulate phases are quite high in activated sludge systems, it appears that Ti has a tendency to adsorb into the biosolids. As the biomass grows through consumption of soluble carbon and other nutrients, the new biomass also appears to accumulate Ti and other influent refractory solids (22, 27). The secondary clarification system efficiently settled the biosolids, as shown by the difference in TSS levels between the aeration basin and secondary effluent samples (Table 1). These WWTP basics explain how and why Ti-bearing materials accumulate in activated sludge biosolids and secondary clarifier settled solids.

To reiterate, association of nanoscale Ti with biosolids must also be critical for their removal during wastewater treatment. Stokes settling rates ($v_s = (g(\rho_p - \rho)d_p^2)/18\mu$) are

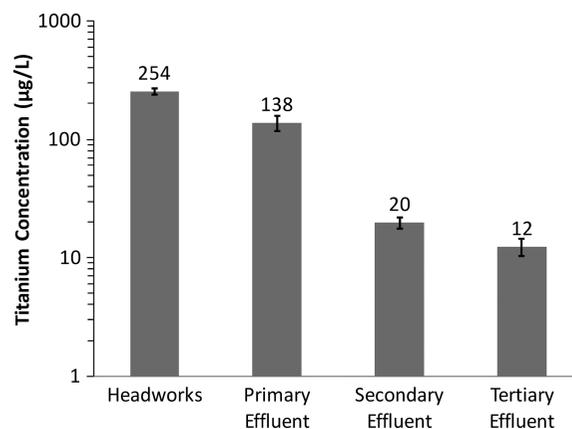


FIGURE 4. January 2009 Ti concentrations of 12:00 p.m. Arizona WWTP headworks and process effluent samples.

extremely slow for submicrometer particles (28). Even for TiO_2 , which has a density of 4.2 g/cm^3 , 10, 100, and 500 nm diameter aggregates require 59 days, 14 h, or 34 min, respectively, to settle only 1 mm in 25°C water.

Figure 4 shows the January 2009 WWTP sampling results, which exhibit the same trends as those seen in June 2008. The Ti concentrations in the plant influent and tertiary effluent were 254 ± 15 and $12 \pm 2 \mu\text{g/L}$, respectively, equating to a 95% removal of Ti from wastewater. This sample set also supports the conclusion from the summer experiment that the majority of Ti associates with solids in wastewater. The average overall removal of Ti for the June 2008 and January 2009 sampling experiments was $82 \pm 21\%$.

During the June 2008 sampling campaign at the Arizona WWTP, finished biosolids were also collected, digested, and analyzed. Ti concentrations in the biosolids ranged from 0.6 to 1.4 mg Ti/g SS , with an average of $1.1 \pm 0.42 \text{ mg Ti/g SS}$. In separate measurements from eight other WWTPs around the United States with varying types of unit processes, the Ti associated with biosolids ranged from 1.8 to 6.4 mg Ti/g SS , averaging $2.8 \pm 1.5 \text{ mg Ti/g SS}$. Triplicate analysis of one biosolid sample showed a high degree of reproducibility ($1.79 \pm 0.02 \text{ mg Ti/g SS}$). The WWTP effluents contained $8\text{--}31 \mu\text{g Ti/L}$ (average $16 \pm 7 \mu\text{g/L}$), which is also consistent with the results from the more intensive study at the Arizona WWTP. The facility with the highest level of Ti in the biosolids also had the highest level in the liquid effluent. A national U.S. EPA study of 83 WWTP biosolid samples observed a range of Ti from 0.018 to 7.02 mg Ti/g SS (29). Overall, these additional points of reference indicate that the WWTP in which we conducted intensive sampling is likely representative of many WWTPs across the United States. Ti levels in biosolids are important because biosolids are land applied as fertilizers, incinerated, disposed to landfills, and used in other applications. A significant fraction of the TiO_2 used in commercial products that enter the sewer system appears likely to accumulate in biosolids and enter the environment via these routes. A smaller fraction of TiO_2 appears to be present in the liquid effluent that enters rivers, lakes, and oceans.

Spectroscopic Analysis of Ti in Wastewater. After the oxidation of organics in biosolids via hydrogen peroxide, SEM in backscatter mode easily detected Ti in biosolids and various commercial products (Figure 5). SEM and EDX analysis confirmed the presence of various sizes, shapes, and compositions of Ti-containing solids. On the basis of EDX analysis at various locations in the SEM image, primary particles of nearly pure titanium and oxygen solids (TiO_x), were observed to occur in small aggregates (Figure 5A,C), while larger, single crystal structures (Figure 5B) were also present. Ti (Figure 5D) was also found to co-occur in other

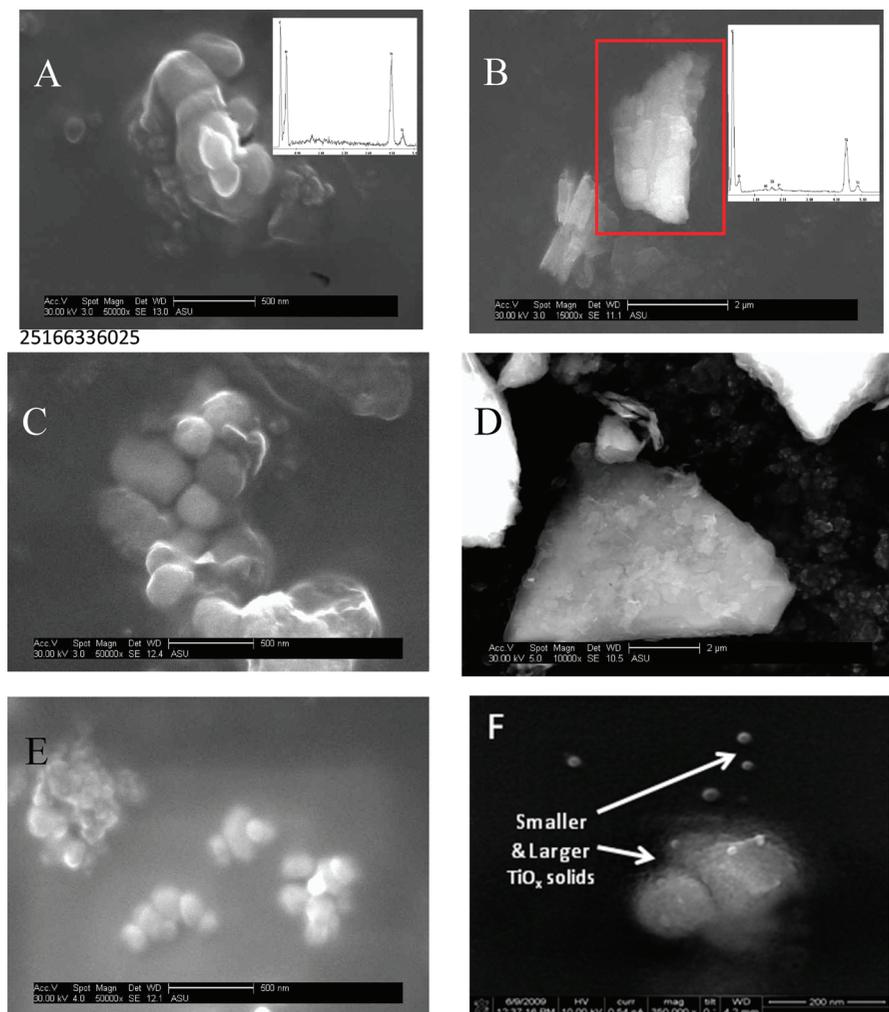


FIGURE 5. SEM analysis of (A) nanoscale TiO_2 , (B) microscale TiO_2 , (C) an aggregate of primary TiO_2 material, (D) mineral-containing Ti in a biosolid sample, (E) TiO_2 in toothpaste as a representative consumer product, and (F) nanoscale TiO_2 in WWTP tertiary effluent. EDX insets were provided for some SEM images, but all solids shown were confirmed to contain Ti and O.

solids with iron, calcium, silica, and oxygen atoms, suggesting a type of silicate or other mineral. Many consumer products contain Ti (5), and several were examined by SEM. For example, in hydrogen peroxide-digested toothpaste, TiO_x was easily identified (Figure 5E) and appeared to be in aggregates of nearly spherical primary nanoparticles. This interpretation is similar to that employed in a study by Powell et al. to analyze aluminum, silicon, and Ti in human gut samples. Three distinct types of microparticles were found to be related to macrophages at the base of gut-associated lymphoid tissue (30): type I, spheres of TiO_2 , 100–200 nm diameter, characterized as the synthetic food additive polymorph anatase; type II, aluminosilicates, <100–400 nm in length, generally of flaky appearance, often with adsorbed surface iron, and mostly characteristic of the natural clay mineral kaolinite; and type III, mixed environmental silicates without aluminum, 100–700 nm in length, and of variable morphology. The study concluded that the TiO_2 was derived partly from food additives and partly from the environment. Beyond the image analysis similarities of the gut and biosolid samples, the Powell study suggests that the metals associated with collagen fibers and plasma cells. This finding is of significance to the investigation of Ti in wastewater because wastewater biomass also contains collagen fibers, suggesting at least one important component of biomass is potentially related to its interaction with Ti solids.

Ti oxides were also present in WWTP effluents (e.g., Figure 5F). These were not always easily found in the WWTP effluent

samples, which contained relatively low concentrations of Ti, but those observed were often aggregates of a few hundred nanometers composed of several primary particles less than 100 nm that were made solely of TiO_x . The primary particles were often spherical in shape, as illustrated by the SEM image shown in Figure 5F. In some cases, single TiO_x nanoparticles were observed (Figure 5F). The shape and size of these Ti materials are consistent with TiO_2 synthesized for industrial/food applications. To our knowledge, this is the first report of Ti concentrations and morphology in both wastewater effluents and biosolids.

Adsorption of Titanium Dioxide with Wastewater Biomass. Bacterial cells can uptake or attach to nanoparticles (31–33). The mechanisms responsible for these observations are poorly understood. To investigate the potential for TiO_2 to sorb onto wastewater biomass, a batch adsorption experiment was performed (Figure SI.1 in the Supporting Information). A control sample (no biomass) contained 0.8 mg/L Ti. As increasing dosages of biomass (in solutions of the same ionic strength) were added to the samples, more TiO_2 was removed from the supernatant; pH was constant and ionic strength increased by <15% at the highest biomass dosage. A comparison of the Ti concentrations in the control and the sample containing 2250 mg/L TSS shows that approximately 85% of the Ti was removed from suspension. Removal data were fit by a Freundlich Isotherm ($q = 0.025C^{0.53}$; $R^2 = 0.90$) (inset of Figure SI.1 in the Supporting Information). This suggests that nonlinear partition models

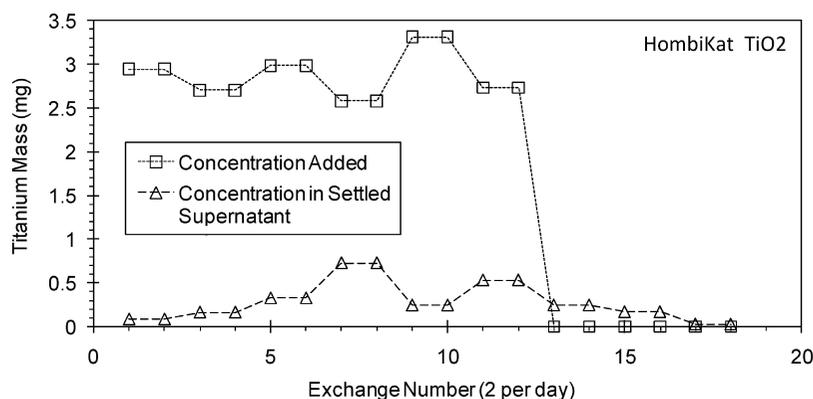


FIGURE 6. SBR experiment using HombiKat TiO₂ with heterotrophic biomass.

may be required to describe nanomaterial biosorption removal in WWTPs, which differs from the linear partition models often used for most organic pollutants.

Titanium in Lab-Scale Reactors. To evaluate the effects of TiO₂ on wastewater biomass and assess sustained TiO₂ removal over time in an actively growing wastewater bacteria culture, continuously operated SBRs containing a feed solution only (no biomass) or feed solution plus biomass (TSS = 3300 mg/L) were supplied with 2.9 ± 0.3 mg Ti/L during each liquid exchange for 9 days (18 exchanges) to manage the HRT and SRT; this equated to adding 4.4 ± 1.0 mg Ti/g TSS. This was followed by an input of feed solution without TiO₂ to evaluate the “washout” of TiO₂ from the reactor. SBRs represent the full-scale WWTP operations of aeration and settling. Over the course of the experiment, a mass balance closure on Ti was maintained (Figure SI.2 in the Supporting Information). The added Ti concentration (2.9 ± 0.3 mg Ti/L) was always greater than that of the settled supernatant (0.28 ± 0.22 mg Ti/L; range 0.03–0.73 mg Ti/L) (Figure 6); Ti concentrations in the suspended solids were 13.2 ± 3.0 mg Ti/g TSS. Consequently, Ti outflow was significantly higher during SRT control (i.e., removal of biosolids from the completely mixed reactor) compared to Ti outflow during HRT control (i.e., removal of settled supernatant). After stopping the TiO₂ feed to the reactor, a gradual washout of Ti from the system was observed (exchanges 12–18 in Figure 6).

Overall, only 12% of the Ti passed through the SBR in the supernatant, while 88% was associated with the biosolid fraction. These experimental results further reveal the high affinity of TiO₂ for biomass. On the basis of the data presented in Figures 2 and 3, these two full-scale unit processes achieved roughly 69% removal of Ti. The lower degree of Ti removal in the full-scale WWTP compared to that in the SBRs may be due to the more complex wastewater matrix in WWTPs. For example, wastewater contains surfactants and natural organic matter, which have been shown to hinder the removal of some nanoparticles from water (34–37). Though not completely equivalent, both systems lead to the conclusion that while significant fractions of Ti will associate with biomass and be present in finished biosolids, a portion of the Ti will also be present in WWTP effluents that enter the aquatic environment.

Implications and TiO₂ as a Sentinel Engineered Nanomaterial

Results of the lab-scale experiments indicated that TiO₂ particles have an affinity for solids, and the majority of TiO₂ in water, on the order of 70–85%, will be removed by wastewater biomass concentrations of around 2000–3000 mg/L TSS. In addition, data obtained from lab experiments were valuable for substantiating the Ti removal observed in

the full-scale WWTPs (both scales achieved comparable percentage Ti removals). For these reasons, development of laboratory experiments that confirm field removal trends will improve our ability to rely on such tests to screen for the ability of WWTPs to remove other, emerging engineered nanomaterials in the future.

Through our field-scale investigation at a municipal WWTP, the concentrations of Ti at each point along the WWTP process train were quantified, thereby revealing the pathways of TiO₂ nanoparticles into the environment as well as their relative significance. Although the majority of Ti sorbed to biomass, we found that 10–100 μg/L Ti still remained in effluents. This study, therefore, defines environmentally relevant concentrations for studying the toxicity of TiO₂ nanoparticles to organisms.

While wastewater effluents are discharged primarily to surface waters (lakes, rivers, streams, oceans) and represent a significant potential point source for nanomaterials into the environment, the presence of TiO₂ and other engineered nanomaterials is likely to be much higher in wastewater biosolids. Biosolids are usually used as agricultural land amendments (fertilizers), placed in landfills, incinerated, or dumped into oceans. Thus, biosolids may represent another point or nonpoint source of TiO₂ release into the environment that is very different from WWTP liquid discharge, and these biosolid releases and resulting ecosystem exposures remain poorly understood.

To the best of our knowledge, this study is the first to provide evidence of the quantities, physical characteristics, and fate of Ti in a municipal WWTP. Given that TiO₂ has been used by the industry for decades, is currently one of the most utilized nanomaterials in consumer products, and is relatively easy to measure and image in complex biological matrices, TiO₂ is a prime candidate to serve as a sentinel, or tracer, for other nanomaterials, especially those of similar size and aggregation behavior, by indicating the possible fate of nanomaterials in a WWTP. By sentinel, we are suggesting that TiO₂ is probably already distributed in the environment (rivers, landfills, agricultural lands, etc.) and that, by examining and monitoring for TiO₂ in these systems, we can have critical knowledge into the potential transport and ultimate disposition of a widely used engineered nanomaterial. Furthermore, monitoring the presence of TiO₂ in the environment may serve to identify locations where other engineered nanomaterials may occur or accumulate in the future. However, because other engineered nanomaterials may have different density, surface coatings, or other properties, their fate could differ from TiO₂.

Acknowledgments

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and RD833322), it has not been subjected to the Agency's required peer and policy review. Therefore, it does not necessarily reflect the views of the Agency, and no official endorsement should be inferred. Additional funding was provided by the Paul L. Busch Research Award from the Water Environment Research Foundation and the Emerging Contaminants Research Group at Arizona State University. Professor Peter Fox at Arizona State University provided valuable ideas regarding the digestion of biosolids using hydrogen peroxide. Collaborators at the U.S. Geological Survey assisted in collecting and shipping wastewater treatment plant liquid and biosolid samples to our laboratory from around the U.S.A.

Supporting Information Available

Figure SI.1 shows removal of Ti from suspension by wastewater biomass, and Figure SI.2 shows the SBR experiment using Hombikat TiO₂ with heterotrophic biomass. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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