An investigation of the fate and behaviour of a mixture of WO₃ and TiO₂ nanoparticles in a wastewater treatment plant

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ABSTRACT

The fate and behaviour of WO₃ and TiO₂ mixture were investigated following the Organisation for Economic Co-operation and Development 303A guidelines. The nanoparticles were found not to influence the chemical oxygen demand removal efficiency which was maintained >80% hence the activated sludge process was not affected. The nanoparticles were eliminated from the wastewater with a greater percentage of 99.8% for TiO₂ and 95.5% for WO₃ found in the sludge. The activated sludge process also had no effect of the polymorphs of the nanoparticles as X-ray diffraction revealed presence of monoclinic WO₃ and anatase TiO₂ which were spiked into the influent. The nanoparticles were mainly removed by bio-adsorption on the activated sludge surface. The total plate count revealed that the bacterial colonies present in the control and the test units were comparable during the gradual introduction of nanoparticles in the chambers. The biomass was >0.75 MLVSS/MLSS (mixed liquor volatile suspended solids/mixed liquor suspended solids) in both the aeration vessels thus a greater proportion of the sludge were the microorganisms. A greater percentage of the Ti and W found in the effluent was mainly due to the nanoparticles adsorbed on the suspended solids with only 3.6% Ti and 28.6% W due to dissolution of nanoparticles.

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Introduction

Wastewater treatment plants have been utilized for the removal and/or degradation of contaminants from wastewater for generations, and they still play a pivotal role in the sustainable wastewater recycling process. The treated wastewater effluent is discharged into the natural water reservoirs that include rivers and lakes. The sludge generated during wastewater treatment has several applications that include use as fertilizer in agriculture. This is achieved through the employment of the activated sludge process which works by sorption of pollutants to its surface while biodegrading pollutants (Peijnenburg et al., 2016).

The increased production and usage of nanoparticles (NPs) have commanded an increased concentration of NPs making their way to municipal wastewater (Xia et al., 2009; Kwak and An, 2016). The main release routes of NPs to surface waters being wastewater effluents and runoff from agricultural sites. Soils are exposed via NPs contained in wastewater treatment plant sludge, direct emissions such as the application of nano-pesticides, NP-induced soil remediation, and diffuse emissions (Peijnenburg et al., 2016). Thus, NPs are among the increasing emerging contaminants in wastewater treatment plants where they have become a major concern leading to an inevitable NP emission into the environment (Sani-Kast et al., 2015). The main drive being the development in nanotechnology resulting in the
increasing integration of NPs into diverse commercial products and manufacturing processes. Metal oxide NPs, such as ZnO, TiO₂, CuO and CeO₂, have attracted much attention due to their amalgamation to a wide range of products like cosmetics, sunscreen, textiles, bactericides, metal catalysts, paints and wastewater treatment (Li et al., 2017; Brar et al., 2010).

Upon release, either intentionally or unintentionally, a complex interaction between nanomaterials, aquatic surfaces and biological species occurs. This interactions lead to aggregation, depending on the interplay between electrostatic and van der Waals interactions (Brar et al., 2010). Abiotic and biotic factors influence transformation, alter bioavailability and the aging process of the NPs. Different hydro-chemical parameters such as pH, ionic strength, and hardness (e.g. Ca²⁺ and Mg²⁺ ions) influence the aggregation kinetics of the NPs in wastewater systems (Dwivedi et al., 2015). Similarly, natural organic matter (NOM), a major constituent of wastewater alters stability through electrostatic and steric interactions even at very low concentrations (Zhang et al., 2009; Zhu et al., 2014; Li et al., 2015).

Several researchers have reported successfully on the fate and behaviour of NPs studied using the prescribed guidelines of the Organisation for Economic Co-operation and Development (OECD). The fate, behaviour and implication of ZnO was studied the Organisation for Economic Co-operation and Development (Zhang et al., 2009; Zhu et al., 2014; Li et al., 2015).

1. Materials and methods

1.1. Collection and characterization of water

The wastewater used in this experiment was collected from Daspoort wastewater treatment works, a Pretoria based treatment plant that mainly handles domestic wastewater. Sampling was conducted after the primary treatment stage with samples stored at 4°C and used within 7 days.

The physical and chemical characteristics of influent and effluent, were measured. The total dissolved solids (TDS), conductivity and pH were measured using Starter conductivity 300C and Starter pH 300 (Starter conductivity 300C and Starter pH 300, OHAUS, USA). The total suspended solids (TSS) were also part of the analysis conducted. For the chemical oxygen demand (COD), a COD kit (1.14541.0001, Merck) equivalent to a German standard DIN EN ISO 7393 was used. The Spectroquant Pharo 300 (Spectroquant Pharo 300, Merck, Ecuador) was used for the COD analysis. Samples were filtered prior to preparation using a 0.45 μm filter and all analyses were conducted in duplicates. A continuous monitoring of both the influent and effluent was ensured every 12 hr throughout the duration of this experiment. The Standard Methods for Examination of Water and Wastewater was followed during the duration and effluent characterization.

1.2. Preparation of NP mixture suspension

Three mixture suspensions of the NPs were prepared by adding equal concentration of WO₃ and TiO₂. To prepare a 5 mg/L mixture suspension 0.1000 g of each NP was dispersed in 1 L of wastewater. The dispersion of the NPs was achieved by sonication (2000 U model, Ultrasonic Power Corp, USA) at 20 kHz for 30 min to break the NP aggregates before marking up to 20 L in the holding tanks. Similar suspensions of 10 and 15 mg/L were prepared by weighing 0.2000 and 0.3000 g, respectively. The WO₃ and TiO₂ NPs used were prepared and characterized as reported by Simelane et al. (2017b) and Mahalela et al. (2017a) respectively.

1.3. Setup and simulation of wastewater treatment plant

The setup of the simulated wastewater treatment plant, inoculation of the aeration chambers with activated sludge were carried out in a similar manner as reported by Simelane et al. (2017a) following the prescription of the OECD 303A guidelines. However, a mean sludge retention time (SRT) of 10 days was used in this study with the dissolved oxygen maintained above 2 mg/L.

Since the microorganisms in the sludge were already acclimatized to domestic wastewater, a steady state was essential in the simulated wastewater treatment plant (SWTP) before adding the NP mixture. Thus, the SWTP was first run with the influent for 3 SRTs to reach the steady state. In the aeration chamber, dissolved oxygen (DO), pH, temperature, mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) were monitored to ensure the survival and activity of the microorganisms. The suspended solids, were analyzed using a glass microfibre filter with 0.6 μm pore size. About 250 mL of the sample was filtered and then dried in an oven for 5 hr at 103–105°C. To obtain the MLVSS, the already dried samples were calcined at 550°C for 20 min (Greenberg et al., 1992). The survival of the microbes was monitored through means of a total plate count (TPC) in aeration chambers upon introduction of the NPs. The agar obtained from Merck was prepared following the manufacturer guidelines and the TPC test was conducted following standard international protocol (Greenberg et al., 1992).

1.4. Functionality of the treatment process

The functionality of the SWTP was assessed by monitoring the capability of the plant to remove organic matter found in wastewater. This was achieved by monitoring the COD levels of the influent and effluent every 12 hr to determine the COD.
removal efficiency. This was done for the steady state establishment and during the addition of the NP mixture. During the addition of the NP mixture, dissolved organic carbon (DOC) was determined for the influent and effluent. The samples were filtered with a 0.22 μm prior to analysis using the total carbon analyser (fusion UV/Persulfate TOC Analyser, TELEDYNE TEKMAR, USA) which was first calibrated using potassium hydrogen phthalate. Other parameters monitored included TSS, TDS and conductivity.

1.5. Determination of WO₃ and TiO₂ in the effluent and sludge

To determine the amount of TiO₂ and WO₃ NPs in the effluent and sludge, the effluent was continuously collected after every 12 hr. The sludge was also continuously withdrawn, dried in the oven at 103–105°C, and later pulvired before being stored. In this study, the digestion method used followed the procedure adopted from Martin et al. (1994).

The concentration of Ti and W was evaluated using inductively coupled plasma optical emission spectrometry (ICP-OES) (SPECTRO ARCOS, Analytical Instruments GmbH, Germany) in the effluent filtered samples to determine solute levels of the metals. The total Ti and W in the effluent and sludge were further determined by digesting the samples with aqua regia. The background Ti and W concentration in the blank solution was determined and subtracted from the concentration of Ti and W in the effluent and sludge dosed with TiO₂ and WO₃ NPs. The levels of calcium, magnesium, potassium, and sodium in wastewater and the sludge were also measured.

1.6. Characterization of NPs and sludge

The polymorph of both pristine and that present in the sludge of WO₃ and TiO₂ was elucidated by using a Philips X’Pert instrument (Panalytical X’Pert PO MPD, Philips, Netherlands) at 40 mA, 40 kV with CuKα radiation at 0.1541 nm wavelength. A monochromatic beam in 2θ was used within the scan range of 10–80° and a step size 0.0170 (2θ) and step time of 175.26 sec was used, respectively. The morphology was investigated using transmission electron microscopy (TEM, Model JEM – 2100F, JEOL, Japan) at an acceleration voltage of 200 kV. The NPs were analysed on a carbon coated copper grid. Elemental analysis of the sludge was conducted using energy dispersive X-ray spectrometry and scanning electron microscopy (SEM) mapping software (Vega 3 LM, TESCAN, Czech Republic) under nitrogen gas on the sludge to ascertain the distribution of the tungsten and titanium elements.

The Brunauer–Emmett–Teller (BET) surface area of the NPs was determined by nitrogen adsorption in a Micrometric ASAP 2020 surface area and pore analyser (ASAP 2020 Plus, Micrometric instruments Corporation, Japan). The samples were degassed at 150°C for 5 hr prior to analysis. Isotherm evaluation was performed at –77 K and pore volume analysis was determined at (P/P₀) of ~0.98, where P is the relative pressure and P₀ is the saturated pressure. Ultraviolet–visible (UV–Vis) spectrophotometer (UV-2450 UV–Vis spectrophotometer, Shimadzu, Japan) operated in diffuse reflectance mode (DRS) was used to measure the absorbance spectra after which Kubelka–Munk transformation was applied. The zeta potential was determined by electrophoretic light scattering (ELS) by means of a Zetasizer (ZetasizerNano ZS, Malvern Panalytical, United Kingdom). Raman measurements were performed with a Raman spectrophotometer (Raman Micro 200, Perkin Elmer, USA) using the 514.5 nm wavelength of an Ar⁺ laser. At the end of SRT 7 in which 15 mg/L of NPs were spikes, the sludge was also sampled and had the volatile suspended solids removed at 550°C for 20 min and had it analysed with Raman and X-ray diffraction (XRD, Panalytical X’Pert PO MPD, Philips, Netherlands) instrument. The Fourier transformed infrared (FTIR) spectra of the sludge was captured using the FTIR spectrophotometer (Spectrum 100 FTIR, Perkin Elmer, USA) with a spectral resolution of 4 cm⁻¹.

2. Results and discussion

2.1. Physicochemical properties of the raw wastewater

The physicochemical characteristics of the raw wastewater were constantly monitored throughout. The obtained data are summarized in Table 1. These parameters were monitored due to their functional effect on the treatment efficiency and to determine the properties of the SWTP. The parameters determined the influent were comparable to the typical untreated raw wastewater profile as reported by Metcalf and Eddy (2004). The major difference in the organic carbon is mainly that the wastewater profile reports total organic carbon which could be dissolved and undissolved carbon material combined. However, the parameters of the influent

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Standard deviation</th>
<th>Effluent limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.14–7.92</td>
<td>–</td>
<td>5.5–9.5</td>
</tr>
<tr>
<td>Conductivity</td>
<td>1768.9</td>
<td>150.84</td>
<td>700</td>
</tr>
<tr>
<td>TDS (mg/L)</td>
<td>881.97</td>
<td>74.01</td>
<td>860</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>3.42</td>
<td>0.51</td>
<td>NR</td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>633.21</td>
<td>177.9</td>
<td>75</td>
</tr>
<tr>
<td>DOC (mg/L)</td>
<td>44.41</td>
<td>4.1270</td>
<td>260</td>
</tr>
<tr>
<td>TSS (mg/L)</td>
<td>270.73</td>
<td>134.39</td>
<td>25</td>
</tr>
<tr>
<td>Salinity (psu)</td>
<td>3.33</td>
<td>0.6724</td>
<td>NR</td>
</tr>
<tr>
<td>Calcium</td>
<td>33.93</td>
<td>0.3960</td>
<td>NR</td>
</tr>
<tr>
<td>Magnesium</td>
<td>12.71</td>
<td>0.0707</td>
<td>NR</td>
</tr>
<tr>
<td>Potassium</td>
<td>18.03</td>
<td>1.1667</td>
<td>NR</td>
</tr>
<tr>
<td>Sodium</td>
<td>44.37</td>
<td>0.5515</td>
<td>NR</td>
</tr>
</tbody>
</table>

NR: not regulated; TDS: total dissolved solids; DO: dissolved oxygen; COD: chemical oxygen demand; DOC: dissolved organic carbon; TSS: total suspended solids; DWA: Department of Water Affairs; psu: practical salinity unit.
were much higher when compared to the South African waste discharge guidelines.

2.2. WO₃ and TiO₂ NPs

The XRD of the NPs confirmed that the WO₃ phase was monoclinic while TiO₂ was anatase as shown by the patterns depicted in Fig. 1a. The phases of WO₃ and TiO₂ were confirmed by ICDD: 04-007-1277 and 00-001-0562 respectively with WO₃ having lattice constants \( a = 7.291 \) Å, \( b = 7.539 \) Å, \( c = 7.688 \) Å and \( \beta = 90.91 \) and TiO₂ \( a = 3.730 \) Å and \( c = 9.370 \) Å. This observation made Raman data in Fig. 1b further complemented the XRD results. The observed bands of WO₃ were sharp and well defined positioned at 806, 717, 326, 269 and 135 cm\(^{-1}\) which are assigned to monoclinic phase of WO₃ (Daniel et al., 1987; Liu et al., 2015) while those of anatase TiO₂ appears at 144, 395, 514, and 639 cm\(^{-1}\).

The crystallite sizes for both m-WO₃ and TiO₂ were 15 nm calculated using Scherer equation. TEM images revealed that the WO₃ NPs were platelets, ovoid, hexagonal and cubic while those of TiO₂ were spherical in shape as depicted in Fig. 2. Other physiochemical properties of the NPs are summarized in Table 2.

2.3. Effect of NP mixture on treatment plant functionality

Prior to the introduction of the NP mixture, a steady state in the efficiency of the SWTP in removing COD was to be established. This was an essential step in establishing a baseline on the performance of the microorganisms present in the activated sludge. The steady state was achieved between SRT 2 and 3 with an average COD removal of 85.2% and 83.7% in the control and test units respectively. These results are depicted in Fig. 3. They are aligned with the validity criteria as stipulated by the OECD 303A guidelines of a set target of 80%. At this point the sludge had adapted to the SWTP thus the NP effect or dosage on the treatment efficiency could be ascertained.

The biodegradation of the COD by the activated sludge was further investigated during the gradual addition of the mixture of NP (WO₃ and TiO₂) from 5 to 15 mg/L. An efficient organic matter removal was observed with the average COD removal ranging from 74.3–81.7 mg/L. The average COD removal efficiency was 86.2%, 86.0% and 89.7% for the test unit during the addition of 5 mg/L (SRT 5), 10 mg/L (SRT 6) and 15 mg/L (SRT 7) NPs, respectively. These results were respectively comparable to the removal efficiency observed in the reference test of 87.6%, 89.4% and 85.9%. Evidence further summarised in Fig. 4a reveals that the NPs had no effect on the functionality of the treatment units as the removal efficiency was maintained above the validity criteria. The DOC analysis of the influent and effluent further complemented these findings. The average DOC of the effluent was 11.8, 11.6 and 11.4 mg/L for the control effluent (CE) while test effluent (TE) was 11.2, 11.5 and 11.6 mg/L for SRT 5, 6 and 7 respectively as depicted in Fig. 4b. Both results are comparable further revealing the effectiveness of both treatment units in tolerating the influent which had an average of 44.4 mg/L dissolved organic carbon.

The effective removal of the COD achieved in both the control and the test units was only possible through a vast population of microorganism present in the sludge this microbe utilizes the pollutants (organic matter) as food while water treatment was achieved. Thus, the total plate count of the aeration chamber revealed presence of bacterial, as shown in Fig. 5. The bacterial colonies present in the control and the test units respectively. These results are depicted in Fig. 3. They are aligned with the validity criteria as stipulated by the OECD 303A guidelines of a set target of 80%. At this point the sludge had adapted to the SWTP thus the NP effect or dosage on the treatment efficiency could be ascertained.

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units were comparable during the introduction of NPs in the chambers. This implied that the NPs had no impact on the microbial population hence the successful removal of the COD. Furthermore, Fig. 5b shows the presence of mould in the sludge which supported that activated sludge was a mixed liquor of different microorganisms.

The conductivity measurements of the effluent samples from the reference unit was 1448.0 ± 23.14 μS/cm while the test unit was 1545.0, 1634.8 and 1720.0 μS/cm for 5, 10 and 15 mg/L, respectively. The total dissolved solids followed a similar trend. The increase in both the conductivity and TDS with an increase of the concentration of added NPs implied dissolution of the NPs. This observation concurs with studies conducted on the dissolution of NPs which revealed that WO3 readily dissolve in aqueous medium with pH > 4 (Liu et al., 2011; Anik and Cansizoglu, 2006) while Mahlalela et al. (2017b) reported the dissolution of TiO2 in a similar simulation setup confirmed through conductivity measurements.

The ratio of MLVSS to MLSS (MLVSS/MLSS) was also used to monitor the survival of the flocs in the aeration chambers. The ratio of MLVSS/MLSS should be greater than 0.75 in a conventional wastewater treatment system (Fan et al., 2015; Wentzel et al., 2002) as it was an indicator of the concentration of the microorganisms constituted in the activated sludge. It was evident that the MLSS were mostly organic matter signifying the population of the microbes in the mixed liquor of the aeration tanks. For both the test and control, the observed ratio for MLVSS/MLSS was much greater than the expected 0.75 in conventional wastewater treatment system as shown in Table 3. The used wastewater had a high organic matter composition as revealed by the COD results ensuring enough food for the flocs for both the test and control units.

As air was forced into the aeration basins, it increased the activity of these microorganisms and helped to keep the organic waste thoroughly mixed. Dissolved oxygen (DO) was added to the aeration basin to enhance the oxidation process by providing oxygen to aerobic microorganisms. For complete metabolism and reproduction, each microorganism should have at least 0.1 to 0.3 mg/L of DO (Emerson Process Management, 2009). Hence the activated sludge remained active thus successful removal of organic matter even when the DO levels were below the 2 mg/L.

2.4. Fate of NPs mixture

An elemental analysis was conducted on the dried sludge of both the control and test units using EDS technique. Fig. 4 shows the presence of a number of elements such as carbon, oxygen, magnesium, calcium, sodium, chlorine and aluminium.

### Table 2 – Other physical properties of the nanoparticles.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>TiO2</th>
<th>WO3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface area (m²/g)</td>
<td>55.7</td>
<td>3.54</td>
</tr>
<tr>
<td>Pore volume (cm³/g)</td>
<td>0.1141</td>
<td>0.0085</td>
</tr>
<tr>
<td>Pore size (nm)</td>
<td>10–20</td>
<td>3–13</td>
</tr>
<tr>
<td>Band gap (eV)</td>
<td>3.20</td>
<td>2.71</td>
</tr>
<tr>
<td>Point of zero charge</td>
<td>5.0</td>
<td>2.5</td>
</tr>
<tr>
<td>TEM particle size (nm)</td>
<td>15</td>
<td>36–255</td>
</tr>
<tr>
<td>Particle size BET (nm)</td>
<td>28</td>
<td>237</td>
</tr>
</tbody>
</table>

in both control and test units. All these elements arose from the chemical used in the treatment process where the sludge was obtained. Both the peaks of tungsten and titanium that appear in the Fig. 6e are a result of the spiked NPs which were also confirmed by elemental mapping as depicted in Fig. 6b–d confirming an even distribution of the elements in the sludges.

Both titanium and tungsten were evident in the sludge, thus the nature of their phase was in question. Therefore, XRD analysis of the sludge originating from the control and test units was conducted and the patterns are shown in Fig. 7a. The observed test sludge patterns revealed presence of both WO3 and TiO2 and these patterns are comparable to those of the pristine mixture. More so, the control showed absence of these peaks with only peaks associated with SiO2 being shown. The presence of SiO2 in the sludge has been widely reported in other studies.

The oxide –SiO2– occurs with other metal oxides such as TiO2, Al2O3, ZnO and Fe2O3 (Lynn et al., 2015; Magdziarz et al., 2016; Chaüque et al., 2016). The presence of both WO3 and TiO2 in the test sludge concurs with other reports that NPs are retained in the sludge. However, the WO3 patterns were more prominent compared to those of TiO2. This could be attributed to the differing densities of the materials which are 7.14 and 3.78 g/mL for WO3 and TiO2 respectively even though the concentrations were equal.

These XRD patterns confirmed the existence of the monoclinic polymorph of WO3 and anatase TiO2 in the sludge as compared to the patterns displayed by the pristine mixture of NPs. Thus, the aerobic activity of the sludge had no negative impact on the polymorph of the NPs. These findings agree with several researchers who reported on ZnO, TiO2 and WO3 (Chaüque et al., 2016; Simelane et al., 2017a).

Furthermore, the vibrational modes of the sludge were investigated with the main aim of determining the presence of the NPs. The control sludge only showed the carbon bands as shown in Fig. 7b. The addition of 15 mg/L of NPs gave only the G-band (1509 cm−1) and D-band (1268 cm−1) of the sludge. However, when the volatile suspended solids were removed from the sludge, Raman bands attributed to the NPs appeared as illustrated in Fig. 7b plus the carbon bands. The bands observed at 385, 487, 667 cm−1 were B1g, A1g and E2g of TiO2 respectively and the bending vibration modes of (O―W―O), stretching vibration modes of (W―O―W) for WO3 NPs were observed at 308 and 739 cm−1, respectively. The Raman bands of the burnt test sludge were complimented by the FTIR spectra which showed two peaks in the absorption region of 1000–500 cm−1 which appeared in the NP mixture spectrum. Thus, the absence of the NP Raman bands in the sludge further complemented by the FTIR spectra which showed no difference in the control and test sludge during addition of NP mixture as depicted in Fig. 7c could be attributed to the high percentage weight of carbon being present in the sludge. These further confirmed the XRD patterns which revealed the presence of NPs in the sludge.

The crystal size was computed using Scherrer’s formula and the XRD data. The obtained crystalline size was 15 nm for both WO3 and TiO2 in the sludge. The obtained crystal sizes were comparable to those of the pristine NPs introduced with the influent. The TEM image in Fig. 8 showed particles in differing particle size further confirming the presence of NPs as indicated with reference to Fig. 2. The morphology of the NPs was not affected by the activity of the activated sludge.

2.5. Distribution and dissolution of NPs in the SWTP

The fate of NPs in wastewater treatment plants is dependent on several parameters such as pH, ionic strength and natural organic matter (NOM). The presence of NOM such as humic acid and dyes has been reported to stabilize both WO3 and TiO2 in wastewater and hence influence their removal by activated sludge treatment (Simelane et al., 2017b; Mahlalela et al., 2017a).

Mahlalela et al. (2017b) reported that 10%–11% of the TiO2 was lost with the effluent while 90% was retained in the sludge. On the similar premise, Simelane et al. (2017a) studying the effect of humic acid on the removal of WO3 nanoparticles in wastewater reported that 80% of the NPs were retained in the sludge. In our findings, these high numbers of NPs reported in the effluent was mainly driven by the suspended solids found in the effluent. A comparison of both Ti and W present in filtered
Fig. 5 – (a) A summary of total plate count of both aeration units and (b) presence of mould in the activated sludge (c and d) indicating microbial population. CA: control aeration; TA: test aeration.

Table 3 – Monitored parameters in the aeration chambers.

<table>
<thead>
<tr>
<th>SRT (day)</th>
<th>Dosage (mg/L)</th>
<th>0</th>
<th>5</th>
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<td>Test unit</td>
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<td>MLSS (mg/L)</td>
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<tr>
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<tr>
<td>pH</td>
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<tr>
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<td>0.47</td>
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SD: standard deviation; MLSS: mixed liquor suspended solids; MLVSS: mixed liquor volatile suspended solids; DO: dissolved oxygen; SRT: sludge retention time.
Effluent samples and the digested samples showed a high correlation as revealed in Fig. 9a–b. About 96.4% and 71.4% of the Ti and W respectively found in the effluent was due to the presence of the suspended solids with only 3.6% and 28.6% due to dissolution. This showed that WO₃ NPs undergoes higher dissolution rate compared to TiO₂. Thus, the high percentage of NPs reported in outflow is mainly due to the NPs adsorbed on the sludge surfaces.

Fig. 9a–b further revealed that about 99.8% of Ti and 95.5% of W was found in the sludge with only 0.2% Ti and 4.5% W found in the effluent. However, it was evident that these results were much lower compared to those reported in literature. This was driven by the fact that, only dissolute titanium and tungsten were considered in the outflow as the suspended solids were filtered out which had the NPs adsorbed on them.

These observations complemented the EDS results which showed the presence of the Ti and W in the sludge.

Several studies done on the fate and behaviour of TiO₂ revealed a wide range of removal efficiencies. A study considering the fate in an industrial dye effluent reported 90% of the TiO₂ NPs being retained in the sludge (Mahlalela et al., 2017b). Similar findings were reported by Johnson et al. (2011) studying the removal of TiO₂ associated with sunscreen products. About 80% of WO₃ was reported to be retained in the sludge (Simelane et al., 2017a). The study of the fate of TiO₂ in industrial dye effluent and that of WO₃ was conducted using synthetic wastewater. The raw wastewater used in this study was found to contain both monovalent and divalent metal ions (Ca, Mg, K and Na) which are also found in increased levels in the activated sludge. The detected average levels of K, Mg, and Na were 131.4, 75.1, and...
106.1 mg/L in the sludge. Several oxides such as Na₂O, K₂O, MgO, ZnO, MnO₂ and CaO among others have been reported to be present in water treatment sludge thus further complementing the detection of the mentioned elements (Ahmad et al., 2016). Divalent especially Ca²⁺ metal ions have been reported to successfully compress the electric double layer of NPs even in the presence of NOM resulting in agglomeration of NPs and by NOM-cation bridge formation achieved through electrostatic interactions (Simelane et al., 2017b; Mahlalela et al., 2017b).

It was therefore hypothesized that the NPs were not only retained in the sludge through adsorption but that the metal ions also played a crucial role in the removal of the NPs from the spiked wastewater. At the working pH ranging from 7.14–7.92, both WO₃ and TiO₂ were negatively charged as their point of zero charge is said to be at pH 2.5 and 5.0 respectively (Simelane et al., 2017b; Mahlalela et al., 2017b). Thus, the metal ions neutralized the electric double layer of the NPs resulting their agglomeration as repulsion forces decrease with an increasing attraction force resulting in NP sedimentation in the clarifier. Furthermore, the presence of NOM and surfactants in wastewater in various concentrations, though binding processes may adsorb or chelate the solubilized metal ions (W⁶⁺ and Ti⁴⁺) resulting in the reduction of the metal concentration in the effluent and hence high removal efficiencies.

More so, the presence of bacteria, a major component of the activated sludge flocs, has a negatively charged surface at pH ranging between 7.4 and 7.8 of its cytoplasm (Dickson and Koohmariae, 1989; Li et al., 2015; Khoerunnisa et al., 2017). Therefore, cations present in the sludge such as W⁶⁺ and Ti⁴⁺ due to NP dissolution will adsorb on the bacterial surface by means of electrostatic interactions resulting in reduced metal ions in the effluents. A greater percentage of the cations were retained with the sludge and only a small percentage leaching out with the suspended solids of those adsorbed.
3. Conclusions

The objectives of this study were to determine the fate and behaviour of a NP mixture of WO₃ and TiO₂ using the OECD 303A guidelines and to ascertain the impact on the functionality of the treatment plant. The NPs had no negative impact on the treatment process as the COD removal efficiency remained above 80% which is the stipulated criteria for validity of results. A large percentage of the NPs settled out with the sludge resulting in 99.8% TiO₂ and 95.5% WO₃ removal, through NP adsorption on the surface of the sludge and NP aggregation.

The XRD and Raman spectroscopy confirmed the presence of anatase TiO₂ and monoclinic WO₃ NPs implying that the activated sludge treatment process had no effect on the crystallinity of the NPs. The NP mixture had no inhibiting effect of the microbial activity as complemented by the bacterial total plate count yet their removal efficiency was high with 3.6% Ti and 28.6% W found in the effluent due to dissolution of the NPs. Thus, to mitigate the possible release of the NPs into the surface water and the environment, other methods should be investigated for the removal of the NPs and metal ions.

Acknowledgments

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REFERENCES


