Supporting information for

**Evaluation of enhanced darkfield microscopy and hyperspectral analysis to analyse the fate of silver nanoparticles in wastewater and biosolids**

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Table S1: Primary physicochemical characteristics of the wastewater mixed liquor and effluent collected from La Prairie's wastewater treatment plant. ORP=oxidation reduction potential; DOC=dissolved organic carbon; TOC= total organic carbon. Analysis was performed by the Ghoshal research group and published previously in Azodi et al., 2016, Environ. Sci. Technol., 50: 13318-13327.

| Wastewater (WW) sample | pH | Conductivity (µS/m) | ORP (mV) | DOC (mg/L) | TOC (mg/L) | Suspended solids (mg/L) |
|---|---|---|---|---|---|---|
| WW mixed liquor (biosolids) (Secondary aeration tank) | $7.13 \pm 0.01$ | $1030 \pm 2$ | $+257 \pm 1$ | 39.7 | 2131.5 | $1823 \pm 60$ |
| WW effluent (Secondary settling tank) | $7.28 \pm 0.01$ | $1002 \pm 1$ | $+264 \pm 2$ | 8.85 | 34.7 | $10 \pm 4$ |

In a control experiment designed to determine if the Ag NP underwent any changes in their spectral profile with time in a simple medium, the spectral profile of the initial sample was used to search subsequent images over 300 minutes. Using a conservative spectral angle mapping of 0.08, it can be seen that the spectral profile did not change significantly over the course of the experiment, indicating that the NP were stable in Milli-Q water.
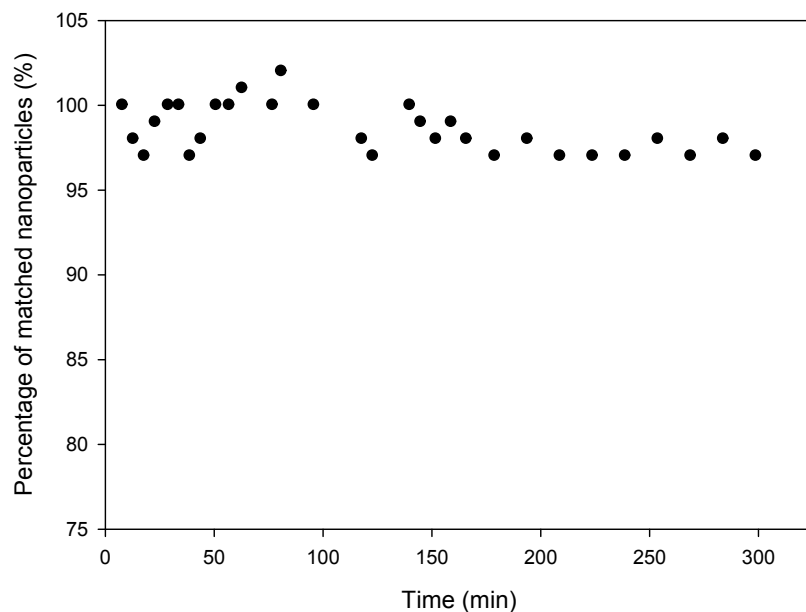


Figure S1. Identification of 2 mg L$^{-1}$ of citrate coated silver nanoparticles (Ag NP (cit)) in Milli Q water as a function of time.
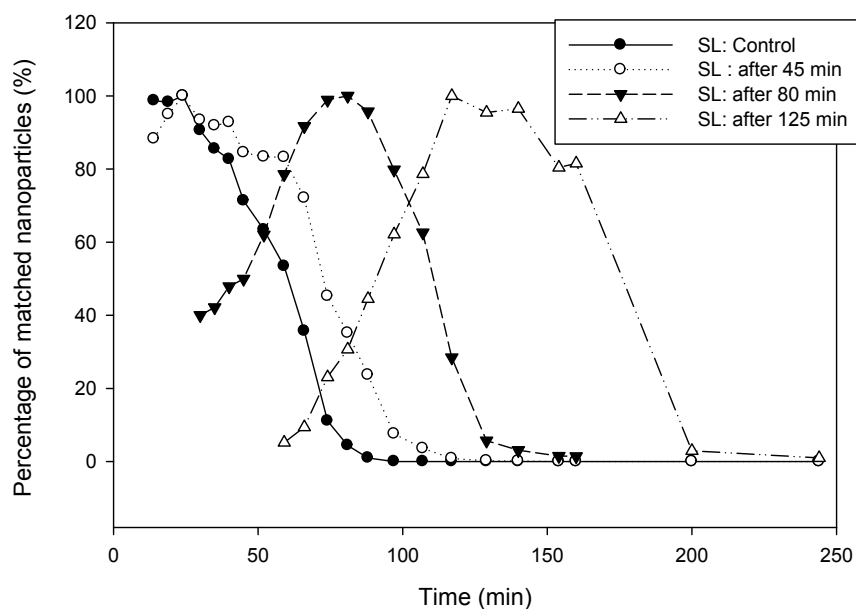
Figure S2. Positively matched pixels of aging 40 nm Ag NP(cit) in a wastewater using different spectral libraries after 0, 45, 80 and 125 minutes.

As shown in the main paper (Fig. 3), the spectral profile of the Ag NP underwent a rapid transformation once the NP were immersed in the wastewater, to the point that the NP could not be detected when using the original spectra generated for Ag NP in Milli Q water. Indeed, for a given image generated from the 40 nm Ag NP(cit) in a wastewater, different proportions of matched pixels were determined, depending upon the spectral library that was used. Indeed, four spectral libraries were generated using Ag NP that were in contact with either Milli-Q water (control) or different contact times in the wastewater effluent. The proportion of positively matched pixels were determined with each of the spectral libraries and results presented in Fig. S2.

Hydrodynamic (DLS) and physical diameters (SP-ICP-MS) for the different Ag NPs are presented in Table S2 as a function of time. DLS results showed a slight increase in the hydrodynamic diameter, whereas SP-ICP-MS showed no significant changes in the physical diameter for any of the Ag NPs, suggesting that there was no homoagglomeration occurring.

Table S2: Distributions of the hydrodynamic diameters (model Rayleigh spheres) and physical diameters of different Ag NPs in filtered wastewater as determined by DLS (0.2 mg $L^{-1}$) and SP-ICP-MS (0.10 µg $L^{-1}$) after 9 min, 181 min. and 16 days.

| | | Time | | | Time | | |
|---|---|---|---|---|---|---|---|
| | | 9 min | 181 min | 16 days | 9 min | 181 min | 16 days |
| Size (nm) | Coating | Hydrodynamic diameter (nm) | | | Physical diameter (nm) | | |
| 40 | cit | 43 ± 2 | 55 ± 2 | 66 ± 3 | 37.4 ± 0.4 | 37.9 ± 0.4 | 37.9 ± 0.4 |
| 40 | PVP | 54 ± 4 | 54 ± 3 | 58 ± 6 | 38.6 ± 0.2 | 38.8 ± 0.2 | 38.7 ± 0.3 |
| 80 | cit | 82 ± 2 | 90 ± 2 | 94 ± 5 | 73.2 ± 0.3 | 73.3 ±0.2 | 73.2 ± 0.2 |
| 80 | PVP | 92 ± 2 | 91 ± 2 | 93 ± 4 | 75.1 ± 0.3 | 75.5 ± 0.3 | 75.8 ± 0.3 |
| 100 | cit | 107 ± 1 | 118 ± 2 | 125 ± 4 | 92.5 ± 0.4 | 92.4 ± 0.3 | 91.6 ±0.3 |
| 100 | PVP | 117 ± 2 | 116 ± 3 | 121 ± 5 | 95.0 ± 0.4 | 95.2 ± 0.4 | 94.6 ± 0.4 |