Occurrence of Transparent Exopolymer Particles (TEP) in drinking water systems

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Abstract
Numerous membrane fouling studies have been conducted to predict and prevent membrane fouling. It was only recently that a new parameter, TEP, was introduced in this research. The deposition of TEP on reverse osmosis (RO) membranes has already been imaged, correlations between ultrafiltration (UF) fouling and TEP concentrations have been reported. Furthermore, TEP deposition takes place in an early stage of aquatic biofilm formation, making TEP one of the accused in search for biofilm initiation factors. After literature reporting about TEP in marine, surface and wastewater, this is the very first research focusing on TEP through in drinking water. Every single treatment step in three completely different drinking water production plants was scored on TEP removal. It could be concluded that TEP concentrations were very dependent of the raw water source but in none of the installations, TEP was able to reach the final drinking water in significant concentrations. The combination of coagulation and sand filtration proved efficient in strongly reducing TEP levels, while the combination of UF and RO could provide a total TEP removal.

Keywords
Biofouling, drinking water, transparent exopolymer particles (TEP)

INTRODUCTION

Transparent exopolymer particles (TEP) are gel-like sticky particles consisting mainly of acidic mucopolycaccharides, ubiquitous in natural waters, ranging in size from 0.05 until a few hundred micrometers (Passow, 2002). They are predominantly formed out of algal exudates, bacterial mucus and particular material from the gelatinous envelopes surrounding phytoplankton and hence they are found abundantly in the ocean as well as in surface- and wastewaters (Alldredge *et al.*, 1993; Grossart *et al.*, 1998; Passow, 2002; de la Torre *et al.*, 2008). Two types of TEP have been described: colloidal TEP (cTEP) ranges between 0.05 and 0.4 µm, while particular TEP (pTEP) are particles larger than 0.4 µm (Villacorte *et al.*, 2009). In most studies, cTEP contributed for up to 90% of total TEP concentrations (Villacorte *et al.*, 2009; Villacorte *et al.*, 2010).

Aquatic biofilm formation. Since the method was developed to stain these hitherto overlooked particles (Alldredge *et al.*, 1993), they have mostly drawn the attention of oceanographers in relation to organic carbon cycling (Passow, 2002). More recently, some membrane technologists recognized the importance of TEP in their field of study. Berman and Holenberg introduced the idea of “sticky TEP”, well suited to induce biofouling on surfaces. Once attached to a membrane, these particles start blocking pores and serve as both an attachment site and nutritious substrate for microbial growth (Berman, 2005). Other researchers noticed that up to 70% of all TEP in influent water would stick on reverse osmosis (RO) membranes (Villacorte *et al.*, 2009). Furthermore, the

efficiency of RO pretreatment systems in preventing TEP from reaching the sensitive membranes was verified. Several combinations of sand and membrane filtration were able to remove pTEP for 30 up to 100% but cTEP was seldom removed for more than 50%. Moreover, this fraction can easily transform to new pTEP (Bar-Zeev *et al.*, 2009; Kennedy *et al.*, 2009; Villacorte *et al.*, 2009; Villacorte *et al.*, 2010). Other researchers showed the correlation between TEP-concentrations and capillary suction time, a common fouling indicator. Multivariate data analysis could relate the critical flux values to TEP, nitrate and temperature in 95% of all cases (de la Torre *et al.*, 2008; de la Torre *et al.*, 2010). Berman *et al.* (2011) stated that early EPS deposition on membranes only originates from TEP in the feedwater instead of being excreted by active bacteria developing in a biofilm. This indicates that TEP can be an important factor for the initiation of biofilms.

Many reported the abundance of TEP in marine, surface, waste- and groundwater but not a single study examined the occurrence in drinking water treatment trains. The limited TEP removal efficiencies reported by Villacorte et al. (2009) suggest the possibility of TEP reaching the drinking water while the conclusions about biofilm formation would have serious safety implications in this case. Waterborne pathogens like *Legionella* species use biofilms both for growth and protection against biocides (Szewzyk *et al.*, 2000; Williams *et al.*, 2003). TEP occurrence in drinking water would give us new insights about biofilm prevention pathways.

This paper is the first one to evaluate the occurrence of TEP in a drinking water environment. The main objectives of this paper were

- To evaluate TEP concentrations in drinking water because of the biofilm formation properties and
- To score common water treatment methods on TEP removal, a possible important pretreatment for sensitive membrane systems

MATERIALS AND METHODS

Sampling

Sampling was done at drinking water production plants (DWPPs) of 3 Belgian drinking water companies: IWVA (‘Plant A’), VMW (‘Plant B’) and Pidpa (‘Plant C’) at respectively February 17, March 11 and Januari 17. Each time, a raw/influent raw rough water sample was taken, followed by a sample after every important step of the treatment process, until the final drinking water. Plant A uses effluent from a wastewater treatment plant (WWTP), Plant B is based on surface water and Plant C uses groundwater and treats it via double sand filtration, extended aeration, chlorination and UV disinfection to drinking water. For the complete configuration of the first two plants and the sampling points, the reader is referred to Figure 1. Additional information about Plant A can be found in Van Houtte *et al.* (2008). Samples were taken in duplicate in plastic 10L drums and were stored at 4°C until further analysis.

TEP measurement

Filtration and quantification. TEP measurements were based on the classic method (Passow *et al.*, 1995), adapted by Villacorte *et al.* (Villacorte *et al.*, 2009). At least three subsamples of each water sample (20mL-2L) were successively filtered over polycarbonate track-eched membranes with pore sizes 0,4 and 0,05µm (respectively it4ip, Seneffe, Belgium and Sterlitech, WA, US) using an adjustable vacuum pump (Knf lab pumps, Aartselaar, Belgium) set at 200 millibar of vacuum and polysulfone filter holders (Nalgene). Subsequently, the membranes were stained and rinsed and the alcian blue stain was extracted in 80% sulphuric acid according to Villacorte *et al.* (2009). Finally, the absorbance of this acid solution was measured at 787 nm with a spectrophotometer (WPA Lightwave II, Biochrom, England). After multiplying this absorbance with a fixed calibration factor, concentrations can be expressed as $\mu g.L^{-1}$ Gum Xanthan-equivalents (X_{eq}).

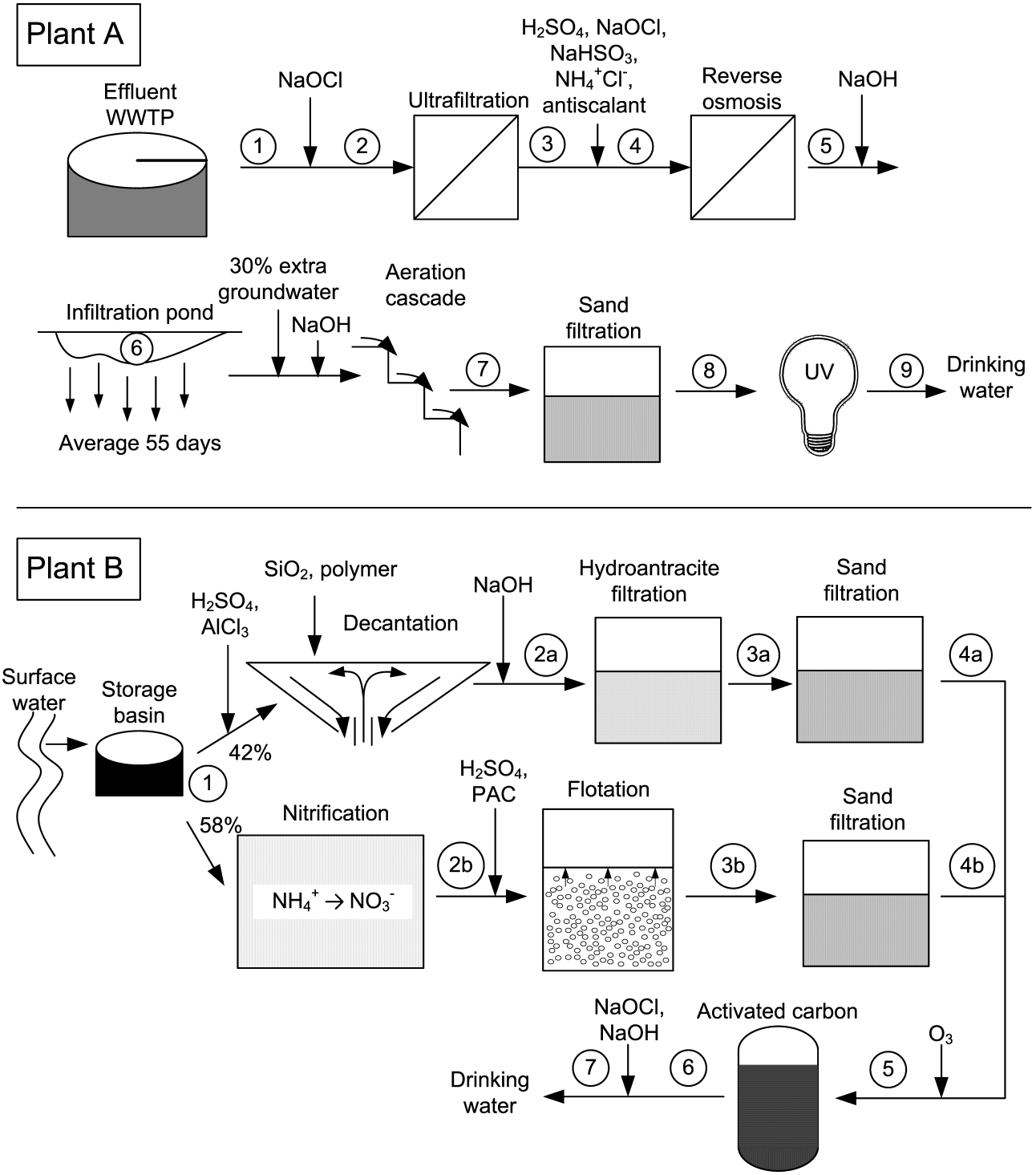

Figure 1: Schematic representation of drinking water treatment plants A and B. The sampling points are indicated by the encircled numbers.

Staining solution. The staining solution, needed for TEP detection, was made by dissolving 150-200 mg of the alcian blue dye (8GX, Standard Fluka) in 100 mL of deionised water and was set at pH 2,5 by addition of acetic acid. This solution was filtered over 0,05 µm track-eched membranes to remove coagulated and non-dissolving stain particles. The alcian blue-concentration of this filtered solution is determined by measuring copper concentrations by flame atom absorbance spectrophotomety (Shimadzu AA-6300) and incorporating the proportional mass of copper in alcian blue (3.84 w%). Next, the solution is diluted with deionised water to set the alcian blue-concentration at 100 ± 2 $mg.L^{-1}$. By doing this, the staining capacity of the each staining solution

was regarded constant. Villacorte *et al.* (2009) declared the staining capacity of the solution as constant whenever alcian blue concentrations remained higher than 85 $mg.L^{-1}$. For this reason, no calibration of the staining solution was performed and the same calibration factor of 114 $\mu g\ X_{eq}$ was used to display the results, which should be regarded as relative. All error bars in figures represent standard deviations to the average of at least 3 samples. When not in use, the solution is conserved at 4°C for maximum one week before renewing.

RESULTS AND DISCUSSION

Raw water TEP concentrations

TEP concentrations were measured using the spectrophotometric method as developed by Passow and Alldredge (1995). Since recent literature revealed that cTEP is a very abundant fraction of total TEP concentrations, this fraction was enclosed in our measurements (Villacorte *et al.*, 2009). In order to incorporate as much variation as possible in the outcome of this study, three very different DWPPs were selected. Both the raw water sources and the main treatment steps varied in these systems, the plant configurations and sampling points are displayed in Figure 1.

Plant A. The first sampled plant ('Plant A') uses wastewater effluent and treats it via membrane filtration and dune infiltration to produce drinking water. The pTEP concentration measured in this raw water was 49 $\mu g\ X_{eq}.L^{-1}$. Only a few studies already addressed the pTEP contents of wastewater effluents before, yielding 270 and 746-4157 $\mu g\ X_{eq}.L^{-1}$ in respectively the Netherlands and Israel (Kennedy *et al.*, 2009; Berman *et al.*, 2010). These notably higher values compared to our results can have multiple explanations: (1) temperature differences, affecting the bacterial activity and TEP production; (2) seasonal differences, resulting in temperature differences; (3) differences in plant configuration. A combination of these factors can be expected since our samples were taken in February while the Dutch samples were taken in April and the Israelian Mediterranean Sea climate is clearly warmer that the Belgian Oceanic climate. No information was provided about the other plant configurations in the cited literature. Besides the pTEP, addressed in the mentioned studies, the cTEP concentration was also assessed in this report. This appeared to be 713 $\mu g\ X_{eq}.L^{-1}$, so that this fraction accounts for 94% of total TEP.

Plant B. In Plant B, a surface water based DWPP, an extremely low pTEP concentration of 7 $\mu g\ X_{eq}.L^{-1}$ could be measured while literature values ranging for freshwater from 36 to 9038 $\mu g\ X_{eq}.L^{-1}$ (Villacorte *et al.*, 2009; Berman *et al.*, 2010; de Vicente *et al.*, 2010). cTEP was more abundant with a measured concentration of 332 $\mu g\ X_{eq}.L^{-1}$. These samples were taken in a water basin where the raw water is stored on average for one year before treatment to drinking water. During this time, self-purification processes take place, resulting in an oligotrophic environment. Furthermore, the sampling took place early March, just during the beginning phytoplankton bloom (9.99 mg $chlorophyll.m^{-3}$) which reached a peak concentration of 72.98 mg $chlorophyll.m^{-3}$ in the beginning of April. It is generally accepted that peak TEP concentrations are usually associated with phytoplankton blooms (Passow, 2002). The higher concentration of cTEP indicates the beginning of a bloom with TEP-precursors, measured as cTEP, being released while physical flocculation to pTEP did not take place yet.

Plant C. The last sampled plant is based on groundwater extracted from a depth of 60m. Berman *et al.* (2010) already reported measurable TEP concentration in the range of 132-417 $\mu g\ X_{eq}.L^{-1}$ in saline groundwater from a well on a depth of 600m. These TEP were attributed to sulfur and iron bacteria present in that specific well. Although bacteria were present in the sampled ground water, no notable TEP concentrations could be found in this extremely oligotrophic water. Since the focus of this study is kept on TEP, none of the further results of the sampling in this installation are included in this report.

TEP evolution in Plant A

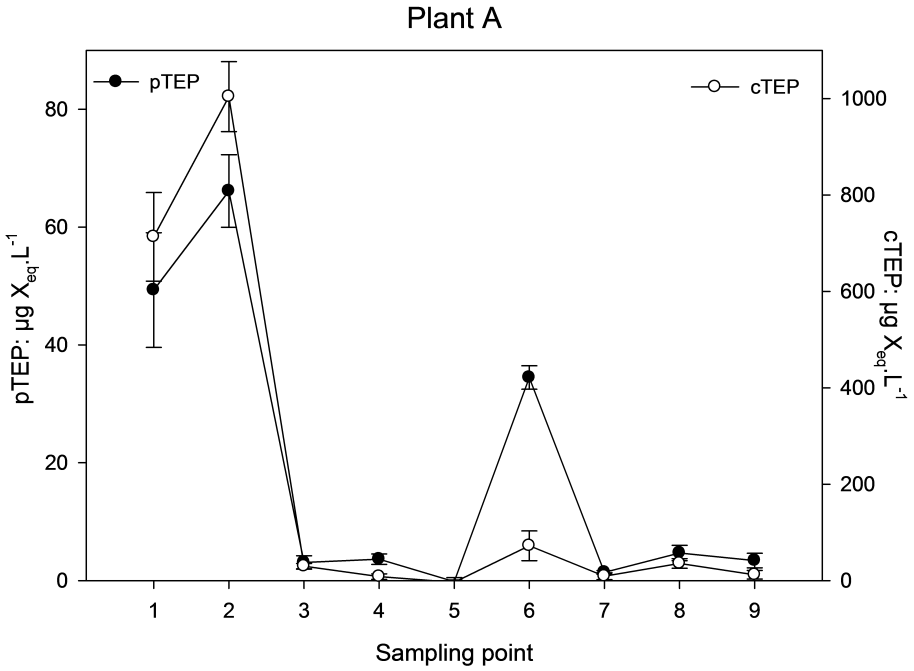

Figure 2. Evolution of cTEP and pTEP concentrations for Plant A. Concentrations are expressed as $\mu g.L^{-1}$ gum xanthan-equivalent. Mind the different axes for pTEP and cTEP.

Chlorination. The TEP evolution of plant A is displayed in Figure 2. It can be seen that chlorination increases both cTEP and pTEP amounts. Ortega-Retuerta *et al.* (2009) showed that another form of oxidative stress, ultraviolet (UV) radiation, promotes the production of TEP in the presence of microorganisms. The TEP release after these aggressive treatments can likely be explained by induced surface mucus and cell lysis.

Ultrafiltration. The following UF was able to remove 95 and 97% of respectively pTEP and cTEP. The efficient pTEP removal by the following UF could be partly expected since similar treatments have been reported to remove pTEP with efficiencies from varying from 27 up to 100% (Kennedy *et al.*, 2009; Villacorte *et al.*, 2009). However, the membranes in these previous studies all had a nominal pore size of 30 nm, while this study comprised UF membranes with a pore size as big as 100 nm. Since TEP are known to be highly flexible they can pass membranes with a nominal pore size smaller than their own diameter, especially when a high pressure is applied. For this reason, a pTEP fraction passing a membrane could not be excluded. Furthermore, it is more surprising that cTEP, with diameters varying from 0.05 to 0.4 µm are retained almost completely by this membrane. This would suggest that the majority of this fraction are particles with diameters well above 0.1 µm.

Reverse osmosis. A total TEP removal by the following RO is consequently seen, as well by a previous author (Villacorte *et al.*, 2009) as now and is to be expected due to the nature of this method. However, this TEP free and oligotrophic water is disposed in the infiltration pond, a open water exposed to external influences. A limited reappearance of nutrients likely leads to microbial and algal regrowth, giving the possibility for TEP reappearance, as can be seen in Figure 2. The following dune infiltration, an extended sand filtration, remineralizes this RO water, transforming it to additional ground water, maintaining the ground water level despite the drinking water extraction (Van Houtte *et al.*, 2008). This transformation comprises a pTEP and cTEP removal of respectively 95% and 87% and minimizes the TEP concentrations in the end of the treatment train. These concentrations are too low to evaluate the effect of the last treatment steps on TEP concentrations.

TEP evolution in Plant B

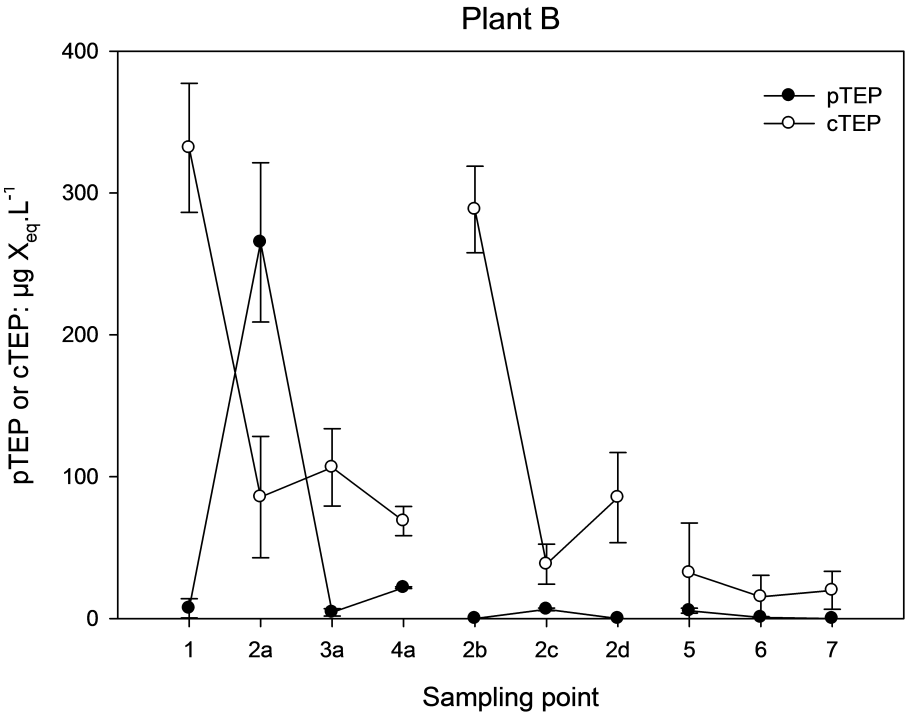

Figure 3. Evolution of cTEP and pTEP concentrations for Plant B. Concentrations are expressed as µg.L^{-1} gum xanthan-equivalent.

Coagulation + sand filtration. The TEP concentrations within Plant B, a plant with two parallel treatment lines using respectively decantation and flotation as main techniques, are displayed in Figure 3. In the first line, a decantation step results in both cTEP removal of 74% and a 37-fold increase of pTEP concentrations, but total TEP concentrations stay about the same. This can likely be explained by the nature of the decantation process. 11 mg.L^{-1} Al^{3+} (as $AlCl_3$) is added to the raw water and these positive charges are able to neutralize the negative charges that are by definition present on TEP. By doing this, Al^{3+} enables the small cTEP to coagulate and form bigger pTEP, that are however not removed by this method. The following double sand filtration (hydroantracite + sand) appears to be a good option to remove these coagulated pTEP (91% efficiency) but is a too rough method to abate the smaller cTEP (20% efficiency). Similar systems with coagulation/sand filtration were reported to remove 20 up to 68% of total TEP (Kennedy *et al.*, 2009; Villacorte *et al.*, 2009) while single sand filtration only showed a few percent up to 12% removal efficiency (Berman, 2005; Bar-Zeev *et al.*, 2009).

Flotation. In the parallel line, where nitrification, flotation and sand filtration are combined, the incoming pTEP amount stays minimal and the cTEP concentration decreases with 70%. An efficient TEP removal was expected here since these particles with an estimated density of 0.7 to 0.84 g.cm^{-3} (Azetsu-Scott *et al.*, 2004) tend to float near the water surface. However, flotation is especially effective for the removal of hydrophobic substances out of the water phase while TEP are regarded as hydrophilic (Passow *et al.*, 1995).

Final treatments. After the confluence of both streams the aggressive ozonation is able to destruct 58% of the cTEP. These concentrations are again cut in two in the activated carbon filtration and stay stable after final chlorination, so limited amounts of cTEP are able to reach the final drinking water.

CONCLUSIONS

- Influent TEP concentrations varied a lot depending on the raw water source; ground water contained no TEP while effluent from a WWTP and surface water both contained several hundreds of µg $X_{eq}.L^{-1}$ of TEP. cTEP is a usually neglected fraction but appeared extremely important since it accounted for 94-98% of total TEP values.
- Few or no TEP reached the final drinking water in the studied systems. However, it should be taken into account that the sampling was performed at moments when relatively low TEP concentrations were to be expected. Further research could proof is these conclusions are valuable all year round.
- A wide range of different water treatment methods was scored on TEP removal efficiency. The combination of coagulation and sand filtration proved effective for a good TEP removal while the combination of ultrafiltration and reverse osmosis resulted in a total TEP removal. However, it should be noted that TEP is a possible important factor in inducing biofouling in membrane systems. Furthermore, TEP concentrations were generally low in the studies systems, higher concentrations can possibly affect the TEP removal efficiencies of these systems.

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