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Polyacrylic acid-based nanoplastics used in cosmetics: a study of biodegradability and effects on heterotrophic and nitrifying microorganisms in the activated sludge

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ABSTRACT

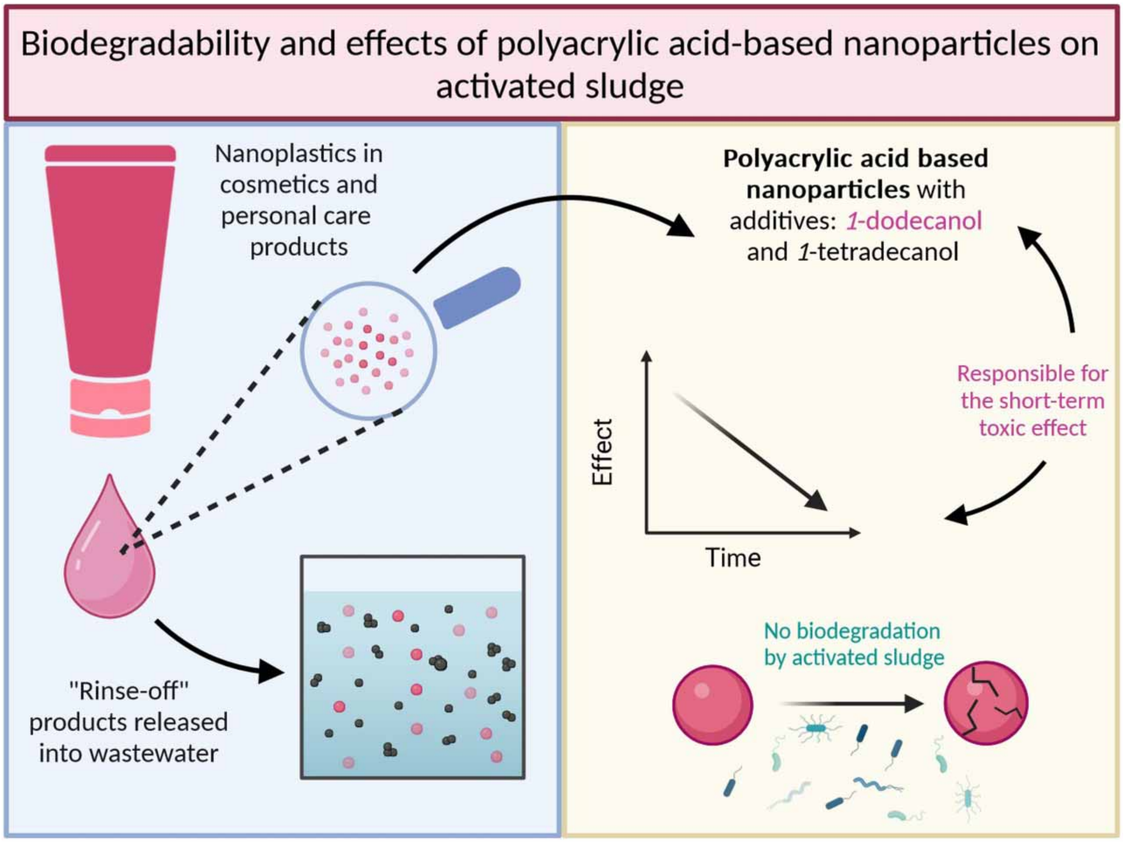
Nanoplastics, commonly used in cosmetics, enter wastewater systems and interact with activated sludge, yet their effects on microorganisms, essential for wastewater treatment, remain poorly understood. The aim of this study was to investigate the effects of polyacrylic acid-based nanoplastics (PANPs) on microorganisms in activated sludge. The PANPs were characterized in terms of their material composition, size, zeta potential, and additive content. Acute (30 min) and prolonged (up to 6 h) toxicity tests were performed to evaluate negative effects on heterotrophic and nitrifying microorganisms in activated sludge. In addition, ready and inherent biodegradability tests were performed to assess their degradation in the environment and within wastewater treatment plants, respectively. The results showed a significant acute inhibition of heterotrophic and nitrifying activity (up to 55 and 72%, respectively) at the highest concentration tested (100 mg/L, 30 min), primarily attributed to the presence of 1-dodecanol detected in the PANPs. This effect decreased with prolonged exposure, likely due to the volatilization of 1-dodecanol. Nevertheless, the PANPs were found to be non-biodegradable in both the ready and inherent biodegradability tests. Although PANPs do not appear to pose a long-term threat to the activated sludge, their persistence in the environment raises concerns about possible accumulation.

Key words: additives, cosmetics, microbial activity, nanoparticles, wastewater treatment, water-soluble polymer

HIGHLIGHTS

- Polyacrylic acid-based nanoparticles (PANPs) are often used in cosmetics.
- The short-term effect of PANPs on microorganisms in activated sludge was observed.
- The effect was related to the detected additive 1-dodecanol.
- No effects of PANPs were observed in prolonged experiments.
- Biodegradation of PANPs did not proceed.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Nanoparticles play a crucial role in the cosmetic and personal care product industry, enhancing product quality. They are utilized as thickeners and rheology modifiers, emulsifiers in lotions, dispersants for pigments, film-forming agents, and to improve water resistance (Chiari-Andréo *et al.* 2019; Gupta *et al.* 2022; Chakraborty *et al.* 2024; Jose *et al.* 2024). Nanoparticles applied in cosmetics can be categorized into inorganic nanoparticles, such as metal-based TiO_2 and ZnO, and organic nanoparticles, including nanoplastics and hydrogels. Nanoplastics are divided into two categories: nanospheres and nanocapsules. Nanocapsules contain a polymer wall with entrapped active ingredient (e.g. UV filters) in an oily or aqueous reservoir, while nanospheres are dispersed in the product together with the active ingredients (Raj *et al.* 2012; Chiari-Andréo *et al.* 2019; Gupta *et al.* 2022). Hydrogels portray another type of nanoplastic material, which expands in water without disintegrating (Chakraborty *et al.* 2024). Nanoplastics can be produced from various solid (Guterres *et al.* 2007; Koltzenburg *et al.* 2014) or water-soluble polymers (WSPs) such as polyacrylic acid or types of hydrogel, which are used as a rheology modifier to stabilize and thicken personal care products by swelling after pH modification (Koltzenburg *et al.* 2014; Rozman & Kalčíková 2021).

According to a report from the United Nations Environmental Programme (UNEP), as of 2018, only five countries outside the EU (Canada, USA, New Zealand, the UK including Northern Ireland, and South Korea) had implemented restrictions on plastic particles smaller than 5 mm (UNEP 2018). A later study confirmed that the situation remained unchanged in 2021 (Anagnosti *et al.* 2021). This changed in 2023, when the European Commission adopted measures to restrict plastic particles intentionally added to products such as cosmetics. But it is important to note that the current EU legislation regulates plastic particles with a lower size limit of 0.1 μm, meaning that nanoplastics smaller than 100 nm are not subject to these restrictions and their release into the environment is hence not regulated by the new restriction (European Commission 2023). These examples show that for most countries, no restrictions are currently put in place and if implemented, they do not necessarily encompass nanoplastics. This is a relevant detail as nanoplastics are used for instance in many 'rinse-off' products facilitating their release into the sewerage and transport to wastewater treatment plants (Roex *et al.* 2013). However, it is still not clear how many nanoplastics enter the wastewater stream, as there are no reliable estimates or monitoring data, which only exist for microplastics (Ali *et al.* 2021; Cavazzoli *et al.* 2023). For example, the minimum detectable size of plastic particles in wastewater is estimated to be as low as 1 μm (Sheriff *et al.* 2024). One major aspect for the inadequate coverage of nanoplastics monitoring in wastewater are allocated to methodological obstacles in their detection in organic-rich samples, such as wastewater (Cavazzoli *et al.* 2023), making them hardly distinguishable from other nanosized particles.

When nanoplastics reach the wastewater stream, the initial step of a wastewater treatment plant is mechanical treatment, where larger debris is removed. The subsequent grit chamber facilitates the sedimentation of, for example, sand and small stones (USEPA 1998; Riffat & Husnain 2022), which also removes large microplastics, for example during grease skimming (as particles are trapped in oil). During the following primary treatment, usually a sedimentation tank, suspended solids (including a significant share of microplastics) settle (Hidayaturrahman & Lee 2019; Iyare *et al.* 2020; Ren *et al.* 2020). However, the efficiency of these latter two treatment steps regarding nanoplastic retention remains unclear (Roex *et al.* 2013; Ali *et al.* 2021). In secondary treatment, where organic matter is utilized by the activated sludge, nanoplastics can be trapped in the sludge flocks, adsorbed or absorbed due to electrostatic attraction and settle in the next stage along with the activated sludge flocs (Rout *et al.* 2022).

Despite the high probability for exposure, data regarding nanoplastics effects on the activated sludge are scarce and mainly focused on the effects of polystyrene (PS) nanoplastics. For example, Alvim *et al.* (2023) investigated the long-term effects of 2 μg/L of PS nanoplastics (100 nm) in a sequencing batch reactor (SBR) and observed no effect on the activity of heterotrophic or nitrifying microorganisms. Nonetheless, a slight shift in the abundance of some bacterial phyla was detected. At concentrations several orders of magnitude above those used by Alvim *et al.* (2023), PS nanoplastics negatively affected nitrification and denitrification (PS, 100 nm, 1 mg/L; Song *et al.* 2022) as well as biological flocculation of the activated sludge (PS, 110 nm, from 500 mg/L; Qu *et al.* 2023). Polytetrafluoroethylene nanoplastics (PTFE, 800 nm, 10 mg/L) significantly reduced oxygen uptake rate, ammonia oxidation rate, nitrite oxidation rate, and nitrate reduction rate in an SBR by inhibiting nitrobacteria and denitrobacteria (Yang *et al.* 2023). Research on other types of nanoplastics could not be found. Furthermore, to our knowledge, no studies are currently available that address the effects of nanoplastics released from personal care products on the activated sludge process.

With the aim to provide a first step toward filling the knowledge gaps, this research investigates the effects of polyacrylic acid-based nanoplastics (PANPs) used in personal care products and cosmetics on heterotrophic and nitrifying microorganisms in the activated sludge. The selection of nanoplastics was motivated by the results of our previous study (Rozman & Kalčíková 2021), in which we investigated the ecotoxicological effects of different types of polyacrylic acid. One of the investigated products contained PANPs and exhibited acute effects on microorganisms in the activated sludge. In this study, we tested the effects of PANPs after prolonged exposure on microorganisms in the activated sludge and their biodegradability under common (i.e. ready biodegradability test) and enhanced (i.e. inherent biodegradability test) conditions, the latter being characterized by a substantially elevated microbial biomass. Ultimately, the results of this study will help to understand potential risks of nanoplastics, as utilized in personal care and cosmetic products, for the effective functioning of secondary wastewater treatment.

2. MATERIALS AND METHODS

2.1. Polyacrylic acid-based nanoplastics

The PANPs originated from a product used as acrylic rheology modifier in a number of personal care products. According to the producer, it was a lightly cross-linked acrylate copolymer. The chemical composition was determined by Fourier-transform infrared (FTIR) spectroscopy. Prior analysis, the product was dried at 70 °C for 24 h. The analysis was done using a PerkinElmer Spectrum Two FTIR spectrometer with an UATR module (Dimond), where background and ATR corrections of the spectra were used. The nanoplastics were visualized with the use of a field emission scanning electron microscope (FE-SEM, ULTRA Plus, Zeiss, Germany), while particle size and zeta potential of a 50 mg/L suspension were determined by Litesizer 500 (Anton-Paar GmbH, Austria).

For analysis of additives, approximately 1 g of the suspension was weighed into a 15 mL PE centrifuge tube with a polypropylene screw cap and around 7 g of methanol (SupraSolv® Methanol for gas chromatography MS, Merck, Darmstadt) was added. The exact mass of the suspension was recorded. The centrifuge tube was shaken and put on vortex for thorough mixing. Extraction was conducted at room temperature for around 100 h. For qualitative analysis, this was used in further procedure. For a quantitative analysis, a drop of methanol spiked with diethyl adipate (DEA) as an internal standard was added to the centrifuge tube. The exact mass of the spiked methanol was recorded. The centrifuge tube was put on vortex and left for an additional 24 h, after which it was centrifuged at 8,500 rpm for 15 min. Due to the poor separation, the emulsion was additionally filtered through a micro-PTFE filter into 0.2-mL GC-MS glass vials with a PTFE/Silicone lid using a PE syringe. A procedural blank with methanol only served as control. For 1-dodecanol and 1-tetradecanol detected in qualitative analysis, a relative response factor against an internal standard DEA was determined by recording a chromatogram of a methanol solution of known ratios. Gas chromatography–mass spectrometry analysis was performed on a 7890B gas chromatograph (Agilent, Santa Clara, CA, USA) coupled with a quadrupole mass detector (5977B). The GC-MS conditions were as follows: column, DB-5 MS Ultra Inert (Agilent, Santa Clara, CA, USA); injected volume, 1 μL; inlet temperature, 250 °C; carrier gas, He; and split ratio, 1:20. Temperature program: initial temperature, 40 °C; hold time, 2 min; ramp rate, 15 °C/min; final temperature, 280 °C. The components were identified based on the mass spectra in comparison with probability-based matching (Agilent, Santa Clara, CA, USA). Quantitative analysis was performed based on the peak area of each component compared with the peak area of the internal standard (i.e. DEA). A relative response factor of detected compounds (1-dodecanol and 1-tetradecanol) to the internal standard was measured and used in the calculations.

2.2. Acute toxicity test with the activated sludge

Acute toxicity tests were performed according to the ISO standard 8192 (ISO 8192 2007), where the effect of a compound on the microorganisms in the activated sludge is assessed by measuring the inhibition of oxygen consumption separately for heterotrophic and nitrifying microorganisms. Four concentrations (0, 1, 10, and 100 mg/L) of PANPs were prepared and introduced in the test vessels, together with peptone (easily degradable carbon source), nutrients, and activated sludge. The concentration of the activated sludge was determined as total suspended solids (TSS) and set at 1,500 $\mathrm{mg_{TSS}/L}$ (ISO 8192 2007). The final volume of the mixture was 50 mL. The blank test only contained the activated sludge, nutrients, and peptone. Additional treatments containing *N*-allylthiourea (ATU) were prepared to inhibit the nitrifying microorganisms and thus to determine the activity of only heterotrophic microorganisms, as oxygen is used solely for the degradation of organic matter and not for nitrification. The prepared mixtures were poured into 250 mL beakers, stirred, and aerated for 30 min. After incubation, the oxygen consumption was measured with the oxygen sensor Seven2Go™ DO portable meter (Mettler Toledo, Switzerland). The values were read off every 30 s for 10 time points (equals a measurement time of 4.5 min in total) and the experiment was repeated seven times. From the measured values, the linear equation was determined for each treatment and oxygen consumption rate (R, mg/L min) was calculated according to Equation (1):

$$R = \frac{\Delta\rho}{\Delta t} \tag{1}$$

where ρ is the concentration of oxygen (mg/L) and t is the time (min).

Afterwards, the inhibition of total oxygen consumption (I_{total}, %), the inhibition of heterotrophic oxygen uptake (I_{H}, %), and the inhibition of oxygen uptake due to nitrification (I_{N}, %) were calculated according to Equations (2)–(4), respectively:

$$I_{\mathrm{total}} = \left[1 - \frac{R_{\mathrm{T}}}{R_{\mathrm{TB}}}\right] \times 100\% \tag{2}$$

$$I_{\mathrm{H}} = \left[1 - \frac{R_{\mathrm{H}}}{R_{\mathrm{HB}}}\right] \times 100\% \tag{3}$$

$$I_{\mathrm{N}} = \left[1 - \left(\frac{R_{\mathrm{T}} - R_{\mathrm{H}}}{R_{\mathrm{TB}} - R_{\mathrm{HB}}}\right)\right] \times 100\% \tag{4}$$

where R_{T} is the oxygen consumption rate in the test sample mg/(L min) by all microorganisms, R_{TB} is the oxygen consumption rate in blank mg/(L min) by all microorganisms, R_{H} is the oxygen consumption rate in the test sample mg/(L min) by heterotrophic microorganisms, and R_{HB} is the oxygen consumption rate in blank mg/(L min) by heterotrophic microorganisms.

2.3. Prolonged toxicity with the activated sludge

The prolonged experiment was prepared according to the conditions of the ISO standard 8192 (ISO 8192 2007), but the activity of heterotrophic and nitrifying microorganisms was evaluated after 1, 3, and 6 h to assess the effect after a longer period of time, which corresponds to the typical hydraulic residence time (HRT) in wastewater treatment plants (Arundel 2000). The test was prepared in beakers of 1,000 mL containing a mixture of peptone, nutrients, activated sludge (1,500 $\mathrm{mg_{TSS}}$/L), and PANPs, and filled up to 400 mL with deionized water. The concentration of PANPs was selected based on the results of the acute toxicity test (100 mg/L; Section 3.2). A control was also included and both control and PANPs treatment were prepared in duplicates. The mixtures were stirred at 200 rpm, aerated continuously at 20 ± 1 °C. Immediately after preparation of the mixture ($t_0 = 0$ h) and after the incubation period ($t_1 = 1$ h, $t_3 = 3$ h, $t_6 = 6$ h), 50 mL were sampled, filtered through filter paper (pore size 12–25 μm, Macherey-Nagel, Germany) and stored frozen at -18 ± 2 °C until analysis of dissolved organic carbon (DOC) and the nitrogen species ($\mathrm{N-NH_4^+}$, $\mathrm{N-NO_2^-}$, and $\mathrm{N-NO_3^-}$) (the description of the analysis is given in Section 2.6). The changes in DOC and nitrogen species in the treatment and control were compared, and consequently, the effects on heterotrophic and nitrifying microorganisms were evaluated.

2.4. Ready biodegradability

The biodegradability of PANPs was determined according to the ISO standard 9408 (ISO 9408 1999) by the measurement of oxygen consumption in a closed respirometer OxiTop® (WTW, Germany). The concentration of the test substance was 33 mg/L, corresponding to a chemical oxygen demand (COD) of 50 mg/L, which was chosen to guarantee non-toxicity based on the results of acute toxicity test (Section 3.2). The concentration of the activated sludge was 30 $\mathrm{mg_{TSS}}$/L. Treatments containing (i) only activated sludge (blank), (ii) PANPs, (iii) reference compound (sodium acetate, $\mathrm{CH_3COONa}$), (iv) PANPs and reference compound, and (v) abiotic control (without activated sludge but with $\mathrm{HgCl_2}$ to sterilize the system) were prepared, as well as treatment containing also ATU to suppress nitrification and evaluate the degradation caused only by heterotrophic microorganisms. Each treatment was prepared in duplicates, poured into dark bottles, sealed, and the oxygen consumption was followed for 28 days at 20 ± 1 °C in the dark. The biodegradation (D_t, %) for each time point was calculated according to Equation (5):

$$D_t = \frac{\mathrm{OD_s} - \mathrm{OD_b}}{\mathrm{COD}} \times 100\% \tag{5}$$

where $\mathrm{OD_s}$ is the oxygen demand (mg/L) of the test substance (reference or PANPs or both combined), $\mathrm{OD_b}$ is the oxygen demand (mg/L) of the blank, and COD is the chemical oxygen demand (mg/L) of the test substance (reference or PANPs or both combined).

2.5. Inherent biodegradability

The inherent biodegradability of PANPs was determined by the Zahn-Wellens test according to the ISO standard 9888 (ISO 9888 1999). The test proceeded under more intensive conditions (i.e. increased concentration of the activated sludge (200 $\mathrm{mg_{TSS}}$/L) and constant aeration) to support microorganisms to degrade the tested compound. Three vessels with a total volume of 1,500 mL were prepared: the blank test (with only activated sludge), the reference test (containing sodium acetate, $\mathrm{CH_3COONa}$), and the test with PANPs. The concentration of PANPs was 66 mg/L (COD = 100 mg/L), as the system is more robust and at this concentration, the activity of heterotrophic microorganisms was not expected to be strongly affected (Section 3.2). Each mixture was incubated for 28 days, at 200 rpm, aerated continuously at 20 ± 1 °C. Every 2–3 days, 55 mL were taken out of every vessel, filtered (pore size 12–25 μm, Macherey-Nagel, Germany) and the filtrate was analyzed for DOC, COD, and nitrogen species ($\mathrm{N-NH_4^+}$, $\mathrm{N-NO_2^-}$, and $\mathrm{N-NO_3^-}$) (the description of the analysis is given in Section 2.6). If the samples could not be examined immediately, they were stored frozen (-18 ± 2 °C). The biodegradation (D_t, %) for each time point was calculated according to Equation (6):

$$D_t = \left[1 - \frac{\rho_{t,t} - \rho_{\mathrm{blank},t}}{\rho_{t,i} - \rho_{\mathrm{blank},i}}\right] \times 100 \tag{6}$$

where $\rho_{t,t}$ is the COD (mg/L) in the test with PANP or reference compound at time t, $\rho_{t,i}$ is the initial COD (mg/L) in the test with PANP or reference compound, $\rho_{\mathrm{blank},t}$ is the COD (mg/L) in the blank test at time t, and $\rho_{\mathrm{blank},i}$ is the initial COD (mg/L) in the blank test.

2.6. Analytical methods

The COD was determined according to the ISO standard 6060 (ISO 6060 1989) with undiluted samples. The DOC was determined according to the ISO standard 20236 (ISO 20236 2018) and prior to the analysis the samples were diluted 1:1 with deionized water and measured by a TOC analyzer (multi N/C 3100 Analytik Jena, Germany). Nitrogen content was determined separately for ammonium ($N-NH_4^+$; 1:2 dilution, Phenate method 4500-NH_3), nitrite ($N-NO_2^-$; 1:2 dilution, Colorimetric method 4500$-NO_2^-$), and nitrate ($N-NO_3^-$; 1:10 dilution, Ultraviolet spectrophotometric method 4500$-NO_3^-$) according to Baird *et al.* (2017).

2.7. Statistics

For statistical analysis and visualization of the results, version 4.1.1 from the R Studio Software was used (R Core Team 2022). Normal distribution was checked by applying the Shapiro–Wilk test. Due to the lack of normal distribution of groups and small sample sizes, the Mann–Whitney *U* test was utilized to identify statistically significant differences (α-level <0.05) between groups.

3. RESULTS

3.1. Characterization of PANPs

The PANPs had a spherical shape (Figure 1(a)) and the FTIR spectrum (Figure 1(b)) contained characteristic peaks for polyacrylic acid (Sahoo *et al.* 2011; Rozman & Kalčíková 2021). Peaks at 2,981 and 2,929 cm^{-1} correspond to the –OH stretching vibrations and intermolecular hydrogen bonding, peaks at 1,728 and 1,700 cm^{-1} were assigned to C = O stretching vibrations,

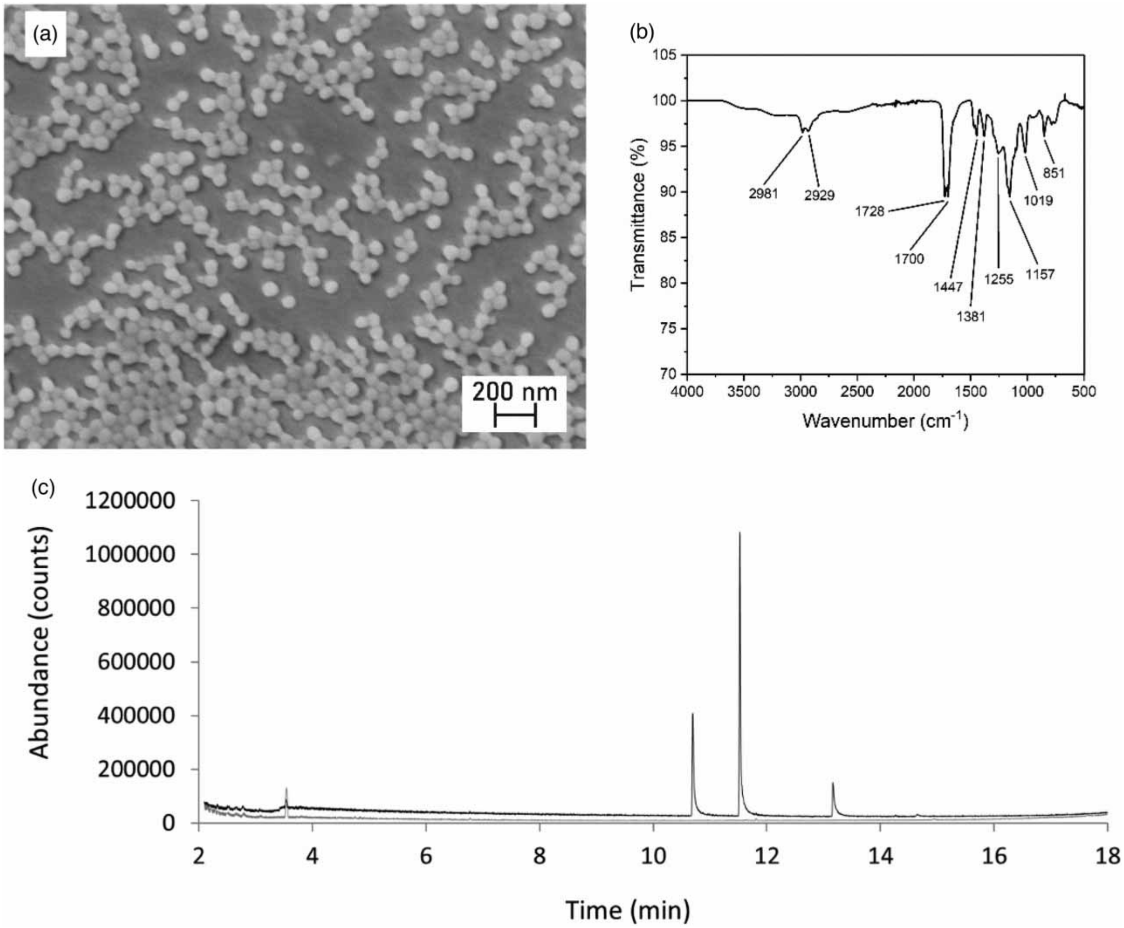


Figure 1 | (a) FE-SEM image (100,000× magnification), (b) FTIR spectrum, and (c) GS-MS chromatogram of the extract of the suspension of PANPs and of the procedural blank.

and peaks at 1,447 and 1,381 cm^{-1} correspond to the stretching of the C–O bond. Peaks between 1,200 and 1,000 cm^{-1} were assigned to the coupling between in-plane O–H bending and C–O stretching in carboxyl groups nearby. In addition, the out-of-plane bending of the $C=CH$ group corresponded to the peak at 851 cm^{-1} (Sahoo *et al.* 2011; Al-Awady *et al.* 2017). The size of PANPs was 104.5 nm, while zeta potential was −43.5 mV. The analysis of additives revealed the presence of 1-dodecanol and 1-tetradecanol (Table 1 and Figure 1(c)).

3.2. Toxicity testing

In the acute toxicity test, concentrations of 1, 10, and 100 mg/L PANPs caused 4.5 ± 10.2%, 27.9 ± 29.3%, and 55.0 ± 21.1% inhibition of heterotrophic microorganisms, respectively, while the effect on nitrifying microorganisms was higher, with 29.1 ± 27.6%, 40.3 ± 43.0%, and 71.5 ± 32.4%, respectively. Note that the variability is caused by high diversity of microorganisms in the activated sludge (the test was repeated seven times), but it can expected as stated in the ISO standard (ISO 8192 2007). Therefore, also the only statistically significant difference was measured for 100 mg/L on nitrifying ($p = 0.0012$) and heterotrophic ($p = 0.0006$) microorganisms. Consequently, the concentration of 100 mg/L was chosen for the prolonged toxicity test.

In the prolonged toxicity test (Figure 2), the concentration of $N-NH_4^+$ increased over time, which is a result of the degradation of peptone (biodegradable compound used in the toxicity tests) and conversion of organic nitrogen to $N-NH_4^+$. The concentrations of $N-NO_2^-$ and $N-NO_3^-$ also increased, which confirmed successful nitrification. However, after 1, 3, and

Table 1 | Detected compounds in the suspension of PANPs

Retention time (min)	Compound	Formula	Mass fraction (%)	Concentration (mg/g)
11.53	1-dodecanol	$CH_3(CH_2)_{11}OH$	0.13	1.3
13.17	1-tetradecanol	$CH_3(CH_2)_{13}OH$	0.04	0.4

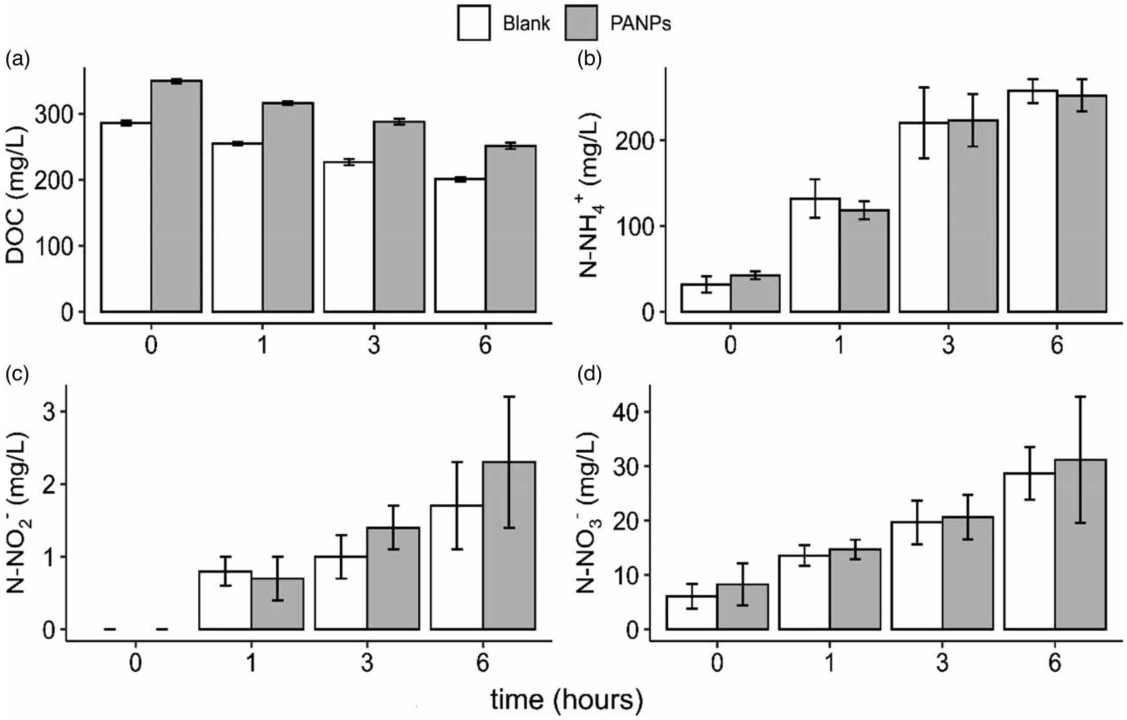


Figure 2 | Prolonged toxicity test and the comparison of DOC (a), $N-NH_4^+$ (b), $N-NO_2^-$ (c), $N-NO_3^-$ (d) at the beginning of the experiment, and after 1, 3, and 6 h of incubation in the blank and test with PANPs.

6 h, the concentrations of $\mathrm{N-NH_4^+}$, $\mathrm{N-NO_2^-}$, and $\mathrm{N-NO_3^-}$ were comparable in the blank test and in the test with 100 mg/L PANPs. Similarly, the DOC removal efficiencies after 1, 3, and 6 h were 11, 21, and 30% for the blank, and 10, 18, and 28% for PANPs, showing comparable values at each sampling time. Thus, PANPs did not affect the removal of organic matter in the activated sludge process over time. Note that the results showed differences in DOC concentrations continuing from the beginning of the experiment. This difference was due to the addition of PANPs, which contributed to the overall DOC.

3.3. Biodegradability testing

In the ready biodegradability test, the reference compound reached 60% degradation after the first 6 days and by the end of the test, i.e. 28 days, it was degraded over 75% confirming the high activity of the heterotrophic microorganisms (Figure 3(a)). The results showed neither degradation of the PANPs nor nitrification, as the treatments with PANPs and PANPs + ATU (Figure 3(a)) were alike and basically close to zero. In the treatment, where PANPs and reference compound were tested together, the curve had a similar pattern to the one for the reference compound only. This confirmed the non-toxic effect of the selected PANPs concentration regarding the activity of heterotrophic microorganisms, which readily degrades organic compounds in the presence of PANPs. The abiotic degradation was monitored and did not proceed (degradation was less than 1%, data not shown).

In the inherent biodegradability test, the reference compound degraded well, and it reached 100% within 5 days (Figure 3(b)). On the other hand, the concentration of PANPs decreased by about 30% after 28 days (Figure 3(b)). The mixtures containing PANPs showed very high foaming, which partially trapped activated sludge in the foam. To examine whether

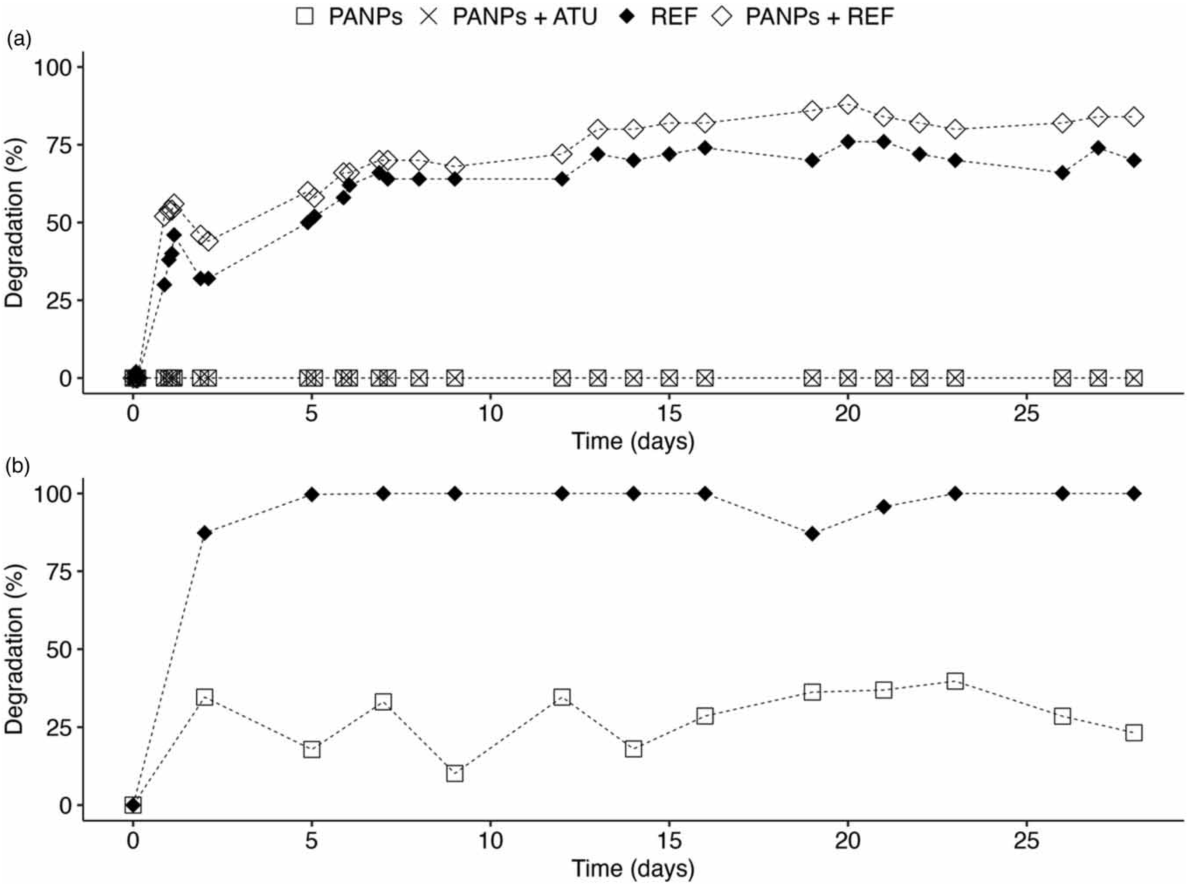


Figure 3 | (a) Biodegradation of PANPs without and with the presence of *N*-allylthiourea (PANPs + ATU), reference compound (sodium acetate, REF), and reference compound in the presence of polyacrylic acid-based nanoplastics (PANPs + REF) in the ready biodegradability test. (b) Biodegradation of PANPs and REF in the inherent biodegradability test.

the nitrification process of microorganisms in the activated sludge is inhibited, different ion species relevant in the nitrogen cycle were measured. The N-NH_4^+ concentration diminished significantly within the first 2 days for all vessels (Figure 4). For the remaining 26 days, the levels stayed relatively constant. Similarly, the N-NO_2^- concentration decreased mostly for the first 48 h. The overall curve progression is similar for all vessels despite minor variations. N-NO_3^- increased over time with initial levels close to zero for all three vessels confirming a viable nitrification process.

4. DISCUSSION

Plastics have been used in personal care products and cosmetics for decades (UNEP 2015). With the development of nanotechnology, nanoplastics have also found their application in this industry and have become an essential component of many products (Somasundaran *et al.* 2006). However, personal care products and cosmetics are often washed out after use and end up in the wastewater stream.

The results of the present study showed that PANPs had an effect on heterotrophic and nitrifying microorganisms in the activated sludge in the acute toxicity test. The acute effect of PANPs was most likely related to the presence of additives, as polyacrylic acid and its derivates are of low toxicity (Lindenschmidt *et al.* 1991; Haselbach *et al.* 2000), unless additives or unreacted monomers are present (Sverdrup *et al.* 2001; Rozman & Kalčíková 2021). This was also indicated by this study, as two additives, i.e. 1-dodecanol and 1-tetradecanol, were detected in the PANPs suspension (Table 1). Indeed, 1-dodecanol has antimicrobial activity (Kubo *et al.* 1995) and expresses high short-term toxicity toward aquatic organisms and in particular to microorganisms (Microtox test, 5 min $EC_{50} = 0.038$ mg/L, 15 min $EC_{50} = 0.043$ mg/L, 30 min $EC_{50} = 0.057$ mg/L, and 48 h $EC_{50} = 2$ mg/L, see Safety Data Sheets by Thermo Fisher Scientific (Thermo Fisher Scientific 2021a, b)). Opposed to this, during the longer exposure period, which can be related to the duration of biological wastewater treatment in the aeration tank, the effects disappeared (Figure 2). The reason for this can be related to the properties of 1-dodecanol, as volatilization from open water can be expected (OECD SIDS (Screening Information Data Set) 2002). Therefore, during the prolonged toxicity tests, 1-dodecanol was most likely stripped out during aeration. Limited long-term effects were also confirmed in the

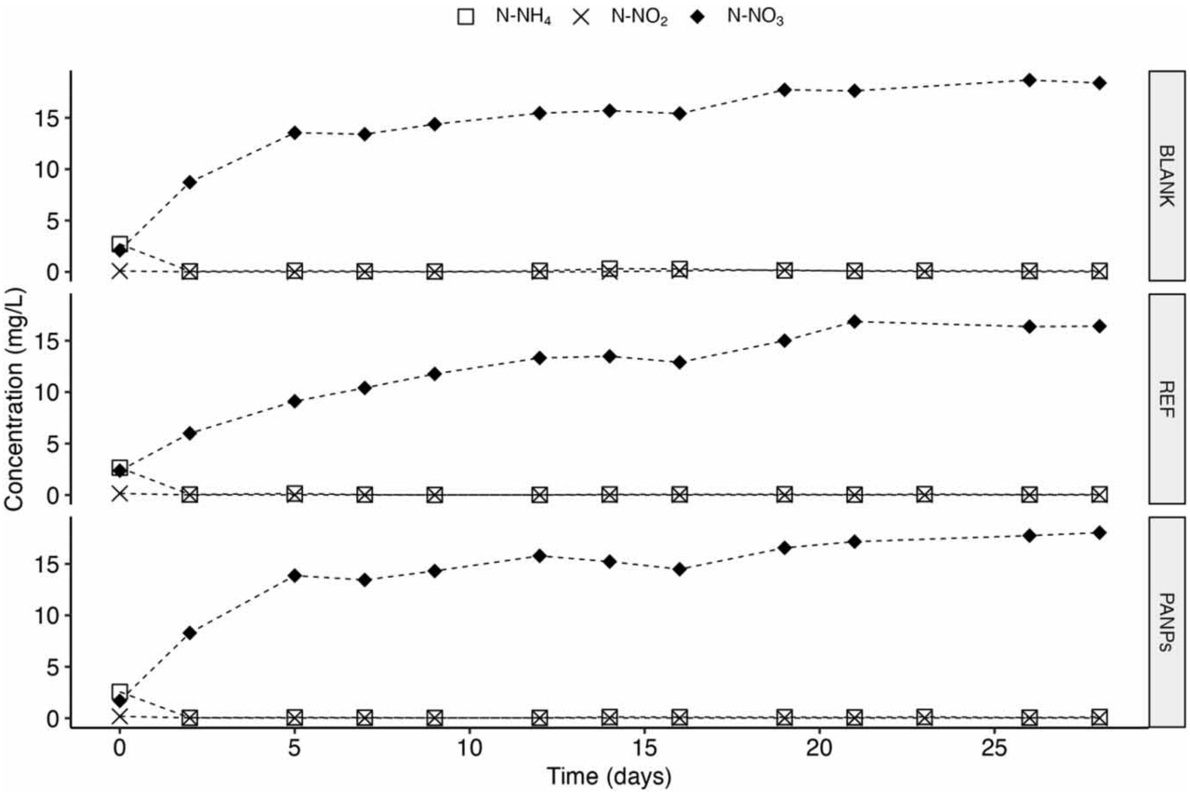


Figure 4 | Comparison of the development of nitrogen species ions during the test run of 28 days for blank, reference (REF), and polyacrylic acid-based nanoplastics (PANPs). The values for NO_3^- on day 23 were neglected due to an unexpected drop in magnitude for all three vessels.

ready biodegradability test: no effects on the activity of heterotrophic microorganisms in the activated sludge were detected, when PANPs were incubated together with the reference compound (i.e. readily biodegradable by the activated sludge). In fact, the degradation curves of the reference compound alone and the reference compound in the presence of PANPs were alike during 28 days test period (Figure 3(a)). Similarly, no effects on nitrifying microorganisms in the activated sludge were observed in the inherent biodegradability test; the nitrification process proceeded comparably in the blank, in the presence of the reference compound, and in the presence of PANPs in 28 days (Figure 4).

However, nanoplastics themselves (without additives) can also affect the microorganisms in the activated sludge. For example, 100 nm polystyrene (PS) NPs (PS-NPs), which is comparable to the size of PANPs used here, induced oxidative stress in activated sludge bacteria, measured by the formation of reactive oxygen species (ROS) and lactate dehydrogenase (LDH) (Tang *et al.* 2022). The leakage of LDH indicates the breakage of the cytoplasmic membrane. In addition, the conversion of nitrogen ions can be attenuated under increasing concentrations of PS-NPs (Tang *et al.* 2022). A reduction in denitrification rates was also detected for even lower PS-NPs concentrations from 0.01 to 1 mg/L (Liu *et al.* 2021). These findings elucidate that the toxicity of nanoplastics greatly vary between particle types and the effect of nanoplastics depends on their composition, functional groups, and thus also on the particle charge (Qian *et al.* 2021). In this study, the PANPs had negative zeta potential (−43.5 mV), indicating good stability (Kumar & Dixit 2017), which means that the tendency to aggregate is rather low. On the other hand, the negative zeta potential is more likely the reason, why PANPs did not significantly affect the bacterial cells over a longer period, as the crossing of the membrane is more probable for positively charged nanoplastics with regards to the anionic phospholipid cell membrane (Dai *et al.* 2022). It can be concluded that the present study points to limited effects in the activated sludge induced by nanoplastics made of polyacrylic acid. Nonetheless, their additives may indeed be of concern.

The results of the ready biodegradability test showed that PANPs are not biodegradable under the tested conditions. These outcomes are in line with the results observed by Jakobi (1984), who showed that the biodegradability of various polyacrylic acids is up to 16% over 30 days. Rozman & Kalčíková (2021) confirmed the low biodegradability of various polyacrylic acids used in cosmetics (<5% in 28 days). In this study, the results of the inherent biodegradability test showed up to 30% removal in 28 days of PANPs, whereas previously the biodegradability of polyacrylic acid was up to 10% in a comparable test setup (HERA 2014). However, during the test with PANPs, intensive foaming was observed, possibly entrapping the nanoplastics in the foam. Furthermore, it is plausible that some parts of PANPs could be removed due to adsorption to the activated sludge. Freeman & Bender (1993) demonstrated that polyacrylate detergent additive was removed through adsorption to the activated sludge (2,500 mg_{TSS}/L), rather than biodegradation, as the increased concentration of the activated sludge correlated with increased removal. Therefore, it is plausible that with increasing sludge concentration, more adsorption proceeded, explaining no removal in the ready biodegradability test (with 30 mg_{TSS}/L of the activated sludge) and 30% removal in the inherent biodegradability test (with 200 mg_{TSS}/L of the activated sludge and intensive foaming due to aeration).

In general, acrylic acid monomers, dimers, and oligomers can be easily degraded by the activated sludge (Gaytán *et al.* 2021). Degradation depends on the molecular weight of the polymer; e.g. oligomers with a molecular weight of 500–700 Mw showed a DOC removal of 75–85%, while compounds with a molecular weight above 1,000 Mw are less degradable (Larson *et al.* 1997). After polymerization, the C–C backbone of polyacrylic acid is recalcitrant (Gaytán *et al.* 2021). In addition, copolymerization, as is the case with the PANPs used in this study, increases the intermolecular forces, which even improves resistance to cleavage and thus thermal, oxidative, and chemical stability (Stivala & Reich 1980). These are all reasons why the degradation of polyacrylic acid-based compounds in the environment is likely to be limited.

5. CONCLUSION

Polyacrylic acid-based polymers are widely used across various products and applications and can also occur as nanoplastics. However, their impact on microorganisms in the activated sludge and, consequently, on biological wastewater treatment processes remains poorly understood. The findings of this study indicated that 1 and 10 mg/L of PANPs exhibit low toxicity, while a high concentration of 100 mg/L caused significant effects on the oxygen consumption of both heterotrophic and nitrifying microorganisms in the activated sludge (55 and 72%, respectively). Albeit, the observed toxicity was attributed to additives, specifically 1-dodecanol, which is known to be toxic to aquatic organisms. However, 1-dodecanol is volatile and dissipates from the water phase during the aeration of the activated sludge, suggesting that the long-term effects on wastewater treatment processes are unlikely. Biodegradability testing revealed that PANPs are not degradable under environmentally relevant conditions, as shown in ready biodegradability tests (~0%), nor under enhanced conditions in inherent biodegradability tests (up to 30%) in 28 days. Consequently, the possibility that PANPs can pass through wastewater treatment plants is viable, while they could persist in the environment for extended periods. This underscores the need to consider their long-term effects and environmental fate in future studies.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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