Inhibitory Effect of Silver and Nanosilver on Activated Sludge Fed with Proteinaceous Feed

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Abstract - This study investigates the response of activated sludge that is fed with proteinaceous feed to silver (Ag⁺) and continuous nanosilver (AgNPs) input. Substrate removal (COD) and respiration of activated sludge were examined in two reactors fed with only peptone as an organic substrate and inorganics. Results showed that substrate removal was not affected by the high concentrations of Ag⁺ and AgNP neither in short- nor in long-term. It was concluded that peptone in the feed formed complexes with silver and reduced its negative effects. Also, in long-term, AgNP lost its effectiveness upon continuous aeration. Moreover, in long-term, biomass protected itself from the inhibitory effect of AgNP by regulating the production of Extracellular Polymeric Substances (EPS). Therefore, no negative impact of silver and nanosilver on substrate removal and change in sludge characteristics would be expected.

Keywords: Activated sludge, Extracellular Polymeric Substances (EPS), inhibition, nanosilver (AgNP), peptone, silver

1. Introduction
Silver is a heavy metal often found in domestic and industrial wastewaters. The major sources of silver are from the photographic and imaging industry, the manufacturing of electronics, silverware and jewellery [1]. The silver ion (Ag⁺) is known to have toxic effects in biological wastewater treatment systems. Today, silver nanoparticles (AgNPs) in municipal wastewater treatment are also of interest. Since AgNPs have antimicrobial properties, they are employed in many consumer goods ranging from personal care products to detergents and cloths. AgNP-containing textiles are reported to emit these nanoparticles into municipal wastewater treatment plants (WWTPs) [2,3]. AgNPs will also be employed in new areas, and an increase in Ag concentration is thus expected in WWTPs [4-6]. Therefore, studies are still needed to assess the fate and impacts of Ag⁺ and AgNPs in WWTPs. Most studies focus on short-term AgNP addition and measure the immediate response of activated sludge. However, a more realistic approach would be to study the impact of AgNP upon continuous and long-term feeding to activated sludge.

The aim of this study is to investigate the inhibitory effect of silver and nanosilver on activated sludge fed with a proteinaceous feed. For this purpose, short-term and long-term experiments with Ag⁺ and AgNP were carried out.

2. Methods
The study consists of two parts:
- Part I: Inhibitory effect of Ag⁺ on an activated sludge fed with a proteinaceous feed in short-term experiments,
- Part II: Inhibitory effect of AgNP on activated sludge fed with a proteinaceous feed in long-term experiments.

In Part I, the respiration of activated sludge was measured with and without Ag⁺ addition. In Part II, AgNP was continuously added to activated sludge in order to observe the long-term effect. Organic carbon removal was followed by Chemical Oxygen Demand (COD) measurements. Also respiration of activated sludges was monitored.

2.1. Operation of Two Reactors
Activated sludge reactors, RP (peptone reactor) and RP_{AgNP} (peptone reactor with AgNP), were operated in parallel for a long time. The RP reactor of 4 L volume served as a control. It was fed with peptone and inorganic substances only. The RP_{AgNP} reactor having a volume of 2 L was started up using the RP sludge, after RP reached steady-state conditions. Then, it was also continuously dosed with AgNP. The details of operation and the composition of synthetic wastewater feed can
be found in previous studies [7,8]. The inhibitory effect of Ag\(^+\) on activated sludge in RP was studied in the range 0–5 mg/L Ag\(^+\). In respiration tests with Ag\(^+\), Fluka Analytical 12818 Silver Standard for ICP solution (1000 mg/L Ag\(^+\)) was used. In the RP\(_{AgNP}\) reactor the long-term effect of AgNP was studied in four phases. The details are given in Table 1.

<table>
<thead>
<tr>
<th>Phase number</th>
<th>Time (days)</th>
<th>Amount of AgNP added in each run</th>
<th>Total AgNP concentration at the end of the phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0-78</td>
<td>RP and RP(_{AgNP}) reactors were first run in parallel without AgNP addition</td>
<td>0 mg/L</td>
</tr>
<tr>
<td>2</td>
<td>79-155</td>
<td>0.3 mg</td>
<td>0.8 mg/L</td>
</tr>
<tr>
<td>3</td>
<td>156-238</td>
<td>2 mg</td>
<td>12.4 mg/L</td>
</tr>
<tr>
<td>4</td>
<td>239-262</td>
<td>10 mg</td>
<td>34.2 mg/L</td>
</tr>
</tbody>
</table>

The stock nanosilver suspension was prepared in the laboratory using the chemical reduction method with minor modifications [9] and the AgNP suspension was characterized by the HACH UV-visible spectrophotometer. Always freshly prepared nanosilver was dosed to the RP\(_{AgNP}\) reactor. Details of the measurement and analyses can be found in a previous study [8].

2.2. Respiration of Activated Sludges without and with Ag\(^+\) and AgNP Addition

Regularly, mixed liquor samples were taken from the two activated sludge reactors (RP and RP\(_{AgNP}\)) to measure their respiration rates. Respiration of sludges was also measured in the presence of Ag\(^+\) and AgNP in order to observe the inhibitory effect. For respiration measurements, the automated Oxymax-ER 10 Gas Respirometer by Columbus Instruments (USA) was used. The principle is based on the continuous measurement of O\(_2\) and CO\(_2\) pressures in gas phase and calculation of O\(_2\) consumption and CO\(_2\) production with respect to time. Details of the respirometry experiments are given in previous studies [10,11].

3. Results and Discussion
3.1. Inhibitory Effect of Silver on Activated Sludge in RP

The inhibitory effect of Ag\(^+\) on RP in short-term was examined by monitoring the respiration of activated sludge for nearly 24 hours. The concentration range was 0–5 mg/L Ag\(^+\). The results of respirometric tests showed that RP sludge was not affected by Ag\(^+\) at any concentration [10]. Even at 5 mg/L Ag\(^+\), the cumulative O\(_2\) uptake was almost the same in the sludge with and without Ag\(^+\) addition. Figure 1 shows the results of an experiment carried out at 5 mg/L Ag\(^+\). The results led to the conclusion that RP sludge was not affected by Ag\(^+\) addition because of feed composition. Additional tests were carried out with the same feed and a different activated sludge. Activated sludge taken from a reactor fed with only glucose as an organic substrate was fed this time with a proteinaceous feed in the presence of 3 and 5 mg/L Ag\(^+\). Results showed that the performance of the sludge was not affected by Ag\(^+\) addition. Most probably, proteins in peptone form complexes with Ag\(^+\) and this reduces the inhibitory effect of Ag\(^+\). As a result, the performance of sludge was not affected.
3.2. Inhibitory Effect of Nanosilver on Activated Sludge in RP_{AgNP}

The results of the first part showed that feeding with proteins changed the characteristics of activated sludge. The sludge became more resistant to Ag^{+}. Complementary to the first study, AgNP was continuously added in a long-term. As mentioned before, the performance of RP and RP_{AgNP} reactors was examined by monitoring the organic carbon removal and respiration of activated sludges. In the RP sludge as well as the RP_{AgNP} sludge a high degree of COD removal was achieved. Even at high concentrations of AgNP, at least 90% of COD was successfully removed. Numerous comparative respiration measurements indicated that oxygen uptake rates and specific cumulative O_2 consumption were very close to each other in both sludges during the whole operation period [11]. An example to respiration of both sludges (226th day of operation) is shown in Figure 2. Results showed that continuous AgNP addition did not negatively affect removal of organic substrate and the performance of the activated sludge.

It was concluded that AgNP did not remain in its original form in long-term; at least some of it agglomerated into silver particles. So, AgNP suspensions lost their stability and effectiveness as an inhibitor. Due to complexation of peptone with silver, Ag^{+} and labile Ag species may have very low concentrations in the reactor. As a result, no inhibition was observed. Additional studies on EPS production revealed an increase in total EPS in long-term RP_{AgNP} operation at higher AgNP
concentrations. As known, metal input usually triggers the production of EPS. Increase in EPS production might enable bacteria to protect themselves from inhibitory effects of silver [8].

4. Conclusion

The study showed the response of activated sludge to silver and long-term nanosilver input when it was fed with peptone as the single organic substrate. The changes taking place in the two activated sludge reactors were examined in a period of nearly 9 months by exposing the biomass to increasing AgNP levels.

The results of the first part showed that RP sludge was resistant to relatively high Ag⁺ concentrations (up to 5 mg/L). A sludge fed with peptone as the single organic substrate may tolerate higher silver concentrations. This indicates that the effect of silver in real biological treatment systems may change with the changes taking place in composition of influent wastewater. In the second part, even though AgNP concentrations were intentionally increased to much higher levels, substrate removal was not affected. Agglomeration of AgNP in long term and complexation of silver forms with the feed components reduced the inhibitory effect of this nanometal. Moreover, the increase in EPS production at higher AgNP concentrations enabled the sludge to cope with the negative effects of AgNP.

Since AgNP concentrations in the influent of a wastewater treatment plant are at micrograms per liter levels, no serious effects are expected on substrate removal. Activated sludge may easily resist the inhibitory effect of this nanometal at realistic concentrations. However, the accumulation of AgNP in sludge phase may be a factor worthy of consideration in sludge digestion and disposal.

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References