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Inhibition of the nitrification process in activated sludge by trivalent and hexavalent chromium, and partitioning of hexavalent chromium between sludge compartments

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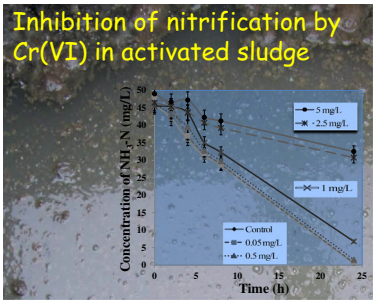
HIGHLIGHTS

- Inhibition of nitrification by Cr(III) and Cr(VI) was studied in activated sludge.
- Quantification of Cr(VI) was performed by speciated ID-ICP-MS.
- Cr(VI) concentration higher than 2.5 mg L^{-1} severely inhibited nitrification.
- Cr was distributed between adsorbed, intracellular and intercellular sludge compartments.
- Reduction of Cr(VI) occurred almost solely within the intercellular sludge compartment.

GRAPHICAL ABSTRACT



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ABSTRACT

The input of wastewater treatment plants (WWTPs) may contain high concentrations of Cr(III) and Cr(VI), which can affect nitrogen removal. In the present study the influence of different Cr(III) and Cr(VI) concentrations towards activated sludge nitrification was studied. To better understand the mechanisms of Cr(VI) toxicity, its reduction, adsorption and uptake in activated sludge was investigated in a batch growth system. Quantification of Cr(VI) was performed by speciated isotope dilution inductively coupled plasma mass spectrometry. It was found that Cr(VI) concentrations above 1.0 mg L^{-1} and Cr(III) concentrations higher than 50 mg L^{-1} negatively affected nitrification. Speciation studies indicated almost complete reduction of Cr(VI) after 24 h of incubation when Cr(VI) concentrations were lower than 2.5 mg L^{-1}, whereas for Cr(VI) added to 5 mg L^{-1} around 40% remained unreduced. The study of the partitioning of Cr in the activated sludge was performed by the addition of Cr(VI) in concentrations of 2.5 and 5.0 mg L^{-1}. Results revealed that Cr was allocated mainly within the intercellular compartments, whereas intracellular and adsorbed Cr represented less than 0.1% of the Cr sludge concentrations. Cr(VI) was reduced in all compartments, the most efficiently (about 94%) within the intracellular and intercellular fractions. The extent of reduction of adsorbed Cr was 92% and 80% for 2.5 and 5.0 mg of Cr(VI) L^{-1}, respectively. The results of present investigation provide a new insight into the toxicity of Cr species towards activated sludge nitrification, which is of significant importance for the management of WWTPs in order to prevent them from inflows containing harmful Cr(VI) concentrations.

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1. Introduction

Municipal wastewater treatment plants (WWTPs) in general receive discharges not only from residential areas but also from industry (Ščančar et al., 2001), often resulting in high metal concentrations in the sewage sludge (Ščančar et al., 2000). Dyeing and tannery wastewaters can contain high Cr(III) concentrations, whereas effluents from metal finishing and the plating industry are polluted with Cr(VI).

The essentiality and toxicity of chromium (Cr) depend primarily on its chemical forms. Cr(III) is considered to be essential for humans, whereas Cr(VI) has carcinogenic and mutagenic properties (Zhitkovic, 2005; Langård and Costa, 2007). Cr(VI) is far more toxic and mutagenic than Cr(III) (Morales-Barrera et al., 2008; Yao et al., 2008). Although Cr can act as a micronutrient at low concentrations (Yetiş et al., 1999), shock loading can inhibit or even stop microbial activity (Gikas and Romanos, 2006) which may cause malfunction of the WWTP. Cr(VI) is usually removed from wastewater by reduction to Cr(III) which precipitates at neutral pH. Cr(VI) can be reduced in both aerobic and anaerobic conditions. In aerobic conditions soluble enzymes use nicotinamide adenine dinucleotide (NADH) as an electron donor for reduction, whereas under anaerobic conditions, a membrane-bound reductase is responsible for Cr(VI) reduction (Stasinakis et al., 2004). Besides enzymatic reduction, Cr(VI) can also enter cells and be reduced by cellular components in the cytoplasm, forming reactive species and free radicals that damage DNA and other biomolecules (Ramírez-Díaz et al., 2008). Cr(VI) can enter cells via the sulphate transport mechanisms due to the similarity of the tetrahedral chromate anion CrO_4^{2-} and sulfate SO_4^{2-} (Nies, 1999), whereas Cr(III) is unable to penetrate membranes due to its insoluble forms at neutral pH (Cary, 1982).

Different parameters can be used to assess Cr toxicity to activated sludge, but in recent years the most popular are respiration activities (Vaňková et al., 1999; Stasinakis et al., 2002; Cecen et al., 2010) growth and growth rate (Gikas and Romanos, 2006), substrate removal (Yetiş et al., 1999; Stasinakis et al., 2003a) and microbial diversity (Stasinakis et al., 2003a; Samaras et al., 2009). The literature data on Cr(III) and Cr(VI) toxicity varies among studies. In general, Cr(III) concentrations above 100 mg L^{-1} severely affect activated sludge and wastewater treatment plant performance, whereas concentrations from 160 mg L^{-1} to 320 mg L^{-1} are considered to be lethal (Gikas and Romanos, 2006). The Cr(III) concentration at which 50% inhibition of nitrification occurs varies greatly and was reported to be 37 mg L^{-1} by Cecen et al. (2010) and 85 mg L^{-1} by Leta et al. (2004), whereas Farabegoli et al. (2004) reported that inhibition of nitrification started at concentrations of Cr(III) higher than 120 mg L^{-1}. The data reported for Cr(VI) toxicity towards nitrification varies even more. Significant inhibition of nitrification was observed at 0.5 mg L^{-1} (Stasinakis et al., 2003a), while Cheng et al. (2011) found that 5 mg Cr(VI) L^{-1} decreases $NH_4^+ - N$ removal from 97% to 58%. Cecen et al. (2010) found that 50% inhibition of nitrification occurred at around 39 mg Cr(VI) L^{-1}. In contrast, Samaras et al. (2009) reported no effect on nitrification in the range of 1–50 mg Cr(VI) L^{-1}. When considering other parameters besides nitrification, even more contradictory results can be found in the literature. There are reports of a decreasing growth rate in the entire range from 0.1 to 11 mg Cr(VI) L^{-1} (Mazierski, 1994), whereas other researchers even found stimulation of growth rate up to 25 mg Cr(VI) L^{-1} (Yetiş et al., 1999; Gikas and Romanos, 2006). Based on the investigation of Stasinakis et al. (2003a), who observed significant inhibition of nitrification at 0.5 mg Cr(VI) L^{-1}, whereas only minor inhibition of substrate removal was observed up to 5 mg Cr(VI) L^{-1}, it can be speculated that autotrophic microorganisms such as nitrifiers are more susceptible to Cr(VI) toxicity than heterotrophic microorganisms. Despite the contradictory data, the lethal dose of Cr(VI) is estimated to be in the concentration range between 80 mg L^{-1} and 160 mg L^{-1} (Gikas and Romanos, 2006).

Although Cr toxicity to activated sludge has been intensively investigated, speciation studies are very scarce. These were performed on the basis of sequential extractions (Álvarez et al., 2002; Solis et al., 2002), which can assess the bioavailability and leachability of Cr, but do not provide information on the Cr species actually present. Imai and Gloyna (1990) reported that Cr(III) cannot be oxidized to Cr(VI) due to kinetic constrains, but Cr(VI) can be adsorbed on the bacterial surface and reduced to Cr(III). Cr(VI) reduction was also reported by other researchers using batch and sequencing batch systems (Stasinakis et al., 2003b; Loo et al., 2012). In contrast, Cecen et al. (2010) who predicted Cr(VI) and Cr(III) speciation by theoretical calculations, suggested that the majority of Cr(VI) was neither reduced nor adsorbed on the negatively charged surface of the activated sludge. Vaiopoulou and Gikas (2012) suggested that an important factor contributing to the contradictory data on Cr toxicity could be Cr(VI) reduction and the decreased mobility of Cr(III) formed due to complexation with humic acids, nucleic acids, sulfhydryl and carboxyl groups and phosphates.

The reported data on Cr(III) and Cr(VI) toxicity are contradictory. There is also lack of information available on Cr speciation and its fate in activated sludge. In order to better understand the mechanisms of Cr(VI) toxicity, the aim of our work was to follow the reduction, adsorption and up-take of Cr(VI) by activated sludge during incubation and to evaluate which concentrations of dissolved Cr(III) and Cr(VI) are toxic towards nitrification. To ensure reliable speciation data, validated analytical procedures and the use of stable isotopes for quantification of Cr(VI) were applied in high performance liquid chromatography–inductively coupled plasma mass spectrometric (HPLC–ICP–MS) determinations.

2. Materials and methods

2.1. Instrumentation

HPLC separations were performed using an Agilent (Tokyo, Japan) series 1200 quaternary pump. For separation of Cr species, a strong anion-exchange FPLC column of Mono Q HR 5/5 (Pharmacia, Uppsala, Sweden) was used. Detection of Cr was performed using an inductively coupled plasma mass spectrometer, model 7700x, from Agilent Technologies (Tokyo, Japan) (Novotnik et al., 2012a).

For determination of nitrate nitrogen (NO_3–N) and ammonia nitrogen (NH_3–N) a DR/2010 (HACH, Düsseldorf, Germany) spectrometer was used. Mechanical shaking was performed with a Vibramax 40 elliptical table shaker (Tehtnica, Železniki, Slovenia). A Hettich Universal 320 centrifuge (Tuttlingen, Germany) was used and a CEM Corporation (Matthews, NC, USA) CEM MARS 5 Microwave Acceleration Reaction System was applied for digestion of activated sludge pellets.

2.2. Reagents and materials

Ultrapure 18.2 MΩ cm water was obtained from a Direct-Q 5 Ultrapure water system (Millipore Watertown, MA, USA). Suprapur acids were purchased from Merck (Darmstadt, Germany). All other chemicals used were of analytical reagent grade.

Enriched ^{50}Cr and ^{53}Cr isotopes in the form of Cr_2O_3 were obtained from Oak Ridge National Laboratory (Oak Ridge, TN, USA) and were used for the preparation of ^{50}Cr(VI) and ^{53}Cr(III) isotopic