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Effect of hexavalent chromium on the activated sludge process and on the sludge protozoan community

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ABSTRACT

The objectives of this study were the determination of chromium effects to the performance of an activated sludge unit and the investigation of the response of the activated sludge protozoan community to Cr(VI). Two bench scale activated sludge reactors were supplied with synthetic sewage containing Cr(VI), at concentrations from 1 up to 50 mg L^{-1}. Protozoan species were identified and were related to the system efficiency. Variations in the abundance and diversity of the protozoan species were observed under various chromium concentrations. High removal rates of organics and nutrients were observed after the acclimatization of the activated sludge, which were related to the initial chromium(VI) concentration. Chromium(VI) removal efficiency was high in all cases. The protistan community was affected by the influent chromium content. Dominance of sessile species was observed in the reactor receiving 5 mg L^{-1} influent chromium, whereas co-dominance of sessile and carnivorous species was observed in the reactors receiving higher chromium concentrations.

1. Introduction

Activated sludge is a widely used process, based on the development of appropriate bacterial aggregates and other associated organisms in an aeration tank; these organisms are easily separated from the aqueous phase during the subsequent sedimentation. Ciliated protozoa play an essential role in the whole process by removing dispersed bacteria through grazing, which otherwise may result in high turbidity effluents (Salvado and Gracia, 1993; Lee et al., 2004). The presence of toxic substances in the influent may induce changes in the whole food web of the activated sludge ecosystems affecting their activity and the performance of the wastewater treatment plant.

Chromium is a common pollutant found in industrial effluents; chromium salts are extensively used in several industrial processes such as tanneries, electroplating, textile, dyeing, and metal finishing industries. Chromium may exist in the trivalent [Cr(III)] and hexavalent [Cr(VI)] state. Hexavalent chromium compounds (chromates and dichromates) are highly toxic and are considered as mutagens and carcinogens (USEPA, 1998). Due to the severe toxicity of Cr(VI), the Agency for Toxic Substances and Diseases Registry (ATSDR) classifies it as the top sixteenth hazardous substance (ATSDR, 2000). Indicative limits for total chromium concentrations in drinking water and reclaimed wastewater for irrigation are 0.05 (WHO, USEPA) and 0.1–1 mg L^{-1}, respectively (EPA, 2004).

Nicolau et al. (2005) investigated the effect of copper in the activated sludge protistan community of a conventional bench scale reactor and found that low concentrations of the metal, 4–8 mg L^{-1}, seemed to stimulate the growth of crawling species. On the other hand, the sessile *Opercularia* sp. was exceptionally tolerant to copper and was related to the low quality of the activated sludge. Abraham et al. (1997a) investigated the ciliate populations in an activated sludge plant, and found significant positive and negative correlations between ciliate species and metal content. Schlenk and Moore (1994) investigated the effect of pH and time on the acute toxicity of copper sulfate to the ciliated protozoan *Tetrahymena thermophila* and found that this protozoan was resistant to copper sulfate toxicity attributed to an intracellular mechanism. Madoni et al. (1996) investigated the toxic effect of heavy metals on the activated sludge protozoan community in a batch reactor and found variability between the sensitivities of the protozoan species to the tested metals. Ciliated species such as *Chilodonella uncinata* and *Trochilia minuta* showed the highest sensitivity to all studied metals, while *Opercularia coarctata* and *Opercularia minima* were the most tolerant species. Abraham et al. (1997a,b) investigated seasonal population variations and significant interspecies correlations, and found that the major ciliate species were able to tolerate sudden rises in metal concentrations, including Fe (>2000 ppb), Zn (>500 ppb), Cu (>60 ppb) and Cr (>100 ppb).

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Ciliated protozoa are commonly found in densities of about 10000 cells mL^{-1} of activated sludge mixed liquor, and constitute approximately 9% of the dry weight of suspended solids in mixed liquor (Madoni, 1994a,b). Ciliated protozoa, together with bacteria play an important role in the performance of activated sludge plants. The correlation of the ciliate sensitivity to a wide number of toxic substances may represent an indicator of the operating conditions of wastewater treatment plants and could provide a useful tool for the assessment of plant performance.

Activated sludge microorganisms or dried waste sludge have been used for metal removal from aqueous solutions (Kargi et al., 2006). Imai and Gloyna (1990) reported the chromium(VI) reduction to chromium(III) in the dissolved phase of a batch activated sludge system. Stasinakis reported the ability of activated sludge to reduce chromium(VI) to chromium(III) in a range of concentrations between 0.5 and 10 mg L^{-1} (Stasinakis et al., 2003). The chromium(VI) reduction was favored in the case of high organic substrate and in the case of the activated sludge system operating in an anoxic–aerobic mode (Stasinakis et al., 2004). Nevertheless, the literature data on the effect of chromium concentration on the activated sludge microfauna are still contradictory. The objectives of this work were the study of the response of the activated sludge protistan community to the exposure of various chromium concentrations, the investigation of the potential effect of chromium on the efficiency of an activated sludge process, their correlation to the effluent quality, and the examination of the ability of the activated sludge microfauna for chromium removal.

2. Methods

Six 2 L glass beakers were used as the bench scale activated sludge reactors at a constant temperature, 25 °C. Continuous aeration was provided by three air pumps using two air diffusers in each system. Start up of the reactor was conducted by the addition of 50 mL of activated sludge collected from the aeration tank of a full scale activated sludge unit with an MLSS content of 4.4 g/L. Each reactor was fed by 1450 mL of synthetic wastewater containing various concentrations of Cr(VI) namely, 0, 1, 5, 10, 20 and 50 mg L^{-1}, respectively. The pH of each system was about 7.5–8.5 and the reactors were initially fed by synthetic wastewater with a low chromium content aiming to the acclimatization of the activated sludge microorganisms to the corresponding experiment conditions. After the acclimatization period, chromium in the desired concentrations was added to the synthetic wastewater and the operation of the units was monitored as a function of time.

Each system was operated in subsequent cycles of four days. At the end of each cycle, sludge sedimentation was taken place by turning off the air pumps. 400 mL of the supernatant water was withdrawn from each system for further analysis, and were replaced by fresh synthetic wastewater containing the desired chromium concentration.

The synthetic wastewater was prepared by the addition of 2.4 g/L sodium acetate CH_3COONa (PANREAC) as carbon and energy source; 60 mg L^{-1} NH_4Cl (PANREAC), 18.7 mg L^{-1} Na_2HPO_4 (PANREAC), 100 mg L^{-1} $MgSO_4 \cdot 7H_2O$ (MERCK) and 1.3 g/L $NaHCO_3$ (PANREAC) as nutrient sources. Trace minerals such as 100 mg L^{-1} NaCl (PANREAC), 20 mg L^{-1} KCl (BAKER), 50 mg L^{-1} $CaCL_2 \cdot 2H_2O$ (MERCK) and 50 mg L^{-1} $FeCL_3 \cdot 6H_2O$ (MERCK) were added in the feeding solution (Kargi et al., 2005). In order to obtain a nutritionally balanced wastewater, the composition of the synthetic wastewater was adjusted to yield an initial chemical oxygen demand (COD) content of 1200 ± 50 mg L^{-1}, a total nitrogen (TN) = 60 ± 3 mg L^{-1} and total phosphorous (P) = 18 ± 2 mg L^{-1} giving a COD/N/P ratio of 100/5/1.5. Potassium dichromate was used as a chromium source at a concentration of 10 g/L; a stock solution was prepared and kept in the refrigerator at 4 °C.

Samples of each reactor were analysed for mixed liquor suspended solids (MLSS) content, COD, ammonia–nitrogen, phosphates, and chromium concentrations; all parameters were measured according to standard methods of analysis (APHA, 1989). Additionally, total chromium concentrations accumulated in the sludge at the end of the experimental period were measured by sludge digestion (APHA, 1989).

For the analysis of protozoan community, aliquots of 200 μL were collected from each reactor at different time periods. Analysis was conducted for the identification of species *in vivo* according to standard methods (Lee et al., 1972) using an optical microscope (Olympus) at 10× 40× and 100× magnification (Jahn et al., 1979; Lee et al., 1972). Small flagellates were counted by placing the sample on a Fuchs–Rosenthal 3.2 μL chamber.

3. Results and discussion

The bench scale activated sludge systems were operated for a total period of 120 days, in order to evaluate the effect of chromium on the operation performance. The removal efficiencies of COD, ammonia–nitrogen, phosphorus and the MLSS content in each system as a function of time are shown in Fig. 1. High COD values were measured in the supernatant from each system during the initial operation stages, decreasing by the operation time and remained almost stable after the 20th day of reactors operation. This might be attributed to the metabolic adaptation of the microorganisms to the corresponding chromium concentration, and the efficient utilization of the carbon source by the sludge microfauna.

The MLSS content of the sludge reactors (Fig. 1b) increased with the operation time, exhibiting significant increase after the 20th day of the operation. The high MLSS value was related to low effluent COD values suggesting the efficient utilization of the organic carbon source provided by the synthetic wastewater substrate. The highest MLSS values, 5000 mg L^{-1}, were observed at the highest chromium concentration of 50 mg L^{-1}, and could be attributed to the increased ATP synthesis, as an additional energy source by activated sludge microorganisms, stimulating their growth. In addition, during the continuous aeration of the aerobic activated sludge reactors, chromium ions might catalyze the extended oxidation of the synthetic wastewater substrate, producing excess energy of that available at normal conditions (Yetis et al., 1999).

Nitrification was more efficient after the 12th day of operation in all systems; however, variations in the effluent concentration of NH_4-N were observed between the 2nd and the 12th day (Fig. 1c). The nitrification process was not affected by the addition of high chromium concentrations. The efficient ammonia–nitrogen removal might be attributed to the use of activated sludge already acclimatized to Cr(VI); thus, the heterotrophic nitrifiers were already adapted to the particular metal.

The efficiency of the systems in removing phosphates is shown in Fig. 1d. During the first days of the experimental procedure the phosphate removal was negligible, but after the acclimatization period of the activated sludge, the phosphate effluent values decreased significantly at about 1.2 mg L^{-1} for the system treating 5 mg L^{-1} Cr(VI). However, after a period of about 2 months for the system fed by 50 mg L^{-1} Cr(VI) concentration, a slowdown in the phosphates removal rate was observed, resulting in higher effluent phosphates values. The efficient removal of phosphates could be attributed to sodium acetate provided in the synthetic wastewater, as the sole carbon source that might take part in the poly-hydroxy-butyrate synthesis during the anoxic (aerobic) phase of a wastewater treatment plant, for generation of large amounts of ATP associated to enhanced phosphate uptake, which in turn is used in polyphosphate formation synthesis during the anoxic phase (Kargi et al., 2005).