ELSEVIER

Journal of Hazardous Materials B133 (2006) 212–217

Journal of Hazardous Materials

www.elsevier.com/locate/jhazmat

Effects of tri-valent (Cr(III)) and hexa-valent (Cr(VI)) chromium on the growth of activated sludge

Petros Gikas *, Pavlos Romanos

Ministry of Environmental Planning and Public Works, General Secretariat of Public Works, Special Service of Public Works for Greater Athens Sewerage and Sewage Treatment, Varvaki 12, Athens 11474, Greece

Received 5 September 2005; received in revised form 1 October 2005; accepted 5 October 2005
Available online 18 November 2005

Abstract

The effects of Cr(VI) and Cr(III) species on the activated sludge growth rate have been assessed for a batch growth system, for a range of chromium concentration between 0 and 320 mg l^{-1}. Cr(VI) was found to stimulate microbial growth for concentrations up to about 25 mg l^{-1}, exhibiting maximum growth stimulation at 10 mg l^{-1}, whilst the lethal dose was found to be between 80 and 160 mg l^{-1}. On the other hand, Cr(III) was also found to stimulate microbial growth for concentrations up to about 15 mg l^{-1}, (with a maximum stimulation concentration at 10 mg l^{-1}), whilst the lethal dose was found to lie between 160 and 320 mg l^{-1}. The results indicate that Cr(VI) is more toxic to biomass at relatively high concentrations (higher than 70 mg l^{-1}) whilst it has a more pronounced growth stimulation effect at relatively smaller concentrations (less than 25 mg l^{-1}), compared with Cr(III).

Keywords: Chromium; Heavy metals; Activated sludge; Toxicity; Inhibition; Stimulation

1. Introduction

In general, heavy metals are considered as toxic compounds that inhibit the growth of micro-organisms [1,2], although their growth is often stimulated by the presence of trace amounts of selected heavy metals [3,4]. The relative response of micro-organisms to the presence of heavy metals is demonstrated quite elegantly by a figure drawn as early as 1964 by McCarthy [5] (Fig. 1), who classifies these effects, in relation with the heavy metal concentration, into three zones: (i) the zone of increasing stimulation, (ii) the zone of decreasing stimulation and (iii) the toxicity zone. The addition of small amounts of heavy metals to the cell environment is usually beneficial to the cell growth, up to the point at which the optimum concentration is surpassed, and a relative decrease of the stimulation effect is observed. Further increase of the heavy metal concentration will have an adverse effect on the cell growth, until the complete reduction of the microbial activity and the failure of the system. The critical point and the shape of the curve in Fig. 1 do not, however, depend only on the type of the micro-organism and on the particular heavy metal, since the micro-organisms have the ability to adapt with time to relatively higher concentrations of heavy metals, according to a phenomenon called acclimation. Acclimation usually involves the use of alternative metabolic pathways, which are not disrupted (or least disrupted to a lesser degree) by the presence of heavy metals. This process is, nevertheless, limited and the cell may not be able to further acclimation at relatively higher concentrations, resulting in complete containment of biological activity [6,7]. When the heavy metals are applied to mixed cultures, such as activated sludge, an even wider tolerance is expected, due to the high diversity of the micro-organisms present in the process. Thus, some species may be significantly affected, whilst others may adapt to the new environment [8]. Despite the fact that the above phenomena have been studied quite extensively, the scientific community has not clearly concluded whether the presence of particular heavy metals inhibit or stimulate microbial growth. An even greater ambiguity exists regarding the establishment of growth-stimulating and toxic doses of heavy metals.

A number of methods has been proposed for measuring metal toxicity in activated sludge systems, the more commonly used ones include the measurement of enzymatic activity [9,10], the measurement of respiratory rate [11,12] and the influence on the micro-organism growth parameters [13,14].

* Corresponding author. Tel.: +30 210 6444410; fax: +30 210 6452753.
E-mail address: petrosgikas@yahoo.gr (P. Gikas).

0304-3894/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2005.10.023

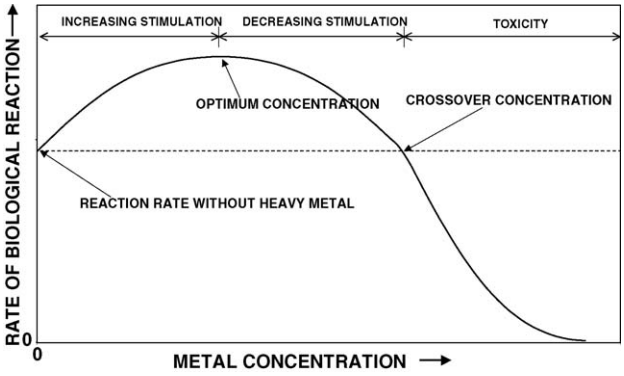

Fig. 1. The effect of heavy metal concentrations on biological reactions (adapted from McCarthy [5]).

Chromium is considered as toxic to aquatic environment. The main sources of chromium are the chemical and electroplating industry and the leather tanneries. Chromium species are encountered in the industrial wastewater in the oxidation stages of III (tri-valent) and VI (hexa-valent). Hexa-valent chromium is regarded as more toxic. Indeed, the tri-valent chromium tends to accumulate in the cell membrane, whilst the hexa-valent chromium is capable of penetrating the membrane into the cytoplasm, where it is reduced to tri-valent chromium and reacts with the intracellular material [15–18].

A number of studies have been carried out on activated sludge to investigate the effects of chromium species on its growth behaviour [4,12,19–21]. A few studies only deal with the effects of both species of chromium to activated sludge, and thus, since activated sludge characteristics exhibit significant variations from study to study, it is hard to draw a clear conclusion about the relative toxicity of the chromium species. This study aims to investigate the effects of Cr(III) and Cr(VI) on unacclimatised activated sludge, with ultimate target being the classification of the toxicity effects of the above Cr species to micro-organisms. Since some of the existing studies indicated that relatively high doses of chromium (e.g. 25 mg l^{-1} of Cr(VI)) had either stimulating [4] or inert [22] effects on biomass growth, a wide range of concentrations have been employed, in order to also identify the lethal doses. It should be pointed out that the actual concentration of Cr species in the growth medium during the experiments may be somehow different than the calculated concentrations, due to the following reasons: (i) due to accumulation of Cr in biomass [23,24] and (ii) due to the biotransformation of Cr(VI) into Cr(III) [15–18]. This work aims to investigate the growth pattern of activated sludge growing in a rich medium at the presence of calculated quantities of Cr(VI) or Cr(III), and not to investigate the fate of Cr species in the microbial culture.

2. Experimental

2.1. Experimental setup

The experimental growth system consisted of 30 borosilicate cylindrical flasks with working volume of 50 ml each, placed in a special rack, and partially submerged in a 30 °C water bath. 30 small air pumps (Resun AC-2600, E.U.) were employed to aerate and to agitate the liquid content of the flasks, by pumping air near the bottom of each flask, via a flexible PVC hose, with nominal diameter of approximately 5 mm. The air flow in each flask was adjusted (using a standard tube clamp) to approximately 20 bubbles of air per minute, a sufficient flow to maintain saturated oxygen conditions in the growth medium.

Table 1
Composition of growth medium used in all trials with the addition of calculated amounts of Cr(III) or Cr(VI) in the form of $Cr(NO_3)_3$ and $K_2Cr_2O_7$, respectively

Constituent	Concentration
Peptone (mg l^{-1})	7500
Dextrose (mg l^{-1})	5000
Yeast extract (mg l^{-1})	5000
KH_2PO_4 (mg l^{-1})	210
K_2HPO_4 (mg l^{-1})	180
NH_4SO_4 (mg l^{-1})	300
$MgSO_4{\cdot}7H_2O$ (mg l^{-1})	100
$NaNO_3$ (mg l^{-1})	25
$Ca(NO_3)_2{\cdot}4H_2O$ (mg l^{-1})	100
$FeSO_4{\cdot}7H_2O$ (mg l^{-1})	50
Triton X-100 (ml l^{-1})	0.1

2.2. Growth medium

The composition of the growth medium used throughout the experiments, with the addition of calculated amounts of Cr(III) or Cr(VI), is described in Table 1. Carbon is supplied to the medium via dextrose, peptone and yeast extract. Peptone and yeast extract are serving as nitrogen and phosphorus sources, the concentrations of which are also supplemented by the addition of ammonia and phosphate salts, making carbon the growth limiting substrate. Magnesium, calcium and iron were also added in sufficient quantities to the growth medium, whilst other micronutrients were provided by the yeast extract. The phosphate salts, apart from providing phosphorus to the microorganisms, also served as buffer to stabilise the pH. Biomass flocculation during growth was controlled by the addition of Triton X-100 (Union Carbide Chemicals Co. Inc, Germany). All growth medium substances were diluted in deionised water, thereafter autoclaved for 20 min at 120 °C, and stored in a refrigerator at 4 °C. The pH of the medium was adjusted to 7.0 by the use of HNO_3.

2.3. Analytical techniques

The microbial growth was monitored by measuring the optical density (ABS) of the mixed liquor at 650 nm, using a U-2000 Hitachi (Japan) spectrophotometer. Experiments with the spectrophotometer showed that aqueous suspensions of activated sludge exhibited a maximum absorbance at 650 nm, whilst pure growth medium with the addition of Cr(III) and Cr(VI) (before seeding) indicated a maximum absorbance at 350 nm, and almost a negligible absorbance at 650 nm, thus omitting interference on the measurement of the microbial concentration from the growth

medium itself. The mixed liquor suspended solids (MLSS) concentration was calculated by means of a calibration curve (Eq. (1)), which was obtained experimentally, by the following procedure. Five 500 ml conical flasks were filled with 200 ml of growth medium, inoculated with activated sludge and placed in a shaker at 30 °C. Several hours later, when sufficient growth was observed, the monitoring of optical densities and biomass concentrations were performed as follows: every hour (for the next 4 h), 30 ml of mixed liquor were extracted from each flask; the optical density was measured using 1 ml of the sample, whilst the rest was immediately filtered through pre-weighed filter paper with a size cutoff of 0.2 μm. The filter was washed twice with 100 ml of deionised water and the filter paper was then weighted, after drying at 105 °C. Optical densities (ABS) were plotted against dry weight, yielding the curve of Eq. (1).

$$\mathrm{MLSS} = 1.1519\mathrm{ABS} + 0.1232 \quad (1)$$

The maximum specific growth rate (μ_{max}) for each growth curve was determined assuming Monod type kinetics, and applying Eq. (2) for the "linear" part of each growth curve.

$$\ln \frac{\mathrm{MLSS}_i}{\mathrm{MLSS}_0} = \mu_{max}(t_i - t_0) \quad (2)$$

MLSS_i is MLSS (mg l^{-1}) at time t_i; MLSS_0, MLSS (mg l^{-1}) at the end of lag phase; μ_{max}, maximum growth rate (h^{-1}); t_i, time at the point of measurement; t_0 is time at the end of lag phase. μ_{max} was calculated by linear regression of all measured values.

2.4. Experimental procedures

Activated sludge cultures were obtained from the aeration tank of the Chalkis municipal wastewater treatment plant. The plant serves approximately 90,000 people from the town of Chalkis in Greece, and has a hydraulic capacity of about 4500 m^3 day^{-1}. During the sampling from the plant which was operated at steady state conditions, the sludge age was approximately 10 days, and the sludge density was measured to be 4.5 g l^{-1}.

Prior to inoculation, the flasks were filled with 29.5 ml of prepared rich growth medium (Table 1). For every experimental run, the concentration of Cr(III) and Cr(VI) in each flask, was adjusted by the addition of calculated amounts of standard aqueous solutions of $Cr(NO_3)_3$ and $K_2Cr_2O_7$, respectively. One series of trials was carried out at Cr(III) concentrations of 0, 5, 10, 20, 30, 40, 80, 160, 320 and 640 mg(Cr) l^{-1}, whilst the other series was carried out at the same concentrations of Cr(VI). Three flasks were used for each concentration and for each chromium species making a total number of trials $2 \times 10 \times 3 = 60$. Inoculation occurred by the addition of 0.5 ml of activated sludge into the flasks which contained the growth medium. The flasks were thereafter placed in the water bath, and aeration was started immediately. Three samples were taken every hour from each flask and the optical densities were measured using the spectrophotometer described above.

2.5. Statistical analysis

The Student's *t*-distribution, with a 0.05 level of significance, was used to reject the statistically extreme values of absorbance. The statistical analysis indicated that 4 measurements out of 60 had to be withdrawn.

3. Results and discussion

3.1. Effects of Cr(VI)

Fig. 2 depicts the growth curves of activated sludge growing at different Cr(VI) concentrations. As the concentration of Cr(VI) increases, the lag time of the culture increases. This phenomenon is more pronounced for Cr(VI) concentrations higher than 30 mg l^{-1}. Biomass growth was sustained even at Cr(VI) concentration of 80 mg l^{-1}. Limited research has been carried out at such high Cr(VI) concentrations; Vankova et al. [25] studied the activated sludge growth under Cr(VI), and have reported growth sustainability at Cr(VI) concentration of 800 mg l^{-1}, after about 25 h of lag time, attributing the phenomenon either to the gradual acclimatization of the activated sludge, or to the reduction of Cr(VI) concentration due to the adsorption in biomass flocks or due to the reaction with other substances which were present in the growth medium.

On the other hand, and despite the relatively extended lag times, the biomass maximum growth rate (μ_{max}) appeared to take higher values at Cr(VI) concentrations up to about 25 mg l^{-1}, compared to the blank, showing a maximum at 10 mg l^{-1}. For Cr(VI) concentrations higher than 10 mg l^{-1}, μ_{max} decreased and reached zero for concentrations greater than 160 mg l^{-1} (Fig. 3). The above results are in relative agreement with those reported by Gokcay and Yetis [4], who worked with a continuous reactor, and determined a continuous activated sludge growth stimulation with increasing Cr(VI) concentration from 0 to 25 mg l^{-1}. However, their system was not tested for higher Cr(VI) concentrations, thus they did not attend to calculate the optimum Cr(VI) concentration for activated sludge growth. Moore et al. [22] have reported biomass growth stimulation with the addition of 5 mg l^{-1} of Cr(VI) and no significant loss of treatment efficiency for Cr(VI) concen-

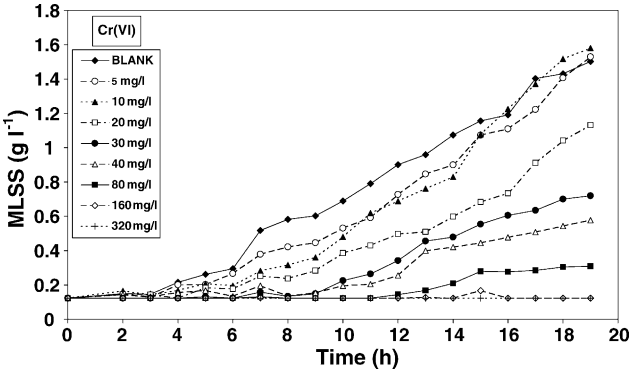

Fig. 2. Growth curves (MLSS vs. time) for activated sludge growth at various Cr(VI) concentrations.

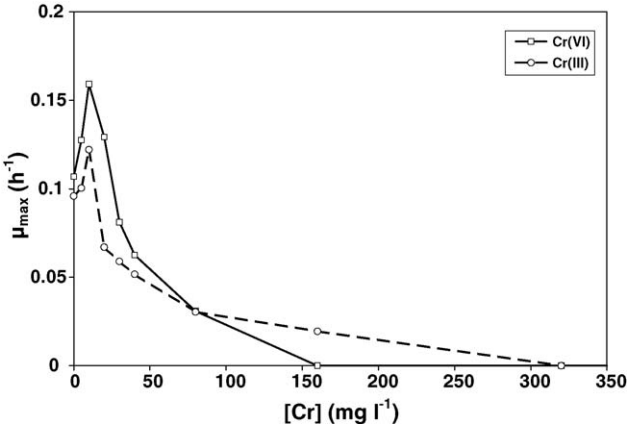

Fig. 3. Calculated values for μ_{max} vs. Cr(VI) or Cr(III) concentration.

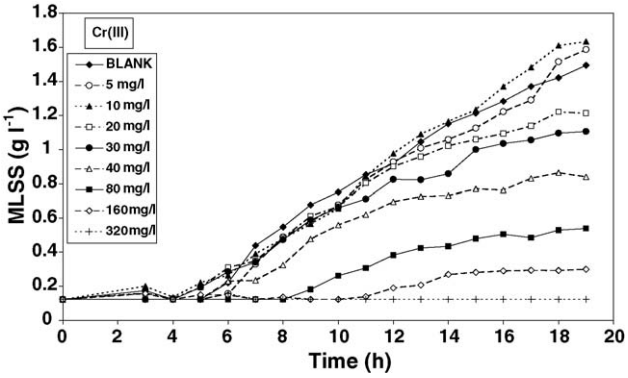

Fig. 4. Growth curves (MLSS vs. time) for activated sludge growth at various Cr(III) concentrations.

trations up to 50 mg l^{-1}. Barth et al. [26] have also reported insignificant loss of treatment efficiency for Cr(VI) concentrations up to 10 mg l^{-1}. On the contrary, Vankova et al. [25], reported that the addition of even 2 mg l^{-1} of Cr(VI), caused inhibition of the activated sludge respiration rate. Stasinakis et al. [20], operated two parallel laboratory scale continuous flow activated sludge plants and demonstrated that the addition of up to 1 mg l^{-1} of Cr(VI) resulted in a non-statistically significant (at a 95% confidence level) reduction of the organic substrate uptake rate, whilst addition of 3 or 5 mg l^{-1} of Cr(VI) resulted to a minor or to a 10% reduction of the organic substrate uptake rate, respectively. In the same study, they reported that addition of 0.5 mg l^{-1} of Cr(VI) in a nitrifying culture, resulted in 74% reduction of NH_4^+ uptake rate, indicating a higher sensitivity of the autotrophic micro-organisms to the presence of Cr(VI). Mazierski [19] worked with activated sludge growing in a chemostat and found a continuous reduction in μ_{max} with the addition of Cr(VI) in concentrations between 0.1 and 11 mg l^{-1}. Despite working with relatively small Cr(VI) concentrations, he did not report growth stimulation effects. Similarly to Stasinakis et al. [20], Mazierski [27] also reported a severe reduction of the growth of nitrifying micro-organisms by the addition of 0.2–0.6 mg l^{-1} Cr(VI). Also Mowat [12], and Lamb and Tollefston [28] reported a steady reduction of culture activity with the addition of up to 20 mg l^{-1} of Cr(VI). Finally, Stasinakis et al. [29], reported that the substrate to biomass ratio, and sludge age may have severe impacts on the growth stimulation or inhibition due to the presence of Cr(VI).

The large divergence of the literature results presented above may be attributed to:

(i) the high diversity of activated sludge behavior,
(ii) the system which was employed for micro-organisms growth (batch, continuous, with or without sludge recycling),
(iii) the concentration of activated sludge during the trial period (since Cr(VI) can be adsorbed by biomass),
(iv) the activated sludge age,
(v) the relative acclimation of activated sludge to Cr(VI), and finally,
(vi) the different measuring methods and techniques used by the researchers.

3.2. Effects of Cr(III)

The growth curves for activated sludge under different Cr(III) concentrations are shown in Fig. 4. Microbial growth has been observed for Cr(III) concentrations up to 160 mg l^{-1}, whilst higher values of Cr(III) concentrations resulted in the reduction of microbial growth. A significantly prolonged lag time is observed for Cr(III) concentrations between 80 and 160 mg l^{-1}, whilst the lag times for Cr(III) concentrations between 0 and 40 mg l^{-1} are shorter and almost in the same range.

Fig. 3 presents the biomass μ_{max} variation with the applied concentration of Cr(III). μ_{max} appears to increase with Cr(III) concentration reaching a maximum at 10 mg l^{-1}, whilst further addition of Cr(III) results in a drastic decrease of μ_{max}. Mowat [12] measured the respiration activity of activated sludge in the presence of Cr(III) and reported a steady decrease of respiratory rate even with the addition of Cr(III) at concentrations below 1 mg l^{-1}. Similarly, Lamb and Tollefston [28], reported a steady reduction of the organic substrate uptake rate with the addition of Cr(III), without growth stimulation at relatively small concentrations. However, the above researchers [12,28] have reported the same trends (absence of growth stimulation doses) for similar experiments utilising Cr(VI) instead of Cr(III). Finally, Chua et al. [30] reported about 18% reduction in COD removal efficiency with the introduction of 0.5 mg l^{-1} Cr(III). The results of the present study are not in agreement with the above reports, since a growth stimulation effect at relatively small Cr(III) concentrations has been observed. The main reasons for the relative discrepancies between the various researches have been analysed at the end of the previous paragraph.

3.3. Comparison between the effects of Cr(VI) and Cr(III)

As stated in the introduction, limited research has been carried out to investigate the effects of both chromium species on the same culture and under the same growth conditions. The conclusions derived from previous work are contradictory, not only

with respect to the quantitative effects of chromium species but also with respect to the qualitative effects. Lamp and Tollefson [28] who worked with activated sludge growing in a continuous reactor setup reported that Cr(VI) is more toxic than Cr(III). More specifically, they monitored that addition of 5 mg l^{-1} of Cr(VI) or Cr(III), resulted in organic substrate uptake rate reduction by 50 and 20%, respectively, whilst for every 2 ppm addition of Cr(VI) or Cr(III), the relative reduction in substrate uptake rate was 5 and 1%, respectively. Similarly, Bieszkiewicz and Hoszowski [31] reported higher toxicity of Cr(VI) compared to that of Cr(III), since they measured the inhibition concentration of Cr(VI) or Cr(III), for activated sludge growth, to be 20 and 1.15 mg l^{-1}, respectively. On the contrary, Mowat [12] investigated that Cr(III) has more toxic effects on activated sludge respiration rate compared to Cr(VI), reporting about 70–80% reduction for Cr(III), and 20–50% reduction for Cr(VI). Vankova et al. [25] attended also to determine the relative toxicities of chromium species on activated sludge, however, their results are incomplete, due to problems with the solubility of Cr(III). They reported the 1-h CE_{50} values for Cr(VI) between 40 and 90 mg l^{-1}, at pH = 7, whilst the 0.5-h CE_{50} value for Cr(III), at pH 5.8, was measured 49 mg l^{-1}.

Fig. 5, depicts the inhibition curves (determined according to Eq. (3)) for Cr(VI) and Cr(III). The graph indicates that Cr(VI) has a more pronounced stimulation effect on μ_{max}, compared with Cr(III). Cr(VI) has been found to stimulate microbial growth for concentrations up to about 25 mg l^{-1}, whilst the relative growth stimulating concentrations for Cr(III) were up to 15 mg l^{-1}. On the other hand, Cr(VI) had more toxic effects than Cr(III) for concentrations exceeding 70 mg l^{-1}. Apparently, the activated sludge was capable of growing at higher concentrations of Cr(III) than for Cr(VI). More specifically, a 100% growth inhibition was achieved at 320 mg l^{-1} of Cr(III), but at 160 mg l^{-1} of Cr(VI).

$$\text{Inhibition}_i\,(\%) = \frac{\mu_{\max 0} - \mu_{\max i}}{\mu_{\max 0}} \times 100 \qquad (3)$$

$\mu_{max\,0}$ is μ_{max} of biomass growth at the absence of chromium and $\mu_{max\,i}$ is μ_{max} at the presence of chromium at i concentration.

The present results thus indicate that Cr(VI) is more potent biomass growth inhibitor compared with Cr(III), whilst the same Cr species, at relatively small concentrations, are also more efficient biomass growth stimulators.

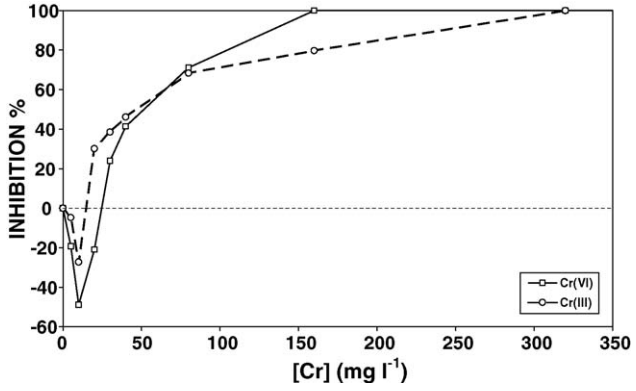

Fig. 5. Inhibition curves for activated sludge growth at the presence of Cr(VI) or Cr(III).

4. Conclusions

The effects of Cr(VI) and Cr(III) in the activated sludge growth rate have been assessed for a batch growth system:

- Cr(VI) was found to stimulate microbial growth for concentrations up to about 25 mg l^{-1}, whilst the lethal dose was found to be between 80 and 160 mg l^{-1}.
- Cr(III) was also found to stimulate microbial growth for concentrations up to about 15 mg l^{-1}, whilst the lethal dose was found to lie between 160 and 320 mg l^{-1}.
- The results indicated that Cr(VI) is more toxic to biomass at relatively high concentrations (higher than 70 mg l^{-1}) whilst it has a more pronounced growth stimulation effect for relatively small concentrations (less than 25 mg l^{-1}), compared with Cr(III).

Acknowledgments

The authors wish to thank Dr. George Sakellaris for the technical and scientific support and for providing unlimited access to the laboratory of the Department of Biological Studies of the National Hellenic Research Foundation for the implementation of the present work.

They also wish to thank Dr. Andrew Ball, from the Department of Biology and Biotechnology of the University of Essex, UK, for the useful conversations and scientific support during the experimental part of the present work.

Finally, they wish to thank Mr. Anastasios Koutsavlis and Mr. Stamatis Kanaris, from the Municipal Wastewater Treatment Plant of Chalkis, for providing the activated sludge samples.

References

[1] J.G. Leduc, G.D. Ferroni, J.T. Trevors, Resistance of Heavy Metals in Different Strains of *Thiobacillus ferroxidans*, World J. Microb. Biotechnol. 13 (1997) 453–462.
[2] P. Madoni, D. Davoli, G. Gorbi, L. Vescovi, Toxic effects of heavy metals on the activated sludge protozoan community, Water Res. 30 (1) (1996) 135–141.
[3] D.K. Wood, G. Tchobanoglous, Trace elements in biological waste treatment, J. Water Pollut. Control Federation 47 (1975) 1933–1948.
[4] C.F. Gokcay, U. Yetis, Effect of chromium (VI) on activated sludge, Water Res. 25 (1) (1991) 65–73.
[5] P.L. McCarthy, Anaerobic waste treatment fundamentals, Part III. Toxic materials and their control, Public Works 95 (11) (1964) 91–99.
[6] M.M. Bagby, J.H. Sherrard, Combined effects of cadmium and nickel on the activated sludge process, J. Water Pollut. Control Federation 53 (11) (1981) 1609–1619.
[7] A. Garcia-Toledo, H. Babici, G. Stotzky, Training *Rhizopus stolonifer* and *Cunninghamella blakesleeana* to copper: cotolerance to cadmium, cobalt, nickel and lead, Can. J. Microbiol. 31 (1985) 485–492.
[8] E.F. Barth, The effects and removal of heavy metals in biological treatment, in: P.A. Krenkel (Ed.), Heavy Metals in Aquatic Environment, Pergamon Press, Oxford, 1975.
[9] S. Sujarittanonta, J.H. Sherrard, Activated sludge nickel toxicity studies, J. Water Pollut. Control Federation 53 (4) (1981) 1314–1322.

[10] C.D. Boswell, R.E. Dick, L.E. Macaskie, The effect of heavy metals and other environmental conditions on the anaerobic phosphate metabolism of *Actinobacter johnsonii*, Microbiology 145 (1999) 1711–1720.
[11] P. Battistoni, G. Fava, M.L. Ruello, Heavy metal shock load in activated sludge uptake and toxic effects, Water Res. 27 (5) (1993) 821–827.
[12] A. Mowat, Measurement of metal toxicity by biochemical oxygen demand, J. Water Pollut. Control Federation 48 (5) (1976) 853–866.
[13] A. Cabrero, S. Fernandez, F. Mirada, J. Garcia, Effects of copper and zinc on the activated sludge bacteria growth kinetics, Water Res. 32 (1998) 1355–1362.
[14] Z. Lewandowski, K. Janta, J. Mazierski, Inhibition coefficient (K_i) determination in activated sludge, Water Res. 19 (5) (1985) 671–674.
[15] K.W. Jannette, The role of metals in carcinogenesis, biochemistry and metabolism, Environ. Health Perspect. 40 (1981) 233–252.
[16] A.E. Martell, Chemistry of carcinogenic metals, Environ. Health Perspect. 40 (1981) 207–226.
[17] H. Shen, Y.-T. Wang, Biological reduction of chromium by *E. coli*, J. Environ. Eng. 120 (3) (1994) 560–572.
[18] A.S. Stasinakis, N.S. Thomaidis, D. Mamais, M. Karivali, T.D. Lekkas, Chromium species behaviour in activated sludge process, Chemosphere 52 (2003) 1059–1067.
[19] J. Mazierski, Effect of chromium (VI) on the growth rate of activated sludge bacteria, Water Res. 29 (6) (1995) 1479–1482.
[20] A.S. Stasinakis, D. Mamais, E.C. Papanikolaou, A. Tsakon, T.D. Lekkas, Effects of chromium (IV) addition on activated sludge process, Water Res. 37 (2003) 2140–2148.
[21] F.B. Dilek, C.F. Gokcay, U. Yetis, Combined effects of Ni(II) and Cr(VI) on activated sludge, Water Res. 32 (2) (1998) 303–312.
[22] W.A. Moore, G.N. Mc Dermott, M.A. Post, M.B. Ettinger, Effects of chromium on the activated sludge process, J. Water Pollut. Control Federation 33 (1961) 54–72.
[23] R.M. Sterritt, J.N. Lester, The influence of sludge age on heavy metal removal in the activated sludge process, Water Res. 15 (1981) 59–65.
[24] M. Ledin, Accumulation of heavy metals by microorganisms-process and importance for soil systems, Earth Sci. Rev. 51 (2000) 1–31.
[25] S. Vankova, J. Kupec, J. Hoffmann, Toxicity of chromium to activated sludge, Ecotoxicol. Environ. Safety 42 (1999) 16–21.
[26] E.F. Barth, M.B. Ettinger, B.V. Salotto, G.N. Mc Dermott, Summary report on the effects of heavy metals on the biological treatment process, J. Water Pollut. Control Federation 37 (1965) 86–96.
[27] J. Mazierski, Effect of chromium (Cr^{VI}) on the growth rate of denitrifying bacteria, Water Res. 28 (9) (1994) 1981–1985.
[28] A. Lamb, E.L. Tollefson, Toxic effects of cuppric, chromate, and chromic ions on biological oxidation, Water Res. 7 (1973) 599–613.
[29] A.S. Stasinakis, D. Mamais, N.S. Thomaidis, T.D. Lekkas, Effect of chromium (VI) on bacterial kinetics of heterotrophic biomass of activated sludge, Water Res. 36 (2002) 3341–3349.
[30] H. Chua, P.H.F. Yu, S.N. Sin, M.W.L. Cheung, Sub-lethal effects of heavy metals on activated sludge microorganisms, Chemosphere 39 (15) (1999) 2681–2962.
[31] E. Bieszkiewicz, A. Hoszowski, Effect of copper and tri- and hexavalent chromium on work of an activated sludge, Acta Microbiol. Pol. 27 (2) (1978) 147–153.