Toxic Effects of Metals on Microbial Activity in the Activated Sludge Process

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Professional paper
Received: September 15, 2001
Accepted: April 9, 2002

The toxic effects of the metals cadmium, zinc and copper on microbial activity in the activated sludge process, are reported. The dissolved metals were added to the process in synthetic wastewater with a COD of 520 mg L^{-1}. Metal toxicity was determined by the reduction in activity levels of variables such as the specific oxygen uptake rate and mass fraction of active cells present in the reactor. The results have permitted establishment of the following toxicity sequence: Cd > Cu > Zn.

Keywords:
Activated sludge, microbial activity, toxicity, heavy metals

Introduction

Activated sludge is a commonly used biological treatment process employed in the removal of colloidal and soluble organic matter present in wastewater. Although, the traditional role of municipal treatment plants was to remove soluble and colloidal organic matter, metals are also frequently present in the municipal sewage due to industrial wastewaters admitted to the sewerage. Metals may originate from industries such as metal finishing, hydrometallurgical refining, battery manufacturing, etc.

The mechanisms by which metals are eliminated in wastewater treatment processes has been widely reported. Of these, the most important are precipitation, adsorption to suspended solids during primary sedimentation,[1,2] or adsorption to extracellular polymers and bacteria in activated sludge.[3,4]

Many factors are involved in metal removal. Some of these, such as the concentration of solids in the mixed liquor, sludge age and organic load, are plant operating quantities; others are related to the metal considered as metal, such as metal speciation, concentration, etc.[3]

Although organisms require a certain concentration of trace metals to achieve optimum growth, we know that these compounds are toxic for the majority of species at specific concentration levels. Many researchers[5,6] have shown that only low concentrations of most metals are required to produce a significant reduction in plant performance and micro-organism activity.

The deleterious effects of heavy metals on biological processes are complex and generally related to the species, the solubility of the metal, the concentration of the toxic material and the characteristics of the influent, such as the pH, the presence and concentration of other cations and/or molecules, suspended solids, etc.[7,8]

Most of the research, relating to heavy metal toxicity has centred on the effect of the latter on the bacterial surface,[9,10] or has studied the nature of the complexes formed between the metals and the extracellular polymers (ECP).[11,12] Various methods have been employed to measure toxicity, the most common being enzyme and nitrification inhibition[13] and effluent turbidity.[14] However, *Kunz* et al.[15] and *Battistoni* et al.,[16] have reported the effects of heavy metals on oxygen uptake rates.

The aim of this research was to determine the toxic effects which the metals zinc, copper and cadmium have on the activated sludge process. These effects will be monitored by control quantities (suspended solids, COD) and by measurements of activity levels (oxygen uptake rate and fraction of active cells).

Material and methods

Laboratory-Scale Activated Sludge Treatment Plant

The experimental bioreactor employed in the laboratory tests is illustrated in Figure 1. It consists of two units: a 3 litre capacity aeration tank and a 2.5 litre capacity sedimentation tank. The sterile feed was pumped continuously into the reactor by peristaltic pump (MASTERFLEX model) and an air compressor provided aeration and homogenization of the reactor contents (TAGUS 2000). A temperature control device was used to maintain the temperature within the activated sludge unit at 25 °C (Gallenkamp FBL-330-010N model). This reactor

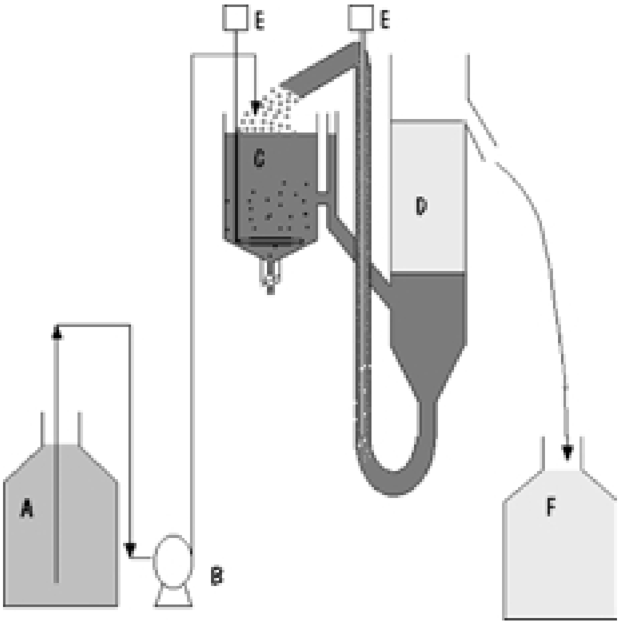


Fig. 1 – *Diagram of the laboratory-scale activated sludge treatment plant. A. Influent. B. Peristaltic pump. C. Aeration tank. D. Sedimentation tank. E. Compressed air pumps. F.*

functioned in continuous regimen for all experiments.

All experimental work has been performed by using activated sludge from Jerez of the Frontera (Cádiz, Spain) activated sludge plant. Influent wastewater was almost entirely domestic.

For start-up, an activated sludge sample was taken from the aeration tank of the wastewater treatment plant of Jerez. A 4.5 L volume of sludge and 2.5 L volume of wastewater was placed in the pilot plant. Steady state conditions were said to exist when all the quantities of the system remained constant over a given time period (5 days).

Continuous reactor

The source for mixed liquor was a lab-scale completely mixed activated sludge system, treating a syntetic wastewater, containing metals compounds, and operated at an STR of 6 days.

Feed Solution

Synthetic wastewater was used in the experiments, with a COD value of 520 mg L^{-1} and composition as detailed in Table 1.

Solutions of cadmium, copper and zinc sulphate were added to the synthetic wastewater in each of the three tests, and feed mass concentration was 2 mg L^{-1} in the case of copper and cadmium, and 3 mg L^{-1} in the case of zinc.

The experiment lasted 8 days.

Table 1 – *Composition of synthetic wastewater*

Compound	Mass concentration γ/mg L^{-1}
Peptone	160
Urea	30
Meat Extract	110
Na Cl	7
$CaCl_2 \cdot H_2O$	4
$MgSO_4 \cdot 7\ H_2O$	2
K_2HPO_4	28

Analytical techniques employed

The techniques employed may be divided into two groups. First, techniques relating to the control of the reactor: determination of suspended solids, COD of the effluent and pH. All of these techniques complied with Standard Methods procedures.[17] Second, techniques to measure activity levels: determination of the specific oxygen uptake rate (SOUR)[17] and determination of the fraction of active cells in the reactor, using the DNA-binding fluorochome 4,6-diamidino-2-phenylindrochlorid (DAPI) and tetrazolium salt 5-cyano-2,3-ditolyltetrazolium chloride (CTC).

Bacteriological count

To control the bacteriological population present in the reactor, we employed the method proposed by *Griebe* et al.,[18] based on the combined use of the tetrazolium salt, CTC, for active microorganisms and the DNA-binding fluorochrome (DAPI) for total microorganisms.

Briefly, the samples of activated sludge were incubated with tetrazolium salt (CTC), at a concentration of 4 mmol L^{-1}; incubation lasted two hours, at ambiental temperature and in darkness. At the end of this time, the reaction was interrupted by the addition of 1mL of w = 37 % formaldehyde.

Because of the way in which bacteria grow in activated sludge, the sample was then placed in an ultrasound bath (6 litre capacity ULTRASONS-H, Selecta) for 15 minutes, to destroy all the bacterial floc without disruption of bacterial cells, and ensure an efficient and reliable count.

Each sample was then diluted to obtain a cell concentration permitting an accurate cell count. DAPI (10 μg mL^{-1}) was added to the diluted samples, which were then left to incubate in darkness for 10 minutes and at room temperature. Thereafter, the stained bacteria were collected by microfiltration using a black 0.2 μm pore-size polycarbonate membrane filter (Millipore GTBP, Ireland). The filters were allowed to air dry and then mounted on glass slides using low fluorescent immersion oil (Laboratory Cargille, Inc.). The microscope count was performed in triplicate, as recommended by *Schaule* et al.[19]

Results

Start up procedure

The metal-free synthetic wastewater in the reactor was inoculated with activated sludge from the municipal wastewater treatment plant in Jerez de la Frontera (Cadiz, SW Spain). The reactor was fed daily with metal-free synthetic wastewater and operated in batch mode until a well established biomass was achieved. Thereafter, it worked continuously until the microorganisms became adapted to the new environmental conditions.

Once acclimatization was complete, as marked by stability in the concentration levels of suspended solids in the mixed liquor and in the COD values of the effluent, we began to add metal to the synthetic feed.

Effect of the metals on the control variables

The evolution of the volatile suspended solids (VSS) in each of the three experiments is illustrated in Figure 2. In the case of zinc and copper, this evolution was similar: a marked decline in the first three days, an increase on the fourth day, a slight drop on the fifth day, and then apparent stabilization until the end of the experiment. This behaviour may be due to the inhibiting effect which zinc and copper have on the reproductive processes of microorganisms in activated sludge. From the third day on, a possible adaptation or acclimatisation by the mi-

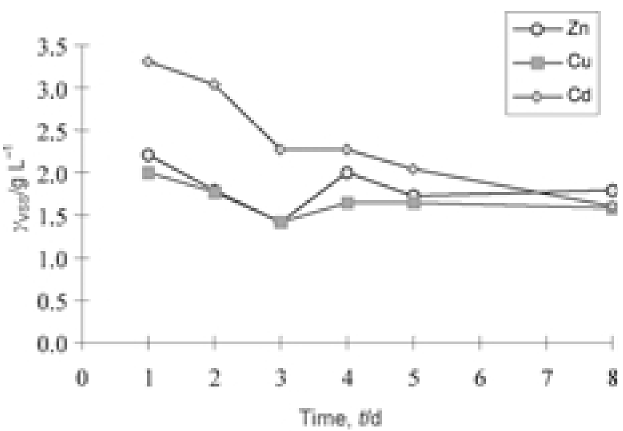


Fig. 2 – *Evolution of the VSS variable in each of the three experiments*

croorganisms to the presence of the metal may explain why the decline in the VSS concentration was less marked than at the start of the experiment.

In the test employing cadmium, the initial VSS concentration in the reactor was greater than in the zinc and copper experiments. It was also slightly greater than is normally found in activated sludge treatment plants. As before, the most significant decline was during the first three days of the test; however, unlike the copper and zinc experiments, no increase was recorded on the fourth day, and the VSS levels in the reactor continued to descend until the end of the experiment, indicating that the process had not stabilized and that, probably, if the test had continued, an even greater loss of solids would have occured.

Figure 3 shows the rate of elimination of organic material in terms of COD fraction for each of the three metals studied.

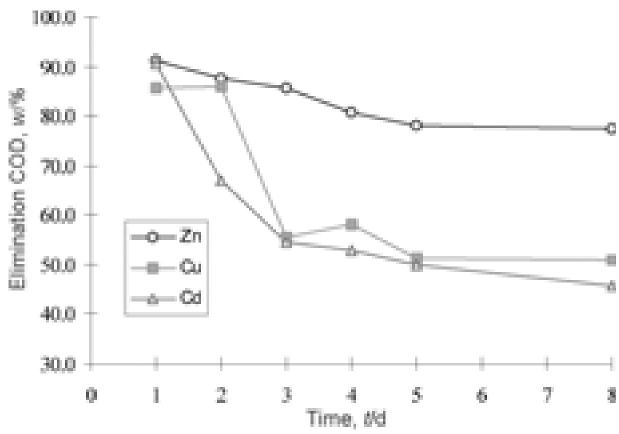


Fig. 3 – *Evolution of the organic material elimination rate in each of the three experiments*

In each case, the presence of the metal leads to a decrease in the rate of organic material elimination throughout the duration of the experiment, reaching values which are below those indicated by legislation (CE Directive 91/271). However, as in the case of VSS, the behaviour of this variable is different for each of the metals studied. Zinc produces the least negative effects, with the elimination rate decreasing from 90 % to 77 % over the 8 days of the test, and with stabilization virtually complete from the fourth day onwards.

Copper and cadmium have similar effects on the elimination rate of organic material, with day one elimination rates of 85.5 % and 90.5 % dropping to 51.5 % and 45.86 %, respectively, by day eight.

This deterioration in reactor performance is no doubt due to the toxic effect which these metals have on the microorganisms, responsible for the elimination of organic material, with floc formation in the activated sludge also affected. And a significant loss of solids by the effluent also results in a deterioration of its quality.

Figure 4 shows how pH values were also affected in all three tests. In the experiment employing zinc, the initial value was 7.36, rising to pH 8.5 on the third day and maintaining this level until the end of the experiment. The rise in pH coincides with the loss of solids in the activated sludge process and, consequently, it may be attributed to the demise of microorganisms present in the unit, which then release their cellular components, basically proteins, into the medium. Since $ZnSO_4$ is an acid hydrolysis which would cause a decrease in the pH of the medium, the excretion of the cellular compounds from dead microorganisms must be sufficiently significant to compensate for the decrease in pH, resulting from the purely chemical effect produced by the dissolution of the metallic salt.

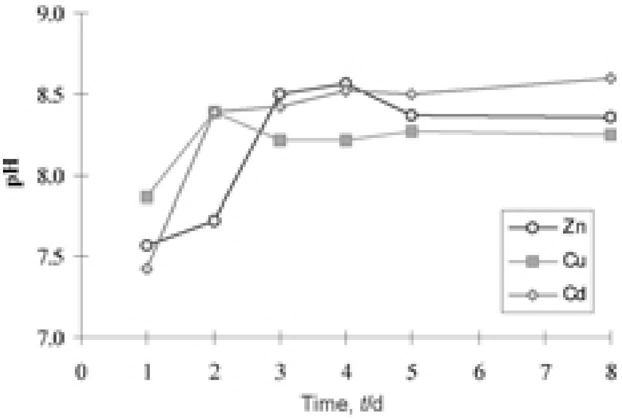


Fig. 4 – *Evolution of the pH of the mixed liquor in each of the three experiments*

During the experiment in which copper was added as a toxic compound, the initial pH value in the aeration tank was 7.9, higher than that recorded in the zinc assay. This value rose rapidly to 8.4 on the second day of the test. Thereafter, pH remained practically constant at 8.2 until interruption of the reaction on the eighth day. The pH variations were less marked than in the zinc experiment, although the speed at which change took place was greater, meaning that the toxic effect of copper is more rapid. The reasons for the increase in pH will no doubt be the same as those described above: death of the microorganisms and liberation of cellular components, principally protein, into the medium.

The effect of the addition of cadmium on pH levels is similar to the two cases already described, although, the increase is slightly greater than in the case of zinc and copper.

Effect of the metals on the levels of microbial activity

In all three assays, microscopic examination showed that as time passed and the concentration of the metal in the activated sludge process increased, the number of species fell considerably, probably due to the toxic and inhibiting effect which metals have on microorganisms.

Figure 5 illustrates the SOUR results obtained in each of the three experiments. In the case of zinc, there was an almost 50% reduction in uptake rate in the first two days, indicating the powerful inhibiting effect exerted on microbial respiratory activity in the reactor. Thereafter the SOUR rate continued practically unchanged for the remainder of the test.

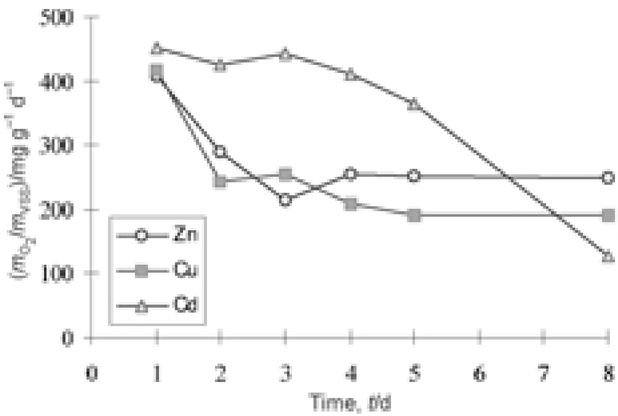


Fig. 5 – *Behaviour of the SOUR variable in each of the three experiments*

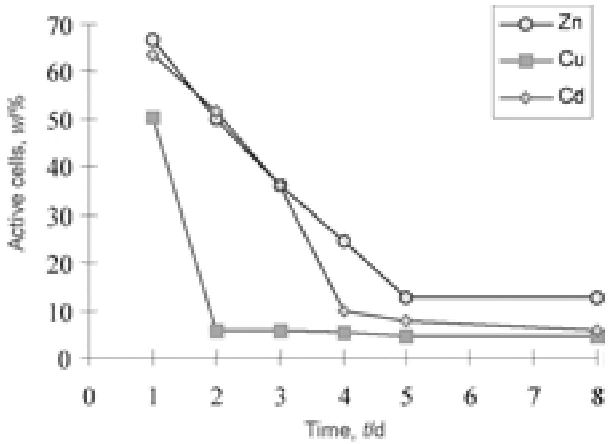
In the case of copper, the effects were more marked. The specific oxygen uptake rate showed a greater reduction on the second day of the test, confirming the more powerful inhibitive effects and lethal consequences, which this metal has on the microorganisms present in the activated sludge process. From the second day on and until the end of the test, the uptake rate remained practically constant, reaching a minimum value of 191.4 mg g^{-1} d^{-1} O_2 inVSS on the eighth day.

Different results were obtained in the experiment to which cadmium was added. Over the first three days, the SOUR rate hardly varied. Probably, this is because inhibited respiratory activity in the microbial community means that a significant number of the microbiota die off, whilst the remaining population maintains its respiratory level. However, from the fourth day onwards the rate dropped, coinciding with the disappearance of the majority of the ciliated protozoa. This decrease continued until the final day of the test, when the lowest value for all three metals was recorded, namely 126 mg g^{-1} d^{-1} O_2 inVSS (Figure 5).

The pattern of specific oxygen uptake rate (SOUR) recorded for these metals closely reflects the results obtained for VSS (Figure 2).

The results obtained in the total and active biomass counts, using epifluorescent microscope, also confirm the SOUR results.

Figure 6 shows the fraction of active cells present in the experiments with each of the three metals. In all three tests, a powerful inhibitory effect is produced, although, as with the other variables, the effects produced by zinc are the least marked.



F i g . 6 – *Results of the fraction of active cells variable in each of the three experiments.*

Copper provoked a strong inhibitive effect from the second day of the test, when the active population was reduced from 50 % to 6 %. There was very little variation from then on until the eighth and final day.

As with the other variables, cadmium provoked greater inhibitory effects than the other metals, reducing the active population present in the reactor by more than 90 % over the eight days of the test—signifying an almost total inhibition of the biomass. However, reflecting again the results with the other variables, the decrease is not as marked as in the case of copper.

Conclusions

The inhibition of microbial activity resulting from metal-bearing activated sludge is well documented, but not fully understood poorly documented and there are few examples of continuous flow. Consequently, we have been unable to compare the results we obtained. The importance of our research lies in the fact that the experiments were carried out on a continuous basis over a period of eight days. This permitted a comprehensive study of the different effects, which the addition of metals produce in the activated sludge process, and reproduced in a more tangible way, the process occurring in a wastewater treatment plant at industrial level.

Moreover, given that the tests performed in this research were carried out with virtually no modifications made to the environmental conditions of the activated sludge, and that the microfauna population was well established, with a bio-sludge index of 10,[20] the results obtained may be extrapolated to other activated sludge treatment plants.

Cardinaletti et al.[21] showed that wastewaters, containing zinc between 0.6 and 1.2 mg L^{-1}, have no negative effects whatsoever on the protozoan communities in activated sludge plants. The experiments performed by *Madoni* et al.[22] revealed that a mass concentration of 0.57 mg L^{-1} of zinc provokes the demise of only certain species, and that concentrations of over 10 mg L^{-1} are toxic for the majority of microorganisms present in activated sludge processes. In our research, the concentration of zinc employed (3 mg L^{-1} Zn) began to produce toxic effects from the third day of the test, when the active microorganisms were reduced from 50 % to 35 %. Our calculations revealed that at that time, the amount of metal absorbed by the bacterial floc surface was 2 mg kg^{-1} Zn in the sludge. Consequently, we can conclude that concentrations greater than this inhibit the development of the microorganisms present in the activated sludge process.

In the case of copper, *Shuttkeworth* and *Unz*,[23] found that concentrations greater than 63.5 μg L^{-1} inhibit entirely the growth of filamentous bacteria. For their part, *Dilek* and *Yetis*,[24] showed that concentrations greater than 10 mg L^{-1} Cu have no effects on the kinetics of the process. By *Madoni* et al,[22] 24 hour exposure to a concentration of 3 mg L^{-1} caused 67% of the population to die off. In our case, using a concentration of 2 mg L^{-1} Cu on a continuous flow, growth was significantly inhibited, with the active population being reduced to just 6% by the second day of the test. This would correspond to a copper adsorption mass fraction in the sludge of 0.6 mg kg^{-1} Cu in the sludge.

With regards to cadmium, only Madoni, in his research[22] determined a LC_{50} value of 0.31 mg L^{-1} for the different species existing in an activated sludge plant. In our research this metal produced the most negative effects on the microbial population, reducing it by over 90 % during the test period.

From our results, we can conclude that cadmium is the most highly toxic metal for the microbial communities present in the activated sludge process, followed by copper, and lastly zinc.

These results match those obtained by other authors using different methodologies to our own.

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