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Copper-Mediated Selective Removal of Nitrification Inhibitors from Industrial Wastewaters

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Current efforts to upgrade wastewater treatment plants focus on biological N and P removal. N removal is often limited by the rate of nitrification, which is sensitive to low concentrations of specific inhibitors present in industrial effluents. Many known nitrification inhibitors act by complexing the copper enzyme ammonium monooxygenase and can be counteracted via Cu(II) addition. We found here, using both a batch inhibition test and bench-scale semi-continuous activated sludge reactors, that the inhibition of nitrification with four industrial wastewaters was greatly reduced when Cu(II) was added and that Cu(I) or even particulate Cu(0) were at times the most effective forms. Conventional remedial additives, such as activated carbon or zeolite, were not effective. Because direct Cu(II) addition would lead to undesirable contamination of waste-activated sludge, a pretreatment process using reusable surface-immobilized forms of copper was sought. Surface-immobilized copper [e.g., particulate Cu(0), Cu(II)-loaded chelating resins] was more efficient than soluble Cu(II) in detoxifying an industrial effluent and model inhibitor solution; XPS analysis suggests a surface complexation mechanism. The immobilized copper phases can be reused repeatedly in a pretreatment contact unit and regenerated upon saturation.

Introduction

Modern wastewater treatment plants rely on the nitrification−denitrification sequence to remove inorganic N. Nitrification is, however, sensitive to some specific organic inhibitors (*1*−*4*) if they are not quickly destroyed during treatment, and this occurs in some industries (*5*, *6*). This causes both environmental damage to receiving water bodies, due to ammonium and the inhibitory substances themselves, and financial penalties to the polluting industries.

The PACT process is typically applied to remedy this situation (*7*). It involves the addition of powdered activated carbon (PAC) directly to the activated sludge basin. Through its high sorptive capacity for poorly soluble weakly polar organics, PAC has been found to be effective in reducing the toxicity of certain types of industrial wastewaters and in easing their biological treatment (*8*, *9*). One disadvantage of the PACT process is its relatively high cost (typically USD 0.5−1/m^3 wastewater) given the high doses needed. An alternative approach has been to increase the sludge retention time (SRT) in order to favor adaptation to and biodegradation of toxic organics (*10*). When successful, this approach may remain impractical in some plants because long SRT (e.g., 40 days) is required to overcome the inhibition. This paper describes two novel pretreatment steps involving surface-immobilized copper that are shown to remove nitrification inhibitors selectively from industrial wastewaters.

Experimental Section

Materials. Samples of a latex wastestream and of equalized final effluents were obtained on a bimonthly basis from two carpet factories (abbreviated B and E) located near Gent, Belgium. The fourth source of samples was a chemical process industry (CPI) located near Gent, which fully inhibits the nitrification in the trickling filters used for water purification. Since the source of nitrification inhibitors had been tracked down to the production unit, we used this particular substream diluted with tap water to obtain the same concentration of inhibitors as in the actual final effluent. The average COD values amounted to 60, 1.3, 3.0, and 5.0 g/L for the latex wastewater samples and the CPI, textile B, and textile E effluents, respectively. TKN was in the range 0.8−1.2 g of N/L in all effluents. The main nitrification inhibitors present in the CPI effluent are dithiocarbamates (thiram) and diethylthiourea, which are being produced in the factory.

The powdered activated carbon (PAC), type Ligno-Sedeg, was purchased from Goldschmidt (Brussels), and the zeolite, a class 4A synthetic aluminosilicate (Vegobond XD), was obtained from Condea Augusta (Milan). The particulate Cu(0) (75-μm particle diameter) was purchased from Aldrich. The iminodiacetate (IDA) chelating resin, type Lewatit TP 207, was manufactured by Bayer (Leverkusen, Germany) and obtained from Caldic (Hemiksem, Belgium). The other chelating resin (Dowex M4195) was a bis(picolylamine) (PLA) functionalized matrix devoid of fixed charges (*11*) and was obtained from Dow Chemical Company (Midland, MI). Both resins had a macroporous polystyrene matrix shaped in 1-mm bead form. Both resins were loaded with Cu(II) by repeated contact with a solution of $CuSO_4$ at pH 4.5 (0.5 g of Cu/L) until saturation, followed by extensive rinsing with distilled water.

Batch Inhibition Test with Stock Nitrifying Culture. A 25-L enrichment stock culture of nitrifiers was maintained in order to provide actively-nitrifying sludge samples for routine batch inhibition tests. The culture was fed once daily with (g/L·d): NH_4Cl (1.2), KH_2PO_4 (0.1), $CaCO_3$ (0.4), and the nutrient supplement Nutrifloc (0.1) (Avecom N.V., Gent, Belgium). The hydraulic retention time (HRT) was 2 days, and the pH was maintained at 7.0 by means of automated addition of 1 M NaOH. Sludge concentration was about 25 g of MLSS/L (2.9 g of MLVSS/L). The nitrifying activity of recently-fed sludge was estimated via O_2 uptake rate (OUR) measurements and amounted to ca. 30 mg of O_2/L·h (the OUR of whole microbial community, including heterotrophs, was ca. 36 mg of O_2/L·h).

A typical inhibition test involved a positive control, a negative control, and the actual test treatments. For the positive control, 50 mL of recently-fed stock nitrifying sludge was mixed with 10 mL of 1 M phosphate buffer (pH 7.0) and 4 mL of a 1-g NH_4Cl-N/L solution and adjusted to 200 mL with tap water. The mixture was then aerated (for a period depending on the exposure time being examined) and transferred to a closed vessel equipped with a magnetic stirrer and an O_2 electrode. Dissolved O_2 measurements (voltammetric O_2 sensor) were continuously computer-recorded, and the slope dO_2/dt, i.e., the total OUR due to both nitrifiers

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and heterotrophs, was calculated for every 50-s interval. Once a stable value for OUR total has been reached, 15 mg/L (final concentration) allyl thiourea (ATU), a specific inhibitor of ammonium oxidation, was added. The dO_2/dt slope measured 5 min after ATU addition was recorded as the OUR by heterotrophs. The difference between total and heterotrophic OUR was taken to represent the OUR by nitrifiers (nitrifying activity). The negative control consisted of the same procedure except that the tap water was replaced by an appropriate dilution of one of the industrial wastewaters. The actual test treatments were carried out like the negative control except that the wastewater had been premixed with the phosphate buffer and a potential remedial additive for 15 min (unless otherwise noted) before being used.

All tests were repeated several times with different batches of the various industrial effluents collected on different dates. The inherent variability of the chemical composition of these different batches resulted inevitably in wide variations of the measured variables. As a consequence, results of representative runs rather than mean results are reported. When repeated with replicate samples of test nitrifying sludge, the measurement of the OUR (by nitrifiers) had a coefficient of variation of 3.5% ($n = 5$). This same CV value rose to 14% when the different sludge samples were exposed to a thiram solution (50 mg/L) that had been fully detoxified with a suspension of 4 g of particulate Cu(0)/L.

Inhibition Test in Semi-Continuously Fed SCAS Reactors. The potentially effective remedial additives, i.e., those identified during the initial screening carried out with the short-term inhibition test, were further tested in longer term experiments with semi-continuous activated sludge (SCAS) reactors. The SCAS reactors were 2-L agitated flasks held at 28 °C and containing 1 L of mixed liquor (MLSS = 2.5−3.0 g/L) initially obtained from a municipal wastewater treatment plant. The pH was kept in the 7.0−8.0 range. The reactors were fed three times a week with skim milk (COD:TKN ratio of 100:5) diluted appropriately to obtain a volumetric loading rate (B_v) of 1 g of COD/L·d with a HRT of 5 d. The sludge wastage rate was adjusted to obtain a SRT of 10 d. Occasionally, the feed solution was spiked with one of the industrial effluents at a final concentration roughly equivalent to an IC_{80} dose, i.e., the concentration necessary to cause an 80% inhibition of the nitrification (as determined in the batch inhibition test). On these occasions, remedial additives were added to and briefly mixed with the feed solutions of various reactors. Five reactors received either PAC (100 mg), zeolite (100 mg), $CuSO_4$ (1.5 mg Cu), PAC (100 mg) combined with $CuSO_4$ (1.5 mg Cu), or no additive (control). Following each toxic shock, the nitrifying activity was determined both by the ATU/OUR method (see above) and by the NH_4^+ and NO_x time courses (50 or 20 mg of NH_4Cl-N was always added together with the toxic pulses for this purpose). Replicate runs of the toxic shocks were performed at different time intervals with the same reactors once these had recovered from the previous inhibition.

Analyses. The surface composition of particulate Cu(0) was analyzed by X-ray photoelectron spectroscopy (XPS) that probes a 3−5 nm thick layer at the surface (*12*). This was performed with a VG ESCA 3 Mk II spectrometer from Vacuum Generators, equipped with an Al anode. The peak position was determined by setting the maximum of the C_{1s} peak at 284.8 eV, typical of carbon bound only to carbon and hydrogen. The atom concentration ratios were determined from peak areas, using Wagner sensitivity factors (*13*); this allowed the computation of the surface concentration of the different elements, expressed as atom fraction (excluding hydrogen). Soluble copper was measured by flame atomic absorption spectroscopy. Ammonium and TKN were determined with automated Kjeltec apparatuses (distillation methods) and COD via the chromate/sulfuric acid method.

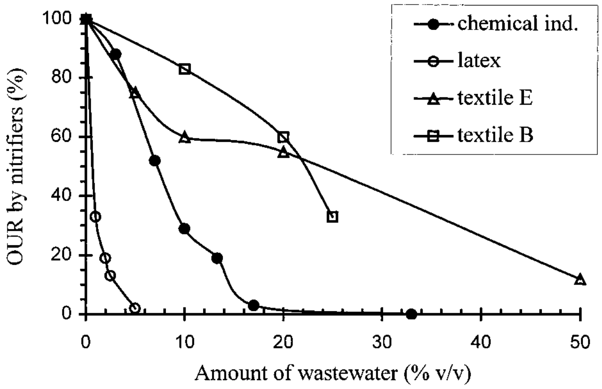

FIGURE 1. Inhibition of the nitrifying activity in a batch inhibition test with stock nitrifying sludge mixed with various amounts of four different industrial effluents.

Results and Discussion

Chemical Inhibition of Chemoautotrophic Nitrification by Various Industrial Wastewaters. The extent to which various industrial wastewaters may inhibit nitrification in wastewater treatment plants was examined by using a short-term batch inhibition test with a stock culture of nitrifying microorganisms. The nitrifying activity after exposing the sludge for ca. 1 h to various amounts of different industrial effluents is plotted in Figure 1. The effluent concentrations causing a 50% inhibition of the nitrifying activity (IC_{50}) were 1, 8, 22, and 24% v/v for the latex, chemical process industry (CPI), textile B, and textile E effluents, respectively. The IC_{90} values were 3, 14, ca. 30, and 50% for the same effluents. While these values were fairly reproducible with different batches of the latex or CPI effluents, considerable variations in IC values were noted with textile effluents sampled on different dates (the values reported are means of three typical effluents). These values are consistent with those reported in previous studies in which nitrification IC_{25} values of various textile factory effluents fell in the range of 11−27% v/v (*6*, *14*).

The data plotted in Figure 1 point toward rather than ascertain potential inhibition because the immediate or short-term effects of inhibitors are not in general a good guide to their influence when applied continuously to activated sludge plants operating under steady conditions. We noted, for example, that the extent of CPI-mediated inhibition increased drastically after several hours exposure. Tomlison et al. (*15*) observed a similar phenomenon with nitrapyrin in a long-term activated sludge system where the inhibition was greater than might have been expected from the results of a short-term toxicity test. Such increases of the inhibition with time of exposure may be linked to the inhibitory effect of certain products formed after microbial attack, as carbon disulfide or cysteine for example (*3*, *16*), or may be linked to the inhibition of growth in the absence of inhibition of activity. The long-term inhibition caused by the latex and textile effluents used in this study was on the other hand much less (see SCAS experiment below) than what was expected from the data plotted in Figure 1. Tomlison et al. (*15*) observed a similar phenomenon with allylthiourea (ATU), the inhibitory effect of which completely vanished after a few hours exposure. Such decreases of the inhibition with time of exposure may be due to biodegradation, sorption, or adaptation.

Despite its poor predictive value of long-term inhibition, the short-term batch inhibition test remains a valuable tool for the rapid screening of large numbers of potential remedial additives. The real efficacy of these additives should, however, be validated in longer term experiments.

TABLE 1. Efficacy of Various Additives To Neutralize Nitrification Inhibitors Present in Various Industrial Wastewaters (Diluted to $IC_{50}-IC_{90}$ Values)

	$CuSO_4$ (3 mg of Cu(II)/L)	zeolite (0.5 g/L)	PAC[a] (0.5 g/L)
textile effluent B	+++[b]	+/−	++
textile effluent E	+	+	++
latex effluent	+++	−	+++
CPI effluent[c]	++	++	++

[a] Powdered activated carbon. [b] +++, ++, and + refer to full elimination, halving, or small reduction of the inhibition, respectively; − refers to a lack of effect on the inhibition. This notation was chosen to account for the relatively large variability among replicate experiments carried out with industrial effluent batches collected on different dates. [c] Chemical process industry.

Screening Potential Remedial Additives. The batch inhibition test was carried out with a final concentration of the various industrial effluents equivalent to an IC_{50-90} dose (as determined in Figure 1). Positive hits during this preliminary screening procedure were compounds that when being pre-mixed with an industrial effluent were able to alleviate the inhibition caused by this effluent. This preliminary screening identified PAC as a potentially effective additive since a reduction by half or more of the extent of nitrification inhibition was achieved with all four industrial effluents when these had been premixed with 0.5 g of PAC/L (final concentration) (Table 1). PAC is indeed known for its ability to remove a wide range of organic toxicants from industrial effluents and owes this ability to its highly porous structure and apolar surface characteristics (*7*). Though less effectively than PAC, zeolite (0.5 g/L) could also counteract to some extent the nitrification inhibitors present in the textile and CPI effluents. Zeolites also possess a high surface area, but given the sub-nanometer size of their pores and large cation exchange capacity, zeolites are conventionally used for the removal of heavy metals and other small cationic species.

The addition of $CuSO_4$ to the industrial effluents before the mixing of the latter with the nitrifying sludge alleviated the inhibitory effect of all four effluents (Table 1). The extent of the copper effect ranged from a slight alleviation, by ca. 25%, of the inhibition caused by one of the textile effluents up to a full protection against the inhibitors present in the other textile effluent or in the latex waste. The maximum effect dose of Cu(II) ranged from 1 to 5 mg/L (final concentration), depending on the nature of the wastewater (Figure 2). As early as 1946, Lees (*17*) found that the addition of $CuSO_4$ to soil columns reverted the inhibition of nitrification induced by various organic compounds. The mechanism of action of Cu(II) may involve an interaction either directly with the bacterial cells or rather with the organic inhibitors present in the wastewater since copper ions are known for their very strong affinity for organic compounds (*18*). The former option may entail, for example, the alleviation of Cu deficiency inasmuch as minute additions of copper have been reported to stimulate the nitrifying activity (*3*). This explanation is, however, unlikely here because Cu(II) alleviated the inhibition of nitrification only when it was premixed with the wastewaters and not when it was added to the sludge immediately after the addition of wastewater. This finding suggested that Cu(II) somehow deactivates the nitrification inhibitors present in the various industrial effluents.

Validation of the Effectiveness of the Various Potential Remedial Additives. As already pointed out, short-term inhibition tests do not necessarily reflect the long-term inhibition that would occur in activated sludge systems operated at steady state because they do not take into account such processes as biodegradation, adaptation, or chronic intoxication. Attempts were made to validate the findings with semi-continuous activated sludge (SCAS) reactors. Different reactors were fed three times weekly with skim milk that occasionnally was spiked with one of the industrial effluents together with a potential remedial additive (except in the control reactor). The industrial effluents were diluted to obtain a final concentration equivalent to an IC_{80} dose (as determined from the data plotted in Figure 1).

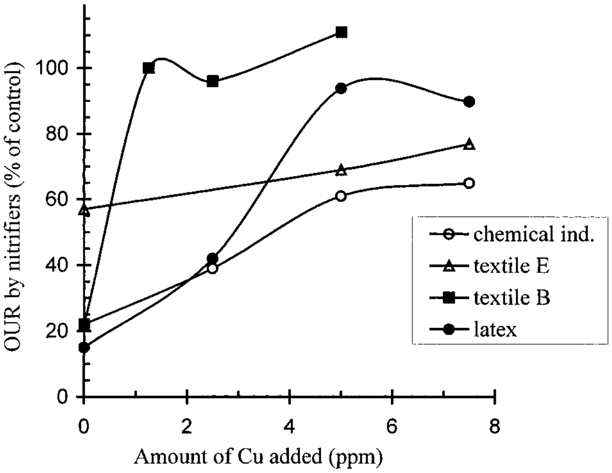

FIGURE 2. Alleviation of the inhibition of nitrification (short-term batch inhibition test) as a function of the amount of $CuSO_4$ added to various types of industrial effluents.

The OUR by nitrifiers measured a few hours after each toxic shock was always severalfold higher in the two reactors amended with copper (1.5 mg of Cu/L of mixed liquor) than in the control reactor (Figure 3). The magnitude of the nitrification enhancement in the Cu-amended reactors relative to the control was 2-, 6-, 6-, and 15-fold ($n = 2-4$) during the toxic shocks with textile B, textile E, latex, and CPI effluents, respectively. The reactors receiving either PAC (100 mg/L of mixed liquor) or zeolite (100 mg/L of mixed liquor) were equally inhibited as the control reactor (Figure 3).

These findings were confirmed by the time courses of NH_4^+ concentration after each toxic shock (NH_4Cl had been spiked together with each industrial effluent in order to follow its disappearance rate). Ammonium diappearance rate does not, however, reflect the nitrifying activity as accurately as OUR measurements do because it is also affected by ammonification and assimilatory ammonium uptake. The general conclusion remains however the same (i.e., the two reactors treated with Cu are always characterized by a faster ammonium disappearance rate than the control reactor whereas PAC and zeolite have no remedial effect on the nitrification inhibition) (Figure 4). The reason the remedial effect of PAC and zeolite could not be confirmed in the SCAS reactors may have been that a smaller amount was added here as compared to the batch inhibition test (0.1 vs 0.5 g/L).

Continuous addition of a soluble Cu(II) salt may eventually lead to the intoxication of the activated sludge. The total Cu concentration required to cause inhibition is however greatly variable because the rate of nitrification is sensitive to the concentration of free copper rather than total copper, with an IC_{50} value of about 0.02 mg of free copper/L of activated sludge (*18*). This explains why activated sludges remain unaffected by copper concentrations several orders of magnitude higher than those inhibiting pure cultures of nitrifiers inasmuch as virtually all the copper dosed to activated sludge binds to organic ligands (*18, 19*). It took, for example, 7 days to inhibit nitrification in a fill-and-draw activated sludge system receiving 10 mg of Cu/L·d (*15*). We

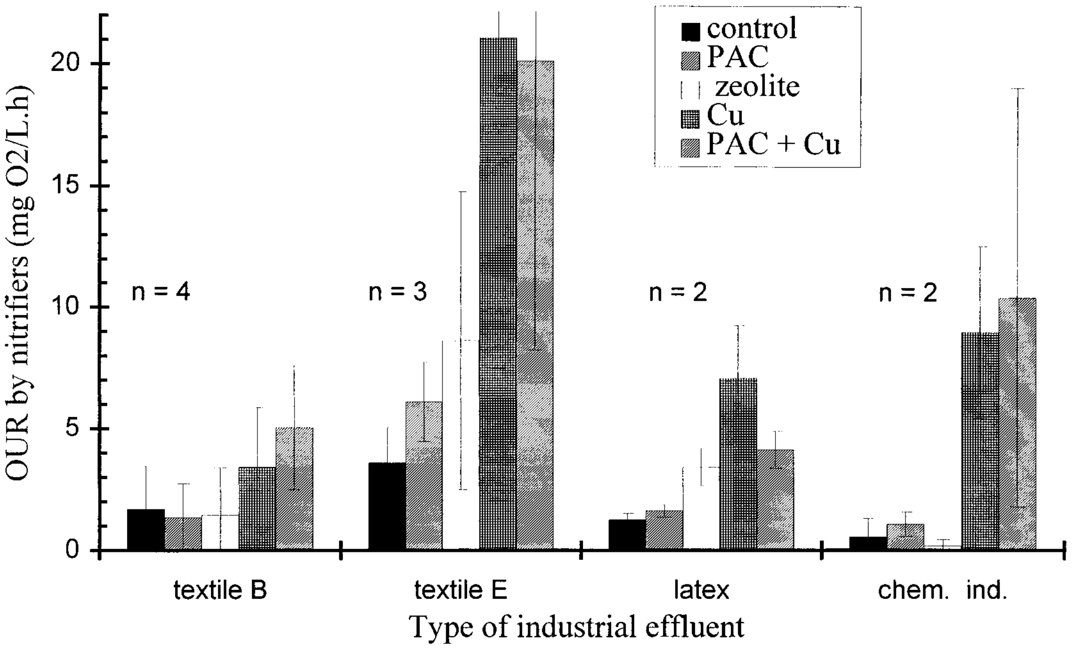

FIGURE 3. Nitrifying activity in semi-continuous activated sludge reactors during toxic shocks with various industrial effluents amended with different remedial additives. Plotted data are mean values of *n* replicate experiments; lines indicate the standard deviation.

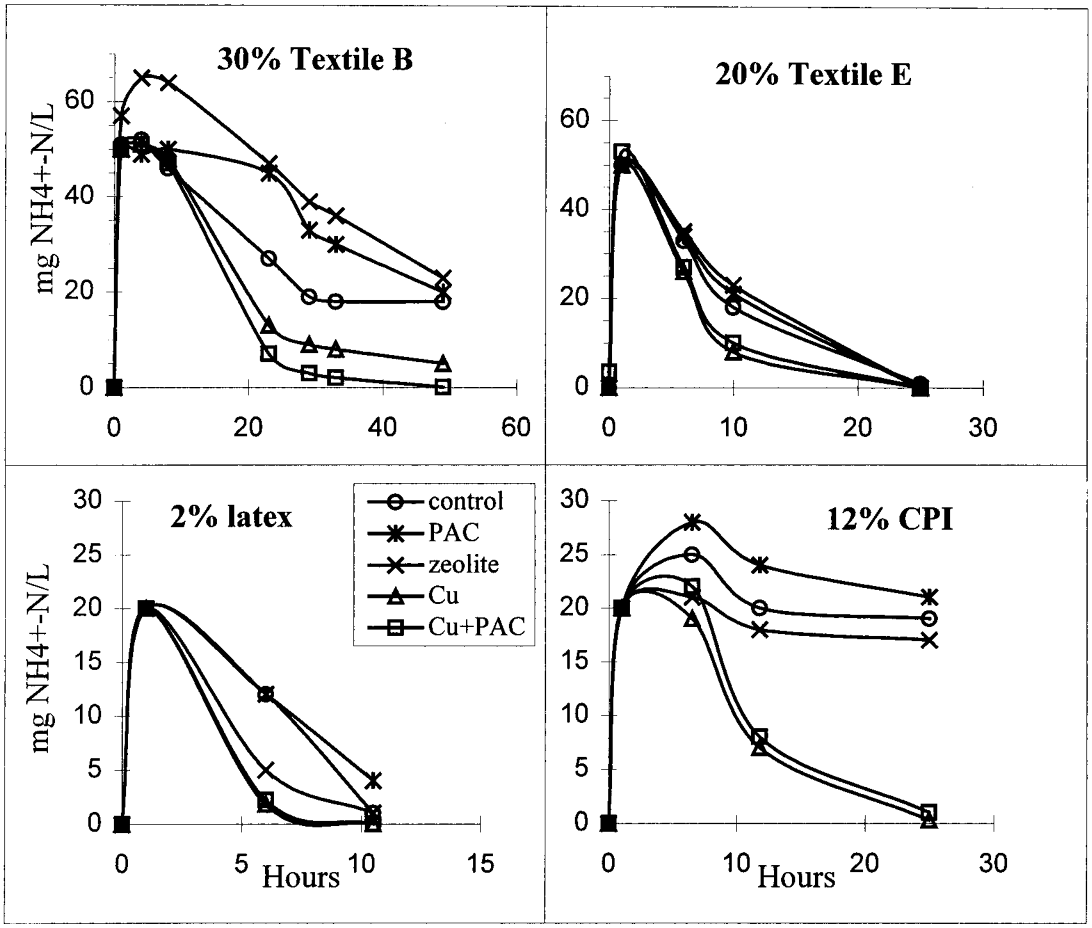

FIGURE 4. Time courses of ammonium concentration in semi-continuous activated sludge reactors during toxic shocks with various industrial effluents amended with different remedial additives. Percent values indicate the final concentration of each industrial effluent. Representative profiles of several replicate experiments are shown.

observed here that the continuous addition of 1 mg of Cu/L·d to the SCAS reactors led to a progressive impairment of COD removal, even though nitrification was enhanced. This together with the undesirable accumulation of copper in the waste sludge makes it impractical to detoxify industrial effluents via direct addition of copper salts.

Mechanism of Cu-Mediated Deactivation of Nitrification Inhibitors. Ammonium monooxygenase (AMO), the enzyme

TABLE 2. Efficacy of Divalent Cu(II) and Monovalent Cu(I) To Neutralize Specific Nitrification Inhibitors[a]

	O_2 uptake rate (OUR) by nitrifiers[b] (% of non-inhibited control; $n = 2$)		
	no Cu	+ Cu(II)	+ Cu(I)
8-quinolinol (10 ppm)	10	94	66
KCN (0.5 ppm)	0	57	51
bipyridyl (50 ppm)	10	41	10
allylthiourea (0.1 ppm)	18	22	17
thiourea (0.1 ppm)	1	6	59
o-phenanthroline (40 ppm)	9	9	43

[a] Cu/inhibitor molar ratio was 3, except for allylthiourea and thiourea for which it was 60). [b] Measured after 1–4-h exposure time to the inhibitors.

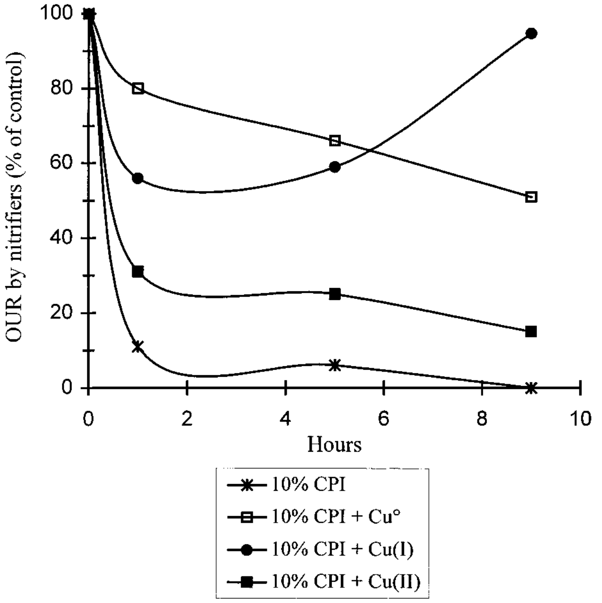

FIGURE 5. Nitrifying activity (batch inhibition test) after different exposure times of stock nitrifying sludge to 10-fold diluted chemical process industry effluent, which had prior to sludge exposure been mixed overnight with either 5 mg of Cu(II)/L, 60 mg of Cu(I)/L, or 5 g of particulate Cu(0)/L. Plotted data are percent values of uninhibited control. A negative control not treated with copper is included.

catalyzing the oxidation of ammonium to hydroxylamine, is thought to be a copper enzyme because it is blocked by copper chelators and because the addition of copper can reverse the inhibition brought about by these chelators (*20*–*23*). For instance, the effect of nitrapyrin, a potent nitrification inhibitor of common usage on agricultural land, i.e., N-Serve, is completely reversed by the addition of Cu(II) (*24*). Along the same line, Lees (*17*) found that Cu(II) reversed the inhibition of the nitrification caused by three organic compounds added to soil columns. In fact, most specific inhibitors of the nitrification are metal chelators, and their mode of action is thought to involve the formation of a complex with the AMO-bound copper leading to the incorporation of the chelator into the overall enzymic structure, thus modifying its configuration detrimentally (*4*, *16*, *21*, *23*, *25*). The weakest chelators cause the weakest inhibition, and the stronger chelators cause the strongest inhibition (*1*). AMO can therefore be viewed as the major vulnerability of chemoautotrophic ammonium oxidizers (*26*).

On the basis of these findings, it seems likely that the mechanism of action of copper as a remedial additive involves the formation of a complex with copper-chelating inhibitors, thereby preventing these to cross the bacterial outer membrane and/or to bind the AMO embedded in the cytoplasmic membrane. This hypothesis was further tested with six model nitrification inhibitors (all metal chelating agents) using the batch inhibition test with the nitrifying sludge. It was found that the inhibitory effect of 8-quinolinol, KCN, and bipyridyl was greatly or completely eliminated when these compounds had been mixed with Cu(II) (at a Cu/X molar ratio of 3) prior to use (Table 2). Copper did not however inactivate allylthiourea, thiourea, and *o*-phenanthroline (Table 2). The two latter compounds were however inactivated upon mixing with monovalent copper (Table 2). This finding suggests that thiourea and phenanthroline may perhaps bind Cu(I) preferentially over Cu(II) [AMO-bound copper cation is thought to switch back and forth between its +1 and +2 oxidation states during the catalytic cycle (*26*)].

If copper counteracts nitrification inhibitors via a complex formation mechanism, it may well be that other metal cations sharing with copper the ability to bind to organic matter could act similarly. The detoxification achieved with Cu(II) could not, however, be reproduced with either Fe(III), Zn(II), or Mg(II) (*15*, *20*). Co(II) on the other hand could somewhat mimick, though to a much lesser extent, the remedial effect of copper (data not shown).

The ability of added copper to complex and thus counteract nitrification inhibitors may be dependent on the solution chemistry. For instance, alternative metal ligands such as OH^-, HPO_4^{2-}, or HCO_3^- may compete with the inhibitors in complexing the added copper. We did not observe however that the presence of phosphate or bicarbonate affected the efficiency of Cu to detoxify the CPI effluent. This was not surprising since organics usually have a stronger affinity for Cu ions than inorganics, and this was certainly the case with dithiocarbamate, a high-affinity copper complexant present in the CPI effluent. On the other hand, zeolite, a cation exchanger, voided the remedial action of Cu(II).

Removal of Nitrification Inhibitors with Particulate Cu(0). As stated earlier, the continuous addition of $CuSO_4$ to wastewaters would be impractical because the accumulation of copper onto the sludge may eventually inhibit water treatment processes and produce contaminated sludge. It was therefore attempted to find a surface-immobilized form of copper sharing the remedial activity of soluble copper. It was shown above that Cu(I) was more effective than Cu(II) at neutralizing certain nitrification inhibitors, but inasmuch as Cu(I) is unstable in water wherein it rapidly undergoes a disproportional reaction yielding Cu(0) and Cu(II), it may well be that insoluble Cu(0) had been the actual active species.

We examined the potential remedial activity of particulate Cu(0) by mixing overnight a 10-fold dilution of CPI effluent with either $CuSO_4$ (5 mg of Cu/L), CuCl (60 mg of Cu(I)/L, insoluble), or particulate Cu(0) (5 g/L) and ran the batch nitrification inhibition test with the settled mixes. While the untreated CPI effluent completely inhibited the nitrification after several hours of sludge exposure, Cu(II)-treated effluent displayed a nitrification rate equivalent to 20% of the non-CPI-amended control (Figure 5). Both Cu(I) and particulate Cu(0) proved more effective than Cu(II), with an average nitrification rate equivalent to 70% of the control. The high effectiveness of Cu(I) is difficult to interpret because Cu(I) decomposes rapidly to the 0 and +2 oxidation states. With the particulate Cu(0), only low levels (0.2–0.6 mg/L) of dissolved copper were measured during serial 2-h agitation periods of 1 g of particulate Cu(0)/L in bicarbonate buffer solutions. XPS analysis (Table 3) of particulate Cu(0) suspended in distilled water reveals a carbon peak with a shape typical of the organic contamination found on high surface energy solids, indicating that about one-fourth of the carbon is bound to a heteroatom (*27*). It also shows a phosphorus peak (P_{2p} peak at 190.6 eV). The C, O, and P

TABLE 3. Surface Compostion of Copper Powder Determined by XPS

	atom fraction (%)					
conditioning	**C**	**O**	**P**	**S**	**N**	**Cu**
distilled water	48.0[a]	40.6	6.4	–[b]	–	5.0
CPI effluent	51.8	16.5	–	12.9	6.2	12.6

[a] 35% carbon bound only to carbon and hydrogen; 13% carbon bound to a heteroatom. [b] –, below detection limit.

concentrations suggest that the surface is coated with phosphate, presumably copper(II) phosphate, and organic matter. With CPI-exposed particulate Cu(0), phosphorus is no longer detected, but nitrogen and sulfur are found with a S/N atom ratio of 2.0, consistent with the formula of the main inhibitor present in the CPI effluent; i.e., the pesticide thiram [or bis(dimethylthiocarbamoyl) disulfide $(CH_3)_4C_2N_2S_4$]. The C_{1s} peak is too broad to provide information on chemical functions. The S_{2p} peak is observed at 161.6 eV, which is characteristic of a low oxidation stage (*28*). These XPS analyses together with the low levels of dissolved copper suggest that the high effectiveness of particulate Cu(0) can be attributed to chemisorption of nitrification inhibitors. Side experiments conducted with pure solutions of thiram demonstrated that this compound was removed from solution during mixing with particulate Cu(0) because this treatment effectively detoxified the thiram solutions (batch nitrification inhibition test).

To be of any practical use in a treatment plant, the particulate Cu(0) would have to be reusable and, moreover, should be amenable to regeneration once the adsorption sites have become saturated. The possibility of reusing the particulate Cu(0) was investigated with five consecutive batches of 10-fold-diluted CPI effluent that were treated with a single batch of particulate Cu(0) (2 g/L). There was no significant decrease of the effectiveness of treatment as determined by the batch inhibition test. The treatment capacity of particulate Cu(0) was further examined by contacting the particles (2 g/L, 3 h, pH 6) with CPI effluent of variable strength and subjecting the supernatant (after a short settling period) to the batch inhibition test with nitrifying sludge. Particulate Cu(0) was decreasingly effective as the strength of the CPI effluent increased (Figure 6). With full-strength effluent, the nitrification was initially fully blocked, but the bacteria were able to gradually recover, up to a level equivalent to 31% of the non-CPI-treated control (Figure 6). A dose of 2 g of particulate Cu(0)/L seems therefore just sufficient to remove the bulk fraction of the nitrification inhibitors, leaving behind a residual amount of inhibitors that the bacteria were able either to adapt to or to degrade. Once the treatment capacity of the particulate Cu(0) has been reached, it must be regenerated. Spent particles were obtained by contacting 2 g of particles with 0.2 g of thiram in 1 L [the sorption capacity was 20−30 mg of thiram/g of particulate Cu(0)]. These particles had lost their remedial activity since nitrification remained fully inhibited by a solution of 20 mg of thiram/L even when this had been contacted with the spent particulate Cu(0). Regeneration was easily achieved by washing the spent particles with 1 N HCl, as shown by the fact that these HCl-washed particles had recovered their ability to fully detoxify a solution of thiram (20 mg/L).

Removal of Nitrification Inhibitors with Cu(II)-Loaded Chelating Resins. Another form of surface-immobilized copper of potential use for wastewater treatment can be prepared with chelating polymers or resins. Copper-loaded resins are routinely used in immobilized metal-ion affinity chromatography and count various emerging applications such as cheap assay kits for glucose level in blood (*29*) or removal of selected wastewater contaminants such as phosphates and organic acids (*11, 30*). The principles of this technology rely on the fact that the coordination number of immobilized Cu(II) is only partially satisfied by the solid-phase chelating groups (cross-linked to the resin matrix) leaving the possibility for soluble chelators to complex the copper atoms.

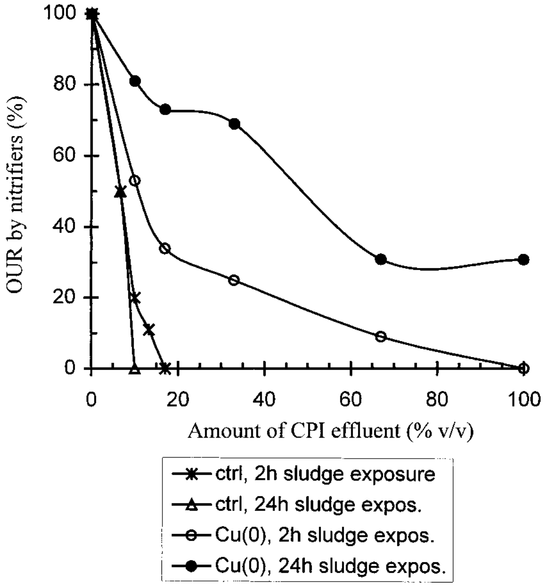

FIGURE 6. Efficiency of removal of nitrification inhibitors by particulate Cu(0) from CPI effluent of increasing strength as determined by the batch inhibition test after 2 and 24 h sludge exposure. The contact time between particulate Cu(0) and the effluent was 3 h at pH 6.

Two types of chelating resins were tested, one functionalized with iminodiacetate (IDA) groups and the other with bis(picolylamine) (PLA) groups. One advantage of these resins relative to the particulate Cu(0) is their high density of available copper atoms: the fully loaded IDA and PLA resins contained 24 and 21.5 g of Cu(II)/L of resin, respectively, as determined by Cu measurements in spent regenerate liquors. Both resin types, after being loaded with cupric ions, were tested for the removal of nitrification inhibitors from full-strength CPI effluent. To this end, the latter was contacted with the resin beads for 2 h in a sequential batch reactor (at pH 7.5 and pH 4.5 for the PLA and IDA resins, respectively), after which the resin beads were recovered by settling and reused. The extent of inhibitors' removal was measured with the batch inhibition test.

Both resin types removed the nitrification inhibitors very effectively from the full-strength CPI effluent and could be reused many times without loss of efficacy (Figure 7). Untreated CPI effluent fully inhibited nitrification (not shown). Leakage of copper from the resins was ca. 1 mg/L (these copper ions could, if necessary, be removed from the wastewater stream by adding some virgin resin to the Cu(II)-loaded resin). The process at hand during the contact period must therefore involve the immobilization of the inhibitors onto the resins. Because the Cu-free resins were unable to remove the nitrification inhibitors from the CPI effluent, this immobilization process must be taking place at the Cu sites. Side experiments, carried out with thiram as a model compound, indicated that the Cu(II)-loaded PLA resin became saturated and ineffective when the molar ratio of applied thiram over loaded copper reached ca. 0.1.

Besides nitrification inhibitors, a broad spectrum of organic ligands will form complexes with the resin-bound Cu atoms (e.g., sugars, organic acids, proteins, hydroxyl,

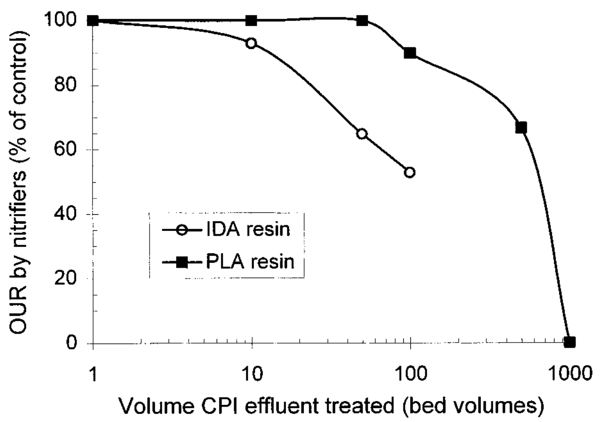

FIGURE 7. Nitrification rate in unadapted sludge after 24 h exposure to 3 vol of CPI effluent pretreated with Cu(II)-loaded resins in a sequential batch reactor. One bed volume corresponds to the volume of resin beads in the contacting vessel; e.g., the data for 100 bed volumes correspond to the 10th 100-mL aliquot being treated with the same 10-mL resin batch or to the first 100-mL aliquot treated with 1 mL of resin (in fact, it was the average of these two).

carbonate, ammonia, phosphate, or water). The weaker ligands are however displaced by the stronger ones by a process of ligand exchange. This was demonstrated with the particulate Cu(0) since the phosphate ions initially present on the copper surface were replaced by organic compounds upon exposure to the CPI effluent (see above). As a consequence of ligand exchanges, the Cu atoms along with resin usage should become gradually saturated with the highest-affinity copper chelators, which are also the most powerful nitrification inhibitors (*1*). Following extended use, the resins' effectiveness started to drop gradually after treating a volume of full-strength CPI effluent equivalent to 500−1000 bed volumes (Figure 7). Regeneration of spent resin was then easily achieved with 2.3 N HCl in the case of IDA resin and 10 N H_2SO_4/7.5 N NH_3 in the case of the PLA resin.

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