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Shoou Yuh Chang

Ju-Chang Huang
Missouri University of Science and Technology

Yow Chyun Liu

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Effects of Cd(II) and Cu(II) on a Biofilm System

By Shoou-Yuh Chang,[1] A. M. ASCE, Ju-Chang Huang,[2] M. ASCE, and Yow-Chyun Liu[3]

Abstract: There is little information available in the literature to describe the effect of heavy metals on a fixed-film biological waste treatment system. The primary objective of this study is to evaluate the toxic effect of Cd(II) and Cu(II) on a fixed-film biological reactor (rotating biological contractor) in treating a sugar waste. Different levels of cadmium (5 and 20 mg/L) and copper (1, 5, 10, 25, and 50 mg/L) were dosed to a three-stage RBC unit treating an influent of 300 mg/L sugar solution. Experimental results indicated that the treatment efficiency was not adversely affected by the presence of copper at a concentration of 10 mg/L or less. However, when the copper concentrations were increased to 25 and 50 mg/L, removal of dissolved organic carbon was reduced by 7 and 10%, respectively. Toxicity was also observed when cadmium was present. Biological treatment efficiency was reduced by about eight percent when the cadmium concentration was either 5 or 20 mg/L. The major portion of the dosed metal was effectively retained by the biofilm. The efficiency of metal removal in the treatment system varied from 85 to 95% for cadmium, and 30 to 90% for copper, depending on their initial concentrations in the feed solution.

Introduction

High concentrations of heavy metals are toxic to most microorganisms and often cause serious upsets in a biological waste treatment system. However, when the metal concentration is not too high, and a proper acclimation process is allowed, a biological system may be used to remove a certain amount of metal without itself being adversely affected.

Toxicity of a metal in a given biological treatment system generally depends upon its species and concentration. Toxicity also depends upon the type of influent, its strength, and the extent of system acclimation. It has been reported that only soluble metal ions are able to cause toxicity (17). The degree of toxicity has also been found to decrease with an increasing organic strength of wastewater (1). The tolerance of a biological system for heavy metals can be greatly enhanced by acclimation. One theory of acclimation is that when a metallic cation causes damage to or inactivation of one or more critical enzymes, additional enzymes can be produced to replace the damaged ones. If the damage becomes too severe, an alternate or a shunt metabolic pathway may be created. Because of the narrow spectrum of anaerobic and nitrifying life forms, they are more susceptible to the toxicity of heavy metals than aerobic

[1]Asst. Prof. of Civ. Engrg., Univ. of Missouri-Rolla, Rolla, MO.
[2]Prof. of Civ. Engrg. and Dir., Environ. Research Center, Univ. of Missouri-Rolla, Rolla, MO.
[3]Grad. Research Asst., Dept. of Civ. Engrg., Univ. of Missouri-Rolla, Rolla, MO.

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heterotrophic microorganisms (7). In an aerobic treatment process, heavy metals have been found to adversely affect several species of microorganisms without affecting the overall efficiency of biological treatment.

The removal of heavy metals in a biological system is mainly attributed to the sorption of soluble and finely-divided metal particulates by biological flocs. It has been found that the microbial removal of heavy metals consists of an initial rapid uptake followed by a slow, but consistent long-term uptake phase. The rate of uptake is greatly affected by the pH of the solution (9). Sludge age as well as the extent of acclimation can also affect the extent of metal removal in an activated sludge system.

Most of the past research on metal toxicities has been directed toward the suspended-growth activated sludge system. The effects of heavy metals on fixed-film systems, such as a rotating biological contactor (RBC), remain relatively unexplored. The objective of this study is to evaluate the effect of cadmium (Cd) and copper (Cu) on the biological stabilization of dissolved organic carbon (DOC) in a laboratory-scale, three-stage RBC unit treating a synthetic sugar waste. Cadmium was spiked to the influent at 5 and 20 mg/L, respectively, in different experimental runs, while copper was spiked separately at 1, 5, 10, 25, and 50 mg/L.

Literature Review

Numerous research studies have been conducted to investigate the toxic effects of heavy metals on biological treatment processes, but most of them were directed toward the suspended-growth activated sludge system. As this study deals with only cadmium and copper, this literature review will present information on the toxic effects of these two metals on various biological systems.

Sierp and Fransemeier (14) investigated the effect of copper on an activated sludge system. They found that copper at a concentration of 1 mg/L had a detectable influence in increasing effluent turbidity and decreasing the extent of nitrification, but showed only a slight impact on the efficiency of BOD removal. Gellman (5) also reported that 1 mg/L of copper effects the activated sludge process. He found that the copper toxicity decreased at a pH between 5 and 6.5. Shumate and Moulton (15) indicated that an acclimated activated sludge system was able to reduce copper by 80 to 85% when the influent copper concentration was 50 mg/L. They also concluded that copper at 45 mg/L was not sufficient to halt the aerobic biological activity. Heukelekian and Gellman (6), as well as Sawyer et al. (12), had found that the toxicity of heavy metals on the activated sludge process was a function of pH, substrate concentration, amount of biomass, and temperature.

McDermott et al. (8) comprehensively studied the effect of copper on an activated sludge pilot plant. Continuous feedings of 10, 15, and 25 mg/L of copper sulfate and 0.4 to 10 mg/L of copper cyanide were undertaken during their study. In all cases, the suppression of the BOD or COD removal was less than 4%; when the copper concentration was 1 mg/L or below, there was no detectable effect on BOD or COD removal efficiency. The removal of copper was found to be between 50 and 75% when the influent copper concentrations were between 0.4 and 25 mg/L. For unacclimated systems, copper cyanide was found to be

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more toxic than copper sulfate. They also observed that slug doses for a few-hour duration with up to 50 mg/L copper as copper sulfate or 10 mg/L copper as copper cyanide had only a slight effect on the treatment efficiency. However, a 4-hr slug dose of copper as copper sulfate in concentrations exceeding 50 mg/L severely reduced the treatment efficiency in an unacclimated system. It took about 100 hrs for the system to return to the normal operating efficiency.

Barth et al. (2) wrote a summary report on the effect of heavy metals on various biological units. Using the data compiled from an activated sludge pilot plant, they indicated that the aerobic biological treatment system could tolerate up to a total heavy metal concentration of 10 mg/L (Cr, Cu, Ni, and Zn), either singly or in combination, with only about a 5% reduction of the BOD removal efficiency. They further concluded that a small dose of metal could noticeably reduce the treatment efficiency, but a much larger dose failed to further decrease efficiency significantly. Nitrifying microorganisms were also reported to be sensitive to heavy metals. The presence of some heavy metals reduced the degree of sludge bulking problems in an activated sludge system. They reported that 5 mg/L of copper in influent sewage was the highest dose that could still allow satisfactory anaerobic sludge digestion.

To investigate the effect of chemical speciation on the toxicity of heavy metals, Sunda et al. (18) used a completely mixed reactor to show that the toxic effects of copper and cadmium to several species of grass shrimp were a function of only the free ion form rather than the total metal concentration. Thus the toxicity could also be reduced through the process of acclimation. Shelley and Sherrard (13) found that 50 mg/L of cadmium in an acclimated activated sludge system caused the same suppression of the COD removal as 10 mg/L in a nonacclimated system. Obviously, an acclimated biological system was able to resist a much higher cadmium shock loading than the nonacclimated one.

The combined effect of two different heavy metals on an activated sludge system was conducted by Bagby and Sherrard (1). They found that toxicity could be minimized by increasing the mean cell residence time and organic loading rates.

Cheng et al. (3) studied the heavy metal uptake by an activated sludge unit. They reported a 74–86% uptake of cadmium and 81–82% uptake of copper when the influent metal concentration varied from 2.1 to 25.5 mg/L. Similarly, Oliver and Cosgrove (10) reported a 60% soluble copper removal in a 6.5 MGD activated sludge plant. Nelson et al. (9) also investigated the fate of heavy metals in an activated sludge system and found that the adsorption of cadmium increased from 15–20% at pH 4 to greater than 90% at pH 10, but the maximum adsorption occurred at a pH of 7 to 8.

Toxic effects of heavy metals have been much less studied on the fixed-film biological system than on the suspended-growth system. Reid et al. (11) employed rotating drums to grow attached slimes and then used a synthetic waste with radioactive phosphorus (P^{32}) to monitor the microbial metabolism rate. They indicated that 1.5 mg/L of cadmium was able to decrease the biological reaction rate by 73.5%. The percentage of heavy metal uptake decreased rapidly as the metal concentration was increased. They also conducted a study on the effects of heavy metals in a pilot trickling filter plant and reported that domestic sewage containing 2 mg/L or less of Cr^{+6} did not significantly affect the BOD removal. A more recent study by Dehkordi (4) indicated that the COD removal increased when the influent copper concentration was increased from 0 to 0.5 mg/L in a six-stage RBC system. The copper concentration could be increased to 10 mg/L without affecting the COD removal efficiency.

Methods and Materials

Biological Reactor Setup.—A bench-scale, three-stage RBC unit was constructed of plexiglass. The unit, which consisted of three semicircular compartments with a total volume of 8.55 L (Fig. 1), provided a four-hour hydraulic detention time when the influent feed was 35 ml/min. Each compartment contained three circular disks (25.1 cm diam) mounted on a stainless-steel shaft. The disks, when rotated at a constant speed of 25 rpm, had an edge velocity of 10 m/min, about one-half the value for full-scale plant operation. The total surface area of the nine disks was 9,120 cm^2, of which 40% was submerged in liquid. One small paddle was attached to each set of three disks to prevent sloughed biomass from being deposited on the bottom of the tank. Other pertinent physical data and operating parameters of the RBC unit are listed in Table 1.

Feed Solution.—A synthetic feed solution was prepared daily with dechlorinated tap water, sucrose, ammonium sulfate, monobasic and dibasic potassium phosphate, and other mineral supplements. The composition of the synthetic solution and other characteristics are shown in Table 2. The substrate solution was prepared and held in a large drum (200-L capacity). A positive displacement pump was used to feed the reactor.

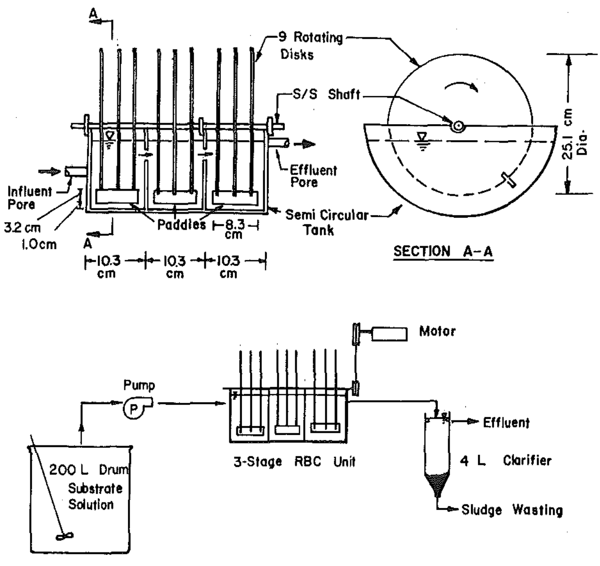

FIG. 1.—Schematic Diagram of Fixed-Film Biological Reactor

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TABLE 1.—Physical Data and Operating Conditions of RBC Unit

Parameter (1)	Value (2)
No. of stages	3
Total liquid volume of all stages	8.55 L
No. of disks per stage	3
Total disk surface in all stages	9,120 cm^2
Flow rate	50 L/day
Hydraulic loading rate	54.8 L/day/m^2 surface
Total detention time	4 hrs
Rotating speed	25 rpm (10 m/min tip speed)
Organic loading rate	6.92 g DOC/m^2-day
Liquid volume/surface area	9.38 L/m^2

Initial Start-Up.—To start biological growth on the disk surfaces, the reactor was seeded with primary effluent from the Southeast Wastewater Treatment Plant in Rolla, MO. During the seeding period, the reactor was operated in a batch mode with a fill-and-draw technique once or twice each day. Batch operation lasted for two weeks before continuous flow operation was started with a synthetic sucrose solution. At this time, monitorings of the dissolved organic carbon (DOC) in both influent and effluent began. As soon as the biological system reached a steady state, as evidenced by a fairly constant DOC removal in the day-to-day operation, either cadmium or copper was added at a controlled rate to determine the effects of these two metals on the DOC removal efficiency.

Testing Procedure.—Levels of dissolved organic carbon were measured with a Beckman infrared carbonaceous analyzer. A Perkin Elmer 305A, atomic absorption spectrometer was used to measure levels of both cadmium and copper. Other chemical analyses were conducted according to standard methods (16). The soluble and insoluble metals were differentiated by filtering the samples through Whatman 934-AH glass microfiber filters. Thus the soluble metal concentrations reported in this study are only operational and may be somewhat higher than the true

TABLE 2.—Feed Solution Characteristics

Constituents (1)	Concentration (mg/L) (2)
Sucrose ($C_{12}H_{22}O_{11}$)	300
$(NH_4)_2SO_4$	150
K_2HPO_4	158
KH_2PO_4	321
$MgSO_4 \cdot 7H_2O$	30
$MnSO_4 \cdot H_2O$	3
$FeCl_3 \cdot 6H_2O$	0.15
Alkalinity	267.4 mg/L $CaCO_3$
Hardness	282.4 mg/L $CaCO_3$
pH	7.2 to 7.4

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solubility of metal ions because of the possible presence of colloidal metallic particulates.

Results and Discussion

Toxic Effect of Cadmium on DOC Removal.—The DOC removal rate was monitored as soon as continuous operation of the RBC was started. After about 6 weeks of operation, the system appeared to have reached a steady state condition. At that time 5 mg/L of cadmium (in the form of $CdCl_2$) was added to the feed solution for 24 hrs. The feeding of cadmium was then stopped to allow the system to recover from the shock of cadmium loading. On the 12th day, at which time the DOC removal efficiency had returned to the pre-shock level, 20 mg/L of cadmium was again spiked for three days before resuming the normal feeding. Fig. 2 illustrates the influent and effluent DOC concentrations and the corresponding DOC removal efficiency of the RBC system. The toxic effect of cadmium on the efficiency of DOC removal is also reflected in Fig. 2. For example, DOC removal efficiency dropped approximately 8% (from 92% to 84%) after 5 mg/L of cadmium was added on the first day. This adverse effect on the removal efficiency persisted for 5 days, after which period the removal efficiency returned to approximately 90%. On the 12th day, after being dosed 20 mg/L of cadmium for 3 days, removal efficiency was reduced from 90% to 82–85% for 4 days, after which it gradually came back to 90% on the 22nd day. Note that the introduction of 5 mg/L cadmium for 1 day induced a toxic effect on the system similar to that produced by the introduction of 20 mg/L cadmium for 3 days. This phenomenon may have been caused by acclimation during the first feeding of cadmium. This would have made the microorganisms considerably more tolerant to the second shock load. During these two dosings, the concentrations of influent soluble cadmium were 1.9 and 2.4 mg/L, respectively, while the corresponding effluent concentrations were

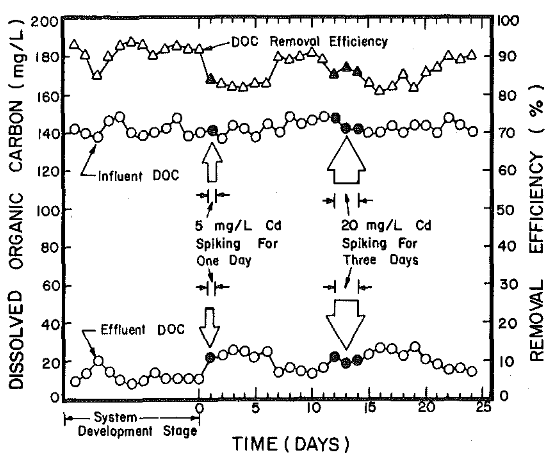

FIG. 2.—Effect of Cd(II) on DOC Removal Efficiencies in RBC Unit

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0.1 and 0.5 mg/L. The color of the RBC biofilm gradually changed from brown to orange after cadmium was added. The orange color persisted for about 2 weeks after 20 mg/L cadmium was fed for 3 days.

Toxic Effect of Copper on DOC Removal.—After the cadmium study was finished, the RBC system was thoroughly cleaned and soaked in chlorine solution for 48 hrs. Then the system was started with primary effluent in order to eliminate any carryover effect from the previous microbial community. During the reseeding process, the DOC removal efficiency was monitored continuously. When the system reached a steady state (fairly constant DOC removal), five concentrations of copper (1, 5, 10, 25, and 50 mg/L in the form of $CuSO_4$) were dosed on different occasions in an increasing sequence. Before each dosing, it was made certain that the RBC system had regained its original treatment efficiency. The influent and effluent DOC concentrations are shown in Fig. 3. Apparently, the DOC removal efficiency either improved slightly or remained practically at the same level when 1, 5, or 10 mg/L of copper was added. This indicated that at these concentrations, copper produced no toxic effect on the RBC system. But when the concentration was increased to 25 mg/L, the effluent DOC level began to increase; the trend continued for about 7 days. The lowest DOC removal was around 70%. The average suppression of the DOC removal efficiency was about 10%. After the 25 mg/L shock, the system was inhibited for about 10 days before its DOC removal efficiency was restored to the original 90%. When the copper dosage was increased to 50 mg/L for another 5 days, the DOC removal efficiency sharply decreased to 75% on the subsequent day. Beyond that, the data appeared to be somewhat erratic, but the average depression of the DOC removal efficiency was about 7%.

At a copper dosing level of 10 mg/L, the biofilm color was observed to change from light brown to dark blue or black, possibly because of the adsorption of copper precipitates. The biofilm was also less adherent, and a lot of sloughing occurred. The volatile fraction of the biofilm decreased because of the entrapment/adsorption of copper precipitates in the biofilm. For example, the VSS/TSS ratio of the biofilm decreased from an average of 0.85 when no more than 10 mg/L of copper was spiked to an average of 0.75 when 25 and 50 mg/L of copper were added.

The soluble copper concentrations in the influent and effluent were also determined so that the copper removal efficiency could be assessed. The influent soluble copper concentration increased from 0.2 mg/L when 1 mg/L of copper was spiked to 0.45 mg/L when the spike was increased to 5 mg/L. As the copper spiking was further increased to 10, 25, and 50 mg/L, respectively, the influent soluble copper concentration was increased to 1.0, 1.1, and 1.2 mg/L. The soluble copper removal efficiency was found to vary from 30 to 90%, depending mainly upon the influent concentration. The actual concentrations of free cupric ion could not be measured because of the low fraction of free cupric ion in a solution having a pH between 7.2 and 7.4.

Examinations of the biomass at 100 magnifications with a light microscope were made before and after 5 mg/L of copper spiking. The population of protozoa and rotifers decreased significantly after the metal was added while the population of bacteria remained approximately the same. Obviously, the higher forms of microorganisms were more sen-

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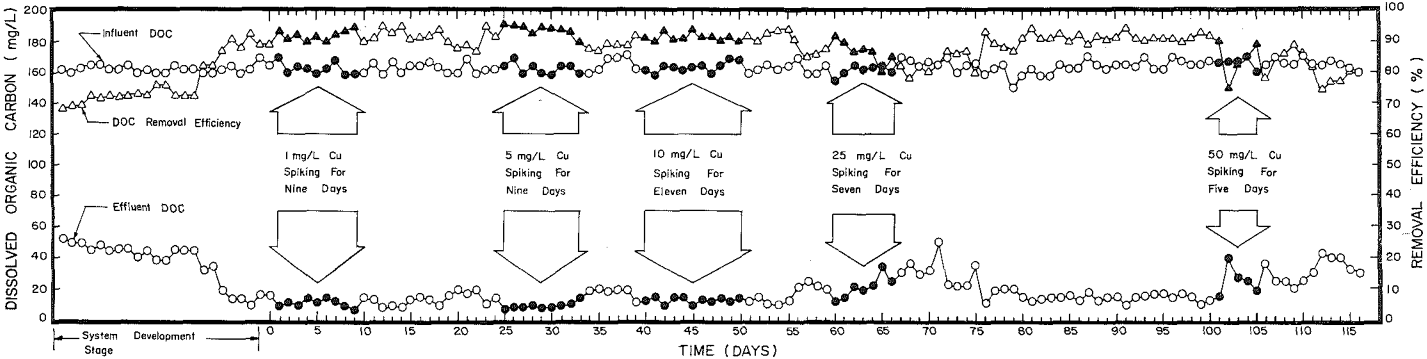

FIG. 3.—Effect of Cu(II) on DOC Removal Efficiencies in RBC Unit

sitive to copper toxicity than the prevalent bacteria in the system.

Effects of Metals in a Parallel Activated Sludge Unit.—Cadmium and copper toxicities in a suspended-growth activated sludge system were also studied. This system was completely mixed and had a hydraulic retention time of 6 hrs. The mixed liquor suspended solids (MLSS) were maintained at 2,500 mg/L, and the sludge age was about 8 days. The activated sludge was given exactly the same sucrose feed with the same spikings of cadmium and copper as those for the RBC unit. The results are shown in Figs. 4 and 5. As shown in Fig. 4, at a cadmium spiking of 5 mg/L for 24 hrs, the DOC removal efficiency dropped from 90% to 85% for the first day. The efficiency dropped further, to 80%, before it came back to 86% at the third day and to 90% at the fourth day. When the cadmium spiking was increased to 20 mg/L, and lasted for 3 days, the DOC removal efficiency dropped sharply from 90% to 78% for the

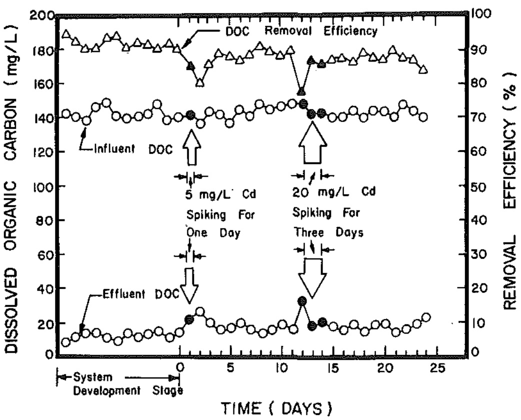

FIG. 4.—Effect of Cd(II) on DOC Removal Efficiencies in Activated Sludge Unit

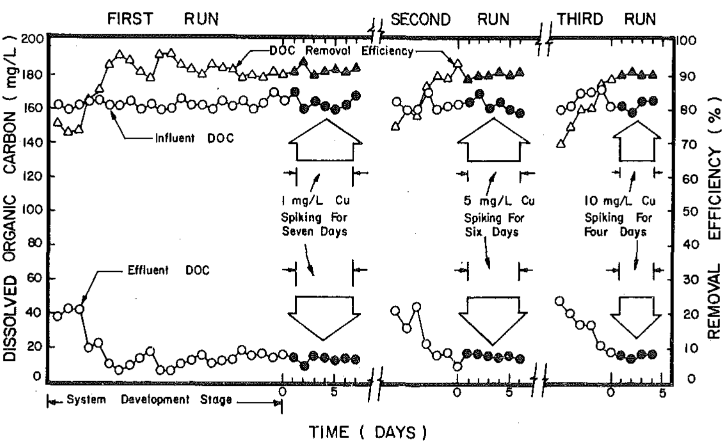

FIG. 5.—Effect of Cu(II) on DOC Removal Efficiencies in Activated Sludge Unit

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first day, but the efficiency returned to 88% the next day. Thus even though the second metal spiking was much higher and lasted longer, the suppression of DOC removal efficiency was not as severe as that for the first time. When these data were compared to the effect of cadmium on the RBC system, the activated sludge unit appeared to recover faster than the RBC system. The average soluble cadmium removal efficiencies in the activated sludge unit were 98% and 36%, respectively, when 5 and 20 mg/L of cadmium were spiked.

As for copper toxicity, the spiking of 1, 5, and 10 mg/L did not appear to reduce the DOC removal efficiency in the activated sludge unit (Fig. 5). These results were similar to those for the RBC unit. However, even though the DOC removal was not affected at these spiking conditions, the biological sludge did not settle well, and a substantial amount of mixed-liquor suspended solids (MLSS) was lost from the system. Because of this situation, it was difficult to maintain an adequate concentration of MLSS in the experimental unit. Thus only three experimental runs were made and the unit was stopped at the fourth day of the 10 mg/L copper spiking. The average soluble copper removal efficiency was between 50% and 55% for both the 1 and 5 mg/L spiking conditions.

Conclusions

The effect of cadmium and copper on the DOC removal efficiency in a fixed-film RBC system was evaluated in this study. Different levels of cadmium (5 and 20 mg/L) and Cu (1, 5, 10, 25, and 50 mg/L) were sequentially spiked in a 300 mg/L sugar solution in a shock load manner with intervals allowed for the recovery of the DOC removal efficiency. Based on the results obtained in this study, the following can be concluded:

1. The first introduction of 5 mg/L of cadmium for 1 day to the RBC system had a similar effect on the DOC removal efficiency as the spiking of 20 mg/L for 3 days after the system recovered from its first cadmium loading. In both cases, the suppression of the DOC removal was 8%.
2. The DOC removal efficiency in the RBC system was not affected when 10 mg/L or less of copper was spiked for 8 to 10 days. Suppression of DOC removal was observed to be 7–10% when the copper concentration was increased to 25 and 50 mg/L.
3. Both cadmium and copper were effectively retained by the biofilm. The average soluble metal reduction varied from 85–95% for cadmium, and 30–90% for copper, depending on the influent metal concentration.
4. In terms of the suppression of the DOC removals, the toxic effects of cadmium and copper to the RBC and the activated sludge systems were much the same. However, in the activated sludge system biological activities seemed to recover faster. On the other hand, the biological sludge was slow to settle when copper was present.

Acknowledgments

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