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The impact of toxicity of metals on the activity of ureolytic mixed culture during the precipitation of calcium

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ABSTRACT

In this article, the inhibitory impact of metals on substrate utilization and microbial carbonate precipitation (MCP) by ureolytic mixed cultures (UMC) was investigated with glucose and mineral medium under batch conditions. The IC_{50} (toxicant concentration eliciting 50% inhibitory effect) values were determined from the BOD values of samples. Inhibition, expressed as the value of 50% inhibitory effect (IC_{50}), was evaluated by the decrease in substrate removal using BOD tests. The effect of toxicity of metals on substrate degradation, IC_{50} values, was found to increase in the following order: Cd(II) > Cu(II) > Pb(II) > Cr(VI) > Ni(II) > Zn(II). Nitrification a possible phenomenon in the biocatalytic process was observed in several samples and this inhibited the precipitation of soluble calcium. During the removal of calcium from industrial calcium-rich wastewater, toxicity of metal at higher metal concentrations and possibility of nitrification at higher sludge ages should be considered.

1. Introduction

Calcium-rich water tends to deposit inside the pipes and on the appliances during water use. Precipitates of calcium are released from young landfill leachates, reverse osmosis concentrates, industrial processes such as bone processing, paper recycling, and sugar processing [1–3] and industrial wastewaters neutralized with lime [4]. Current classic chemical crystallization reactors are based on the addition of a base [NaOH or $Ca(OH)_2$] in the presence of nucleation sites (e.g., sand grains). These reactors are highly effective but often require complex monitoring and control and consequently, can give rise to high alkaline effluents [5], which requires neutralization for the following biological treatment.

A nowel method for the removal of biological calcium from industrial wastewater, microbial carbonate precipitation (MCP) process based on microbial urea hydrolization, was reported by a research group [3–7]. Microorganisms have long been known to catalyze the precipitation of $CaCO_3$ under natural environments such as oceans, soils and saline lakes, in a process referred to as MCP [8,9]. The generally accepted mechanism of MCP involves an increase in pH and dissolved inorganic carbon (DIC) of a given environment through normal physiological activities [9]. Under aerobic conditions, heterotrophic microbial urea hydrolysis (one of the known MCP processes) occurs, processes, in which 1 mol of urea is hydrolyzed by the urease enzyme to 2 mol of ammonia and 1 mol of carbon dioxide. These products can subsequently react to form ammonium and carbonate ions, which can further react and precipitate as $CaCO_3$ in the presence of soluble calcium ions. In addition to these factors, precipitation process of $CaCO_3$ is favoured by the presence of nucleation sites, e.g. sand, suspended solids, bacteria in the medium.

Numerous industries, such as automotive, metal producing, electroplating, battery manufacturing, mining, electric cable manufacturing, tannery, steel and textile, release various concentrations of heavy metals like cadmium, lead, chromium and copper into wastewaters. These heavy metals are toxic to aquatic ecosystems and human health and also get accumulated in organisms beyond tolerance levels [10]. Heavy metals can exert stimulatory, inhibitory, or even toxic effects on biochemical reactions depending on their concentrations. Zero-valent heavy metals are considered as having no biological activity, however, simple or complex forms of ionized heavy metals can dramatically affect the performance of biological systems. Trace amounts of the so-called essential heavy metals (such as Fe, Zn, Ni, Cu, Co) have been found to stimulate microbial growth, while no beneficial biochemical role has been assessed, up to now, for other ones (like Hg, Ag, Cd, As and Au), which are considered as non-essential substances [11–14].

Many metal ions can exert toxicity on biological systems through multiple biochemical pathways simultaneously. The various mechanisms of metal toxicity in microorganisms are (1) substitutive ligand binding, (2) redox reactions with sulphur groups, (3)

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Table 1
Experiment set up composition and conditions

Composition/conditions	Pb(II)	Cd(II)	Cr(VI)	Zn(II)	Cu(II)	Ni(II)
Experiment periods (h)	120	238	216	156	160	134
Sludge VSS (mg VSS L^{-1})	199	101	197	204	232	220
Sludge SS (mg SS L^{-1})	241	251	250	252	252	250
Metal concentration (mg L^{-1})	0–64	0–128	0–128	0–128	0–256	0–512
Effective volume (mL)			97			
Glucose-COD (mg L^{-1})			750			
Urea (mg L^{-1}; 10 mM)			600.6			
Ca^{2+} (mg L^{-1}; 10 mM)			400.8			
Mineral medium			As described in text			
Initial pH			7.00 ± 0.10			

Fenton-type reactions, (4) inhibition of membrane-transport processes, and (5) electron siphoning [15]. Workentine et al. [16] reported that the correlations are different for biofilm and planktonic cells; therefore, the chemical mechanisms of toxicity are concluded to be different for the two modes of cell growth. Biofilms may reduce the toxicity of the metal by altering the physiology to ensure protection of the sensitive chemical targets of the reactive metal species. The primary mechanism of metal removal involves a metabolism-independent process (passive uptake) through ion-exchange phenomena, complexation with negatively charged groups, and adsorption and precipitation by extracellular polymeric substances (EPS) [17]. Harrison et al. [15] have identified several phenomena that protect biofilm cells from toxic metal species. The various components of the resistance and tolerance of biofilm are as follows: (1) metabolic heterogeneity introduced by population structure, (2) extracellular signaling events affecting the physiology of the biofilm, (3) immobilization of metal by biosorption, (4) bioinorganic reactions of metal ions with the metabolites of biofilm, (5) adaptive responses to metal ions, (6) persister cells and genetic rearrangements, and (7) mutations and phenotypic variations.

MCP based on microbial urea hydrolization is a novel method for the removal of calcium from calcium-rich waste such as young landfill leachates, reverse osmosis concentrates, bone processing, paper recycling, and sugar processing. pH and alkalinity increase as ureolytic mixed culture uses COD as carbon source and urea as nitrogen source. Then, Ca in wastewater precipitates as $CaCO_3$. However, MCP process has not been studied extensively with the consideration of environmental variables. This study was basically carried out to determine the inhibitory impact of heavy metals on the ureolytic mixed cultures (UMC) in synthetic calcium-rich wastewater.

2. Materials and methods

2.1. Synthetic wastewater

The synthetic wastewater was prepared by considering the simulating medium strength of municipal and liner paper manufacturing wastewater proposed by Holakoo et al. [18], and Kim et al. [19], respectively. The medium had the following composition in mg L^{-1}: glucose-COD (750); urea (600); $CaCl_2$ (17); $MgSO_4{\cdot}7H_2O$ (1541); KH_2PO_4 (132); $FeCl_3{\cdot}6H_2O$ (19); $CuSO_4{\cdot}5H_2O$ (0.118); $MnSO_4{\cdot}H_2O$ (0.123); $ZnCl_2$ (0.229), $CoCl_2{\cdot}6H_2O$ (0.404); Na_2CO_3 (477) and $NaHCO_3$ (378). Sulphuric acid was used to maintain a pH of 7.00 ± 0.10. Composition of synthetic wastewater resulted in COD/N/P of 100/37/4 ratio. Urea of excessive concentration (600.6 mg L^{-1}) was used in the medium to stimulate MCP process. During batch experiments, $CaCl_2$ was also added to the medium as total Ca^{2+} of 400.8 mg L^{-1}.

2.2. Sludge production and experimental set up

Cultures were obtained from a fed-batch reactor receiving synthetic wastewater and devoid of calcium and used as the initial inoculum for the batch experiments. The sludge retention time (SRT) and biomass concentration in this reactor were aproximately 10 days and 2000 mg L^{-1}, respectively, calculated as mixed liquor volatile suspended solids (MLVSS). Dissolved oxygen concentrations during sludge production were found to exceed 2 mg L^{-1}. The produced sludge had a VSS/SS ratio of 0.80 and a sludge volume index (SVI) of 100 mL g^{-1}.

The inhibitory effect of metals on bacterial urea hydrolysis was evaluated on the basis of the removal of BOD during the experimental period and differences in parameters such as alkalinity, pH, NO_3^-, NH_4^+, SS, and VSS after the experimental period. Eighteen BOD bottles were used and their samples were analyzed for every metal. Experimental conditions and medium composition are shown in Table 1. Experiments were carried out in an incubator, where the medium was mixed using a magnetic stirrer at 20 °C.

2.3. Respiration–inhibition test

Respiration–inhibition tests based on BOD measurements were carried out in the bottles (500-mL volume) of WTW (Germany) Oxi Top system. The synthetic wastewater containing the mineral medium mentioned above was added to the bottles. Starting from a stock solution, heavy metals were added to obtain the target concentration of metals. Duplicate experiments were performed in the bottles containing metals and non-metals. The consumption of oxygen was monitored at specified times and compared with the control samples. Inhibition was defined as the decrease in oxygen consumption compared with the control samples. The inhibitory effect of metal (percentage inhibition) at each concentration was calculated as

$$\%I = \frac{R_B - R_{24}}{R_B} \times 100$$

R_B, R_{24}: respiration rates calculated from BOD measures in the bottle of blank control and the tested concentration of metals mg O_2 L^{-1} day^{-1}.

The IC_{50} and IC_{25} values were derived after plotting percentage inhibition against concentration. IC_{50} and IC_{25} represent the concentrations of metal eliciting 50 and 25% inhibitory effect, respectively, measured after 24 h (mg L^{-1}).

2.4. Analytical methods

Samples were removed from the mixed liquor medium after incubation time and were centrifuged at 5000 rpm for 10 min to remove suspended solids from the medium. Clear supernatants were investigated to analyze alkalinity and ammonium, Ca^{2+} con-