Separation and Purification Technology 62 (2008) 337–341

Contents lists available at ScienceDirect

Separation and Purification Technology

journal homepage: www.elsevier.com/locate/seppur

Nickel inhibition of calcium precipitation by ureolytic mixed microorganisms under batch conditions

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ARTICLE INFO

Article history:
Received 2 January 2008
Received in revised form 2 February 2008
Accepted 8 February 2008

Keywords:
Ureolytic
Calcium
Inhibition
Ni(II)
Mixed

ABSTRACT

The respirometric assessment of the inhibitory impact of Ni(II) on substrate utilization and microbial carbonate precipitation (MCP) by ureolytic mixed microorganisms was investigated with a glucose containing mineral medium under batch conditions over an incubation period of 134 h. The IC_{25} was determined as 224 mg Ni(II) L^{-1} from the BOD values of samples. The interpretation of kinetic data showed that the substrate removal rate fitted a zero-order at the beginning of the incubation period and first-order during the last period, for a range of Ni(II) concentrations between 0 and 512 mg L^{-1}. Increasing Ni(II) concentrations from 0 to 512 mg L^{-1} reduced the substrate degradation rate constant from 10.8 to 5.3 mg L^{-1} h^{-1} for zero-order rate constant (k_0), and from 0.015 to 0.002 h^{-1} for first-order rate constant (k_1). The zero- and first-order reaction rates during incubation period were equalized to the reaction rate of the Monod equation in order to determine the kinetic constants, half saturation concentration (K_S) and maximum substrate removal rate (R_{max}). BOD removal rate was inhibited accordingly to mixed inhibition model with increasing Ni(II) concentrations during the calcification process. Also, the inhibition of calcium precipitation was observed at a higher Ni(II) concentration because of inhibition of ammonium production in these samples.

1. Introduction

Water containing high concentration of calcium tends to deposits scale on the inside of pipes and on the appliances during water use. Also, calcium-rich water and wastewater is one of the problems in the water and wastewater treatment processes. Precipitates of calcium are associated with young landfill leachates, reverse osmosis concentrates, industrial processes such as bone processing, paper recycling, and sugar processing [1–3] and industrial wastewaters containing lime, which is used as an inexpensive neutralization [4]. Existing classic chemical crystallization reactors are based on the addition of a base [NaOH or $Ca(OH)_2$] in the presence of nucleation site (e.g. sand grains). Such reactors are, however, often expensive, complex, and sometimes give rise to highly alkaline effluent [5] requiring neutralization before biological treatment.

A new biological approach for calcium removal from calcium-rich industrial wastewater was also reported by a research group who proposed microbial carbonate precipitation (MCP) process based on microbial urea hydrolization [3,5–7]. Microorganisms have long been known to catalyze the precipitation of $CaCO_3$ in natural environments such as oceans, soils and saline lakes, in a process referred to as MCP [8,9]. The generally accepted mechanism of MCP is the increase in pH and dissolved inorganic carbon (DIC) of a given environment through normal physiological activities [8]. Under aerobic conditions, one of the known MCP processes is heterotrophic microbial urea hydrolysis processes, in which 1 mol of urea is hydrolyzed by the urease enzyme to 2 mol of ammonium and 1 mol of carbon dioxide. These products can subsequently react to form ammonium and carbonate ions, which, in the presence of soluble calcium ions, can react and precipitate as $CaCO_3$. Urea hydrolysis provides simultaneously a pH and CO_2 increase, both of which are responsible of $CaCO_3$ production. In addition to these factors, precipitation process of $CaCO_3$ needs nucleation site, e.g. sand, suspended solids, and bacteria in the medium.

The input of heavy metals into the aquatic environment has increased parallel with the rapid industrialization of our world during the past 150 years. Heavy metals can be stimulatory, inhibitory, or even toxic in biochemical reactions depending on their concentrations. A trace level of many metals is required for activation or function of many enzymes and co-enzymes. Excessive amounts, however, can cause inhibition or toxicity. This is mostly due to the chemical binding of heavy metals to the enzymes, resulting in the disruption of enzyme structure and activities [10,11]. Many industries, such as automotive, metal producing, electroplating, battery manufacturing, mining, electric cable manufacturing, tannery, steel and textile, release various heavy metals like nickel, cadmium, lead,

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doi:10.1016/j.seppur.2008.02.002

chromium and copper in wastewaters. These heavy metals are toxic to aquatic ecosystems and human health, get accumulated in organisms beyond tolerance levels [12] and are also non-competitive inhibitors to substrate utilization [13]. A number of methods have been proposed for measuring metal toxicity in biological systems. The more commonly used ones include the measurement of enzymatic activity, the measurement of respiratory rate, influence on the microorganism growth parameters and the use of fluorescent and bioluminescence methods [14].

Ni(II) is a heavy metal frequently occurring in raw wastewater streams from industries such as non-ferrous metal, mineral processing, paint formulation, electroplating, porcelain enameling, copper sulphate manufacturing and steam-electric power plants [15]. While the Ni(II) ion concentration in plating rinse can approach 2–900 mg L^{-1}, wastewater from paint and ink formulation, porcelain enameling, copper sulphate manufacture industries record effluent Ni(II) ion concentrations that varies from 0–40, 0.25–67 and around 22 mg L^{-1}, respectively [16]. Ni(II) which belongs to the so-called "essential" metals has been identified as a component in a number of enzymes, participating in important metabolic reactions, such as: ureolysis, hydrogen metabolism, methane biogenesis and acidogenesis [14].

Calcium removal from industrial wastewater is a new proposed method and it has not been studied extensively by considering environmental variables. In this study, the inhibitory effect of Ni(II) on ureolytic microbial community was studied by evaluating BOD change and Ca precipitation under batch conditions.

2. Materials and methods

2.1. Synthetic wastewater

The synthetic wastewater was prepared by considering the composition of liner paper manufacturing and medium strength municipal wastewater proposed by Kim et al. [17], and Holakoo et al. [18], respectively. The wastewater contained (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); $NaHCO_3$ (378). Sulphuric acid was used to maintain a pH of 7.00 ± 0.10. Composition of synthetic wastewater resulted in glucose-COD/N/P ratio of 100/37/4. Urea was used in a higher concentration than what is necessary for growth in the medium to serve MCP process. $CaCl_2$ was also added to the medium as total Ca^{2+} of 400 mg L^{-1} during batch experiments.

2.2. Sludge production and experimental setup

A mixed culture was obtained from a fed-batch reactor receiving the synthetic wastewater described above and used as the initial inoculum for the batch experiments. The sludge retention time and biomass concentration in this reactor were approximately 10 d and 2000 mg L^{-1} as mixed liquor volatile suspended solids (VSS), respectively. Dissolved oxygen was measured above 2 mg L^{-1}. The sludge can convert of 0.6 g urea L^{-1} d^{-1} to ammonia. The produced sludge had a VSS/suspended solids (SS) ratio of 0.82 and a sludge volume index (SVI) of 92 mL g^{-1}.

The experiments performed in duplicate, were carried out to determine the Ni(II) inhibition to urea hydrolyzing bacteria via BOD measurement during the period and change of other parameters such as, calcium, alkalinity, pH, SS, and VSS after experimental period of 134 h. Eighteen BOD bottles were used, twice for each of the nine tested Ni(II) concentrations. The effective volume and initial sludge concentration were 97 mL and 220 mg VSS L^{-1}, respectively, in the bottles. Experiments were carried out in an incubator in which bottles were mixed with magnetic stirrer at 20 °C.

2.3. Respiration–inhibition test

Respiration–inhibition tests based on BOD measurements were carried out in bottles of WTW Oxi Top system. The synthetic wastewater containing the mineral medium given above was added to bottles. Ni(II) concentration varying between 0 and 512 mg L^{-1} in the bottles were adjusted by stock solution of 800 mg Ni(II) L^{-1} as $NiCl_2{\cdot}6H_2O$. Control samples were performed in the bottles containing no Ni(II). Oxygen consumption was monitored at specified times and compared to the control samples. Inhibition was defined as a decrease in oxygen consumption compared to the control samples. The inhibitory effect of Ni(II) (percentage inhibition) at each concentration was calculated as

$$I(\%) = \frac{R_B - R_{24}}{R_B} \times 100 \quad (1)$$

Where R_B and R_{24} are respiration rates of blank control, and of the tested concentration of Ni(II) for initial 24 h, respectively (mg BOD L^{-1} h^{-1}). IC_{50} and IC_{25} are Ni(II) concentrations (mg L^{-1}) eliciting a 50% and 25% inhibitory effect after 24 h, respectively, IC values were derived after plotting percentage inhibition against concentration and by assuming linearity.

2.4. Analytical methods

Samples were withdrawn from the mixed liquor medium after incubation time, and were centrifuged at 2375 × *g* for 10 min to remove SS from the medium. Clear supernatants were analyzed for alkalinity, ammonium, and Ca^{2+} concentrations of samples at the end of the incubation period. Standard kit and spectrometric method described in the guide of Merck-Spectroquant were used for ammonium. VSS, SS, SVI, alkalinity and Ca^{2+} were analyzed as specified in standard methods [19]. The pH and DO were measured by using with WTW pH330i/SET and WTW Oxi340i/SET, Germany, respectively.

3. Results and discussion

3.1. Inhibition level of substrate removal

The effect of Ni(II) on the substrate removal was monitored using BOD data by adding various concentrations of Ni(II) ions into the assay. The response of bacteria to higher Ni(II) concentration was depicted in Fig. 1. Ni(II) up to concentrations of 128 mg L^{-1} had no apparent effect on substrate removal. IC_{50} and IC_{25} were calculated as 525 and 224 mg L^{-1} from the plot of Ni(II) concentration versus inhibition % (Fig. 1). Ni(II) showed an inhibitory effect on BOD degradation by ureolytic mixed microorganism in a concentration-dependent manner. For samples containing 0 and 512 mg Ni(II) L^{-1}, the BOD values decreased from 250 to 140 mg L^{-}, respectively, during the incubation period of 24 h. As substrate was almost consumed by ureolytic mixed bacteria, the rate of substrate consumption gradually decreased with respect to Monod kinetic that is clarifies substrate-limiting growth. BOD data clearly show that Ni(II) concentrations which are higher than 128 mg L^{-1} inhibited the substrate oxidation rate of ureolytic mixed bacteria.

The BOD values increased when the Ni(II) concentration increased from 0 to 64 mg L^{-1}, and thereafter decreased gradually to the lowest value (at concentrations higher than 128 mg L^{-1}). The data indicates that Ni(II) stimulates the ureolytic mixed bacteria at concentrations below approximately 64 mg L^{-1} while at higher