

EFFECTS OF MOLYBDATE ON SULFATE REDUCTION AND BENZOATE DEGRADATION

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ABSTRACT: Molybdate inhibited both sulfidogenesis and benzoate degradation in anaerobic treatment of wastewater, but the impact was more severe to the former reaction. In batch reactors, molybdate inhibition increased progressively with concentration; biogranules lost 50% of methanogenic activity treating wastewater containing 48 mg/L of molybdate. In continuous experiments the molybdate toxicity had a threshold level. Below 0.5–0.8 mM bacterial activities were unaffected, but above this level both sulfidogenic and benzoate degradation were severely inhibited. However, the molybdate toxicity was not permanent. Biogranules were able to gradually regain their bioactivities once molybdate was removed from the wastewater.

INTRODUCTION

Sulfate could adversely affect the efficiency of the anaerobic treatment of wastewater. In some extreme cases it could even result in a complete system failure (Parkin et al. 1990). Sulfate-reducing bacteria (SRB) not only compete with syntrophic acetogenic bacteria for substrates but also compete with methanogens for hydrogen as an electron donor (Isa et al. 1986). In addition, SRB convert sulfate into hydrogen sulfide, which is malodorous, corrosive, and inhibitory to methanogenesis (Maillacheruvu et al. 1993). Karhadkar et al. (1987) reported that molybdate was able to selectively inhibit sulfidogenesis without affecting methanogenesis in anaerobic wastewater treatment, but Hilton and Archer (1988) reported that molybdate inhibited both sulfidogenesis and methanogenesis. This study was thus conducted to clarify the effect of molybdate on sulfate reduction and anaerobic degradation of organic substrate. Benzoate, a key intermediate for the degradation of aromatic chemicals, was chosen as the model substrate. The recovery of bacterial activity after molybdate inhibition was also examined.

MATERIALS AND METHODS

Experiments were conducted in both batch and continuous reactors. Two types of biogranules were used in the batch experiments: benzoate-degrading (BD) and benzoate-degrading/sulfate-reducing (BD/SR). The former was sampled from an upflow anaerobic sludge blanket (UASB) reactor treating sulfate-free wastewater containing 5,040 mg/L of benzoate equivalent to 10,000 mg/L of chemical oxygen demand (COD), whereas the latter was sampled from another UASB reactor treating wastewater contained 10,000 mg-COD/L of benzoate and 1,000 mg/L of sulfate. Both biogranules consistently removed over 99% of COD at 37°C in UASB reactors at 40 h of hydraulic retention, corresponding to a daily loading of 6 g-COD/L.

All batch experiments were conducted in duplicate at 37°C in 157-mL glass serum bottles containing 100 mg of biogranules, plus 100 mL of feed solution comprising 2,500 mg-COD/L of benzoate, nutrient, vitamins, trace metals (Fang and Chui 1993), and molybdate (the concentration of which varied from

nil to 20 mM). The feed solution for the BD/SR biogranules contained an additional 1,000 mg/L of sulfate. The effect of molybdate was quantified by comparing the specific methanogenic activity (SMA), COD removal, and sulfate reduction of biogranules in each reactor to the corresponding control reactors without molybdate. The pH of all feed solutions was buffered at 7.2–7.6 by bicarbonate. At the end of each batch test, which lasted 15 d, residual benzoate, intermediate volatile fatty acids (VFA), and sulfate were analyzed.

Continuous experiments were conducted at 34°C for 420 d in two 150-mL upflow reactors (Fang et al. 1997), each packed with 150 mL of BD/SR biogranules equivalent to 2,910 mg of volatile suspended solids (VSS). The wastewater contained benzoate equivalent to 5,000 mg/L of COD, plus nutrient and trace metals. At 12 h of hydraulic retention, the average loading rate was 10 g-COD/(L·d). Wastewater treated in reactor A contained 3,000 mg/L of sulfate, except in the first 60 d when it was only 2,000 mg/L, and molybdate at increased concentrations up to 0.8 mM. Wastewater treated in reactor B contained 2,000 mg/L of sulfate and molybdate up to 0.5 mM.

Compositions of biogas (i.e., methane, carbon dioxide, H₂S, and nitrogen) and mixed liquor were analyzed by two separate gas chromatographs (Fang et al. 1995), and sulfate concentration was measured by an ion chromatograph (Fang et al., 1997). Sulfide and VSS were analyzed according to *Standard Methods* (American Public Health Association 1989).

RESULTS AND ANALYSIS

Batch Experiments

Table 1 summarizes the bioactivities of both biogranules in batch reactors at various molybdate concentrations. Strictly speaking, COD measurement has two components: organic and sulfidous. In this study, the organic-COD were calculated

TABLE 1. Results of Batch Treatment

Molybdate concentration (mM)	Benzoate-Degrading Biogranules		Benzoate-Degrading/Sulfate-Reducing Biogranules		
	Organic-COD removal efficiency (%)	Specific methanogenic activity [g-CH ₄ -COD/(g-VSS·d)]	Organic-COD removal efficiency (%)	Specific methanogenic activity [g-CH ₄ ·COD/(g-VSS·d)]	Sulfate-reducing efficiency (%)
(1)	(2)	(3)	(4)	(5)	(6)
0	96	0.73	94	0.52	32.9
0.5	78	0.34	71	0.38	3.2
2	33	0.24	13	0.30	N/D ^b
5	28	0.23	21	0.29	N/D
10	20	N/A ^a	11	0.26	N/D
20	15	0.15	11	0.20	N/D

Note: COD represents chemical oxygen demand; VSS stands for volatile suspended solids.

^aN/A: not available.

^bN/D: not detectable.

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from concentrations of residual benzoate and individual VFA based on gas chromatographic analyses. BD biogranules in control reactors removed 96% of organic-COD and 100% of benzoate (residual benzoate was below the detectable limit of 1 mg/L), whereas BD/SR biogranule controls removed 94% of organic-COD and 99% of benzoate. Organic-COD removal efficiency decreased drastically with the increase of molybdate concentration for both biogranules; however, the impact was more severe for the BD/SR biogranules.

The sulfate-reducing efficiency of BD/SR biogranules also deteriorated drastically with the increase of molybdate concentration, reducing from 32.9% for the controls to 3.2% with molybdate at 0.5 mM, and there was no sulfate reduction at all when molybdate was at 2 mM or higher. Overall, molybdate inhibition is more severe toward sulfate reduction than organic-COD removal.

Table 1 also shows that the SMA of BD and BD/SR biogranules in the control reactors was 0.73 and 0.52 methane-COD/(g-VSS·d), respectively. The latter biogranules had lower methanogenic activity because they contained SRB, which competed with methanogens for substrate and hydrogen. An increase of molybdate concentration lowered the SMA and cumulative methane production of both biogranules. On the other hand, the effect was more severe for the BD biogranules. However, molybdate at concentrations as high as 20 mM did not completely inhibit the methanogenic activity of both biogranules.

The $C_{1.50}$ value, i.e., the concentration at which biogranules lose 50% activity, of Mo^{+6} (as MoO_4^{-2}) for BD biogranules in the present study is 48 mg/L. Fang and Chan (1997) investigated the toxicity of five electroplating metals on the bioactivity of BD biogranules obtained from the same UASB reactor of this study. They reported that the $C_{1.50}$ values for the BD biogranules were Cd^{+2} , 150 mg/L; Cr^{+3} , 250 mg/L; Cu^{+2} , 175 mg/L; Ni^{+2} , 100 mg/L; and Zn^{+2} , 110 mg/L. Having a lower $C_{1.50}$ value, molybdate was more toxic than those electroplating metals.

Continuous Experiments

The effluent pH of the two upflow reactors were steady, ranging pH 7.1–7.8 in reactor A and pH 7.2–8.0 in reactor B. Both reactors did not experience sludge washout. The VSS contents in the effluent were 76 ± 29 mg/L in reactor A and 73 ± 18 mg/L in reactor B. Fig. 1 illustrates the key results of reactor A throughout the 420-d experiment, including the influent molybdate concentration, organic-COD removal efficiency, sulfate-reducing efficiency, soluble sulfide in the effluent, and the methane production rate. Fig. 2 illustrates the corresponding results of reactor B.

Organic-COD Removal and Sulfate-Reducing Efficiencies

Fig. 1(a) illustrates there was no molybdate initially in wastewater treated in reactor A, but it was increased stepwise up to 0.8 mM. Fig. 1(b) illustrates that the organic-COD removal efficiency was consistently 99.5% for molybdate concentration up to 0.65 mM. As the molybdate was further increased to 0.8 mM on day 328, the organic-COD removal efficiency dropped drastically below 46% by day 357. However, as the molybdate dosing was suspended on day 360, the organic-COD removal efficiency gradually recovered, reaching 95% by day 383.

Figs. 2(a) and 2(b) illustrate reactor B was able to remove 99.8% of organic-COD initially in the absence of molybdate. Molybdate was dosed to the wastewater starting at a concentration of 0.5 mM on day 29. One month later, the organic-COD removal efficiency began to drop and was lowered to only 8% by day 90. After suspending molybdate addition on

day 91, organic-COD removal efficiency slowly increased, returning to the normal level of 99% by day 171. The dosing of molybdate resumed on day 176 from 0.1 mM initially to 0.35 mM on day 236, and then to 0.5 mM on day 267. During days 176–266, organic-COD removal efficiency averaged 99%. However, as soon as the molybdate was raised to 0.5 mM, it dropped immediately to as low as 9% by day 353. However, the efficiency gradually recovered, similarly to reactor A, when the molybdate addition was suspended on day 359, slowly reaching 75% by day 420, the day which the experiment was terminated.

The organic-COD in the effluent of both reactors was almost solely attributed to residual benzoate. The average residual acetate in effluents of reactors A and B throughout the study were 7.0 and 23.7 mg/L, respectively, whereas the average residual propionate and butyrate were less than 0.35 mg/L in both reactors. When bioactivities were severely inhibited by molybdate at 0.8 and 0.5 mM in reactors A and B, residual VFA in both reactors was below the detectable limit of 1 mg/L. These results seem to suggest that molybdate adversely affected mainly the conversion of benzoate to fatty acids, but not on the conversion of VFA to methane.

Fig. 1(c) illustrates the sulfate-reducing efficiency of biogranules in reactor A in the absence of molybdate gradually increased from 40 to 68.8%. The efficiency was reduced immediately to 52.1% when 0.1 mM of molybdate were added to the wastewater on day 90. It was improved over time as the biomass was acclimated to the molybdate. The average efficiency was 64% for molybdate concentrations at 0.35–0.65 mM. However, when molybdate was increased to 0.8 mM on day 328, the sulfate-reducing efficiency dropped drastically from 72 to 17% in 30 d. However, biogranules were also able to regain their sulfate-reducing efficiency when molybdate addition was suspended. The efficiency reached 66% by day 420.

Similar results are found in Fig. 2(c) for reactor B. The sulfate-reducing efficiency drastically decreased as soon as molybdate reached 0.5 mM, the same level that COD removal bioactivity was inhibited, but was able to recover when the molybdate dosage was suspended.

Figs. 1(d) and 2(d) illustrate the concentration of total soluble sulfide in the effluent of reactors A and B, respectively. Figs. 1(e) and 2(e) illustrate the methane production rates in the respective reactors. As expected, the sulfide concentration in either reactor varied corresponding to the reactor's sulfate-reducing efficiency and the methane production rate to the COD removal efficiency.

Molybdate Toxicity

The inhibition effects of molybdate on benzoate degradation and sulfate reduction in the continuous reactors were not progressive, unlike in the batch reactors. Instead, it appears that there was a threshold toxicity level of 0.5–0.8 mM. Below this level, molybdate had no adverse effect, but above this level, molybdate almost completely inhibited benzoate degradation and sulfate reduction. However, the toxic effect was not permanent. Biogranules were able to gradually regain their bioactivity once the molybdate was suspended from the wastewater.

Analytic results showed that the maximum levels of total soluble sulfide and H_2S in this study were 620 and 198 mg-S/L. They were below the respective tolerable levels of 769 and 234 mg-S/L reported in a recent study (Fang et al. 1997).

Sulfur and COD Balances

The fate of sulfur (as sulfate) in the wastewater was residual sulfate and soluble in the effluent, H_2S in the biogas, precipitated metal sulfides, and those accumulated in biomass.

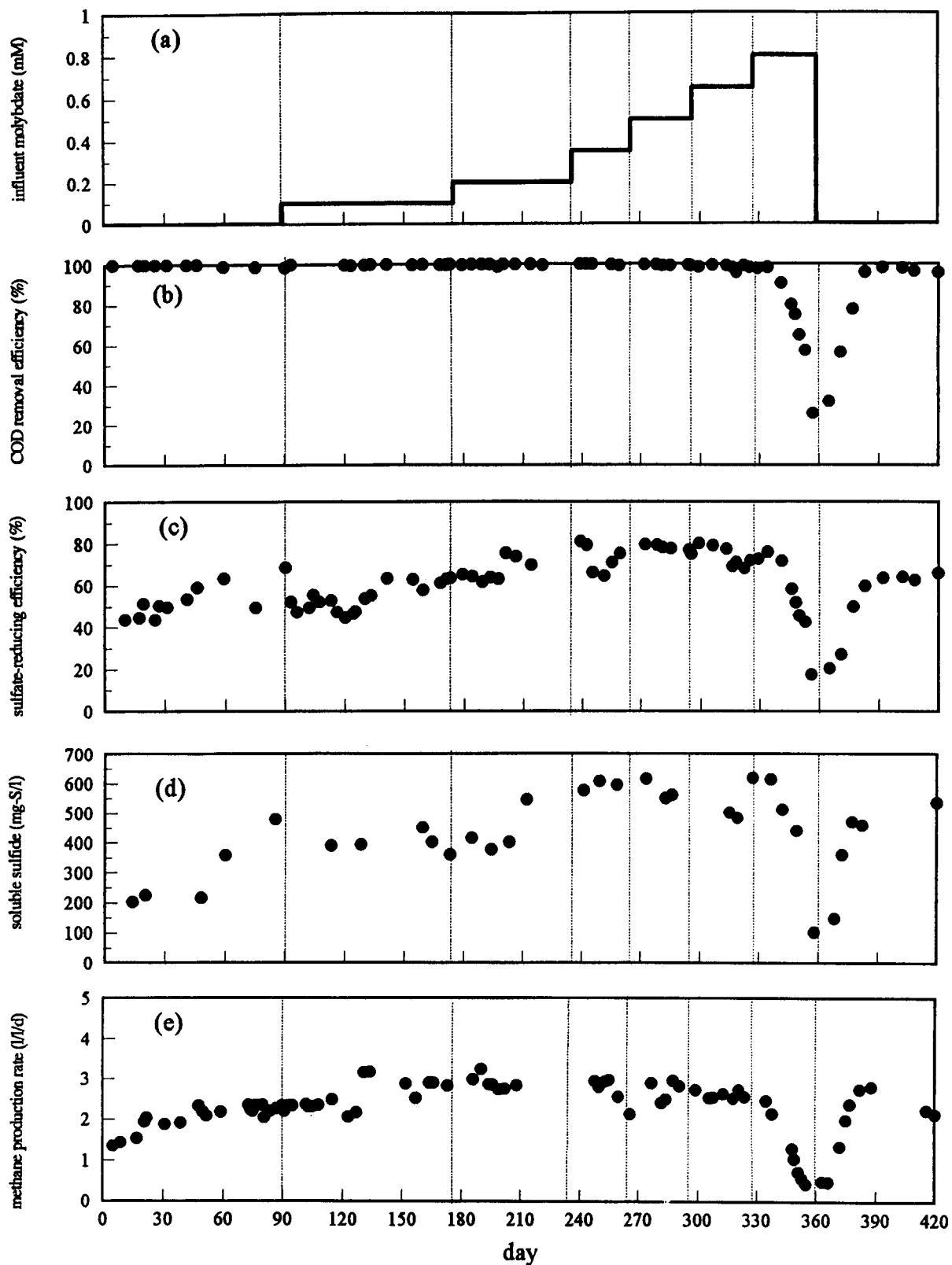


FIG. 1. Performance of Upflow Reactor A: (a) Influent Molybdate Concentration; (b) Organic-COD Removal Efficiency; (c) Sulfate-Reducing Efficiency; (d) Soluble Sulfide in Effluent; (e) Methane Production Rate

Among them, the first three were measured routinely, and the fourth could be estimated assuming all the metals in the feed-water were precipitated as metal sulfides. Those in the biomass were difficult to estimate but presumably negligible. Fifty complete sets of data were collected for reactor A, and 44 sets were collected for reactor B. Based on these data, the sulfur balances were $97.0 \pm 8.7\%$ in reactor A and $100.0 \pm 7.8\%$

in reactor B. Similarly, the principle of conservation also applied to COD in anaerobic systems. COD (as benzoate) in the wastewater was converted to an organic fraction comprised of VFAs, methane and those in the washed-out solids, and a sulfidous fraction (including soluble sulfide in the effluent, H_2S in the biogas, and precipitated metal sulfides). Accordingly, the COD could be balanced from the experimental data. Re-

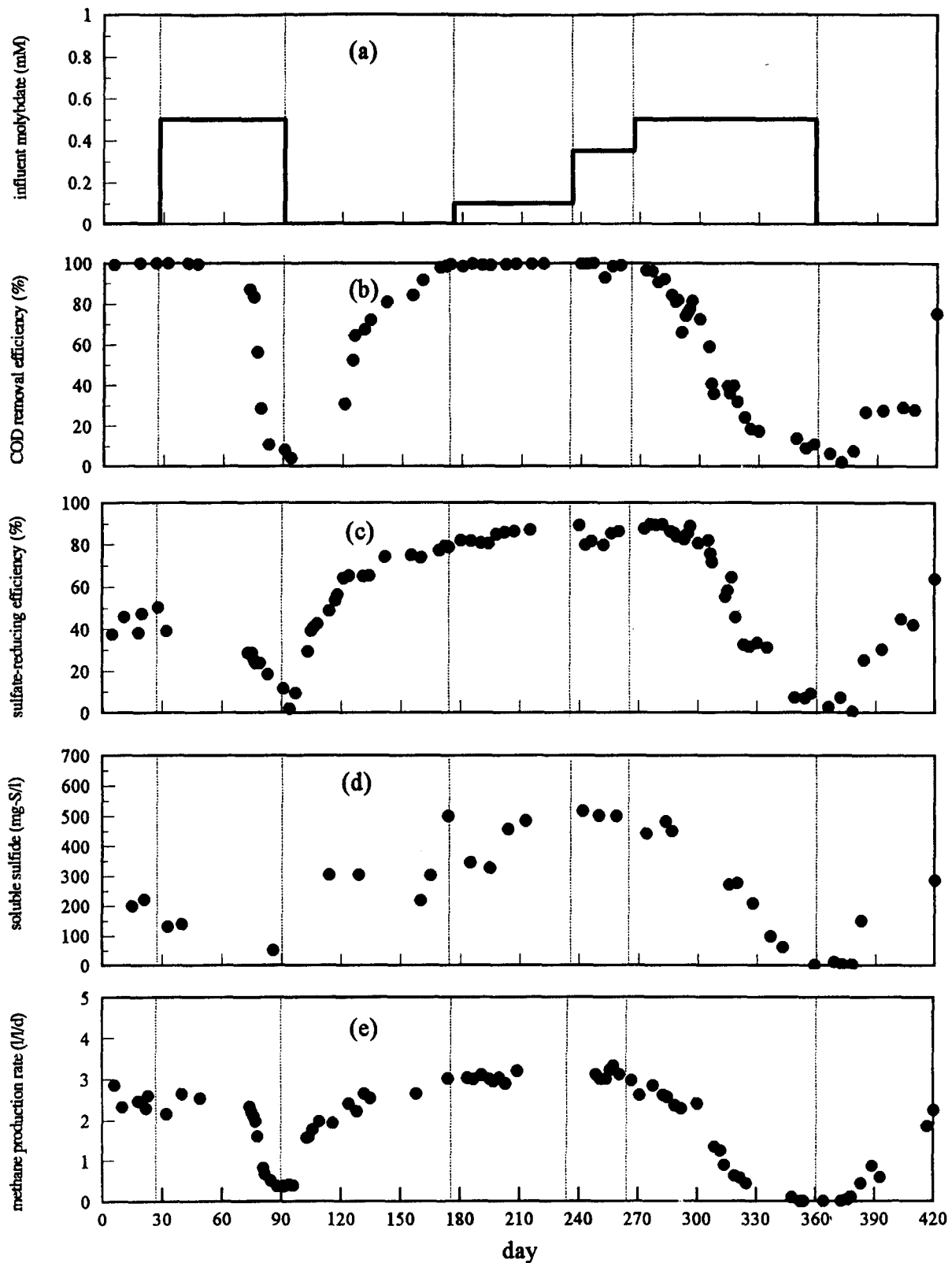


FIG. 2. Performance of Upflow Reactor B: (a) Influent Molybdate Concentration; (b) Organic-COD Removal Efficiency; (c) Sulfate-Reducing Efficiency; (d) Soluble Sulfide in Effluent; (e) Methane Production Rate

sults showed that the COD balances were $91.7 \pm 7.4\%$ for reactor A and $97.8 \pm 5.8\%$ for reactor B.

CONCLUSIONS

The study was conducted to clarify the confusing results in the literature regarding the effect of molybdate on sulfate re-

duction and anaerobic degradation of organic substrate. Based on results of this study, it is concluded that molybdate inhibited both sulfidogenesis and benzoate degradation, although the impact was more severe to the former. In batch reactors molybdate inhibition increased progressively with concentration; biogranules lost 50% of methanogenic activity treating wastewater containing 48 mg/L of molybdate. However, in

continuous experiments the molybdate toxicity was not progressive. Instead, it had a threshold level. Below 0.5–0.8 mM bacterial activities were unaffected, but above this level both sulfidogenic and benzoate degradation were severely inhibited. The toxic effect of molybdate was not permanent. Biogranules were able to gradually regain their bioactivities once molybdate was suspended from the wastewater.

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