Inhibition by chromium and cadmium of anaerobic acidogenesis

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Abstract The effects of chromium (III) and cadmium on the anaerobic acidogenesis of a simulated dairy waste were examined using serum vials. At Cd dosages less than 20 mg/l, the acidogenesis process was enhanced by the dosage, resulting in a higher degree of acidification, protein conversion, and hydrogen production than the control. At dosages over 20-mg/l, Cd inhibited the acidogenesis. The Cr (III) dosage of 5 mg/l reduced overall volatile fatty acid and alcohol generation, degree of acidification, conversions of lactose, lipid and protein, and total biogas production, with the exception of accumulation of hydrogen and propionate. At dosages exceeding 5 mg/l, Cr (III) had a severe inhibition on the acidogenesis. The Cd concentrations which caused a 50% reduction in total volatile fatty acid and alcohol production, degree of acidification and cumulative gas production were higher than the corresponding values caused by Cr (III), suggesting that Cr (III) was more toxic to acidogenic bacteria than Cd.

Keywords Acidogenesis; cadmium; chromium (III); dairy waste; inhibition

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Introduction

Heavy metals are present in significant concentrations in some industrial wastewaters and municipal sludge, and are often found to be the leading cause of anaerobic reactor upset and failure when the reactor is treating industrial wastewaters and municipal sludge (Lester *et al.*, 1983; Stronach *et al.*, 1986). The effects of heavy metals on the anaerobic digestion process have been widely studied over several decades (Fang, 1997; Fang and Chan, 1997; Fang and Hui, 1994; Kugelman and McCarty, 1965; Lin, 1992; Mosey, 1976). Most of these studies tended to examine the effect on the overall performance rather than on the individual stages, i.e., acidogenesis and methanogenesis. The results have shown that the severity of metal inhibition depends upon factors like metal concentration in a soluble, ionic form in the solution, type of metal species, and amount and distribution of biomass in the digester. For example, Fang and Hui (1994) found that heavy metals inhibited the methanogenic activity of anaerobic starch-degrading granules in the order: Zn > Ni > Cu > Cr > Cd, and that granular sludge had higher toxicity-resistance than flocculent sludge, due to the layered structure.

Many researchers believe that the methanogenic bacteria are the most sensitive to toxic material in the waste being treated among the anaerobes (Kugelman and McCarty, 1965; Mosey, 1976). However, two studies suggested that some of the acid forming bacteria were more severely affected by the presence of heavy metals than the methanogens (Hickey *et al.*, 1989; Lin, 1993). Hickey *et al.* (1989) investigated the effects of Cu, Zn and Cd on methane production and on hydrogen and carbon monoxide levels, and found that some trophic groups of organisms within the anaerobic consortia of digesters might be more severely inhibited by a pulsed addition of heavy metals than the methanogenic populations. Lin (1993) also demonstrated that Cu and Zn were more toxic to acidogens than to methanogens.

In view of the results reported by Hickey *et al.* (1989) and Lin (1993), it is apparent that there is a need for a further investigation into the effects of heavy metals on the acidogenic

phase. The objective of this work was to investigate the influence of two heavy metals, Cd and Cr (III), on the acidogenic phase of anaerobic digestion through examining the conversion of substrate to volatile fatty acids (VFA). These two heavy metals are frequently present in industrial wastewaters and municipal sludge. A milk-based wastewater was used as substrate.

Methods

A laboratory-scale upflow anaerobic sludge blanket (UASB) reactor was used to supply inocula for the serum vial tests. The UASB reactor was 2.8 l in volume with an internal diameter of 84 mm and a height of 500 mm. On top of the reactor was a gas-liquid-solid separator with an internal diameter of 114 mm and a height of 250 mm making a filled volume of 2.0 l. The UASB reactor was water-jacketed and operated at a constant temperature of 37ºC. When the reactor was steady at 12.0 g-COD/l . d for 30 days, sludge samples were taken from the UASB reactor for metal inhibition tests.

The UASB reactor was fed with a synthetic dairy wastewater, prepared by using full-cream powered milk supplied by Nestle. This milk contained casein, lactose and butterfat as protein, carbohydrate, and lipid, respectively. The influent chemical oxygen demand (COD) of the UASB reactor was kept at 4000 mg/l. Since the milk contained enough nitrogen, minerals and vitamins for microorganisms, only 20 mg-P/l was supplemented.

Experiments were conducted in glass serum vials with 157 ml working volume. The protocol used for the serum vial tests was developed by Owen *et al.* (1979). A detailed description of the procedures used have previously been reported by Fang and Chan (1997). Sludge of about 100 mg taken from the UASB reactor was added to 157-ml serums along with 100 ml feed solution, plus various dosages of heavy metals. Ten serum vials were used for each metal test. Nine serum vials were dosed with one metal of 5, 10, 20, 40, 80, 150, 200, 300, and 400 mg/l, respectively. The tenth vial was prepared as control with no heavy metal dosage. The vials were immediately flushed with nitrogen and then sealed with a rubber septum and aluminum cap. The vials were placed in a shaking water bath with temperature controlled at 37ºC. The volume of biogas production was measured by a syringe, and the composition was analyzed by a gas chromatograph (GC). Both the volume and composition of the biogas were monitored at regular intervals for 7 days, by then the biogas production was nearly exhausted. At the beginning and the conclusion of each test, contents of all serums were analyzed for mixed liquid volatile suspended solids (MLVSS), COD, and VFAs, including acetate (HAc), propionate (HPr), butyrate (HBu), isobutyrate (i-HBu), valerate (HVa), isovalerate (i-HVa), caproate (HCa) and alcohols, including methanol, ethanol, propanol and butanol.

The contents of H_2, CH_4, CO_2 and N_2 in the biogas were analyzed by a GC (Hewlett Packard, Model 5890 Series II) equipped with a thermal conductivity detector and a 2 m $\times$ 2 mm (inside diameter) stainless-steel column packed with Porapak N (80–100 mesh). Injector and detector temperatures were respectively kept at 130ºC and 200ºC, while column temperature was increased from 90ºC to 110ºC. The concentration of VFAs and alcohols, were determined by a second GC (Hewlett Packard, Model 5890 Series II) equipped with a flame ionization detector and a 10 m $\times$ 0.53 mm HP-FFAP fused-silica capillary column. Samples were filtered through a 0.2 μm filter, acidified by formic acid, and measured for free acids. The initial temperature of the column was 70ºC for 4 minutes and then 140ºC for 3 minutes, and finally 170ºC for 4 minutes. The temperatures of injector and detector were both 200ºC. Helium was used as the carrier gas at a flow rate of 25 ml/min.

Lactose and protein were measured by the phenol-sulfuric method (Herbert *et al.*, 1971), and the Lowry-Folin method (Lowry *et al.*, 1951), respectively. Lipid was extracted by the Bligh-Dyer method from the acidified sample, and was then measured gravimetrically after

Table 1 Characteristics of the UASB sludge

Acidification degree (%)	Specific VFA production rate[a] (mg/mg-VSS·d)			Specific component conversion activity[b] (mg/mg-VSS·d)		
	HAc	HPr	HBu	Lactose	Protein	Lipid
51.5	0.057	0.033	0.022	0.103	0.131	0.049

[a] Specific VFA production rate as mg individual VFA produced per mg biomass per day
[b] Specific component conversion activity as mg individual component fermented per mg biomass per day

the solvent was evaporated at 80ºC (APHA, 1992). Measurements of COD, pH and MLVSS were performed according to the *Standard Methods* (APHA, 1992).

Results and discussion

The parameters used in measuring the effects of the metals were VFA and alcohol production, biogas production and specific substrate conversion. Inhibition was quantified by determining the metal concentration which caused a 50% reduction for the above parameters compared with that of a fed control.

Characteristics of the UASB sludge

The characteristics of the UASB sludge are summarized in Table 1.

Effect on VFA and alcohol production

VFAs and alcohols are the main products of anaerobic acidogenesis of organic matter. Hence, the extent of inhibition on acidogenesis can be evaluated through examining the production of total VFAs and alcohols. In Figure1, relative VFA and alcohol concentration, defined as the ratio of total VFA and alcohol concentration for metal dosage to that for the control, is illustrated as a function of metal concentration. The general trend was that the relative VFA and alcohol concentration decreased with increased Cr concentration. On the other hand, the relative VFA and alcohol concentrations were greater than 100% as Cd dosages were 5 and 10 mg/l, respectively. This showed that the acidogenesis process was enhanced by the Cd dosage rather than being inhibited. However, for the dosage of 20-mg/l of Cd, the relative VFA and alcohol concentration dropped to 95%, indicating that this level addition of Cd inhibited the acidogenesis. These results were partially contradictory to Lin's findings (1993). Lin found that even 3-mg/l of Cd was toxic to acidogenesis. This contradiction may be due either to the difference in the substrates (mixture of carbohydrate, protein and lipid as opposed to glucose) or to the different sludges used.

Effect on degree of acidification

The degree of acidification can be quantified using the percentage of the initial substrate concentration converted to VFAs and other fermentation products (e.g., hydrogen and alcohols). The initial substrate concentration was measured in mg-COD/l and quantity of acidogenic products was converted to the theoretical equivalent in mg-COD/l, i.e., the nominal COD exerted by the mixture of the products:

$$Acidification = \frac{COD_{VFA} + COD_{alcohol} + COD_{H_2}}{COD_{\inf}} \times 100\% \quad (1)$$

The relationship between the relative degree of acidification as compared to the control and metal concentration is illustrated in Figure 2. The relative degrees of acidification were lower than 100% at all the Cr dosages, while they exceeded 100% at Cd concentrations less

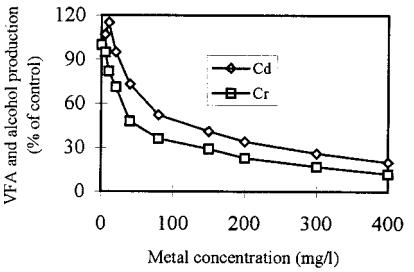

Figure 1 Effect of metals on VFA generation

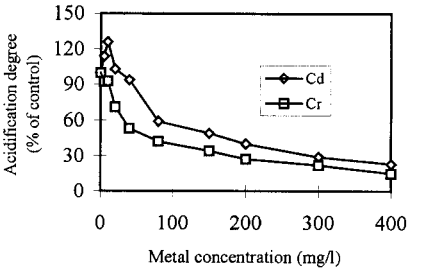

Figure 2 Effect of metals on acidification degree

than 20 mg/l; the maximum acidification occurred at 10 mg/l of Cd concentration; as Cd over 20 mg/l was added, there was a gradually decreasing acidification degree.

Effect on VFA production

When the sludge was taken from the UASB reactor for the metal-inhibition tests, HAc, HBu and HPr were the main VFAs produced in the UASB reactor, while the levels of i-HBu, HVa, i-HVa, HCa and alcohols were relatively lower. Therefore, this study focused on the effects of Cd and Cr on the concentrations of HAc, HBu and HPr, while the concentrations of i-HBu, HVa, i-HVa, HCa and alcohols are not shown in the paper. The relative concentrations of HAc, HBu and HPr are plotted against Cd or Cr concentrations in Figures 3 and 4, respectively.

As seen in Figures 3 and 4, a very low dosage of either Cd or Cr decreased both HAc and HBu concentrations. The relative HAc concentration was slightly lower than that of HBu. The relative HAc and HBu concentrations generally decreased with increased metal dosage. On the other hand, the relative HPr concentration did not follow this pattern; the addition of Cd and Cr resulted in a significant accumulation of HPr at low metal dosages; only after the dosages of Cd and Cr exceeded 150 and 40 mg/l, respectively, did the HPr concentration began to decrease; even when the dosage of Cd was 400 mg/l, the HPr concentration was still 54% of the control, while the relative HAc and HBu concentrations were only 5% and 4%, respectively. Lin (1993) did not examine the HPr concentraion so there is no way of comparing the present study with his study. Yenigun *et al.* (1996) reported that Zn and Cu both had a more severe inhibition on HPr production than on HAc or HBu production. However, the present results about HPr production were in agreement with the results of Chacin and Forster (1995) and Ahring and Westermann (1983). Chaci and Forster (1995) found that the HPr concentrations at dosages of 32 mg/l Cu and 104 mg/l Pb were 268% and 224% of the control respectively. Ahring and Westermann (1983) also reported the enhanced HPr production as a result of metal inhibition for thermophilic sludges.

Effect on specific substrate conversion

Figure 5 illustrates the relative conversions of the three components (lactose, protein and lipid) of the substrate at various Cd concentrations. In the control, the conversions of lactose, protein and lipid were 97%, 88% and 54%, respectively. In the serum with 10 mg/l dosage of Cd, the lactose conversion was 98% of the control, showing that low dosage of Cd had a very small effect on the degradation of lactose; the relative protein conversion exceeded 100%, suggesting that degradation of protein was even encouraged; the relative lipid conversion was 71%, indicating that Cd had the greatest effect on lipid degradation. After the Cd dosage was greater than 150 mg/l, the conversions of all the three components were less than 50%, indicating the micro-organisms responsible for the acidogenesis of the three components were severely inhibited.

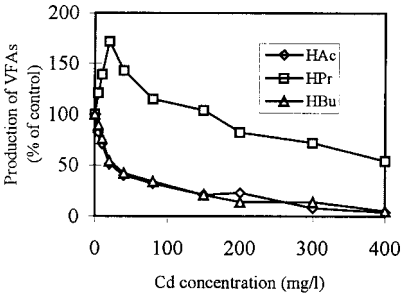

Figure 3 Effect of Cd on VFA concentrations

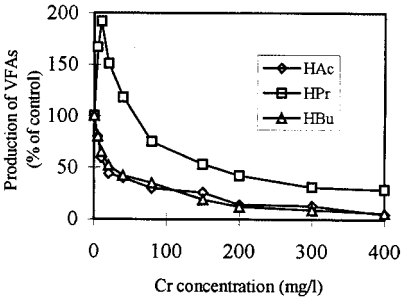

Figure 4 Effect of Cr on VFA concentrations

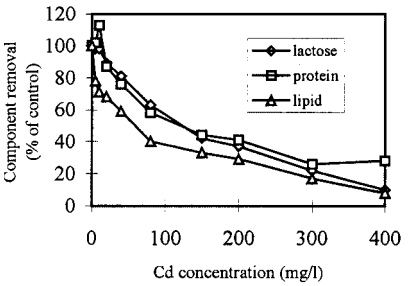

Figure 5 Effect of Cd on component conversion

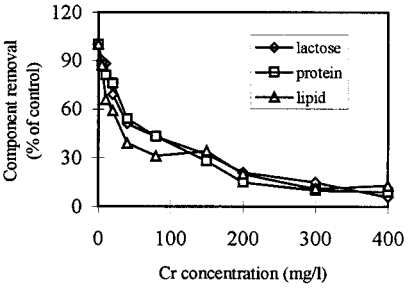

Figure 6 Effect of Cr on component conversion

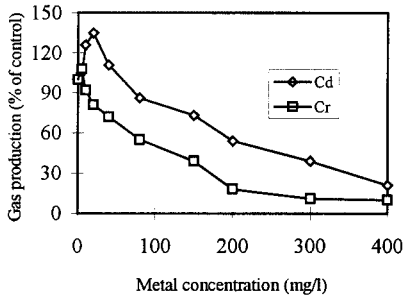

Figure 7 Effect of Cd and Cr on gas production

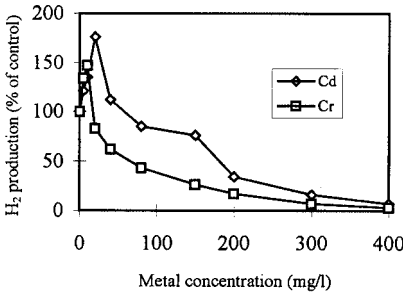

Figure 8 Effect of Cd and Cr on H_2 production

Figure 6 shows that Cr exhibited a similar influence on the conversions of lactose and lipid; the relative protein conversions were less than 100% at all the dosages. At the same concentration of Cd and Cr, the conversions of lactose, protein and lipid in the Cr-added serums were lower than the corresponding conversions in the Cd-added serums.

Effect on biogas production

Gas production is a useful indicator for monitoring an anaerobic digestor suffering from toxicants (Parkin and Speece, 1982). Compared to a conventional single-phase anaerobic process, the acidogenic reactor produced a much lower amount of gas and had a significantly different gas composition. Figure 7 presents the relative cumulative biogas production for 7 days as a function of metal dosage. For Cd addition, the cumulative biogas production at the dosages between 5 and 40 mg/l were more than that in the control, showing that low-dosage Cd increased gas production; the biogas production decreased with the increased dosage after exceeded 80 mg/l. For Cr addition, only at 5-mg/l dosage, the relative

biogas production was greater than 100%; as Cr over 5 mg/l was added, there was a gradually increasing inhibition.

Hydrogen is an important product from anaerobic acidogenesis. Hydrogen is produced during the fermentation of carbohydrate, protein and lipid and in the subsequent degradation of propionic acids and other higher molecular weight volatile fatty acids to acetic acid. Figure8 illustrates the relative hydrogen production in the gas headspace as a function of metal dosage. The effect of Cd and Cr addition on the hydrogen production was similar to that on the biogas production, with an exception at 10-mg/l of Cr, the relative hydrogen production was beyond 100% but the relative total biogas production was below 100%.

It is commonly observed that hydrogen concentration would increase sharply when single-phase anaerobic sludge was exposed to toxic matter (Hickey *et al.*, 1989). The reason for the increased hydrogen concentration was that the methanogens responsible for hydrogen removal were inhibited by toxicants and that hydrogen could not be utilized by the methanogens. In the present study, methanogens in the UASB sludge were already heavily inhibited, but the low dosage of Cd still enhanced hydrogen production. The results in Figures 3 and 8 suggest that, at low Cd concentrations, e.g., 5–40 mg/l, the increase in hydrogen production coincided with the accumulation of HPr. This result is reasonable. In an anaerobic system, the hydrogen pressure is a crucial factor governing the distribution of acidogenic products. The type and concentration of the various acidogenic products formed are regulated by the hydrogen concentration (McInerney, 1988). The regeneration of NAD from NADH is essential to promote the degradation of substrate. When hydrogen partial pressure prevails the equilibrium of this reaction is strongly in favor of NADH formation (Thauer *et al.*, 1977). In this study, at low Cd dosages, hydrogen accumulated and resulted in an altered catabolic pathway where proton reduction was not used to dispose of the generated electrons. Substrate was catabolized to more reduced products such as HPr rather than HAc.

It is evident from Figures 4, 6 and 8 that the low dosages of Cr increased hydrogen and HPr productions, but that the protein conversion was slightly reduced. This might suggest that, at low Cr concentrations, there was no direct relationship between protein degradation with hydrogen and HPr productions. However, such a direct relationship seemed to exist for the low dosages of Cd.

Inhibition index

An index, C_{50}, can been adapted to describe the inhibition caused by metals. In this study C_{50} was defined as the metal concentration which caused a 50% reduction in VFA and alcohol production, acidification degree and gas production by metal dosage. This index was also used to compare the relative toxicity of the metals. The values of C_{50} for Cd and Cr to the above parameters are listed in Table 2. The value C_{50} was dependent upon the type of metal species, substrate used and seed sludge (Chacin and Forster, 1995; Lin, 1992). The comparison between the values of C_{50} in Table 2 suggests that Cr was more toxic to acidogenic bacteria than Cd. This was in good agreement with the findings of Lin (1993), although different substrates and seed sludge were used in these two studies.

Table 2 The C_{50} values for Cd and Cr (in mg/l)

			Individual VFA generation			Specific component conversion				
	VFAs + alcohols	Acidification	HAc	HPr	HBu	Lactose	Protein	Lipid	Total gas	H_2
Cd	90	155	20	>400	28	110	100	60	280	170
Cr	38	50	17	160	25	42	55	3	115	72

Conclusions

1. At concentrations less than 20 mg/l, Cd enhanced the acidogenesis process, resulting in higher HPr generation, acidification degree, protein conversion, and hydrogen production than the control. At concentrations over 20-mg/l, it inhibited the acidogenesis, as evidenced by reduced VFA and alcohol generation, acidification degree, component conversion, and biogas production.
2. At a concentration of 5 mg/l, Cd (III) decreased overall VFA and alcohol generation, acidification degree, component conversion, and total biogas production, with the exception of accumulation of hydrogen and HPr. At concentration sover 5 mg/l, it had a severe inhibition on the acidogenesis.
3. The Cd concentrations which caused a 50% reduction in total VFA and alcohol production, acidification degree and cumulative gas production were 90, 155, and 280 mg/l, respectively; while the corresponding Cr concentrations were 38, 50, and 115 mg/l, respectively. The comparison between the values of C_{50} suggests that Cr was more toxic to acidogenic bacteria than Cd.

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