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ScienceDirect
Chemosphere 67 (2007) 668–673
CHEMOSPHERE
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# Inhibition of heavy metals on fermentative hydrogen production by granular sludge

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Received 26 June 2006; received in revised form 30 October 2006; accepted 1 November 2006
Available online 19 December 2006

## Abstract

This study was conducted to investigate the toxicity of six electroplating metals on the $H_2$-producing activity of a granular sludge sampled from an $H_2$-producing upflow reactor treating sucrose-containing wastewater. The $H_2$ production activities of the sludge were measured in serum vials using wastewater containing not just sucrose and proper nutrient, but also individual heavy metals at concentrations ranging 0–5000 mg $l^{-1}$. The relative toxicity to $H_2$ production was found in the following order: Cu (most toxic) $\gg$ Ni $\sim$ Zn $>$ Cr $>$ Cd $>$ Pb (least toxic). The $C_{I,50}$ values, at which the bioactivity of the sludge was reduced to 50% of the control, for individual heavy metals were Cu 30 mg $l^{-1}$, Ni and Zn 1600 mg $l^{-1}$, Cr 3000 mg $l^{-1}$, Cd 3500 mg $l^{-1}$, and Pb >5000 mg $l^{-1}$. Compared with the literature data, $H_2$-producing sludge exhibited in general higher resistance to metal toxicity than methanogenic granular sludge.

*Keywords:* $C_{I,50}$; Heavy metals; Hydrogen; Inhibition; SHA; Toxicity

## 1. Introduction

Heavy metals are present in significant concentrations in some industrial wastewaters and municipal sludge, and are often the leading cause for the upset of the wastewater treatment process (Lester et al., 1983; Stronach et al., 1986; Fang and Chan, 1997). 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 lead to 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 (Vallee and Ulmer, 1972).

Sludge and concentrated wastewater are conventionally treated by anaerobic processes leading to the production of methane (Gallert et al., 2003). More recently, a new anaerobic technology has been developed to convert organic wastes into $H_2$ (Li and Fang, 2007). Recent research has shown that the $H_2$-producing sludge may agglutinate into granules, similar to methane-producing sludge, in upflow reactor to facilitate $H_2$ production with high biomass concentration (Li et al., 2006). Although the effects of heavy metals on the anaerobic methane-producing process have been widely studied (Fang and Hui, 1994; Fang and Chan, 1997; Lin and Chen, 1999), little is known on their effects on fermentative $H_2$ production.

A series of batch experiments were conducted in this study to investigate the inhibition effect of heavy metals on the production of $H_2$ from a sucrose-containing wastewater by granular sludge. The six heavy metals selected were those commonly found in electroplating effluents, including Cd, Cr, Cu, Ni, Zn, and Pb.

## 2. Materials and methods

### 2.1. Inoculums

$H_2$-producing granular sludge was sampled from a packed-bed upflow reactor which has been operated at

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0045-6535/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2006.11.005

26 °C treating sucrose-containing wastewater for over 500 d. This sludge had a $H_2$ production yield and rate of 1.22 mol-$H_2$ mol$^{-1}$-sucrose and 6.17 l-$H_2$ l$^{-1}$ d$^{-1}$, respectively. Detailed characteristics of this granular sludge have been reported in a previous study (Li et al., 2006).

### 2.2. Batch tests

Six series of batch tests were conducted in duplicate for Cd, Cr, Cu, Ni, Zn and Pb, respectively, in 250 ml glass serum vials. Each vial was filled with 150 ml of synthetic wastewater containing 10 g l$^{-1}$ of sucrose (equivalent to 11.2 g l$^{-1}$of COD) as substrate along with proper dosages of nutrient and trace metals using the formulation reported before (Fang et al., 2006). Each vial was then added with 1.0 g (wet weight) of $H_2$-producing sludge, corresponding to 35 mg of biomass as measured by the volatile suspend solids (VSS) content, plus individual heavy metals. The dosages of heavy metal varied from 20 to 5000 mg l$^{-1}$, plus one for each set without any metal dosage to serve as the control. All vials were then capped with butyl rubber, completely purged with nitrogen, and incubated in a constant temperature chamber at 26 °C with constant mixing device. The pH of the mixed liquor during the $H_2$ production process was controlled twice a day at pH 5.5 by adding either HCl or NaOH. $H_2$ production data were measured over 10 d for each batch until the production leveled off. The inhibition effect of an individual metal at a given concentration was quantified by the decreases of $H_2$ potential, the specific $H_2$-producing activity (SHA), and the degradation sucrose relative to the controls.

### 2.3. Analysis

The biogas production from each vial was monitored daily using a syringe. The composition of biogas and concentrations of volatile fatty acids (VFA) and alcohols in the effluent were determined daily (Fang et al., 2006). Sucrose concentration was measured using the anthrone-sulfuric acid method (Gaudy, 1962). COD and VSS were measured according to the Standard Methods (APHA, 1992).

### 2.4. Kinetic modeling

The cumulative $H_2$ production in the batch experiments followed the modified Gompertz equation (Lay et al., 1997; Fang et al., 2006):

$$H = P\exp\left\{-\exp\left[\frac{R_{\mathrm{m}}e}{P}(\lambda - t) + 1\right]\right\} \qquad (1)$$

where $H$ is the cumulative $H_2$ production (ml), $\lambda$ is the lag time (h), $P$ is the $H_2$ production potential (ml), $R_{\mathrm{m}}$ is the maximum $H_2$ production rate (ml h$^{-1}$) and $e = 2.718281828$.

Values of $P$, $R_{\mathrm{m}}$ and $\lambda$ for each batch were estimated using the solver function in Excel (version 5.0, Microsoft) with a Newtonian algorithm. The SHA (in l-$H_2$ g$^{-1}$-VSS d$^{-1}$) was calculated by dividing the $R_{\mathrm{m}}$ by the initial VSS in the reactor. The $H_2$ yield (ml g$^{-1}$-sucrose) was calculated by dividing $P$ by the sucrose content of the feedstock.

## 3. Results and discussion

In all experiments, the biogas produced contained only $H_2$ (35–56%), $CO_2$ (44–65%) and residual $N_2$ from the initial purging. The biogas was free of methane due to the lack of methanogenic activity in the sludge. The VFA and alcohol residues in the mixed liquor were mainly acetate, butyrate, ethanol and propanol, plus small amounts of butanol, valerate and caproate.

Inhibition of individual metal to $H_2$ production was represented by concentration $C_{\mathrm{I},50}$, at which the $H_2$-producing activity of the sludge was reduced 50% relative to the controls.

### 3.1. Effect of heavy metals on hydrogen production

In all batches of this study, $H_2$ production data satisfactorily fitted Eq. (1) with $R^2 > 0.95$, as illustrated in Fig. 1, using Zn as an example. In general, the lag time $\lambda$ of $H_2$ production, increased with the metal concentration. For the 12 control batches, the average $H_2$ yield was $171 \pm 7$ ml, corresponding to $114 \pm 5$ ml-$H_2$ g$^{-1}$-sucrose or $1.6 \pm 0.1$ mol-$H_2$ mol$^{-1}$-sucrose. The maximum slope of specific $H_2$ production represented the SHA of the sludge. For the control batches, the SHA averaged $3.5 \pm 0.2$ l-$H_2$ g$^{-1}$-VSS d$^{-1}$, corresponding to $2.3 \pm 0.1$ g-$H_2$-COD g$^{-1}$-VSS d$^{-1}$.

Table 1 summarizes the kinetic parameters of the modified Gompertz equation at various Zn concentrations. It shows that the lag time increased with Zn concentration, from as low as 4.6 h for the control to 29.9 h for 5000 mg l$^{-1}$ of Zn. Low dosages of Zn (80–400 mg l$^{-1}$) slightly increased the $H_2$ potential from 171 ml up to 188 ml, and the $R_m$ from 4.9 to 5.4 ml h$^{-1}$, corresponding to an SHA from 3.5 to 4.0 l g$^{-1}$-VSS d$^{-1}$. Further increases of Zn dosage (from 1000 to 5000 mg l$^{-1}$) adversely affected both the $H_2$ potential and the SHA.

Similar trends of inhibition as shown in Fig. 1 and Table 1 were observed for the other five metals although to different degrees. Fig. 2 illustrates the inhibition effects of individual metals for concentrations up to 5000 mg l$^{-1}$: (a) changes of $H_2$ production potential, $P$, and (b) changes of SHA, as compared to the controls. Fig. 2a illustrates that $H_2$ production potential decreased with the increase of heavy metals except Zn. Low concentrations (80–400 mg l$^{-1}$) of Zn actually resulted in the increase of $H_2$ production by 8–10%. Fig. 2b illustrates SHA decreased similarly with the concentration increase of heavy metals, except Zn and Pb. Low concentrations of Zn (80–400 mg l$^{-1}$) and Pb (80 mg l$^{-1}$) resulted in the increase of SHA. Of the six heavy metals tested, Cu appeared to be most toxic to $H_2$ production. Fig. 2 illustrates that Cu at

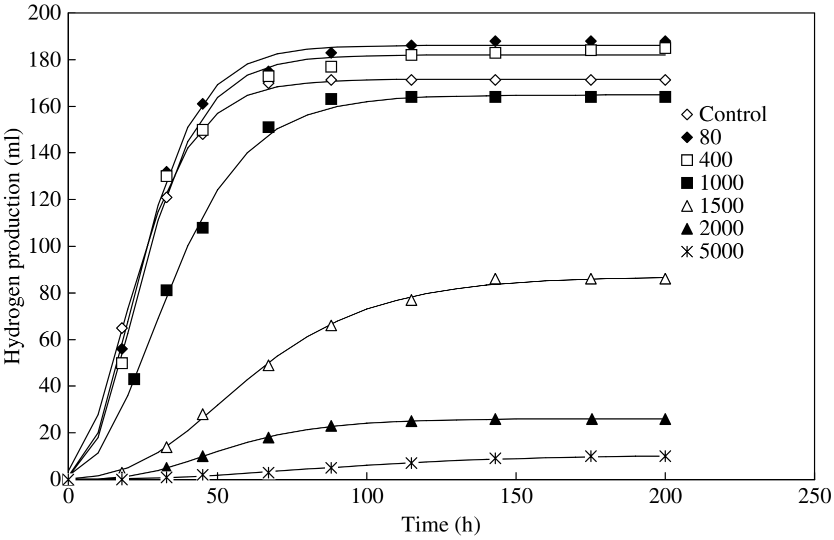

Fig. 1. Cumulative $H_2$ production at various Zn concentrations (in mg $l^{-1}$).

Table 1
Kinetic parameters of $H_2$ production at various concentrations of Zn

| Zn (mg $l^{-1}$) | $\lambda$ (h) | $R_m$ (ml $h^{-1}$) | $P$ (ml) | SHA (l-$H_2$ $g^{-1}$-VSS $d^{-1}$) |
|---|---|---|---|---|
| 0 | 4.6 | 4.9 | 171 | 3.5 |
| 80 | 7.4 | 5.4 | 188 | 4.0 |
| 400 | 7.9 | 5.2 | 185 | 3.8 |
| 1000 | 9.7 | 3.4 | 164 | 2.5 |
| 1500 | 21.4 | 1.1 | 87 | 0.8 |
| 2000 | 22.1 | 0.4 | 26 | 0.3 |
| 5000 | 29.9 | 0.1 | 10 | 0.1 |

concentrations as low as 40 mg $l^{-1}$ reduced the $H_2$ production potential and SHA by over 90% as compared to the controls.

### 3.2. Effect of heavy metals on sucrose conversion

In all the control batches, sucrose was completely degraded in the absence of heavy metals. However, residual sucrose at various concentrations was found in batches dosed with heavy metals. Fig. 3 illustrates the relative conversion of sucrose at various concentrations of individual heavy metals. Similar to Fig. 2, Fig. 3 shows that, of the six heavy metals tested, Cu was the most inhibitive and Pb was the least. For the other four metals, sucrose degradation was adversely affected by high concentrations of metals, but was little affected at low concentrations (20–1000 mg $l^{-1}$).

### 3.3. Comparison of inhibition effects of heavy metals

The inhibition effect of each individual metal may be expressed by $C_{I,50}$, the concentration at which the $H_2$ production parameter was 50% relative to the control (Lin, 1993; Fang, 1997; Fang and Chan, 1997). The values of $C_{I,50}$ for the six heavy metals to $H_2$ production potential, sucrose conversion and SHA are summarized in Table 2. The $C_{I,50}$ values in this study ranged from 10 to over 5000 mg $l^{-1}$, indicating the inhibition effect by individual metals varied substantially. The inhibition effect on $H_2$-producing activity was in the following order: Cu (most toxic) $\gg$ Ni $\sim$ Zn $>$ Cr $>$ Cd $>$ Pb (least toxic). The reason that Cu had considerably lower $C_{I,50}$ values for hydrogen production compared to the other five metals is unclear. A further investigation is warranted.

Table 2 also lists the limited $C_{I,50}$ data for $H_2$ production in the literature for comparison. Zheng and Yu (2004) reported that Cu (with a $C_{I,50}$ of 350 mg $l^{-1}$) was more inhibitive than Zn ($C_{I,50} > 500$ mg $l^{-1}$) in converting glucose to $H_2$. Similarly, in two other studies of $H_2$ production from dairy wastewater, Cu ($C_{I,50}$ 65 mg $l^{-1}$) was found more inhibitive than Zn ($C_{I,50}$ 120 mg $l^{-1}$) (Yu and Fang, 2001a), and Cr ($C_{I,50}$ 72 mg $l^{-1}$) more than Cd ($C_{I,50}$ 170 mg $l^{-1}$) (Yu and Fang, 2001b). The inhibitive effect of these metals was in the order of Cu $>$ Cr $>$ Cd $>$ Zn, slightly different from that observed in this study.

Also listed in Table 2 are the corresponding $C_{I,50}$ data for methanogenic activities of granular sludge degrading starch (Fang and Hui, 1994), benzoate (Fang and Chan, 1997) and VFA (Lin and Chen, 1999). Heavy metals inhibited the methanogenic activity of starch-degrading sludge in the order of Zn $>$ Ni $>$ Cu $>$ Cr $>$ Cd, while the corresponding orders for benzoate- and VFA-degrading sludge were, respectively, Ni $>$ Zn $>$ Cd $>$ Cu $>$ Cr and Cu $>$ Cr $>$ Cd $\sim$ Zn $>$ Ni $>$ Pb. Results indicate that the inhibition effect was substrate dependent.

Overall, with the exception of Cu, the $C_{I,50}$ values in this study were substantially higher than those reported in the literature, indicating that heavy metals were less inhibitive to the $H_2$-producing sludge than to other granular sludge producing either $H_2$ or methane. This may be

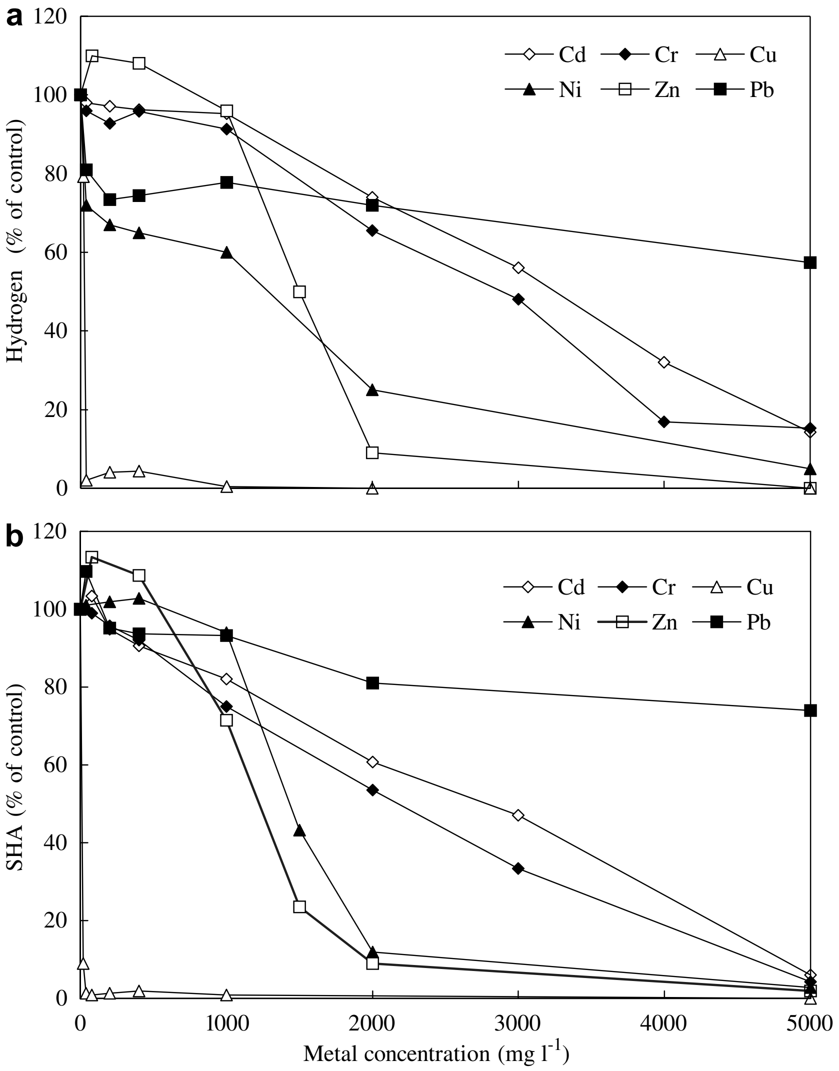

Fig. 2. Effects of heavy metal concentration on (a) $H_2$ production potential and (b) SHA.

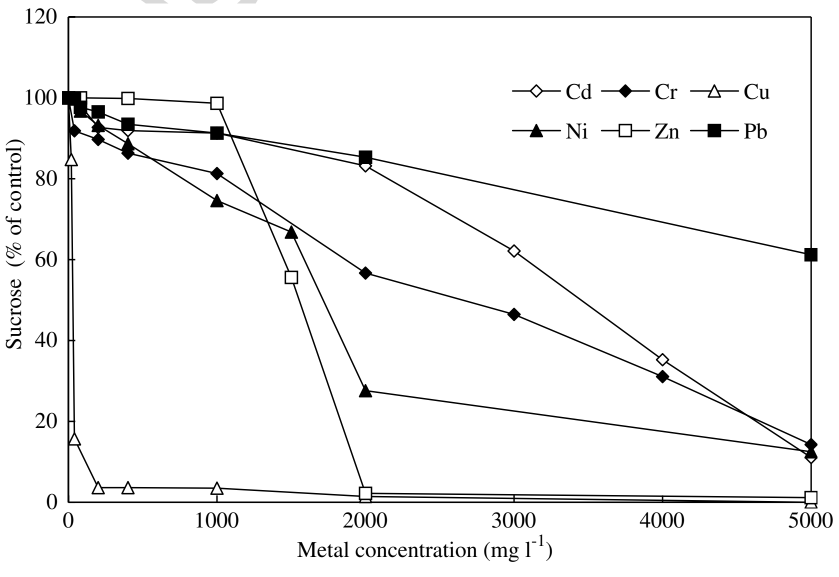

Fig. 3. Effects of heavy metal concentration on sucrose conversion.

Table 2
Comparison of $C_{I,50}$ values of six heavy metals

| Basis of bioactivity parameter | Target product | Carbon source | $C_{I,50}$ (mg $l^{-1}$) | | | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | Cd | Cr | Cu | Ni | Zn | Pb | |
| Hydrogen production potential | Hydrogen | Sucrose | 3300 | 3000 | 30 | 1300 | 1500 | >5000 | Present study |
| Hydrogen production potential | Hydrogen | Glucose | N.A. | N.A. | 350 | N.A. | >500 | N.A. | Zheng and Yu (2004) |
| Hydrogen production potential | Hydrogen | Dairy wastewater | 170 | 72 | 65 | N.A. | 120 | N.A. | Yu and Fang (2001a,b) |
| Methane production potential | Methane | VFA | 330 | 250 | 130 | 1600 | 270 | 8000 | Lin and Chen (1999) |
| Substrate conversion | Hydrogen | Sucrose | 3400 | 2500 | 30 | 1600 | 1500 | >5000 | Present study |
| Substrate conversion | Hydrogen | Dairy wastewater | 110 | 42 | 37 | N.A. | 135 | N.A. | Yu and Fang (2001a,b) |
| Substrate conversion | Methane | VFA | 180 | 140 | 130 | 440 | 200 | 3200 | Lin and Chen (1999) |
| SHA | Hydrogen | Sucrose | 2800 | 2200 | 10 | 1400 | 1200 | >5000 | Present study |
| SMA[a] | Methane | Starch | >550 | 630 | 158 | 118 | 97 | N.A. | Fang and Hui (1994) |
| SMA[a] | Methane | Benzonate | 150 | 210 | 175 | 100 | 110 | N.A. | Fang and Chan (1997) |

[a] Specific methanogenic activity.

attributed to the higher concentrations of extracellular polymeric substances (EPS) in the $H_2$-producing sludge (Fang et al., 2002; Liu and Fang, 2002), and their dense distribution at the outer layer of the granular sludge (Zhang and Fang, 2004). As such, EPS protect the $H_2$-producing cells against the harsh external environment (Liu and Fang, 2003). EPS were found crucial to the flocculation and dewatering of sludge (Liu and Fang, 2003) as well as to the microstructure of methanogenic and $H_2$-producing granular sludge (Schmidt and Ahring, 1996; Fang et al., 2002). Their effects on the resistance of toxic metals and other chemical on $H_2$-producing sludge warrant a further study.

## 4. Conclusion

Inhibition of six heavy metals commonly found in electroplating effluent inhibited the bioactivity of $H_2$-producing sludge in the following order: Cu (most toxic) $\gg$ Ni $\sim$ Zn $>$ Cr $>$ Cd $>$ Pb (least toxic). The $C_{I,50}$ values for individual heavy metals were Cu 30 mg $l^{-1}$, Ni and Zn 1600 mg $l^{-1}$, Cr 3000 mg $l^{-1}$, Cd 3500 mg $l^{-1}$, and Pb >5000 mg $l^{-1}$. $H_2$-producing sludge exhibited in general higher resistance to metal toxicity than methanogenic granular sludge.

## Acknowledgements

The authors wish to thank the Hong Kong Research Grant Council for the financial support of this project (HKU7007/02E), and Chenlin Li wishes to thank HKU for the postgraduate studentship.

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