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Inhibition effects of heavy metals (copper, nickel, zinc, lead) on anaerobic sludge

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ABSTRACT

The inhibition effects of heavy metals (Cu, Ni, Zn, Pb) on to anaerobic sludge were investigated using glucose (3000 mg/l COD) as carbon source and electron donor with a batch experiments (serum bottle assays). The seed sludge was obtained from a UASB (upflow anaerobic sludge blanket) reactor treating yeast wastewater of the Pakmaya Yeast Factory in İzmit, Turkey. The heavy metal concentrations tested were copper (1–1000 mgCu/l), nickel (1–3000 mgNi/l), zinc (1–1000 mgZn/l, and lead (1–10,000 mgPb/l). According to relative inhibition effects of heavy metals on methane producing activity of anaerobic sludge and COD removal in serum bottles experiments during incubation time (12 d) were Cu(most toxic) > Ni ~ Zn > Pb(least toxic). In general, cumulative methane production (ml) decreased with increasing heavy metal concentrations above 10 mg/l. Inhibition effects of heavy metal values (1–10 mg/l) were low on COD removal. Heavy metals measurements in influent and effluent of serum bottles were also compared. Effluent heavy metal concentrations from low to high were found to be Pb, Cu, Zn and Ni.

Keywords: Inhibition; Heavy metal; Anaerobic sludge; COD; Methane production

1. Introduction

Anaerobic treatment by methanogenesis is widely used for the stabilization of municipal wastewaters sludges and municipal solid wastes. Methane fermentation of high-strentgh industrial wastewaters is also widely prefered [1]. Degradation and stabilization of organic materials under anaerobic conditions results in the formation of mixture of carbon dioxide and methane called biogas and biomass [2,3]. An upflow anaerobic sludge blankent (UASB) process has high treatment efficiency and a short hydraulic retention time (HRT) [3,4].

Heavy metals can be present in municipal sewage and sludge. Heavy metals such as copper, nickel, lead, zinc are the most common pollutants found in industrial effluents [5]. They are discharged by several industries such as electro-plating, metal finishing, textile, storage batteries, mining, ceramic and glass [5,6,7]. Selection of the best treatment method is depended on concentratin of heavy metals and cost of treatment. Determination of level of heavy metal concentration for inhibition/toxicity is important because of inhibitory/toxicity of them on to anaerobic sludge.

Heavy metal toxicity and/or inhibition on biological sytems has been reported. However, heavy metal toxicity/inhibition on to UASB processes has been considered rarely [4,8,9]. Heavy metals are not biodegradable and anaerobic process failure can occur for several concentrations of heavy metals [3,8]. Chemical forms of heavy metals are precipitation as sulfide, carbonate and hydroxides; sorption onto either biomass or inert particulate matter and formation of complexes in system [3].

The objective of this study has been to determine inhibition effect of heavy metal concentration on anaerobic sludge. Therefore four heavy metals, copper (II)

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(1–1000 mg Cu/l), zinc (II) (1–1000 mg Zn/l), nickel (II) (1–3000 mgNi/l) and lead (II) (1–10,000 mg Pb/l) have been chosen for investigation of inhbition/toxicity effects on anaerobic sludge.

2. Materials and methods

2.1. Batch experiments and experimental procedure

A 500 ml glass serum bottles were used by sealing with a rubber screw cap. Experiments were performed with 300 ml of working volume by a batch test. Each of the serum bottles consisted of anaerobic sludge (as 3000 mg MLVSS/l) taken from UASB reactor treating the wastewaters of Pakmaya Yeast Factory, in İzmit Turkey, 3000 mg COD/l of glucose and necesarry Vanderbilt mineral medium . This mineral medium contains the following inorganic composition (in mg/l): NH_4Cl, 400; $MgSO_4 \cdot 7H_2O$, 400; KCl, 400; $Na_2S \cdot 9H_2O$, 300; $(NH_4)_2HPO_4$, 80; $CaCl_2 \cdot 2H_2O$, 50; $FeCl_3 \cdot 4H_2O$, 40; $CoCl_2 \cdot 6H_2O$, 10; KI, 10; $(NaPO_3)_6$, 10; L-cysteine, 10; $AlCl_3 \cdot 6H_2O$, 0.5; $MnCl_2 \cdot 4H_2O$, 0.5; $CuCl_2$, 0.5; $ZnCl_2$, 0.5; NH_4VO_3, 0.5; $NaMoO_4 \cdot 2H_2O$, 0.5; H_3BO_3, 0.5; $NiCl_2 \cdot 6H_2O$, 0.5; $NaWO_4 \cdot 2H_2O$, 0.5; Na SeO_3, 0.5 [10]. Substrate, metal ions, and distilled water were added into serum bottles. The alkalinity and neutral pH were kept constant by addition of 5000 mg/l $NaHCO_3$. The initial pH values were adjusted to 7.0 with NaOH (2M) and H_2SO_4 (2M) solutions.Temperature controlled incubator was used at 35 °C for all batch experiments. The serum bottles were shaken at 120 rpm during determined intervals. The syringe was used to take supernatant samples from bottles. The mixed liquors samples were centrifuged for analysis of heavy metals and chemical oxygen deman (COD).

A control, without heavy metal sample, was used to determine and compare COD measurements in all batch serum bottles. COD removal and heavy metal experiments in all batch study were performed in duplicate to control the accuracy of the experimental results. Experimental data was detected both from COD and heavy metal measurements. Methane gas to COD conservation was considered as 395 ml methane gas produced from the removal of 1 g of COD^{-1}.

2.2. Analytical procedure

TSS (total suspended solid) and TVS (total volatile solid) of anaerobic sludge were measured using the Standart Methods of American Public Health Association [11]. Bicarbonate alkalinity and COD measurements was determined by titrimetric method [11]. Methane production was measured by using a sodium hydroxide solution (3%, w/v) displacement system [12]. Heavy metal measurements were carried out in 5 ml samples removed from supernatants of serum bottles during the experimental study. Nova 60 Model Spectrophotometer was used for heavy metal measurements. The samples were centrifuged at 5000 rpm for 20 min.

3. Results and discussion

3.1. Effects of heavy metal concentrations (Cu, Ni, Zn, Pb) on COD removal

TSS and TVS of anaerobic sludge were determined as 22,555 mg/l and 18,000 mg/l, respectively. Heavy metal solutions were added to serum bottles at the beginning of the incubation time. The samples were drawn at 24 h intervals with a syringe.

In this study, glucose was used as a co-substrate for carbon and energy source for microbial growth. Fig. 1 illustrates the effect of initial copper concentrations on the COD removal efficiency of samples during incubation time (12 d). As shown from Fig. 1, as the copper ion concentration increased, COD removal efficiency decreased.In general, increase of inhibition/toxicity at higher heavy metal concentrations was found for the four studied (Cu, Zn, Pb, Ni) heavy metals based on COD removal efficiency (Fig. 1, Figs. 3–5). Cumulative methane gas productions in batch reactors (Fig. 2) were determined for each of serum bottles (data not shown, except Cu).Cumulative methane gas production decreased as the heavy metal concentrations increased. It could be considered that high concentrations of heavy metal caused inhibition of anaerobic degradation of glucose. Thus lower methane gas was produced at high heavy metal concentrations.

As seen in Fig. 1, when the copper concentrations increased from 250 to 1000 mg Cu/l, COD removal efficiency decreased rapidly. Similar to COD measurements, high concentrations of copper ions (500–1000 mg

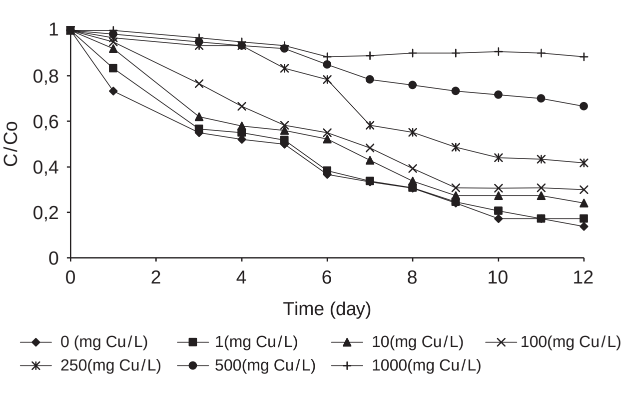

Fig. 1. The effect of initial copper concentrations on the COD removal efficieny of samples during incubation time (Co = 3000 mg/l).

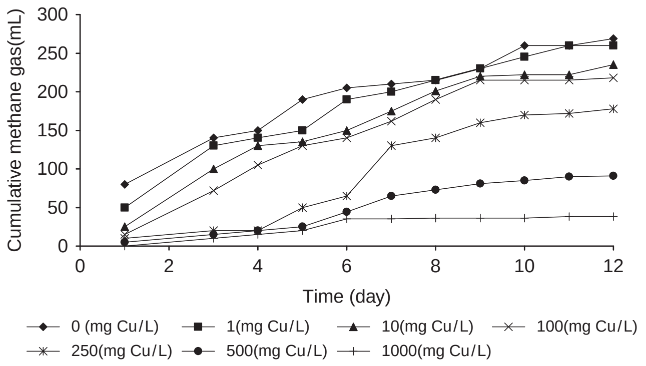

Fig. 2. Effect of copper concentrations on cumulative methane production during the incubation time.

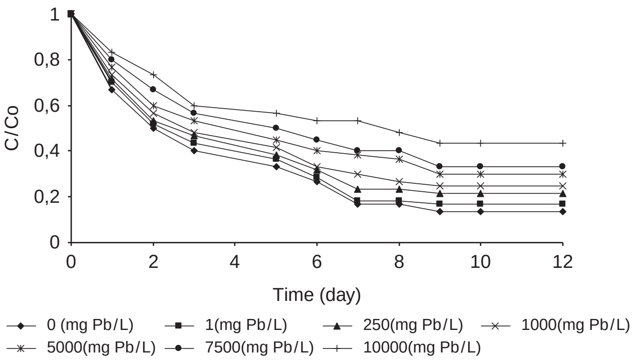

Fig. 4. The effect of initial lead concentrations on the COD removal efficiency of samples during incubation time (Co = 3000 mg/l).

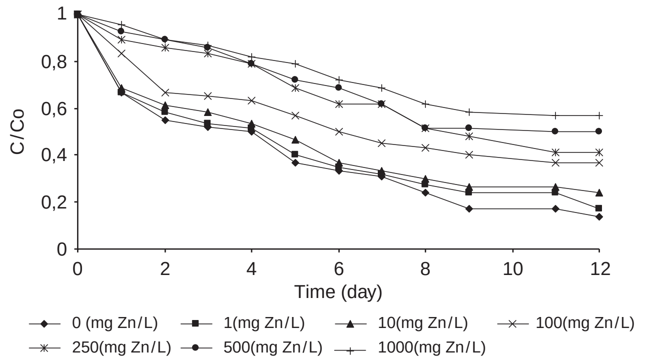

Fig. 3. The effect of initial zinc concentrations on the COD removal efficiency of samples during incubation time (Co = 3000 mg/l).

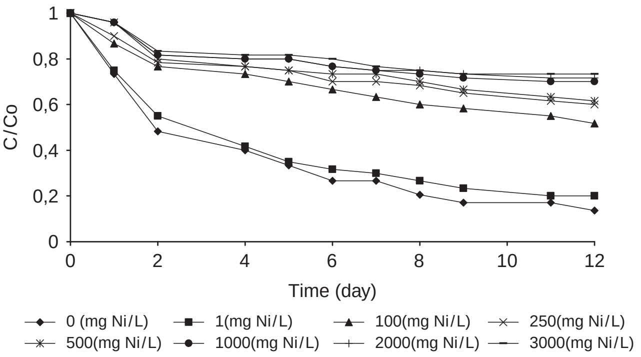

Fig. 5. The effect of initial nickel concentrations on the COD removal efficiency of samples during incubation time (Co = 3000 mg/l).

Cu/l) lead to decreasing of methane gas production (Fig. 2). Cumulative methane gas production was found to be 275 ml in control (without copper). It was observed to be 91–38 ml for 500–1000 mg Cu/l concentrations at the end of incubation time, respectively.

Addition of the zinc ions as 100–250–500–1000 mg Zn/l into serum bottles inhibited COD removal efficiency compared to addition of 1–10 mg Zn/l (Fig. 3). Cumulative methane gas production in serum bottles was measured as 152 and 120 ml, respectively for 500–1000 mg Zn/l.

When compared the effect of Cu and Zn ions in batch serum bottles studies, it could be thought that copper was more toxic than zinc on to anaerobic sludge.

Lead showed a low toxic effect at high concentrations (1000–5000 mg/l) (Fig. 4). Lead produced an effect which was less marked than other three metals. It could be said that copper had the greatest impact.

In general, cumulative methane production (ml) decreased with increasing heavy metal concentrations above 10 mg/l. Inhibition effects of heavy metal values (1–10 mg/l) were low on COD removal. The relative toxicityof four metals to the anaerobic sludge was found to be $Cu^{2+} > Ni^{2+} \sim Zn^{2+} > Pb^{2+}$ (Fig. 5).

According to literature studies, although results of batch and continuous experimental studies for toxicity of several heavy metals gave some differences , lead was least toxic effect onto anaerobic bacteria among copper, zinc and nickel heavy metals.The effects of other heavy metals (Cu, Zn, Ni) on anaerobic sludge were found to be different depended on experimental conditions and sludge characteristics [3,4,5,15,16,17].

3.2. *Effluent heavy metal concentrations (Cu, Ni, Zn, Pb) during the ıncubation time*

As shown from effluent heavy metal concentrations (Cu, Ni, Zn, Pb) during the incubation time (Figs. 6–9), soluble heavy metal concentrations rapidly decreased during the batch study. Chemical forms of heavy metals are important in order to determine toxic effects of them

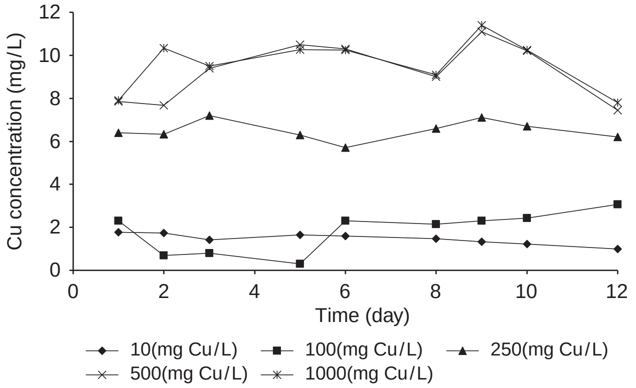

Fig. 6. Copper concentrations in effluent of serum bottles during the incubation time.

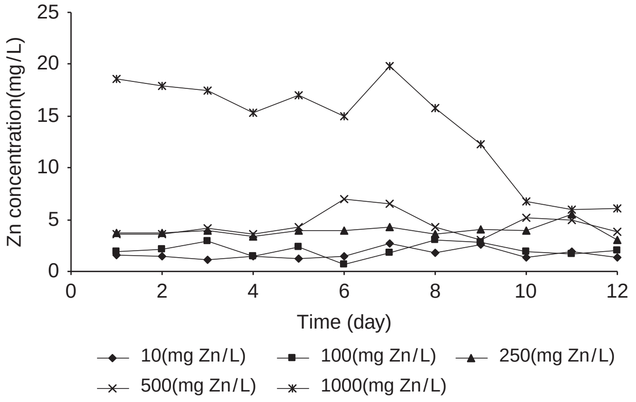

Fig. 7. Zinc concentrations in effluent of serum bottles during the incubation time.

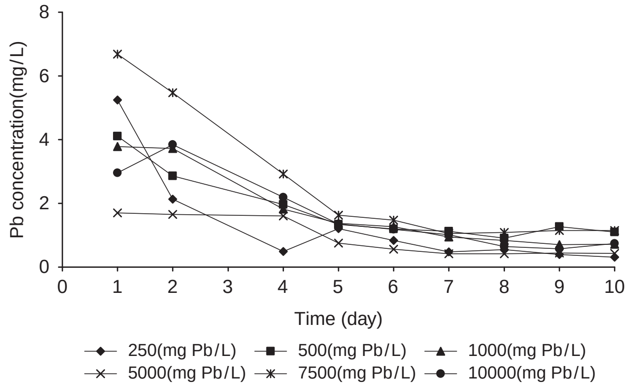

Fig. 8. Lead concentrations in effluent of serum bottles during the incubation time.

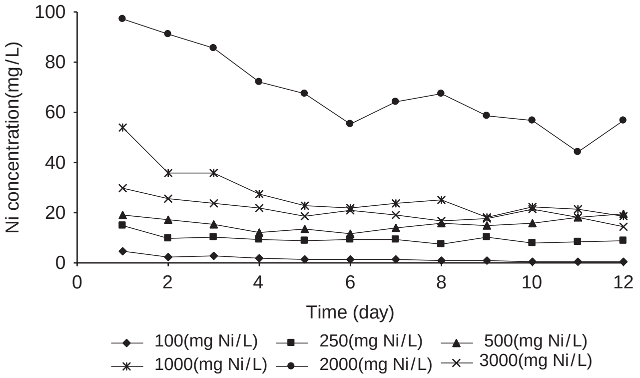

Fig. 9. Nickel concentrations in effluent of serum bottles during the incubation time.

onto anaerobic sludge. Chemical forms of them are precipitation (as sulfide, carbonate and hydroxides), sorption on to solid form, either biomass or inert particulate matter [3,13]. Metal attachment to anaerobic sludge can occur by precipitation in the form of carbonates or sulfides or by linkage to organic liquids in several ways [14]. The reason of this has been attributed to probably precipitation and sorption mechanism on the anaerobic sludge. Although influent lead concentration to serum bottles was high, effluent of them during the incubation time was so low. It could be attributed to precipitation and adsorbed form of lead [18].

The highest initial heavy metal concentrations were readily tolerated by anaerobic sludge. The results of this study showed that anaerobic bacteria had high resistance to heavy metal inhibition/toxicity even at high concentrations of them.

Effluent heavy metal concentrations from low to high were found to be Pb, Cu, Zn and Ni. Adsorption capacity of studied heavy metal in anaerobic reactor was found to be Pb > Cu > Zn > Ni by Leightan and Forster [19]. Smilar to this study, the affinity order of anaerobic biomass for the four studied metals has been established as Pb > Cu > Ni > Cd [18].

4. Discussion

There is complex mechanism (physical-chemical-biological) between added metals into serum bottles and anaerobic granules. Especially, precipitation of metal hydroxides for higher pH values is important in

Table 1
Parameters for the binding strength of heavy metals [18]

Heavy metal	Charge, z	Ionic radius, r_{ion}(A°)	Hydrated ion radius, r_{hyd}(A°)	Equilibrium constant, pK^d
Pb	2	1.19	4.01	7.7
Cu	2	0.73	4.19	7.7
Ni	2	0.69	4.04	9.9
Zn[a]	2	0.74	–	–

[a] Ref. [20].

Table 2
Relative toxicity limits for heavy metals

Bioactivity parameter	Product	Carbon source	Relative toxicity/Inhibition	Reference
COD removal potential	Methane	Glucose	Cu(most toxic) > Ni~Zn > Pb	Present study
Methane production potential	Methane	Glucose	Zn > Cr > Ni ~ Cd	[5]
Methane production potential	Methane	VFA(Volatile Fatty Acid)	Cu > Cr~Zn > Cd > Pb	[4]*
Specific Methanogenegic activity	Methane	Starch	Zn > Ni > Cu > Cd > Cr	[16]*
Hydrogen production potential	Hydrogen	Sucrose	Cu > Ni~Zn > Cr ~ Cd > Pb	[21]*
Hydrogen production potential	Hydrogen	Glucose	Cu > Zn	[22]*
Hydrogen production potential	Hydrogen	Dairy wastewater	Cu > Cr > Zn > Cd	[23]*, [24]*
Specific Methanogenegic activity	Methane	VFA	Cd > Cu > Zn > Pb	[9]*

*Ref. [5].

the serum bottles. Therefore, it is difficult to understand and interpret the heavy metal behaviour in this study.

The binding capability of heavy metal ion to anaerobic sludge is dependent on hydration and hydrolysis effects [18] (Table 1). From the literature findings [15,18] and present study result it was understood that lead has the highest affinity for anaerobic sludge because of its lowest hydration radius or largest ionic radius compared with those of other metals. This behaviour may be explained that larger ions interact with different groups simultaneously in the anaerobic sludge. The statement mentioned are in good agreement with the results given in literature [15,18]. As can be seen figures (Figs. 6–9) effluent heavy metal concentration follows the scale: (lowest)Pb^{+2} < Cu^{+2} < Zn^{+2} < (highest)Ni^{+2}.

Results of among the studied heavy metals's inhibition/toxicity effects on anaerobic sludge are quite different in literature. The reason for this can be attributed to some cases: The carbon sources used for anaerobic methabolism (glucose, VFA and so on), measured evaluation parameter (methane or hydrogen production potential, COD removal etc.), operation of experimental study (batch or continuous), characteristics of anaerobic sludge, binding strength of a heavy metal ion to the anaerobic sludge (sorption, precipitation) were different in each study and evaluated in the literature by different ways (see Table 2) [3–5,9,15–24].

5. Conclusion

The effects of copper, zinc, nickel and lead on the methanogenic anaerobic sludge taken from UASB were investigated by using serum bottles.

In general, results of this study showed that anaerobic bacteria exhibited high resistance to studied heavy metals in batch study. The inhibition of the heavy metals were in the following order: copper (most toxic) > nickel > zinc > lead (least toxic). Copper was the most toxic metal tested. Effluent heavy metal concentrations from low to high were found to be Pb, Cu, Zn and Ni.

Increase of inhibition/toxicity at higher heavy metal concentrations was found for the four studied (Cu, Zn, Pb, Ni) heavy metals based on COD removal efficiency in batch reactors.

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References

[1] B.E. Rittmann and P.L. McCarty, Environmental Biotechnology: Principles and Applications, McGraw-Hill International Editions, 2001.
[2] B.P. Kelleher, J.J Leahy, A.M. Henihan, T.F. O'Dwyer, D. Sutton and M.J. Leahy, Advances in poultry litter disposal technology—a review, Biosource Technol., 83 (2000) 27–36.
[3] Y. Chen, J.J. Cheng and K.S. Creamer, Inhibition of anaerobic digestion process: A review, Biosource Technol., 99 (2008) 4044–4064.
[4] C.Y. Lin, and C.C. Chen, Effect of heavy metals on the methanogenic UASB granule, Water Res., 33/2 (1999) 409–416.
[5] L. Altaş, Inhibitory effect of heavy metals on methane-producing anaerobic granular sludge, J. Hazardous Mater., 162/2–3 (2009) 1551–1556.
[6] K.K. Pand, G. Prasad and V.N. Singh, Copper (II) removal from aqueous solutions by fly-ash, Water Res., 19/7 (1985) 869–873.
[7] M. Sarioglu, Ü.A. Atay and Y. Cebeci, Removal of copper from aqueous solutions by phosphate rock, Desalination, 181 (2005) 303–311.
[8] C.Y. Lin, Effect of heavy metals on volatile fatty acid degradation in anaerobic digestion, Water Res., 26 (1992) 177–183.
[9] C.Y. Lin, Effect of heavy metals on acidogenesis in anaerobic digestion, Water Res., 27 (1993) 147–152.
[10] R.E. Speece, Anaerobic Biotechnology for Industrial Wastewaters, In: Nashville Tennessee, Archae Press, USA (1996).

[11] APHA-AWWA-WPCF, Standard Methods for Examination of Water and Wastewater , 21st ed., Am. Public Health Ass., Washington, DC, USA (1989).

[12] M. Jawed and V. Tare, Microbial composition assesment of anaerobic anaerobic biomass through methanogenic activity tests, Water SA, 25 (1999) 345–350.

[13] H.S. Shin, S.E. Oh and C.Y. Lee, Influence of sulfur compounds and heavy metals on the methanization of tannery wastewater, Water Sci. Technol., 35/8 (1997) 239–245.

[14] I.R. Leighton and C.F. Forster, Tech., The effect of heavy metals on a thermophilic methanogenic upflow sludge blankent reactor, Biosource 63 (1998) 131–137.

[15] J.C. Codinc, M.A. Munoz, F.M. Cazorla and A. Perez-Garcia, The inhibition of methanogenic activity from anaerobic domestic sludges as a simple toxicity bioassay, Water Res., 32/4 (1998) 1338–1342.

[16] H.H.P. Fang and H.H. Hui, Effect of heavy metals on the methanogenic activity starch-degradig granules, Biotechol. Lett., (1994) pp. 1091–1096.

[17] M.L. Lin, Effects of Heavy Metals an UASB Process, M.S. Thesis, Feng China University, Taichung, 1994.

[18] A.H. Hawari and C.N. Mulligan, Effect of the presence of lead on the biosorption of copper, cadmium and nickel by anaerobic biomass, Process Biochem., 42 (2007) 1546–1552.

[19] I.R. Leighton and C.F. Forster, The adsorption of heavy metals in an acidogenic thermophilic anaerobic reactor, Water Res., 32/12 (1998) 2969–2972.

[20] C.C. Liu, M.K. Wang, C.S. Chiou, Y.S. Li, C.Y. Yang and Y.A. Lin, Biosorption of chromium, copper and zinc by wine-processing waste sludge: Single and multi-component system study, J. Hazardous Mater., 171 (2009) 386–392.

[21] C. Li and H.H.P. Fang, Inhibition of heavy metals on fermentative hydrogen production by granular sludge, Chemosphere 67 (2007) 668–673.

[22] X.J. Zheng and H.Q. Yu, Biological hydrogen production by enriched anaerobic cultures in the presence of copper and zinc, J. Environ. Sci. Health A 39 (2004) 89–101.

[23] H.Q. Yu and H.H.P. Fang, Inhibition on acidogenesis of dairy wastewater by zinc and copper, Envir. Tech., 22 (2001) 1459–1465.

[24] H.Q. Yu and H.H.P. Fang, Inhibition by chromium and cadmium of anaerobic acidogenesis, Water Sci. Technol., 43 (2001) 267–274.