

CHAPTER VI

THE MAXIMUM CONCENTRATION OF HEAVY METALS IN WASTEWATER THAT WILL NOT CAUSE SYNERGISTIC INHIBITION TO ANAEROBIC WASTEWATER TREATMENT

6.1 Introduction

Prevention of biological inhibition or toxicity in an anaerobic treatment system is a complex phenomenon that is slowly being understood. In this study, the optimum reactor conditions for simultaneous control of sulfate (SO_4^{2-}) and heavy metals in complex wastewater streams were provided to enhance the system performance. A mixed-bacterial culture from a brewery wastewater treatment plant with a high potential for effectiveness was used in the study in order to facilitate the use of sulfate reducing bacteria (SRB) that utilize SO_4^{2-} as an electron acceptor for cell growth, resulting in the reduction of SO_4^{2-} to sulfide (S^{2-}) and the transformation of organic substances to methane (CH_4), with concurrent lowering of sulfide and reduction of the toxic risk from heavy metals through metal precipitation as metal sulfides. To control the level of SO_4^{2-} reduction, in order to encourage the mutualistic condition between the SRB and the methane producing bacteria (MPB) and to support the high specific methanogenic activity of the MPB, the optimum COD:S ratio of 9 was provided in the reactors. The effects of Cd, Cu, and Zn when present as a single metal and as a mixture of combined metals on CH_4 production, COD removal, and VFA degradation and accumulation were investigated.

The extent to which metals are concentrated in wastewater can directly affect the system. This study illustrates the effects of heavy metal(s) on anaerobic processes. Previous studies have focused on a single metal system. However, the reactors present a more complex situation and it is more usual in practical situations to find a mixture of heavy metals. For the design of a treatment system and for the operation of an anaerobic reactor, it is useful to determine heavy metal levels in soluble forms in the influents that could be expected to cause inhibition.

6.2 Purpose of this Chapter

This study investigated the toxic effect of the soluble metal(s), which was mitigated by metal sulfide precipitation in order to reduce metal bioavailability. The effects of a single metal and that of combined metals on the bioactivities as monitored by CH₄ production, COD removal, SO₄²⁻ reduction, and VFA accumulation in the system were examined. The synergistic and/or antagonistic effects of combined metals were studied in order to understand how to prevent biological inhibition. Inhibition by combined metals was studied at the given pH value and COD:S ratio and they were included in the model. An activity factor for bacteria was the key variable to quantify the extent of inhibition in reactors. The criterion model for the bioreactor utilized for metal removal and wastewater treatment was developed. Then the model was validated.

Such heavy metal concentration in wastewater that would adversely affect the anaerobic biological treatment were studied in the form of the soluble added metals before the metal sulfide precipitation and the soluble metal that left in the effluent after treatment.

6.3 Materials and Methods

Procedure for the assessment of inhibitory effects of heavy metals toward anaerobic microorganisms as well as the experimental method are provided in Chapter 3 (Section 3.3). The laboratory scale batch apparatus for this study was shown in Figure 3.6.

A series of completely-mixed batch experiments was carried out in reaction bottles with 100 ml working volumes. These bottles, equipped with a separate gas collection system, were placed in a shaking water-bath with temperature controlled at 35±1 °C.

The seed sludges obtained from a mesophilic brewery wastewater treatment plant were acclimated in 6-liter completely mixed reactors for more than 2 months at room temperature (30±2°C). The glucose substrates used in the acclimation and during the batch test also contained sufficient inorganics, with their components applied from Leighton and Forster (1997) (Section 3.2 Table 3.2). The hydraulic

retention time (HRT) for the fill-and-draw operation type seed sludge reactors was 20 days.

The reaction bottles were initially purged with N_2 and then seeded with 80 ml acclimated steady-state seed sludge. Proper amounts of 32,000 mg COD/l glucose synthetic waste and distilled water were added into the gassed bottles to make the initial COD around 3,000 mg/l in order to mimic industrial wastewater. The real value of COD from synthetic waste commingling with filtrated seed sludge was analyzed and recorded as influent COD. The initial biomass concentration was around 10,000 mg/l. The initial loading factor (COD/MLVSS) in each bottle was similarly controlled. The proper amount of the Na_2SO_4 solution was added in order to give rise to the optimum COD:S ratio of 9 in the reactors. Cd, Cu, and Zn as aqueous nitrate forms were added to the reactors in single metal soluble forms and in a combined metal form. The concentration range of each heavy metal was determined from the solubility of that heavy metal under the expected hydroxide concentrations in the wastewater at the operating pH (7.0 ± 0.5). Various ranges of metal concentrations used in the test were selected based on the differing levels of inhibition shown by different metals, which affect the critical process variables. The concentrations of Cd, Cu and Zn used in this study were ranged between 0-20, 0-2 and 0-30 mg/l, respectively.

Optimum conditions were provided in this operation. Operational parameters of initial pH 7 and temperature of $35^{\circ}C$ were adjusted in order to facilitate anaerobic reaction. After a preliminary operation of 5 minutes for minimizing the effects of environmental changes and gas phase difference, the experiment started. The contents were sampled every 6 to 12 hours with a syringe to determine MLVSS, SO_4^{2-} , and heavy metal. Before sampling mixed liquor, for the purpose of measuring CH_4 production, the total gas production was recorded (at room temperature) and collected at times 1, 3, 6, 12, 24, 48, 72, 96, and 120 hours. The mixed liquors were drawn with a syringe at times 1, 72, and 120 hours and filtered for MLVSS determination and supernatants were taken for SO_4^{2-} , metal and COD analysis. Samples for COD analysis had been acidified and shaken for 2 hours to remove sulfide. The initial and final CODs were analyzed. The final pH of the effluent was measured.

The CH₄ content was then determined by a Shimadzu GC-14B gas chromatograph equipped with a thermal conductivity detector (TCD). Sulfate was analyzed according to the procedure of Standard Methods (APHA, 1992).

Assays were run for a 120 hour period. Inhibition was quantified by determining the doses of added metal(s) and soluble metal(s) left in the effluent that inhibit methanogenesis in terms of CH₄ production and COD removal (or both) during the anaerobic reaction over a fixed period of time. The parameters used in measuring the effects of metal(s) were CH₄ production and the effluent COD. The gas volumes were calculated to the standard temperature (0⁰ C) and pressure (760 mm Hg) (STP) and recorded at this condition. An activity factor (AV) was used to indicate the extent of inhibition. The role of heavy metal(s) on SO₄²⁻ reduction and VFA accumulation was also monitored.

The data obtained from the experiments were analyzed. A criterion model for preventing system inhibition was developed. Then another set of experiments was performed to verify the model.

6.4 Results and Discussion

The results showed a uniform drop in CH₄ production as well as in the COD removal efficiency, providing information that could be used to determine the portion of remediation that could be attributed to biological activity. To quantify how much the biological activity was reduced, the amount of CH₄ production and COD removal were observed in the same period and are shown as the activity factor (AV) in the unit of % of control. The production activity (AV) was defined as the fraction of CH₄ production in 120 hours with metal present compared with a no-metal control.

$$AV (\%) = (\text{CH}_4 \text{ production m} / \text{CH}_4 \text{ production c}) \times 100$$

CH₄ production m = amount of CH₄ production in 120 hours with metal dosed.

CH₄ production c = amount of CH₄ production in 120 hours without metal (control)

Similarly with COD, the COD removal activity (AV) was defined as the fraction of COD removal in 120 hours with metal present compared with a no-metal control.

$$AV (\%) = (\text{COD removal m} / \text{COD removal c}) \times 100$$

COD removal m = amount of COD removal in 120 hours with metal dosed.

COD removal c = amount of COD removal in 120 hours without metal (control).

The toxicity of the metal(s) was depicted by the reduction of methanogenic activity and the reduction of the COD removal, as compared to the controls. VFA's were monitored to elucidate the step that was affected by the presence of heavy metal. The inhibitory effects of heavy metal(s) on the microorganisms were assessed. The system performance in the removal of COD, SO_4^{2-} and heavy metal was reported.

The results were divided into 6 main parts.

6.4.1 Inhibition of Methane Production by Metal

There is no universal definition of the toxicity of heavy metals because toxic effects may vary according to the organisms affected and the particular situations. In this study, **the inhibition level** is defined as a 20% decrease in CH_4 production observed. Thus, in this case, the production activity (AV) for methane production is 80%. **The toxic level** is defined as a 50% decrease in CH_4 production. Thus, the production activity (%AV) as the fraction of CH_4 , is reduced to 50%.

6.4.1.1 Inhibition of Methane Production by a Single Metal

Cd, Cu and Zn resulted in a decrease in the percentage of CH_4 production and/or a delay in production. Figure 6.1 (Table H-1) depicts the percentage of CH_4 production in an experiment taken as an example. Nevertheless, differences in inhibition of individual metals and their concentrations were observed.

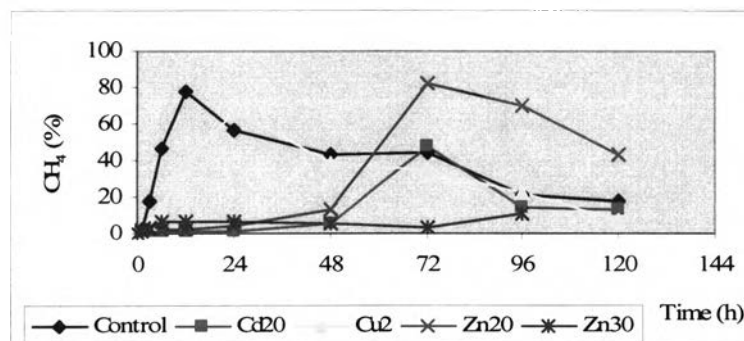
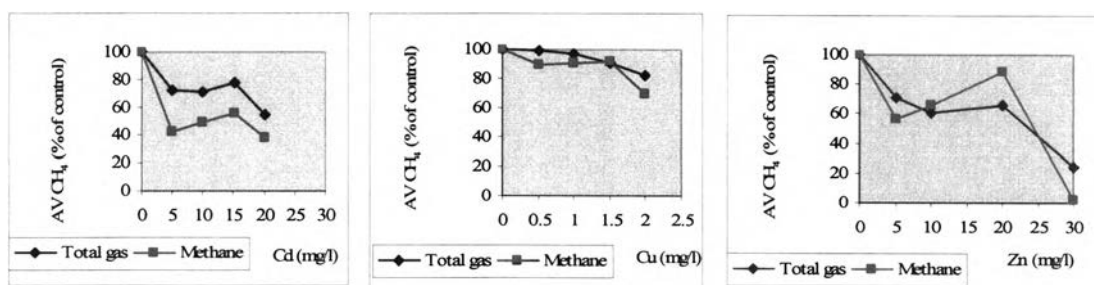


Figure 6.1 The percentage of methane profile when system contains a **single metal**.

The figure reveals that metal could affect either the CH_4 content or cause a delay in CH_4 production, or both. Cd at 20 mg/l caused both a delay and an inhibition

in producing CH₄. Cu at 2 mg/l caused both a slight delay and inhibition in CH₄ production. Zn at 20 mg/l caused a delay in CH₄ production but the CH₄ content was still as high as that of the control. While Zn at 30 mg/l caused inhibition in the CH₄ production. The highest CH₄ content which was obtained after dosing Zn at 30 mg/l was only 10 per cent.



(A) (B) (C)
Figure 6.2 Relationship between methane production activities (AV) and a single metal dosed (A) Cd , (B) Cu, and (C) Zn

Methane production activity decreased with increasing metal concentration, but the experiments showed differences in the inhibition by individual metals as a function of its concentration. Figure 6.2 reveals the role of initial Cd, Cu, and Zn concentration on the methanogenic activity normalized with respect to the control in assays with glucose synthetic waste. Results illustrate that heavy metals, in general, inhibit the bioactivity. The 20% inhibition concentrations of Cd, Cu and Zn with regard to CH₄ production were 2, 1.7 and 3 mg/l, respectively (Figure 6.2 and Table H-2). The relative toxicity in terms of CH₄ production was Cu > Cd > Zn. It is of interest that the presence of Zn at concentrations lower than 5 mg/l inhibits CH₄ production activities but Zn at higher concentrations between 5-20 mg/l, increases activities. However, when the Zn concentration was higher than 20 mg/l, the activities significantly decreased. The reason of this varying effect on the degree of inhibition by Zn concentrations is not well understood and should be studied. Lin and Chen (1999) found that at metal concentration of 1 mg/l, for several metals except lead, CH₄ production activity was reduced by 10-20%. Moreover, they found that the relative toxicities were different for the same metals depending on the sludge affinity.

Lin (1992) reported results similar to those of this study (the relative toxicity was Cu>Cd>Zn) when that researcher studied the effect of heavy metals on the

degradation of volatile fatty acids. Lin and Chen (1999) studied the metal toxicity by considering biogranules from a UASB reactor and reported the relative toxicity as $Cu > Cd > Zn$ and $Cu > Zn > Cd$ for sludge at hydraulic residence time, HRT, 1 and 2 day, respectively. They found that the effects of the metal depended on the HRT and on sludge affinity. Various degrees of sludge affinity with the metals was considered to be one of the reasons why the relative toxicities were different for same metals. The sludge affinity depends on sludge sorption, the mechanism of which was believed to be chemisorption by weakly acidic organic functional groups (Gould and Genetelli, 1984).

6.4.1.2 Inhibition of Methane Production by Combined Metals

Combined metals caused more inhibition to the system than a single metal. Figure 6.3 (Table H-3) reveals the effect of combined metals from an experiment taken as an example of the combined metal effect on the percentage of CH_4 production. Methane production activities were reduced substantially when combined metals were added in the system.

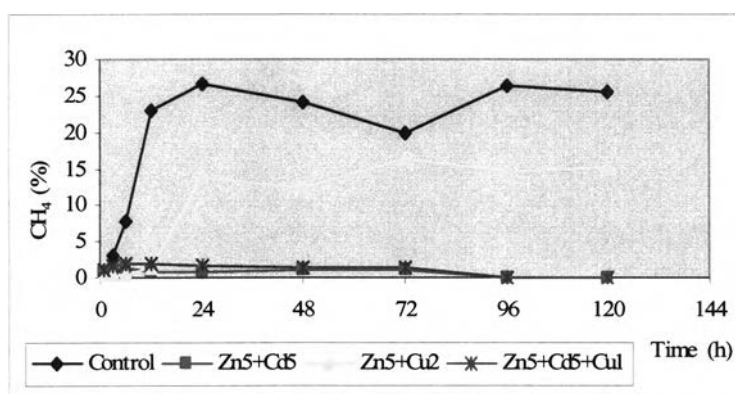


Figure 6.3 The percentage of methane profile when system contains **combined metals**.

Combined metals at some concentrations caused synergistic inhibition on CH_4 production activities (Figures 6.4 to 6.6 and Table H-4). For example, Figure 6.4(A), Zn (20 mg/l) caused 11 % inhibition, while Cd (5 mg/l) caused 58 % inhibition. When Zn (20 mg/l) was combined with Cd (5 mg/l), the percentage of inhibition was 98.

Figure 6.5(B), Zn (10 mg/l) caused 35 % inhibition, while Cu (2 mg/l) caused 31 % inhibition. When Zn (10 mg/l) was combined with Cu (2 mg/l), the percentage of inhibition was 99.

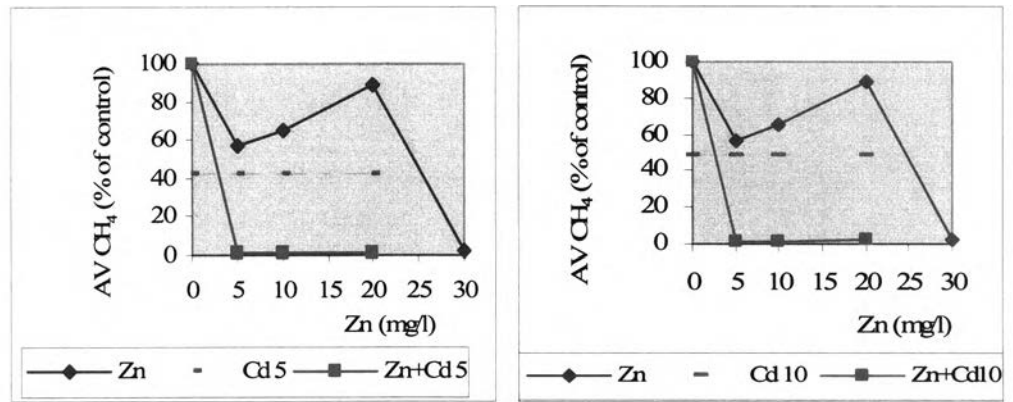


Figure 6.4 Effect of **combined metals** on the pattern of inhibition of methane production (A), Zn at various concentrations + Cd (5 mg/l); and (B), Zn at various concentrations + Cd (10 mg/l).

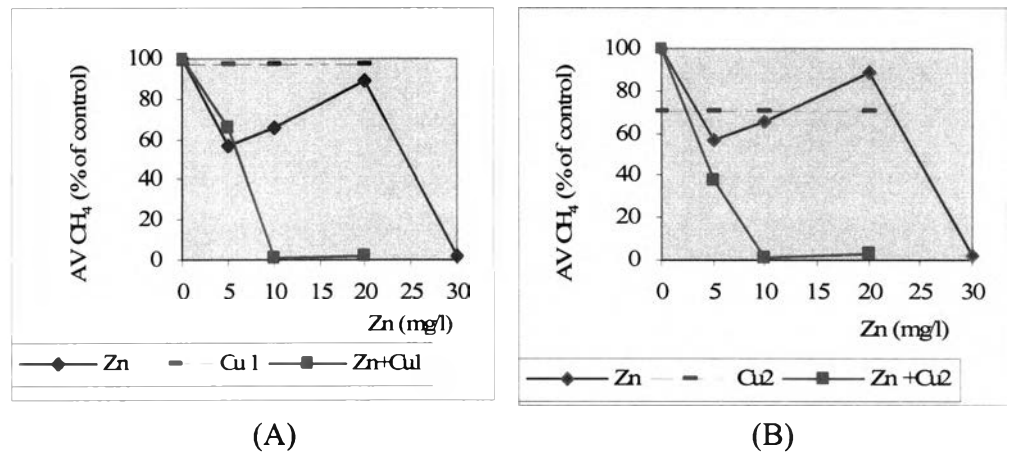


Figure 6.5 Effect of **combined metals** on the pattern of inhibition of methane production (A), Zn at various concentrations + Cu (1 mg/l); (B), Zn at various concentrations + Cu (2 mg/l).

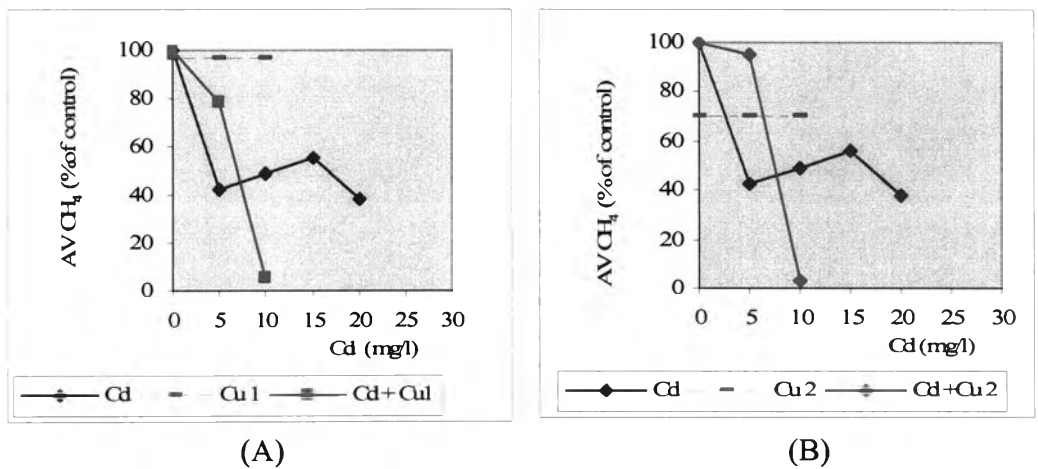


Figure 6.6 Effect of **combined metals** on the pattern of inhibition of methane production (A), Cd at various concentrations + Cu (1 mg/l); (B), Cd at various concentrations + Cu (2 mg/l).

Figure 6.6(A), Cd (10mg/l) caused 52 % inhibition, while Cu (1mg/l) caused 3% inhibition. When Cd (10 mg/l) was combined with Cu (1 mg/l), the percentage of inhibition was 95.

Interestingly, while combinations of metals were more toxic than some single metals, on the other hand combinations of metals at some smaller concentrations reduced the inhibition effect on CH₄ production activities. For example, as shown in Figure 6.5 (A) toxicity of Zn (5 mg/l) was mitigated by addition of Cu (1 mg/l). Another example involved Cd. Figure 6.6 (A) and (B) show that toxicity of Cd (5mg/l) was reduced in inhibition if the cadmium was commingled with Cu (1 – 2 mg/l). However, the phenomena were not found when Zn was combined with Cd (Figure 6.4 (A) and (B)). This suggested that Cu and Zn at small concentrations may support the activity factor in terms of CH₄ production. When inhibition is being caused by an excess of a specific metal, the inhibition can sometimes be antagonized by the addition of one or more of the other metals. The studies on the type and degree of the antagonistic effects is not well understood and should be studied further.

6.4.2 Inhibition of the COD Removal by Metal

Similar to the CH₄ production activity, the inhibition level for COD removal is defined as a 20% decrease in COD removal. Thus, in this case, inhibition is defined as occurring when the AV is 80% or less, as measured by COD removal.

6.4.2.1 Inhibition of the COD Removal by a Single Metal

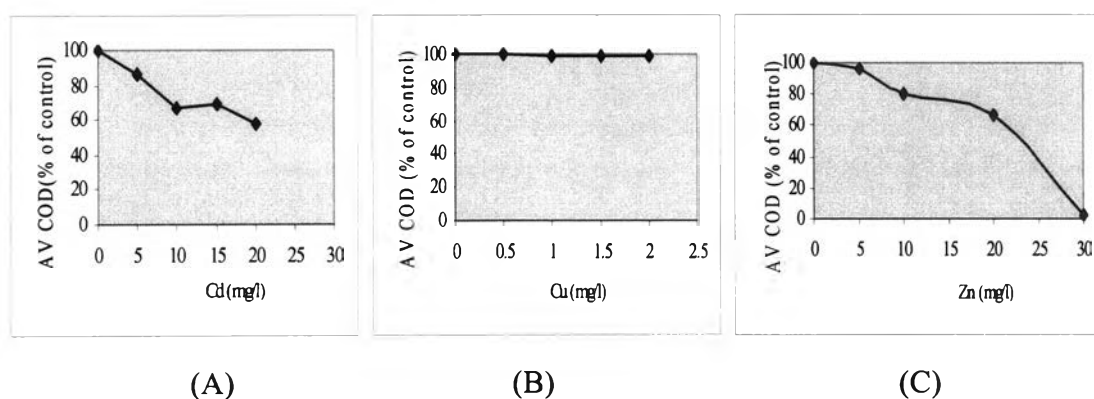


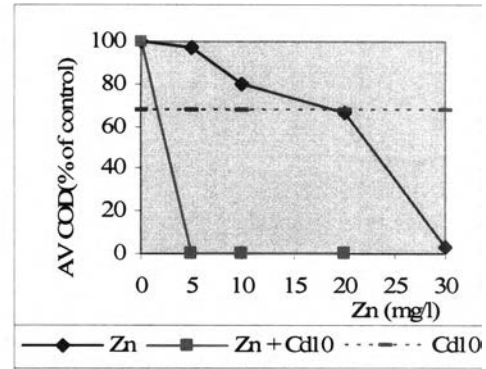
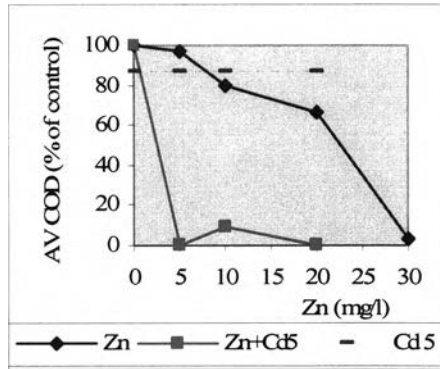
Figure 6.7 Relationship between COD removal activities (AV) and a **single metal** dosed (A) Cd, (B) Cu, and (C) Zn.

COD removal activity decreased with increasing metal concentration but with differences in the degree of inhibition by any individual metal and its concentration. Figure 6.7 (Table H-5) reveals the role of initial Cd, Cu, and Zn concentration on the COD removal activity normalized with respect to the control in assays with glucose synthetic waste. A single metal showed the potential to inhibit organic waste biodegradation. The 20% inhibiting concentration of Cd and Zn on COD removal was 6 and 10 mg/l, respectively (Figures 6.7 (A) and (C)). Cu in the range of this study showed some degree of inhibition but did not exceed 20%. COD removal activities were reduced continually as metal concentration increased when Cd was >6 mg/l and Zn was >10 mg/l. Contrary to the pattern of CH₄ inhibition in Figure 6.2 (C), Zn was not observed to stimulate the activity in terms of COD removal. This implied that Zn played a role in inhibiting the bioactivities of SRB (which use COD in the SO₄²⁻ reduction reaction) that was more than played in inhibiting MPB. MPB and SRB exhibited high resistance toward Zn. The relative toxicity of the heavy metals in terms of COD removal was Cd>Zn.

6.4.2.2 Inhibition of the COD Removal by Combined Metals

Combined metals caused synergistic inhibition of COD removal activities (Figures 6.8 - 6.10 and Table H-6). For example, as shown in Figure 6.8 (A), Zn (5 mg/l) caused 3.2% inhibition in terms of COD, while Cd (5 mg/l) caused 13.4% inhibition. When Zn (5 mg/l) was combined with Cd (5 mg/l), the percentage of inhibition was 100. As shown in Figure 6.9 (B), Zn (5 mg/l) caused 3.2% inhibition in terms of COD removal, while Cu (2 mg/l) caused 1.7 % inhibition. When Zn (5 mg/l) was combined with Cu (2 mg/l), the percentage of inhibition was 20.

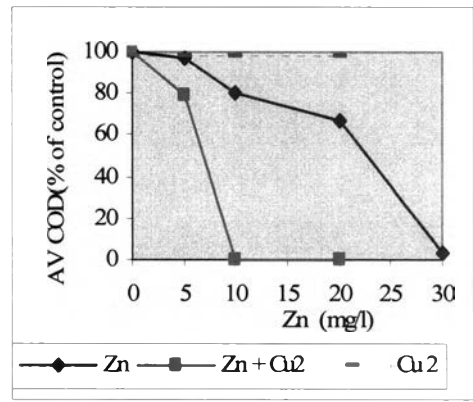
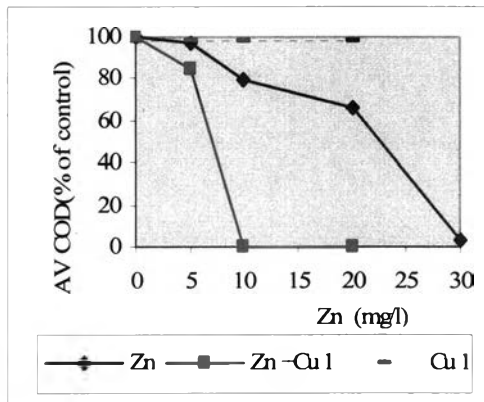
Mitigation of the toxicity of combined metals in terms of COD removal activity was not observed. However, it can be noticed that the trend of the synergistic inhibition occurred only when the combined metals were at high concentrations.



(A)

(B)

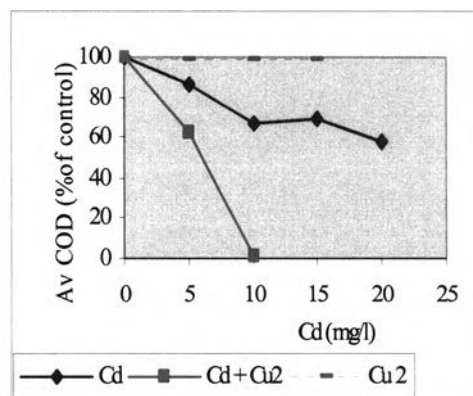
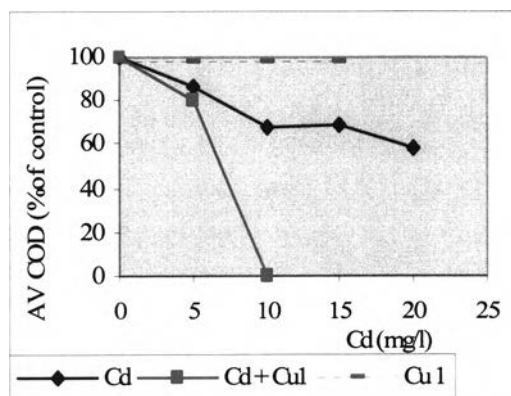
Figure 6.8 Effect of **combined metals** on the pattern of inhibition of COD removal: (A), Zn at various concentrations + Cd (5 mg/l); (B), Zn at various concentrations +Cd (10 mg/l).



(A)

(B)

Figure 6.9 Effect of **combined metals** on the pattern of inhibition of COD removal: (A), Zn at various concentrations + Cu (1 mg/l); (B), Zn at various concentrations + Cu (2mg/l).



(A)

(B)

Figure 6.10 Effect of **combined metals** on the pattern of inhibition of COD removal: (A), Cd at various concentrations + Cu (1 mg/l); (B), Cd at various concentrations + Cu (2 mg/l).

Combination of the three metals was studied (Figure 6.11 and Table H-7). There was no activity in any of the reactors in the range of the test even at the lowest combined metal concentrations of this test (Zn5+Cd5+Cu1, which gave rise to the metal loading 20.4 meq/kg MLVSS). Hence, the intriguing thing to be determined is to find the metal loading at which the synergistic inhibition first becomes apparent.

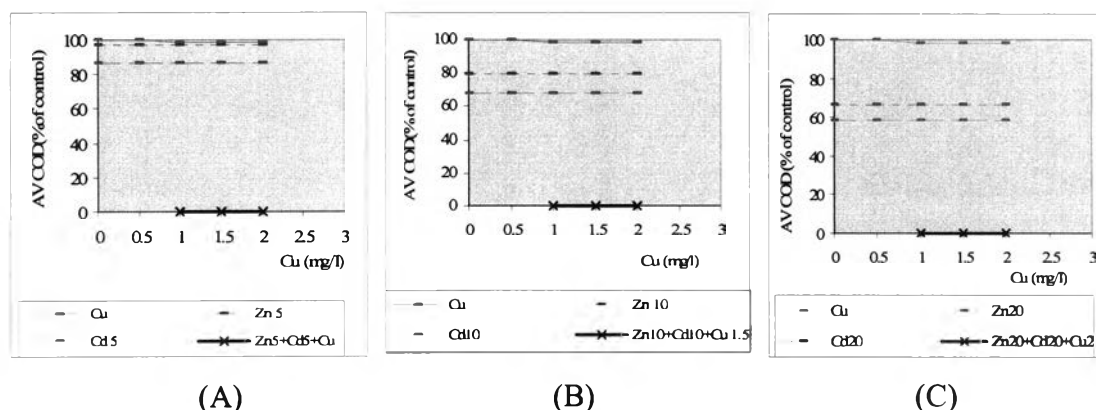


Figure 6.11 Effect of **three combined metals** on the pattern of inhibition of COD removal (A), Cu at various concentrations + Cd (5mg/l)+Zn (5mg/l)
 (B), Cu at various concentrations + Cd (10mg/l)+Zn(10mg/l)
 (C), Cu at various concentrations + Cd (20mg/l)+Zn (20mg/l).

6.4.3 Criterion Model of Inhibition and Validation

6.4.3.1 A Criterion for Diagnosis of Inhibition

It is important to determine a universal way to define toxicity for this type of application and for this reason the following two levels are proposed:

- **Inhibition level**, when a decrease in gas production and COD removal efficiency first become apparent (e.g. 20% decrease).
- **Toxic level**, when gas production and COD removal efficiency both are reduced to 50%.

The method of predicting the effect of combined metals on an anaerobic system was developed under the assumption that 1) the neutralizing agents (e.g. sulfide or organic components of sludge solids) are likely to react stoichiometrically with all the heavy metals and therefore the concentrations of the metals (Cd, Cu and Zn) are expressed using an added equivalent weight basis (milliequivalents-meq/l)

and 2) the ability of the wastewater biodegradation process to tolerate heavy metals is directly proportional to the MLVSS concentration.

Table 6.1 expresses the results in terms of CH₄ production and COD removal activity of reactors that contained a single metal of different types and concentrations while Table 6.2 expresses the results of combined metals. The observed results in terms of synergy was also described. Based on the above assumptions, the criterion model was proposed using the data obtained from the tests. The use of a criterion factor, K (in meq/kg MLVSS), which is defined as the ratio of the sum of heavy metal concentrations (in meq/l) divided by the MLVSS content of the biodegrading wastewater, W (kg/l), was proposed. The concentration of heavy metal(s) was expressed in meq/l. MLVSS was expressed in kg/l, and the metal loading (K) was expressed in the unit of meq/kgMLVSS. The relationship between the activity factors in terms of CH₄ production (%AV) and the metal loadings (K) was plotted in order to establish a correlation and the plots are shown in Figures 6.12 - 6.14. The relationship between the activity factors in terms of COD removal (%AV) and the metal loadings (K) was also plotted in order to establish a correlation and is shown in Figures 6.15 - 6.17.

In most cases, the combined metals caused synergistic inhibition. It was noticed that when the added combined metals were less than 15 meq/kgMLVSS the CH₄ production efficiency (Figure 6.13) and the COD removal efficiency (Figure 6.16) were reduced less than 20 per cent (% AV = 80). Moreover, synergistic inhibition was not observed.

In this study another important point was also an approach to justify the soluble combined metals (after metal sulfide precipitation) that were biologically toxic (Figures 6.14 and 6.17). In these both figures, soluble metal per MLVSS (soluble metal loading) is plotted with the activity factor (%AV). The curves (Figures 6.14 and 6.17) generated are similar to those of the added metal loading (Figures 6.13 and 6.16). From these curves, it can be concluded that the soluble combined metals in the system should not exceed 0.5 meq/kg MLVSS to avoid affecting organism.

Table 6.1 Compilation of data from reactors that contained **a single heavy metal** at various types and concentrations and the observed results in terms of methane production and COD removal activities.

Heavy Metals (mg/l)	Heavy Metals (meq/l)	MLVSS (kg/l)	Metal Loading (meq/kg MLVSS)	CH ₄ (ml @STP)	AV CH ₄ (% of control)	COD(mg/l)			AVCOD (% of control)
						Influent	Effluent	Removed	
	0	0.01004	0.00	42.25	100	3200	517	2683	100
Cd 5	0.0890	0.01374	6.47	-	-	3200	876	2324	86.62
Cd 10	0.1779	0.01422	12.51	-	-	3200	1394	1806	67.31
Cd 15	0.2669	0.01614	16.54	23.53	55.69	3200	1354	1846	68.80
Cd 20	0.3558	0.01373	25.92	16.13	38.17	3200	1633	1567	58.40
Cu 0.5	0.0157	0.01274	1.23	38.01	89.97	3200	517	2683	100
Cu 1	0.0314	0.01492	2.11	38.21	90.44	3200	562	2638	98.32
Cu 1.5	0.0472	0.01466	3.22	38.71	91.63	3200	562	2638	98.32
Cu 2	0.0629	0.01394	4.51	-	-	3200	562	2638	98.32
Zn 5	0.1529	0.01374	11.13	23.82	56.39	3200	602	2598	96.83
Zn 10	0.3058	0.01256	24.35	27.61	65.36	3200	1061	2139	79.72
Zn 20	0.6116	0.01338	45.71	-	-	3200	1422	1778	66.27
Zn 30	0.9174	0.01178	77.88	0.77	1.82	3200	3129	71	2.65

Table 6.2 Compilation of data from reactors that contained **combined heavy metals** at various types and concentrations and the observed results in terms of methane production and COD removal activities.

Combined Metals (mg/l)	Initial Metals (meq/l)	MLVSS (kg/l)	Metal Loading (meq/ kg MLVSS)	Soluble Metal Loading (meq/ kg MLVSS)	CH ₄ (ml@STP)	AV CH ₄ (% of control)	COD(mg/l)			AVCOD (% of control)	Observed Result
							Influent	Effluent	Removed		
Control	0	0.0127	0.0	0.0	9.194	100	3200	422	2778	100	
Zn 5 + Cd 5	0.2419	0.0130	18.7	0.756	0.137	1.49	3200	3200	0	0.00	S
Zn 10 + Cd 5	0.3948	0.0137	28.8	0.764	0.125	1.36	3200	2951	249	8.96	S
Zn 20 + Cd 5	0.7006	0.0127	55.2	1.300	0.155	1.69	3200	3200	0	0.00	S
Zn 5 + Cd 10	0.3308	0.0094	35.3	1.898	0.067	0.73	3200	3200	0	0.00	S
Zn 10 + Cd 10	0.4837	0.0129	37.5	2.028	0.069	0.75	3200	3200	0	0.00	S
Zn 20 + Cd 10	0.7896	0.0144	54.7	1.914	0.188	2.04	3200	3200	0	0.00	S
Zn 5 + Cu 1	0.1844	0.0119	15.5	NA	6.02	65.48	3200	843	2357	84.85	No
Zn 10 + Cu 1	0.3373	0.0103	32.8	1.689	0.089	0.97	3200	3200	0	0.00	S
Zn 20 + Cu 1	0.6431	0.0125	51.4	2.600	0.163	1.77	3200	3200	0	0.00	S
Zn 5 + Cu 2	0.2158	0.0125	17.3	0.897	3.701	40.25	3200	996	2204	79.34	No
Zn 10 + Cu 2	0.3687	0.0129	28.5	1.405	0.089	0.97	3200	3200	0	0.00	S
Zn 20 + Cu 2	0.6745	0.0139	48.5	2.381	0.264	2.87	3200	3200	0	0.00	S
Cd 5 + Cu 1	0.1204	0.0114	10.5	0.467	7.806	84.90	3200	958	2242	80.71	No
Cd 10+ Cu 1	0.2094	0.0133	15.8	0.997	0.539	5.86	3200	3200	0	0.00	S
Cd 5+ Cu 2	0.1519	0.0131	11.6	0.789	9.407	102	3200	1456	1744	62.78	No
Cd 10 + Cu 2	0.2408	0.0132	18.3	NA	0.314	3.42	3200	3181	19	0.68	S
Zn 5 + Cd 5 + Cu 1	0.2733	0.0134	20.4	1.142	0.198	2.15	3200	3200	0	0.00	S
Zn 10 + Cd10 + Cu1.5	0.5309	0.0140	37.9	1.398	0.07	0.76	3200	3200	0	0.00	S
Zn 20 + Cd 20 + Cu2	1.0304	0.0127	81.3	1.861	0.603	6.56	3200	3200	0	0.00	S

NA = Not analyzed

S = Synergistic inhibition

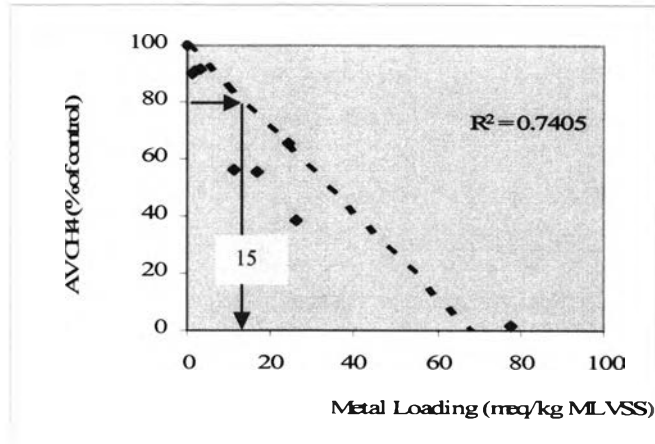


Figure 6.12 The relationship between %AV in terms of **methane production** and the metal loadings of reactors in a **single metal system**.

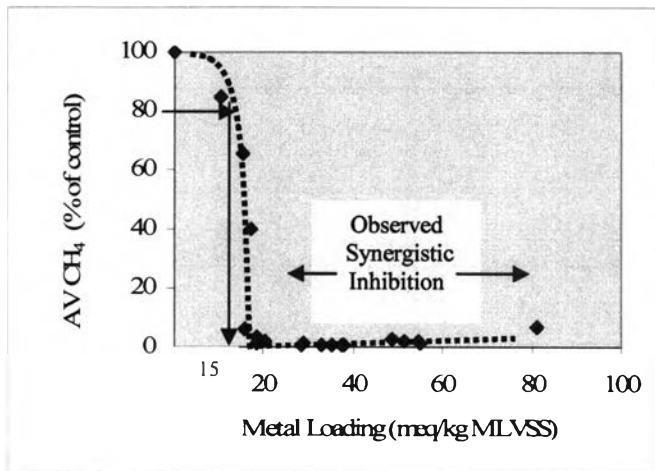


Figure 6.13 The relationship between %AV in terms of **methane production** and the metal loadings of reactors in a **combined metal system**.

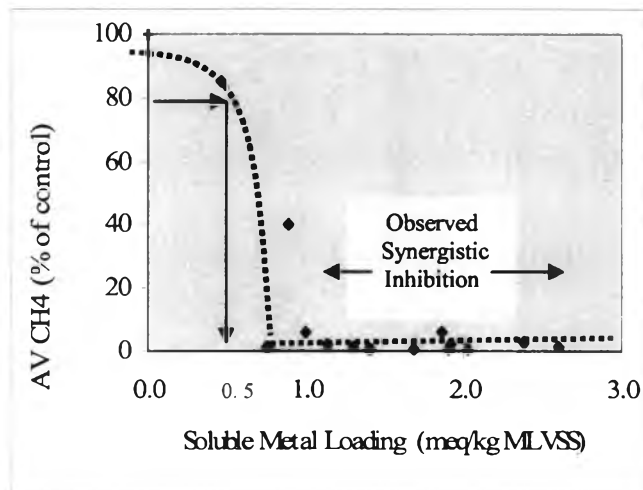


Figure 6.14 The relationship between %AV in terms of **methane production** and the soluble metal loadings of reactors in a **combined metal system**.

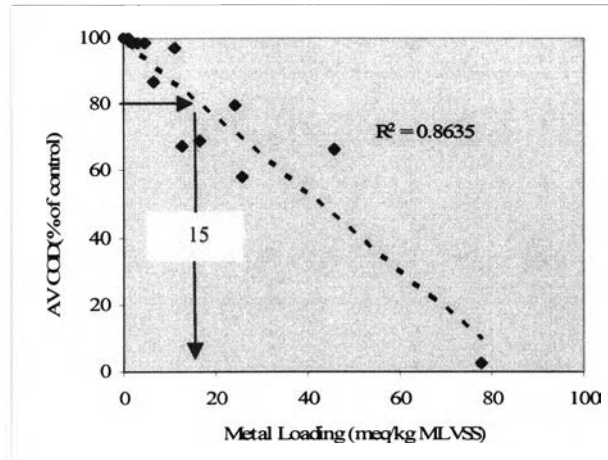


Figure 6.15 The relationship between %AV in terms of **COD removal** and the metal loadings of reactors in **a single metal system**.

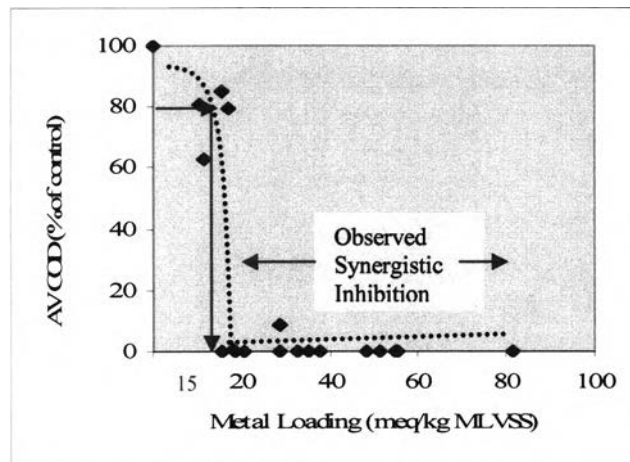


Figure 6.16 The relationship between % AV in terms of **COD removal** and the metal loadings of reactors in **a combined metal system**.

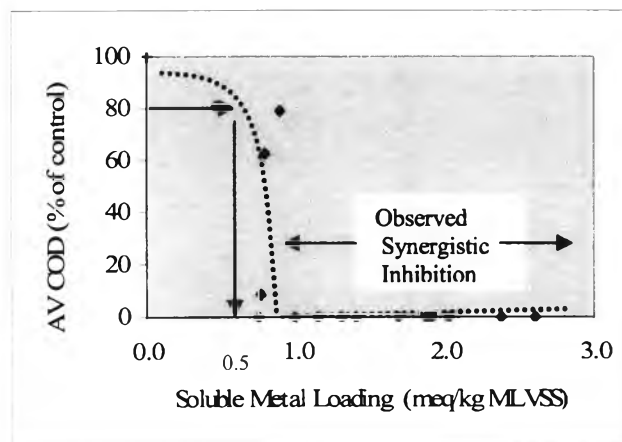


Figure 6.17 The relationship between % AV in terms of **COD removal** and the soluble metal loadings of reactors in **a combined metal system**.

The conclusion that can be obtained from data are:

1. The concentration of the combined metals at which the synergistic effect began was less than the concentration required to produce inhibition if any one of the metals was present alone (Figure 6.12 compared with Figure 6.13, while Figure 6.15 compared with Figure 6.16).

2. At low added metal loading between 0-15 meq/kg MLVSS, the synergistic effect was not observed (Figures 6.13 and 6.16). In the other word, the soluble metal loading between 0-0.5 meq/kg MLVSS, the synergistic effect was not observed (Figures 6.14 and 6.17)

6.4.3.2 The Proposed Model

The experimental data (Table 6.2 and Figures 6.13 and 6.16) show that the maximum added metal loading that will not cause synergistic inhibition is a value that is not higher than 15 meq/kg MLVSS. Also the soluble metal loading to avoid biologically toxic of not higher than 0.5 meq/kg MLVSS is proposed (Figures 6.14 and 6.17). If K is the metal loading, the criterion model provided is

$$K = (Zn/32.7 + Cd/56.2 + Cu/31.8) / W$$

(Note in each case that the metal concentration is divided by the equivalent weight of the element.)

Application of this proposed model could prevent system inhibition and maintain the optimum reactor conditions for simultaneous control of SO_4^{2-} and heavy metals in complex wastewater streams. K is the ratio of the sum of Zn, Cd, and Cu (in meq/l) divided by the MLVSS, W (in kg/l). The COD:S ratio was 9. The value of K in wastewater should not exceed 15 meq/kg MLVSS in order to avoid inhibition. Zn, Cd, and Cu are the concentration of the metal in solution in mg/l.

In the other word, the soluble metal loading (after safeguard by metal sulfide precipitation) in the wastewater treatment system should not exceed 0.5 meq/kgMLVSS to avoid biologically toxic.

This model was validated by another set of experiments. The 19 reactors were set. Combined heavy metals at various metal loadings were provided to the reactors in the range around 0-30 meq/kg MLVSS. The results were as recorded in Tables H-8 and H-9.

6.4.3.3 Results from the Experiment Set to Verify the Proposed Model

Results from the verified study were shown in terms of CH₄ production and COD removal. Residual SO₄²⁻ and heavy metals were also reported. The effect of combined metals at the various metal loadings (around 0-30 meq/kgMLVSS) on the CH₄ production and COD removal activity was shown in Figures 6.18- 6.21 (Tables H-8 and H-9), respectively.

The proposed model satisfactorily predicted the system inhibition and the synergistic inhibition concentrations of metal combinations in the reactors. The data in Figures 6.18 and 6.19 (the curve of which was regressed linearly and the coefficient of relationship, R², was higher than 0.7) indicates that if the metal loading (K) was between 0 (control) and 15 meq/kg MLVSS (soluble metal loading was less than 0.5 meq/kg MLVSS), the CH₄ production decreased by less than 20%, whereas when K was higher than 15 meq/kg MLVSS (soluble metal loading was higher than 0.5 meq/kg MLVSS), the CH₄ production decreased by a larger degree. The data in Figure 6.20 indicates that when K was between 0 (control) and 15 meq/kgMLVSS, the COD removal activity was nearly constant, whereas when K was higher than 15 meq/kg MLVSS the COD removal activity was remarkably decreased.

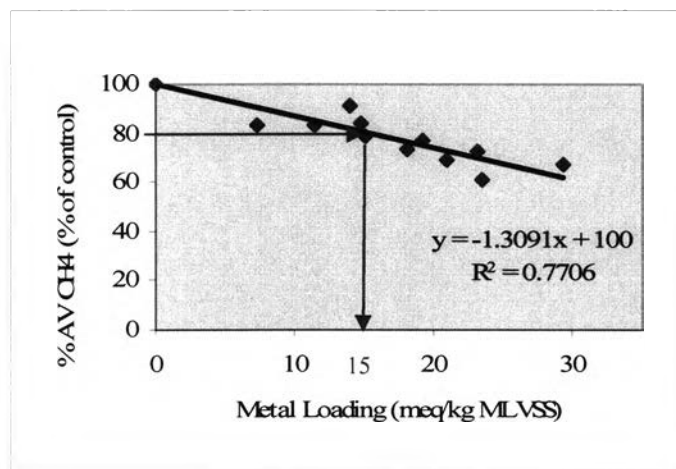


Figure 6.18 Effect of combined metals on methane production activity.

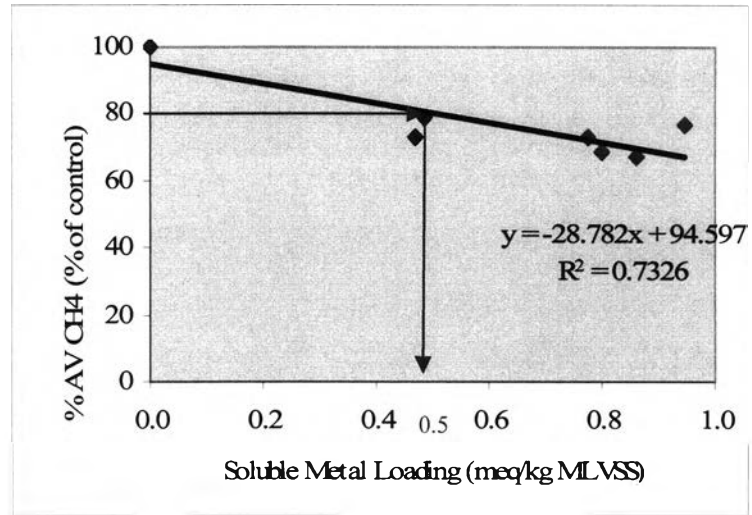


Figure 6.19 Effect of soluble combined metals on methane production activity.

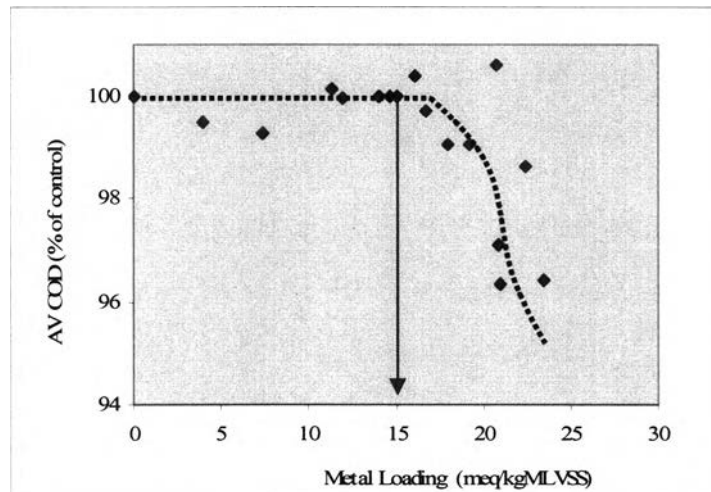


Figure 6.20 Effect of combined metals on COD removal activity.

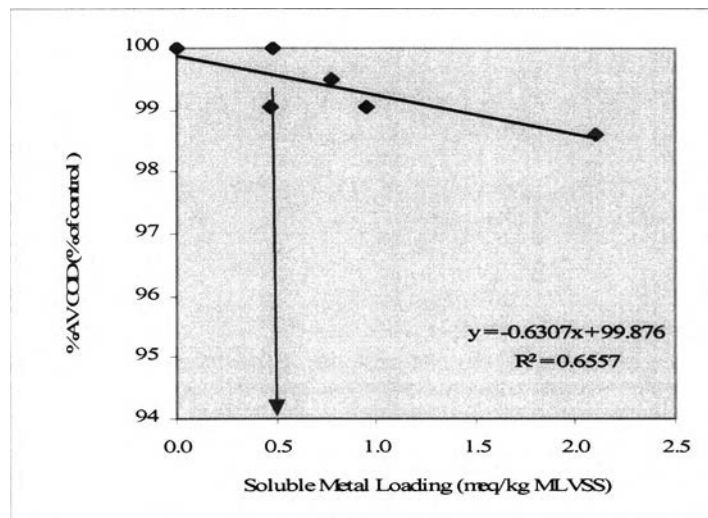


Figure 6.21 Effect of soluble combined metals on COD removal activity.

Mosey (1976) studied the maximum concentration of heavy metals in crude sewage that would not inhibit the anaerobic digestion of sludge and reported inhibition results and a predictive equation that are in some ways analogous to the results reported here. His proposed criteria allowed K not to exceed 168 meq/kg of dry solids. The K value from this study is much lower than that of Mosey. There are differences in the approach and objectives. The main data that Mosey used were obtained mostly from laboratory tests in which the added metals were in soluble form and some data were gathered from the anaerobic digester. In some of these cases, the metal content in the digester was in the form of total metal. Another reason for the difference is that the data that he obtained refers to inhibition by individual metals, hence these data had a large diversion in inhibition levels due to the appropriation to a more complex situation for digesters. Any material to be biologically active must be in solution. In the present study, the metals were in the soluble form at the beginning. To mimic the real world of an anaerobic reactor that contains SO_4^{2-} , in which hydrogen sulfide had to be produced and the metal sulfide would be precipitated, SO_4^{2-} at an optimum amount had been added. The metal loading (K) from soluble metals (after or during metal sulfide precipitation) that could pass through the cell wall and therefore adversely affect the microorganisms was reported in order to propose the criterion model to maintain the system performance.

The objective of this proposed model is to predict the maximum concentration of heavy metals in the wastewater that would not cause synergistic inhibition to anaerobic wastewater treatment while the objective of Mosey was to predict the maximum concentration of heavy metals in crude sewage which would not inhibit the anaerobic digestion of sludge. Data obtained from this study related to the combined metals which caused synergistic inhibition. From the Mosey assessment, the levels of toxic materials in the sludge gradually increased within the environment of the wastewater treatment system during the course of the wastewater treatment process. Metals were taken up by bacteria in the reaction tank of a wastewater treatment plant and they were accumulated in the bacterial cell wall or in the cell fluids before the bacteria reached the digestion system. Many organisms were metal-acclimated. Thus, the amount of the metals that was taken into consideration had to be much higher than in this study. For this study, metals were fed and taken up by bacteria in the system

directly. There was not enough time for acclimation and the treatment process failed more easily. In this study, the approach was set to mimic the real world of anaerobic treatment for wastewater. To prevent the anaerobic treatment system to be inhibited from metal toxicity we proposed the formula for K of 15 meq/kg MLVSS. If the combined metal concentration that is directly fed is high, the metal loading to microorganisms can be decreased by increasing the biomass concentration.

6.4.4 Assessment of Inhibitory Effects of Heavy Metals to Anaerobic Microorganisms via the VFA Monitoring

VFA's were monitored to elucidate the mechanism of inhibition. The time course of butyric, propionic and acetic acid production when the system was dosed by a single metal of Cd (0-20 mg/l), Cu (0-2 mg/l), and Zn (0-30 mg/l) is shown in Figure 6.22 (A)-(C), Figure 6.23 (A)-(C) and Figure 6.24 (A)-(C), respectively (Table H-10). There were differences in the VFA patterns when the system was dosed by an individual single metal. Cd, Cu, and Zn inhibited different processes.

Cd adversely affected acetic acid formation (Figure 6.22 (C)). This indicated that Cd inhibited the acetic acid forming bacteria (acetogens). Thus the activities in CH₄ production and COD removal were reduced (Section 6.4.1.1 and 6.4.2.1).

Cu in the range of the study (0-2 mg/l) caused a reduction of butyric acid formation (Figure 6.23 (A)) and it caused propionic acid accumulation (Figure 6.23 (B)). However acetic acid accumulation was not observed (Figure 6.23(C)). This could explain why Cu at low concentrations had the least effect on the system inhibition (Section 6.4.1.1 and 6.4.2.1). However, Cu causes an adverse effect on butyric acid formation, the first step of VFA formation. This could be attributed to a condition where the presence of Cu at high concentration could greatly affect the system by inhibiting butyric acid forming bacteria.

Zn at 30 mg/l significantly affected acetic acid accumulation (Figure 6.24(C)). This indicated that Zn(at high concentration) directly inhibited methane producing bacteria (MPB or methanogens).

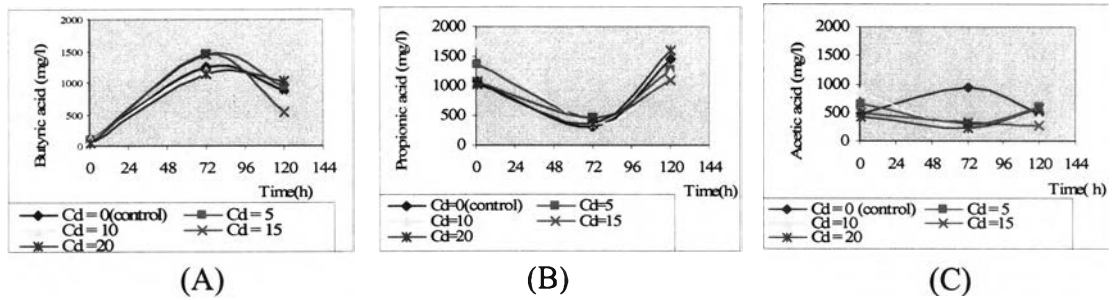


Figure 6.22 The time course of butyric, propionic and acetic acid when Cd in the range 0-20 mg/l was provided.

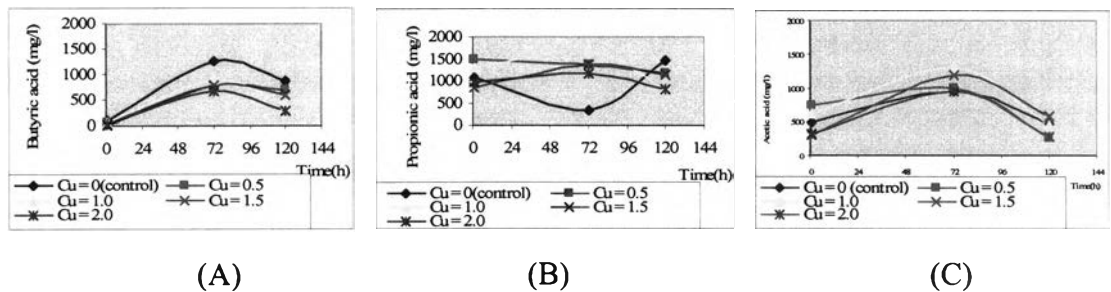


Figure 6.23 The time course of butyric, propionic and acetic acid when Cu in the range 0-2 mg/l was provided.

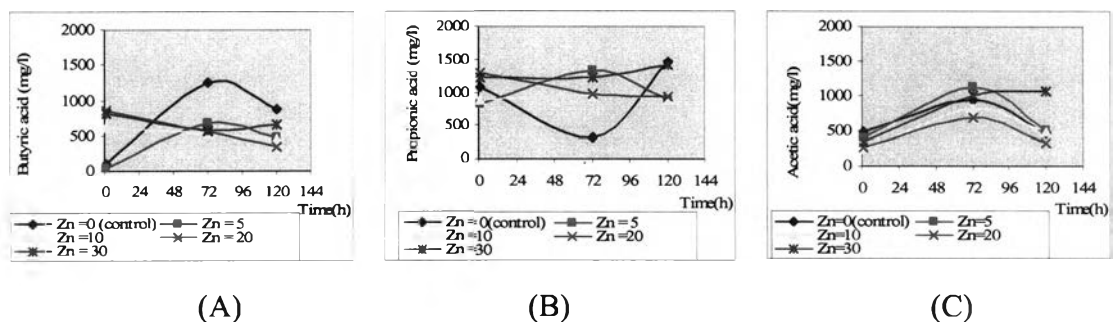


Figure 6.24 The time course of butyric, propionic and acetic acid when Zn in the range 0-30 mg/l was provided.

The time course of butyric, propionic and acetic acid when the system was dosed by combined metals at different metal loadings (0-80 meq/kg MLVSS) is shown in Figures 6.25 (A)-(R) (Table H-11).

Some combined metals significantly affected VFA accumulation, such as shown in Figures 6.25(B), (E), (F) when Zn was combined with Cd. On the other hand, in another combined metal situation (Figures 6.25(H), (K), (L), (N), (O)), when Zn or Cd was combined with Cu at low concentration, VFA degradation increased compared with a no-metal control. The results elucidated that some combined metals

(i.e. Cd+Zn) caused VFA accumulation but the others (i.e. Cd+Cu, or Zn+Cu at low concentration) can mitigate the VFA accumulation. This depended on the type and concentration of metals. This results support the results from Section 6.4.1.2 (Figures 6.5(A), 6.6(A) and 6.6(B)).

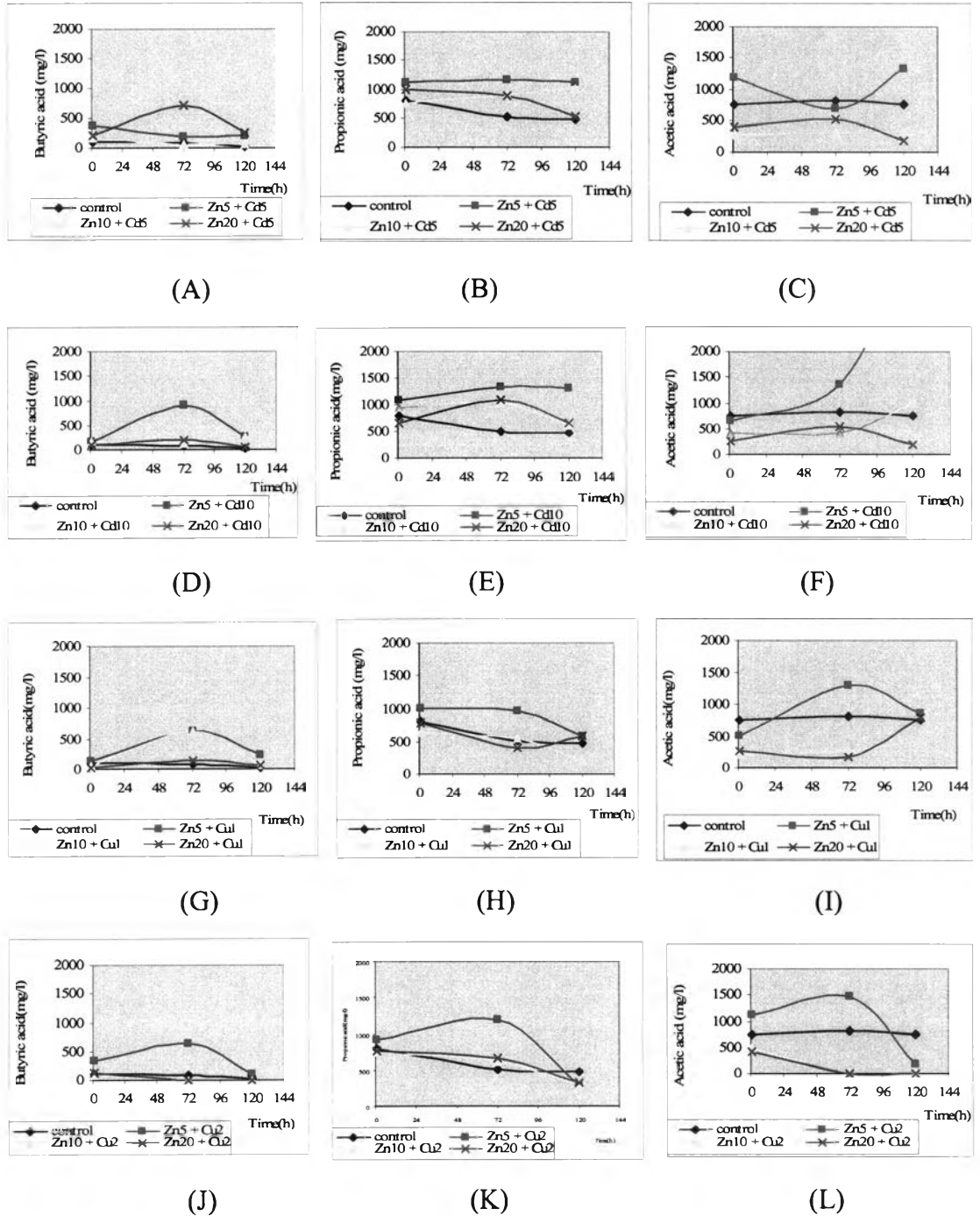


Figure 6.25 The time course of butyric, propionic and acetic acid when metals were combined at various concentrations.

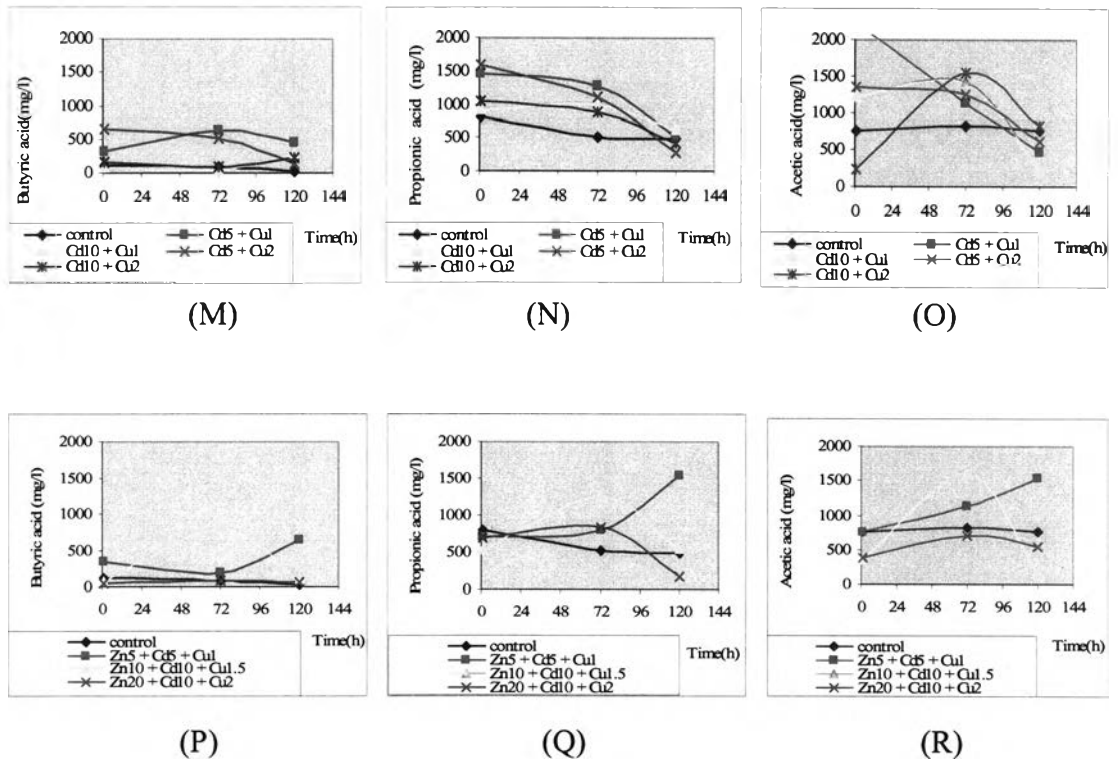


Figure 6.25 (-Continued-) The time course of butyric, propionic and acetic acid when metals were combined at various concentrations.

Data from another set of experiments (Table H-12) were gathered to observe the effect of combined metals on the VFA's. When different combined metals at various loadings (K between 0-30 meq/kg MLVSS) were provided to the system, the VFA formation and its degradation were quite similar to that of the no metal-control.

6.4.5 Assessment of Inhibitory Effects of Heavy Metals to Anaerobic Microorganisms via Sulfate Monitoring

A single metal at high loading adversely affected the SO_4^{2-} reduction reaction. Single metals at different loadings (around 0-80 meq/kg MLVSS) were studied and it was found that the adverse effect depended on the type of metal and its concentration (Table H-13). Cd at concentrations higher than 15 mg/l (0.267 meq/l) and Zn at 30 mg/l (0.917 meq/l) showed an adverse effect to the SO_4^{2-} reduction reaction (Figures 6.26(A) and (C)). This could be attributed to the fact that Cd and Zn at this concentration inhibited SRB. However, from this study it was found that Cd in the range of 0-10 mg/l (0.178 meq/l), Zn in the range of 0-20 mg/l (0.612 meq/l), and Cu in the range 0-2 mg/l (0.063 meq/l) did not affect SO_4^{2-} reduction.

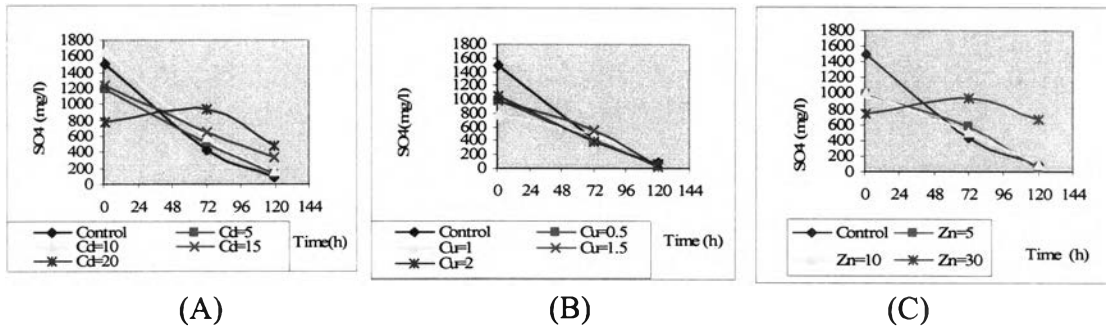


Figure 6.26 The time course of sulfate when a **single metal** at various concentrations was provided (A) Cd, (B) Cu, and (C) Zn.

Combined metals adversely affect the SO_4^{2-} reduction reaction. When the system contained combined metals at different loadings (around 0-80 meq/kg MLVSS, all experiments showed that the SO_4^{2-} reduction reaction was inhibited (Figures 6.27 (A)-(G), Table H-14).

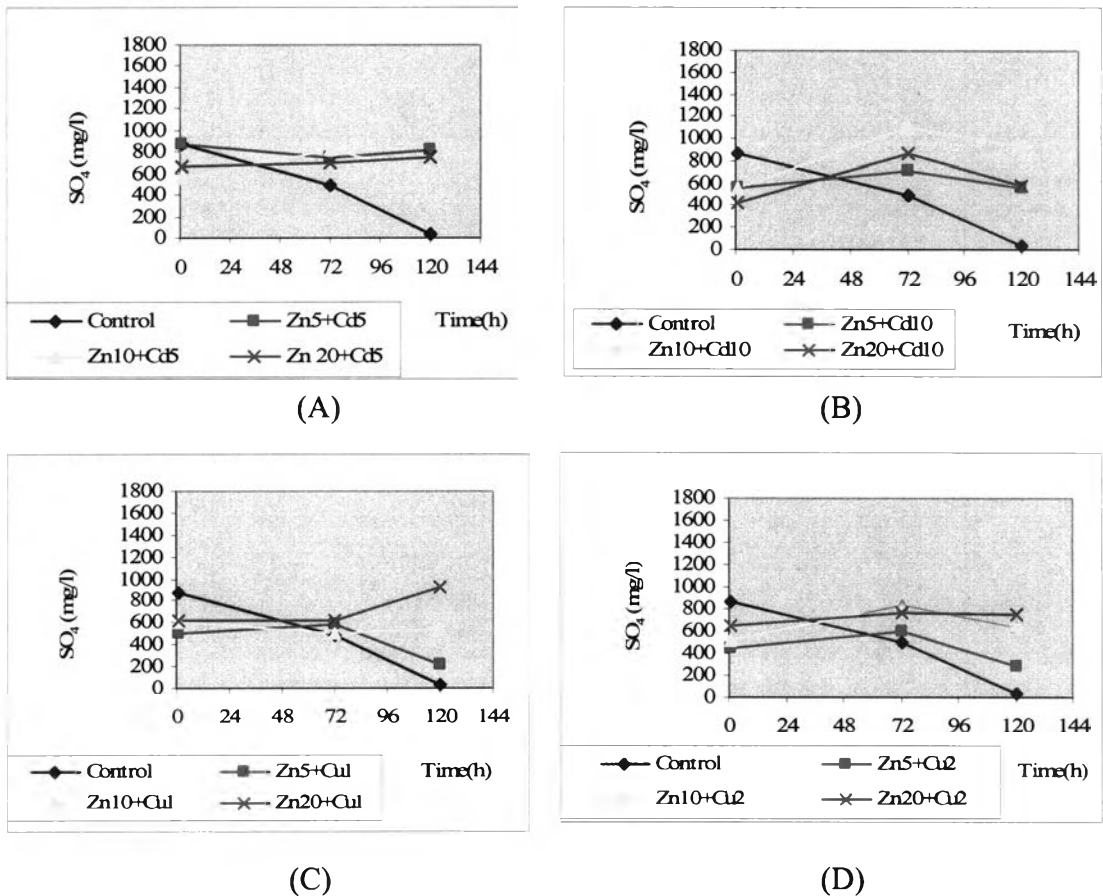


Figure 6.27 The time course of sulfate when **combined metals** of various types and concentrations were provided.

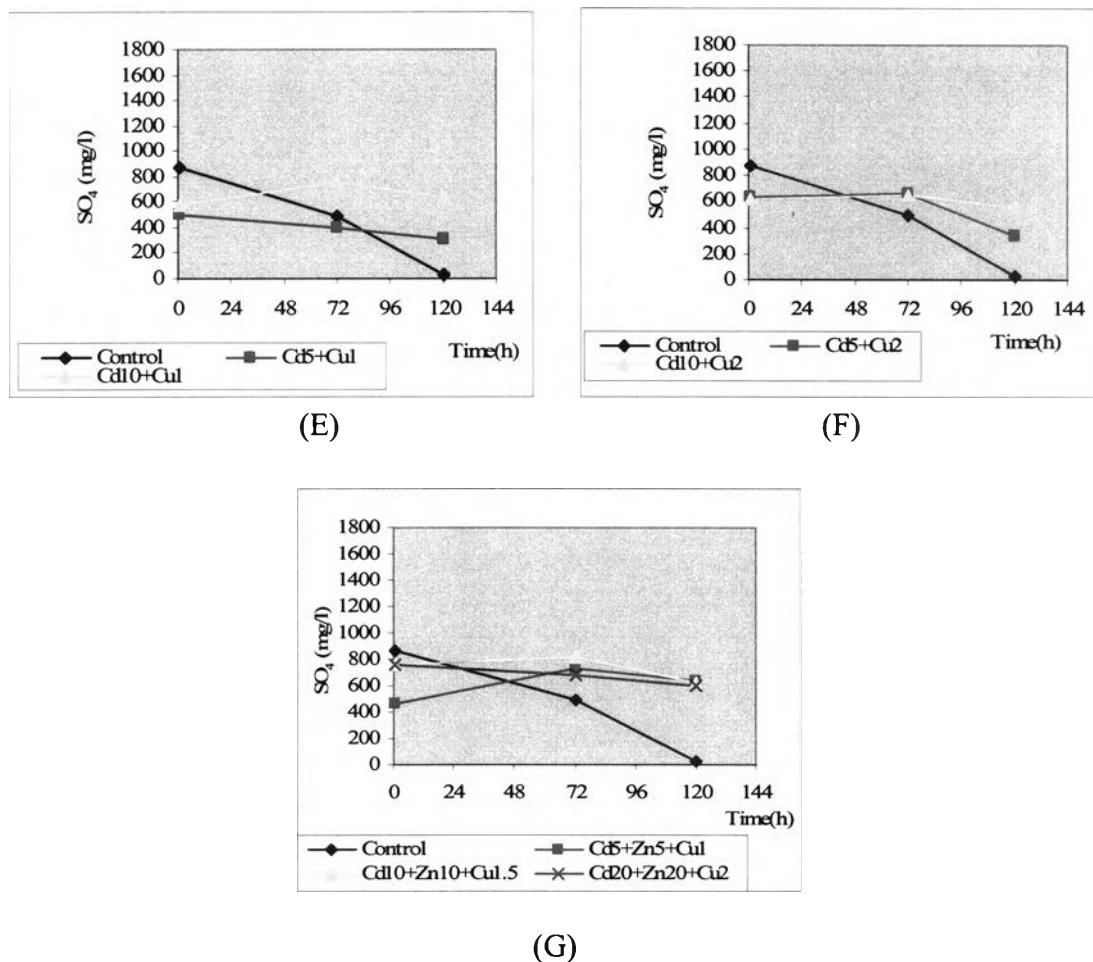


Figure 6.27 (-Continued-) The time course of sulfate when **combined metals** of various types and concentrations were provided.

Combined metals caused adverse effect to SRB. Their effect was higher than the effect caused by a single metal at the same metal loading. The decrease of the SO_4^{2-} reduction reaction causes a decrease in the production of sulfide. Thus, soluble metals were accumulated affecting the system performance.

The effect of heavy metal(s) at the range of K between 0-80 meq/kg MLVSS on the SO_4^{2-} reduction reaction is depicted in Figures 6.28 (A) and (B) (Tables H-13 and H-14). The higher the heavy metal loading (K), the lower the percentage of SO_4^{2-} removal. Combined metals (Figure 6.28 (B)) show a greater effect than that of the single metal at the same metal loading, K.

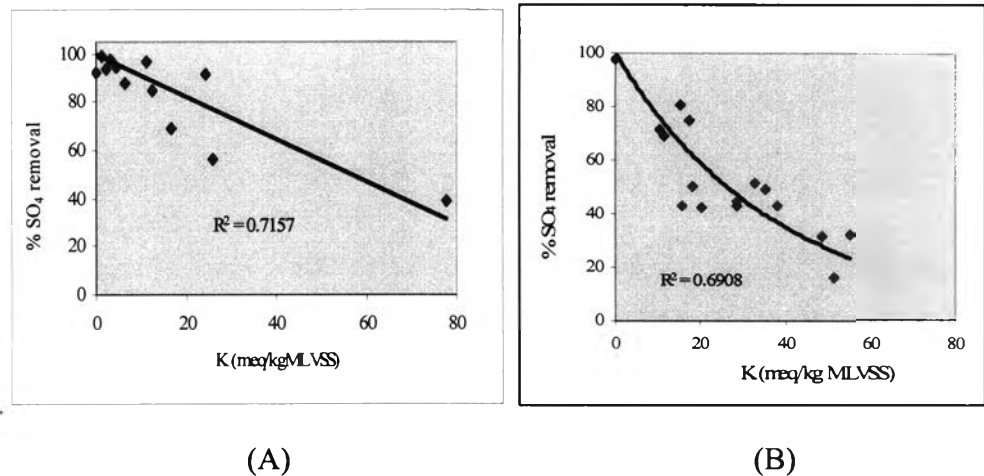


Figure 6.28 Metal affecting sulfate reduction (A) a single metal (B) combined metals.

6.4.6 The Performance of the Bioreactor

The reactor performance was investigated by examining the COD, SO₄²⁻ and heavy metal removal efficiencies throughout the reactor operation. In addition, the pH of the influent and effluent was also measured.

6.4.6.1 COD Removal

For the study of a **single metal** at metal loading around 0-80 meq/kg MLVSS, the COD removal efficiencies were reduced as the metal concentration increased, but differences among metals were found (Figures 6.29 (A)-(C) and Table H-15). For influent Cd concentrations between 0-20 mg/l, the COD removal efficiencies varied between 83 (control)-48 % (Figure 6.29 (A)). For influent Zn concentrations between 0-30 mg/l, the COD removal efficiencies varied between 83 (control)-2 % (Figure 6.29 (C)). While with influent Cu concentrations between 0-2 mg/l, the COD removal efficiencies varied in a narrow range between 83 (control) - 82 % (Figure 6.29 (B)).

For the study of **combined metals** at metal loading around 0-80 meq/kg MLVSS, the COD removal efficiencies decreased from 86% (control) to 0%. Most reactors faced this problem. In only 6 out of 20 reactors could that system be maintained (Table H-6). Hence, it would be interesting to examine the value of metal loading under which the system could be maintained.



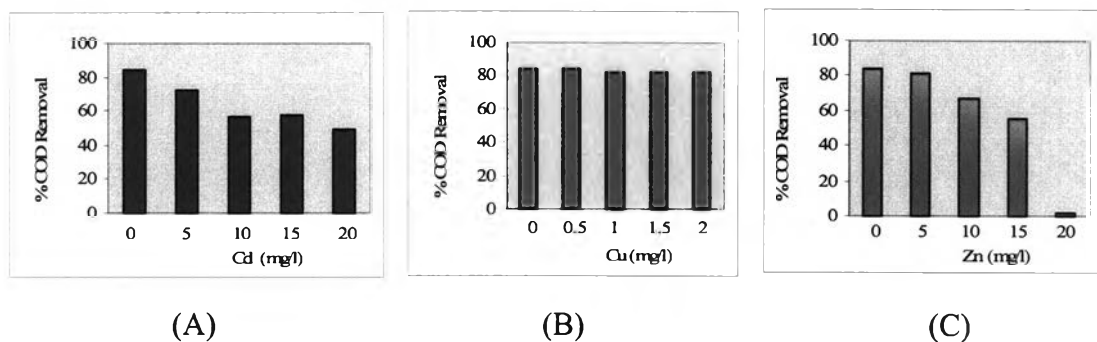


Figure 6.29 The percentage of COD removal in reactors with single metal dosed (A) Cd 0, 5, 10, 15, and 20mg/l, (B) Cu 0, 0.5, 1, 1.5, and 2 mg/l, and (C) Zn 0, 5, 10, 20, and 30 mg/l.

The observation on COD removal efficiencies led to another set of reactors with dosing of various types and concentrations of combined metals in the range of 0-30 meq/kg MLVSS. The conditions provided were the same as that of the first two sets of the experiment, except that the initial COD was a bit higher. The influent COD and the MLVSS was around 5,300 and 10,000 mg/l, respectively. Sulfate was added to give rise to COD:S of 9. COD removal was found to vary between 96 % (control) to 92 % during the 120 hour operation (Table H-9). The average COD in the effluent was 257 mg/l. The average percentage COD removal was 95%.

Anaerobic reactors containing combined metals at metal loading less than 30 meq/kg MLVSS could maintain the high COD removal percentage at 95%. Although the desired removal efficiencies were achieved, the CH_4 production was not accomplished (Section 6.4.1.2). This may be attributed to the use of COD mostly for SO_4^{2-} reduction.

6.4.6.2 Sulfate Removal

During the study of **single metals** at metal loading around 0-80 meq/kg MLVSS, the SO_4^{2-} removal efficiencies were reduced as the metal concentration increased (Table H-13), but differences among metals were found. For influent Cd concentrations between 0-20 mg/l, the SO_4^{2-} removal efficiencies varied between 92 (control) to 56 %. For influent Zn concentrations between 0-30 mg/l, the SO_4^{2-} removal efficiencies varied between 92 (control) to 39 %. Cu within the range of the study did not affect the system.

For the study of **combined metals** at metal loading around 0-80 meq/kg MLVSS, the SO_4^{2-} removal efficiencies were reduced as the metal concentration increased (Table H-14), the SO_4^{2-} removal efficiencies decreased from 97% (control) to 15%. The average percentage of SO_4^{2-} removal was 46.

However from another set of experiments, when the metal loadings were less than 30 meq/kg MLVSS (Table H-16), the average percentage of SO_4^{2-} removal was 93. There was no inhibition of the SO_4^{2-} reduction reaction when this range of metals was provided. Results of this study also indicate that combined metals, in general, inhibit the bioactivities of SRB more than a single metal is observed to do. This phenomenon affected the anaerobic treatment system because the production of sulfides during the SO_4^{2-} reduction reaction has to be accounted in part for the higher tolerance of organisms to metal toxicity. Thus, to operate the system with the aim to remove SO_4^{2-} , the metal loadings on the system should be considered.

6.4.6.3 Heavy Metal Removal

For a **single metal** study at metal loading around 0-80 meq/kg MLVSS, the heavy metal removal efficiencies were higher than 93 % (Table H-17).

For the study of **combined metals** at metal loading around 0-80 meq/kg MLVSS, the average percentage of heavy metal removal achieved was 95 % (Table H-18). However from another set of experimental results, when the metal loadings were less than 30 meq/kg MLVSS, as could be expected, the average percentage of metals removal was 96 % (Table H-19).

The percentage of heavy metal removal was not the only indicator to assess the performance of the system. Although the desired removal efficiencies were achieved, CH_4 production, COD removal and SO_4^{2-} removal were not accomplished satisfactorily when the system contained the high metal loadings (Section 6.4.1, 6.4.2 and 6.4.6.4). This phenomenon can be explained by the realization that when metal(s) is provided at low loading (not exceeding 30 meq/kg MLVSS) the mechanism of metal removal was concomitant metal sulfide precipitation and adsorption by the organisms. However, in the case where metal(s) was provided at high loading (i.e. up to 80 meq/kg MLVSS), metal(s) was available to inhibit the SO_4^{2-} reduction reaction. Thus, the reaction leading to metal sulfide precipitation decreased. The metal

removal mechanism by bacterial adsorption would be dominating. Hence, the system was more inhibited.

In this study, to investigate the amount of heavy metals that will not inhibit the SO_4^{2-} reduction it is very important to realize that the sulfate reduction reaction is necessary in order to result in sulfide precipitation. The crucial mechanism step is that the metal should be eliminated as metal sulfide before bacterial adsorption mechanisms become fully operational. The finding from this study at metal loadings less than 30 meq/kg MLVSS was that the SO_4^{2-} removal efficiency was 93% (Section 6.4.6.2), the heavy metal efficiency was higher than 96 %, and the average percentage of COD removal was 95%. However, the CH_4 production activity was reduced to 60% (Figure 6.18). This could be attributed to a situation where the mechanism involved concomitant metal sulfide precipitation and bacterial adsorption. Residual metals were not detectable in the effluent.

Nelson et al.(1981) reported that the amount, or percentage, of a particular heavy metal precipitated depends on several factors; pH, concentration of organic material, and the presence of other metals and or pollutants. In general, sludge will concentrate the metal by a factor of 2,000 to 10,000 times over that of the surrounding liquid, heavy metal concentration (Kalinski, 1981).

The initial and final pH of the mixed liquors in these studies were recorded to ensure that pH would not cause any inhibition and to monitor the behavior of the reactors after providing the metal(s). Figure 6.30 depicts the pH of various reactors that contained a single different metal at metal loading 0-80 meq/kg MLVSS (Table H-20). Figure 6.31 depicts the pH of various reactors that contained combined metals at metal loading 0-80 meq/kg MLVSS (Table H-21). Figure 6.32 depicts the pH of various reactors that contained combined metals at metal loading 0-30 meq/kg MLVSS (Table H-22). Table H-23 shows the reactor number and the type and concentration of the single and combined metals in each reactor.

Influent pH was adjusted to around a value of 7. After dosing a single metal in the range 0-80 meq/kg MLVSS, the pH values gradually rose to around 7.9 (but not exceeding 8) in the effluent (Figure 6.30). This higher pH value was not harmful to the system and was appropriate for metal sulfide precipitation.

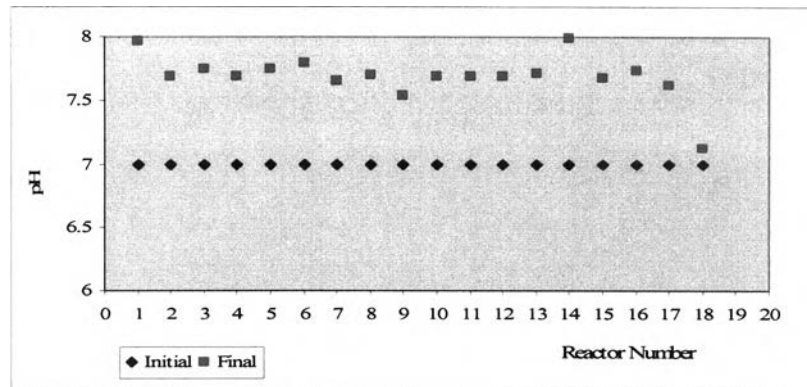


Figure 6.30 The influent and effluent pH in various reactors which contained **a single metal** in various types and concentrations at the metal loadings 0-80 meq/kg MLVSS.

Different results were obtained when dosing **combined metals** in the range 0-80 meq/kg MLVSS to reactors (Figure 6.31). Most of the effluent pH values were decreased to around 6.8, which were lower than the initial pH. From Figure 6.31, reactor number 1 was the no-metal control, and reactor numbers 8, 11, 14 and 16 were reactors containing the low metal loading not exceeding 15 meq/kg MLVSS (Table H-21). This shows that the higher pH values were obtained from the reactor containing the metal loading at less than 15 meq/kg MLVSS. The pH measurement elucidated the results that the combined metals caused VFA accumulation (Figure 6.25). The decrease in pH values indicated that the system was not appropriate for metal sulfide precipitation. The pH value was as an indicator of reactor imbalance.

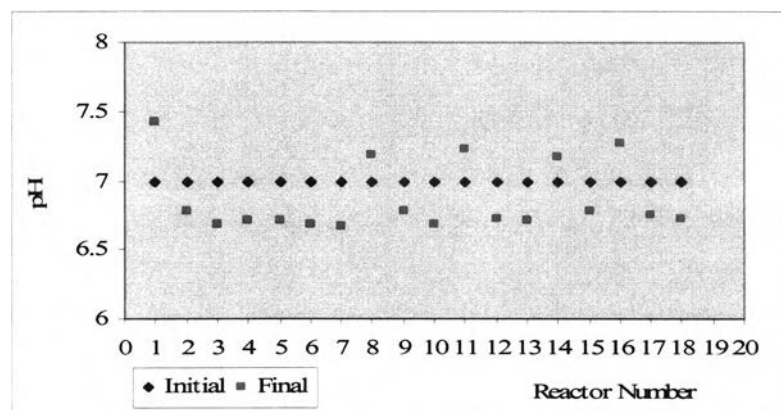


Figure 6.31 The Influent and effluent pH in various reactors which contained **combined metals** in various types and concentrations at the metal loadings 0-80 meq/kg MLVSS.

However, when the combined metal loadings were less than 30 meq/kg MLVSS, the effluent pH increased (Figure 6.32). The average pH was 7.4, which was still appropriate for metal sulfide precipitation.

In anaerobic reactors at a slightly alkaline pH, most of the sulfide is precipitated as heavy metal sulfides, in which state it is harmless to microorganisms and their environment. To support detoxification, heavy metals were induced to precipitate chemically. Metal ion contaminants in wastewater can be removed.

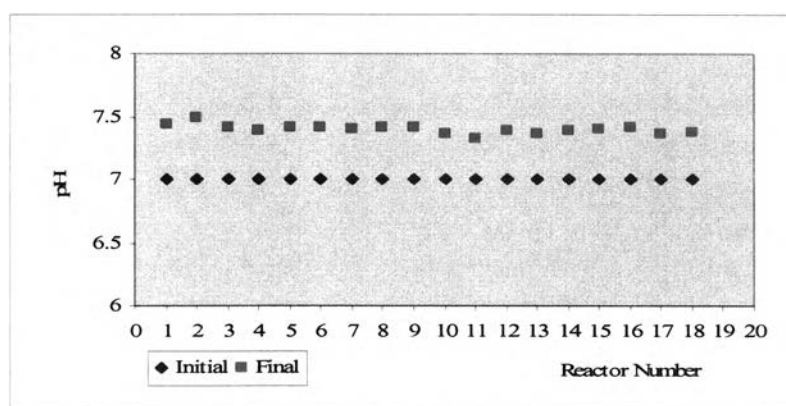


Figure 6.32 The Influent and effluent pH in various reactors which contained **combined metals** in various types and concentrations at the metal loadings less than 30 meq/kg MLVSS.

6.4 Conclusions

Heavy metals can negatively impact the activities of methane producing bacteria (MPB) and sulfate reducing bacteria (SRB) in anaerobic sulfate reducing bioreactors. The relative toxicity of heavy metals for a sludge from a brewery wastewater treatment plant as demonstrated in degradation of the organics was $Cu > Cd > Zn$. Mixtures of the combined heavy metals caused either synergism or antagonism, or both on the bacterial activities depending on the type and concentration of heavy metal. For the system that contained either Cd or Zn at less than 5 mg/l, the inhibition could be antagonized by Cu for which the concentration was not more than 1 mg/l. The antagonism was not observed when Cd combined with Zn. In most cases, combined heavy metals caused synergistic inhibition on the bacterial activity factor. The activities in CH_4 production and COD removal were substantially decreased when the system contained a mixture of combined metals.

Under this condition, the SO_4^{2-} reduction reaction was reduced. Hence, metals were in the soluble form which caused more inhibition. A tool for the design and operation of anaerobic reactors and for treating heavy metals and high SO_4^{2-} wastewaters is proposed as a model to prevent synergistic inhibition by the mechanism of metal sulfide precipitation before or during biological uptake. While the COD:S ratio was at 9, the metal loading, K, was $= (\text{Zn}/32.7 + \text{Cd}/56.2 + \text{Cu}/31.8)/W$. The value of K in wastewater has to be less than 15 meq/kg MLVSS. Where K is the ratio of the sum of Zn, Cd, and Cu (in meq/l) divided by the MLVSS, W (in kg/l). Zn, Cd, and Cu are the concentration of the metal in solution in mg/l. The model satisfactorily predicted the system inhibition and the synergistic inhibition concentrations of metal in the reactors. At this metal loading of 15 meq/kg MLVSS, the relative CH_4 production activity was not lower than 80% and synergistic inhibition was not observed. The recommended permissible combined heavy metals concentration that will not cause cellular toxicity is proposed. When considering the soluble combined heavy metal that adversely affect to bacteria, the soluble metal loading (K) should not exceed 0.5 meq/kg MLVSS. The average SO_4^{2-} reduction and heavy metal reduction were 93 and 96 per cent, respectively. At these conditions, VFA's did not accumulate in the reactor. The final pH was elevated but did not exceed 8. These experimental results elucidated the performance of the reactor involved in sulfide detoxification and heavy metal remediation processes. The metal loading that could prevent a negative effect on the sulfate reduction reaction from this study is proposed as a K less than 30 meq/kg MLVSS. At this value, the average COD removal efficiency was 95% although the CH_4 production activity was reduced to 60%.

6.6 References

- Gould, M. S., and Genetelli, E. J. 1984. Effects of complexation on heavy metal binding by anaerobically digested sludges. Water Research 18, :123-126.
- Kalinski, A.A. 1981. Extracting heavy metals and toxic organics from sludge. Water Engineering and Management Reference Handbook 81: R148-151.
- Leighton, I.R. and Forster, C.F. 1997. The adsorption of heavy metals in an acidogenic thermophilic anaerobic reactor. Water Research 31:2969-2972.

- Lin, C.Y. and Chen, C.C. 1999. Effect of heavy metals on the methanogenic UASB granule . Water Research 33: 409-416.
- Lin, C.Y. 1992. Effect of heavy metals on volatile fatty acid degradation in anaerobic digestion. Water Research, 26: 177-183.
- Mosey, F.E. 1976. Assessment of the maximum concentration of heavy metals in crude sewage which will not inhibit the anaerobic digestion of sludge Water Pollution Control 75: 10-19.
- Nelson, P.O., Chung, A.K., and Hudson, M.C. 1981. Factors affecting the fate of heavy metals in the activated sludge process. Journal Water Pollution Control Federation 53: 1323-1333.
- APHA, AWWA, and WPCF. 1992. Standard Methods for the Examination of Water and Waste Water 19 ed. Washington, D.C.