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Chemical speciation of heavy metals in excess sludge treatment by thermal hydrolysis and anaerobic digestion process

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ABSTRACT

Adequate treatment of excess sludge from municipal wastewater treatment plants (WWTP) is a critical issue nowadays in WWTP management. Thermal hydrolysis and anaerobic digestion are the methods often used to reduce the organic content of the excess sludge. However, these two methods have very limited effects on heavy metals' removal. As the toxicity of heavy metals is closely related to not only their quantity but also in their chemical speciations, more in-depth studies on heavy metals speciation distribution are needed. In this paper, the speciation of eight kinds of heavy metals before and after thermal hydrolysis and anaerobic digestion were investigated using a modified BCR (Bureau Communautaire de Référence: a four-step sequential extraction process proposed by the European Community Bureau of Reference in 1992) sequential extraction process. The results showed that the stable content of eight kinds of heavy metals increased by various extents after thermal hydrolysis. The chemical speciation of heavy metals during thermal hydrolysis and anaerobic digestion exhibited significant changes from non-stable to relatively stable state. The existence of H_2S can reduce the activity of anammox bacteria, and thus can lead to the deterioration of anaerobic biological treatment system. However, the toxicity of H_2S and heavy metal ions can be significantly inhibited simultaneously by the interaction between heavy metals and H_2S without secondary pollution, which is beneficial for excess sludge disposal.

Keywords: Activated sludge; Thermal hydrolysis; Anaerobic digestion; Heavy metals; H_2S

1. Introduction

Excess sludge from municipal wastewater treatment plants (WWTP) has become an outstanding environmental dilemma over the recent years [1]. It is often a struggle for plant management to find a cost-effective and environmentally acceptable method to properly handle the excess sludge generated everyday [2].

Heavy metals in excess sludge, such as Cu, Cr, As, Hg, and Pb are very difficult to be dealt due to their high mobility in the nature. If excess sludge cannot be properly handled, the heavy metals it carried can easily cause secondary pollution to the surrounding environment [3]. Therefore, immobilize heavy metals

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in excess sludge is an important part in excess sludge handling [4,5].

At present, using thermal hydrolysis in conjunction with anaerobic digestion is a promising technology to achieve safe disposal of excess sludge. When it comes to the removal of heavy metals, many researchers firstly tried to reduce the content of heavy metals. Raf Dewil found out that, acid thermal hydrolysis can reduce the heavy metal content in the filter cake except for Cu, Hg, and Pb, while alkaline thermal hydrolysis releases Cu, Pb, and Cr [6]. Furthermore, it is also realized that the severity of the harm caused by heavy metals depends on not only their quantity, but also on their speciations [7]. In the study of heavy metals' speciation, the modified BCR sequential extraction process is widely used [8–10]. There are four kinds of heavy metal speciations as defined by BCR: acid soluble, reduced, oxidation, and residual. Among these four states, acid soluble, and reduced are categorized as unstable state, while oxidation and residual are categorized as stable. The modified BCR is often used to extract heavy metals from sludge or soil accurately [11,12], and it is believed that the results obtained by modified BCR have more higher repeatability than the other recognized speciation methods [13].

Recent studies have focused on the speciation distribution of heavy metals during anaerobic digestion. The stability of heavy metals in sewage sludge before and after anaerobic digestion has already been studied [14,15]. Liu [7] studied the change of heavy metal speciation distribution with the influence of changing the physicochemical properties of the medium in the course of anaerobic digestion on the concentrations, bioavailability, or chemical forms of Cu, Zn, Cr, and Ni. However, currently there is no report on sludge heavy metal speciation change during thermal hydrolysis.

The purpose of this study was to: (1) investigate the mobility potential of sludge heavy metals during thermal hydrolysis and anaerobic digestion process and (2) investigate the effect of thermal hydrolysis and anaerobic digestion process on the stability of sludge heavy metals.

Specifically, the speciation distribution of eight kinds of heavy metals before and after the thermal hydrolysis and the anaerobic digestion was investigated. Modified BCR (a four-step sequential extraction process proposed by the European Community Bureau of Reference in 1992 [16]) sequential extraction process was employed to extract heavy metals from the sludge. Finally, the interaction between heavy metals and H_2S was discussed.

2. Materials and methods

2.1. Experimental setup

The flow diagram of the thermal hydrolysis process is shown in Fig. 1. The thermal hydrolysis apparatus was composed of a vapor generator, a thermal hydrolysis reactor, and a decompression tank with a treatment capacity of 1 t/d. The thermal hydrolysis process was controlled at 170℃ for 30 min (based on the previous experiment on optimizing thermal hydrolysis processes [17]). After the temperature and pressure of the decompression tank became normal values, the sludge samples were taken and then stored at 4℃ in a dry and clean beaker pre-soaked in dilute HNO_3 and washed by distilled water).

The flow diagram of the anaerobic digestion process is shown in Fig. 2. The anaerobic digestion apparatus was made of four glass bottles (each of 1 L): the reaction bottle, the displacement bottle, the catchment bottle, and the supplement bottle. The reaction bottle was placed in a water bath, while the displacement bottle and the supplement bottle were filled with supersaturated sodium bicarbonate solution.

The thermally hydrolyzed sludge was initially placed into the reaction bottle to produce gas (CH_4 and CO_2). The generated gas was introduced into the displacement bottle to replace the sodium bicarbonate, and then discharged to the catchment bottle. The supplement bottle was designed to provide make-up water to the displacement bottle. The stable operating parameters of anaerobic digestion reactors are shown in Table 1. The anaerobically digested sludge was sampled from the reaction bottle after 10-d reaction,

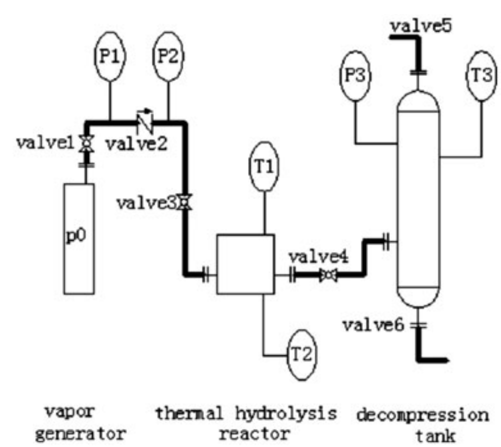

Fig. 1. Flow of thermal hydrolysis (P_0–P_3: piezometer; T1–T2: thermometer; valve 2: one-way valve; valve 1, valve 3–valve 6: control valve).

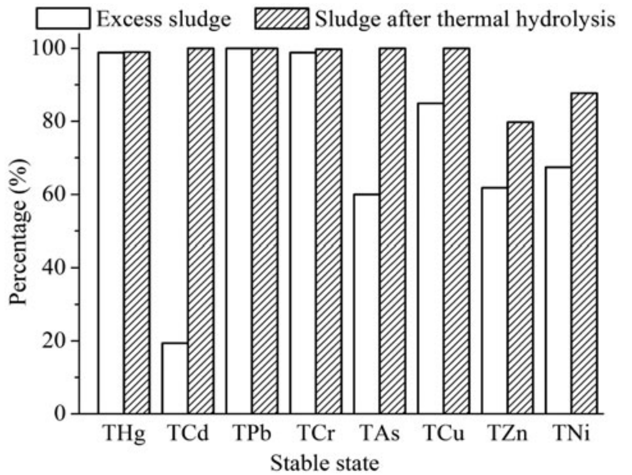

Fig. 2. Change in stable state of heavy metals before and after thermal hydrolysis.

and then stored at 4℃ in a dry and clean beaker pre-soaked in dilute HNO_3 and washed by distilled water.

2.2. Experimental sludge

The excess sludge was taken from the sludge dewatering unit of the Yu Liangzhou Municipal WWTP in Xiangyang, Hubei Province, China. The biological process of this plant is oxidation ditch.

2.3. Experimental methods

The raw excess sludge, thermally hydrolyzed sludge, and anaerobically digested sludge were taken separately and centrifuged (8,000 rpm, 5 min). Then, they were heated to a constant weight at 105℃ in a baking oven (HZ-2014B), and were finally put in a dryer for preservation. For the analysis of heavy metal speciation, the modified BCR extraction procedures proposed by reference [8] were adopted to extract heavy metals (Hg, Cd, Pb, Cr, As, Cu, Zn, and Ni) from these sludge samples.

Firstly, the content of Zn, Cu, Ni, and Hg were determined by spectrophotometer in the extract from the modified BCR extraction procedures. Then the sludge sample was digested by HNO_3–$HClO_4$ and extracted by methyl-isobutyl-ketone. After that the Pb and Cd were determined from the extraction by spectrometer. The content of Cr and As was determined with Diphenylcarbohydrazide and Ag-DDC spectrophotometry, respectively. Among these eight kind of heavy metals, Cd, Pb, Cr, Cu, Zn, and Ni were determined by atomic absorption spectrometer (GBC AVANTA M, the minimum detection value is 0.06 μg/L), whereas Hg and As were determined by atomic fluorescence spectrometry (AFS-930, D.L. As < 0.01 μg/L; Hg < 0.001 μg/L). All tests were repeated three times.

3. Results and discussion

3.1. Concentration change of heavy metals during thermal hydrolysis and anaerobic digestion

The concentrations of heavy metals in different sludge samples alone with the corresponding standards (level A/level B) for agricultural disposal of sludge from sewage treatment plant are listed as follows.

The thermal hydrolysis and anaerobic digestion process did not have a significant effect on reducing the content of heavy metals in the sludge (Tables 1 and 2). Almost all the measured heavy metals in the tested samples increased slightly after the process of thermal hydrolysis and anaerobic digestion. This phenomenon could be explained in that the total content of heavy metals remained constant after anaerobic digestion, whereas the mass of total solid decreased with the combustion loss of organic matter.

3.2. Speciation change of heavy metals after thermal hydrolysis

The speciation of eight kinds of heavy metals in the sludge before and after thermal hydrolysis was comparatively studied, and the percentage changes of heavy metal ions in different speciations are shown in Table 3.

The result indicates that Hg in the raw excess sludge basically took the state of stable residual. After the thermal hydrolysis, the macromolecular organic compounds were degraded into smaller organic molecules. During the thermal hydrolysis, smaller organic molecules and sulfur in the excess sludge were acting

Table 1
Stable operating parameters of anaerobic digestion reactor

Temperature (℃)	pH	Alkalinity (mg/L)	Ammonia nitrogen (mg/L)	VFA (mg/L)
40	7.9–8	9,000–10,000	3,000–3,600	1,100–1,300

Table 2
Heavy metal concentrations of the tested samples (mg/kgDS)

Heavy metal		Hg	Cd	Pb	Cr	As	Cu	Zn	Ni
Excess sludge		2.8	1.7	153	125	7.1	203	1,085	78
Sludge after thermal hydrolysis		2.9	1.9	158	133	7.9	202	1,145	84
Sludge after anaerobic digestion		3.3	2.2	179	162	9.0	207	1,393	107
CJT309-2009	A	3	3	300	500	30	500	1,500	100
	B	15	15	1,000	1,000	75	1,500	3,000	200

Table 3
Percentage changes of heavy metals in sludge before and after thermal hydrolysis

Heavy metal		Hg (%)	Cd (%)	Pb (%)	Cr (%)	As (%)	Cu (%)	Zn (%)	Ni (%)
Excess sludge	Acid soluble state	0.32	0.00	0.00	0.00	17.32	7.19	6.93	16.62
	Reduced state	0.72	78.43	0.00	2.77	19.49	13.64	29.14	20.32
	Oxidation state	0.88	16.34	56.52	55.98	15.50	63.53	30.35	21.02
	Residual state	97.05	8.76	42.09	38.69	44.78	17.87	32.40	44.41
	Recovery percentage	98.97	103.53	98.61	97.44	97.09	102.23	98.82	102.37
Sludge after thermal hydrolysis	Acid soluble state	0.87	0.00	0.00	0.31	0.00	0.00	6.65	11.44
	Reduced state	0.40	0.00	0.00	0.00	0.00	0.00	17.21	3.21
	Oxidation state	48.23	94.57	0.00	47.77	9.11	80.26	49.34	62.72
	Residual state	56.77	6.96	100.59	50.43	86.57	17.71	25.42	20.35
	Recovery percentage	106.27	101.53	100.59	98.51	95.68	97.97	98.62	97.72

Note: X state percentage = X state content/total content; recovery percentage = acid soluble state percentage + reduced state percentage + oxidation state percentage + residual state percentage.

as ligands to combine with Hg. As a result, the content of Hg in residual state was quickly reduced and Hg in oxidation state was increased significantly at the same time.

Cd in the raw excess sludge was basically in the form of reduced state (about 78%), which might bring hazardous effects to local environments during its migration. However, its content of oxidation state increased rapidly, while its reduced state declined to 0 after thermal hydrolysis. This change clearly illustrated that the toxicity of Cd could be restricted effectively by thermal hydrolysis.

It was also observed that the contents of Pb and Cr, which was initially in stable oxidation and residual state, remained nearly unchanged after thermal hydrolysis. Meanwhile, 63.53% of Cu in the raw excess sludge was in the form of oxidation state, but its content increased to 80.26% after thermal hydrolysis. Based on this, it is concluded that the unstable state of Cu disappeared completely after thermal hydrolysis.

At the same time, the content changes of Zn and Ni in the oxidation state also showed an ascending trend. It is known that Zn has negative environmental implications because of its migration potential. After thermal hydrolysis, the content of Zn decreased in the reduced state, while increased in the oxidation state significantly.

The content change of eight kinds of heavy metals in their stable state before and after thermal hydrolysis is shown in Fig. 2.

The content of heavy metals is one of the most important factors that can influence the sludge utilization. When thermal hydrolysis was applied to sludge treatment, the existing form of heavy metals would inevitablly change. As it can be seen from Fig. 2, Pb still maintained in 100.59% stable state after thermal hydrolysis, and the content of Hg and Cr in stable state had a slight increase, while the percentage of Cd, As, Cu, Zn, and Ni in stable state all increased greatly. Especially, the heavy metals with strong toxicity, such as Hg, Pb, Cd, and As, all stayed in the stable state. Among these heavy metals, the content of Cd in the stable state increased 76.43% and its increasing trend was the most obvious one.

When thermal hydrolysis occurs at 170℃, fat, carbohydrate, and protein will be hydrolyzed into smaller molecular substances. In this way their bonds (COO–, OH–, C=O, etc.) with heavy metals will break down and the heavy metals will be released into supernatant [18].

However, the released heavy metals can combine with inorganic ligands or organic ligands (such as some amino acids) in solution instead of being dissociated as free ions. In contrast, it is easier for heavy metals to enter into solid phase where they can be absorbed by hydrolyzed metal oxides and form polyphosphates by combining with uncompleted hydrolyzed amino compounds or hydrolyzed phosphorus compounds. These forms represent 75–100% of heavy metals that are stored in the form of solids, which is helping to achieve high stability in the sludge disposal.

3.3. Change of heavy metals after anaerobic digestion

The speciation of eight kinds of heavy metals in sludge during the stable operation of anaerobic digestion reactor was comparatively studied, and their percentage changes are investigated in Table 4.

Changes in stable state of eight kinds of heavy metals before and after anaerobic digestion are also observed in Fig. 3.

The stability of Cd, Pb, As, and Cu in sludge samples before and after anaerobic digestion basically did not change, while the stability of Hg, Cr, Zn, and Ni slightly changed (Table 4 and Fig. 3), which indicated that the heavy metals are intended to remain in stable state.

These findings can be explained from two aspects. Firstly, sulfate reducing bacteria and acidifying bacteria can promote the decomposition of sulfate and sulfur containing organic matter. Under this theory, S^{2-} can be produced during anaerobic digestion and form stable sulfides with heavy metals [19]. For this reason, the ratio of heavy metals (such as Hg, Zn, Ni, and Cr) in stable state may be increased. Secondly, the change of environmental conditions (temperature for example), the complexity with inorganic or organic compounds as well as the other heavy metals can induce the transformation of heavy metals from exchangeable ionic state into the other semi stable or stable state by micro-organisms.

3.4. Relationship between heavy metals and H_2S

H_2S can reduce the activity of anammox bacteria, and thus can lead to the deterioration of anaerobic biological treatment system [20]. Excess sludge generated from wastewater treatment system can contain various forms of sulfide, mainly the organic sulfur, sulfate, sulfur, and sulfur hydrogen ions. The organic sulfur and sulfate in sludge can be degraded, and thus producing H_2S under anaerobic condition.

The percentage changes in sulfide and organic binding state (oxidation state) heavy metals before and after thermal hydrolysis and anaerobic digestion are shown in Fig. 4.

Table 4
Percentage changes in heavy metals of sludge before and after anaerobic digestion

Heavy metal		Hg (%)	Cd (%)	Pb (%)	Cr (%)	As (%)	Cu (%)	Zn (%)	Ni (%)
Sludge after thermal hydrolysis	Acid soluble state	0.87	0.00	0.00	0.31	0.00	0.00	6.65	11.44
	Reduced state	0.40	0.00	0.00	0.00	0.00	0.00	17.21	3.21
	Oxidation state	48.23	94.57	0.00	47.77	9.11	80.26	49.34	62.72
	Residual state	56.77	6.96	100.59	50.43	86.57	17.71	25.42	20.35
	Recovery percentage	106.27	101.53	100.59	98.51	95.68	97.97	98.62	97.72
Sludge after anaerobic digestion	Acid soluble state	0.00	0.00	0.00	3.61	0.00	0.00	6.54	5.65
	Reduced state	0.00	0.00	0.00	0.22	0.00	0.00	12.21	7.02
	Oxidation state	9.42	79.04	16.17	65.20	41.14	82.64	55.68	58.26
	Residual state	86.58	16.33	81.22	32.68	62.01	17.15	29.22	30.87
	Recovery percentage	96.00	95.37	97.39	101.71	103.15	99.79	103.65	101.80

Note: X state percentage = X state content/total content; recovery percentage = acid soluble state percentage + reduced state percentage + oxidation state percentage + residual state percentage.

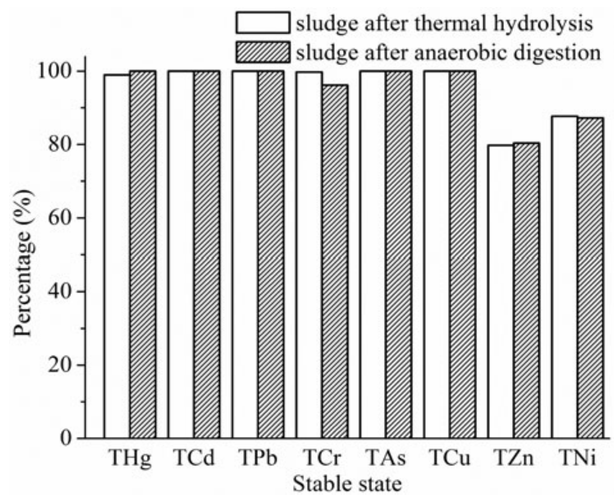

Fig. 3. Change in stable state of heavy metals before and after anaerobic digestion.

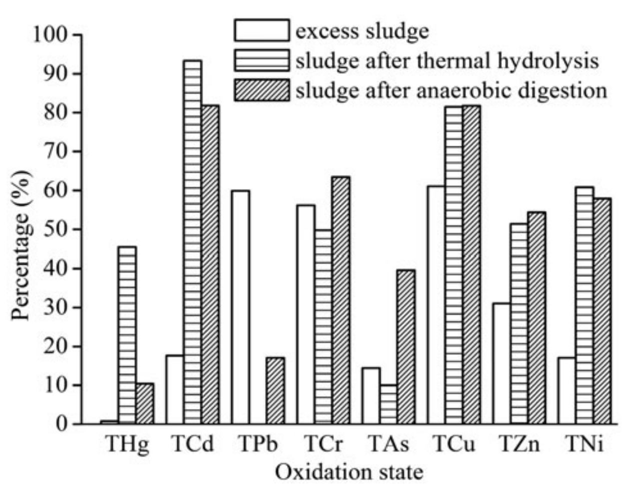

Fig. 4. Change in oxidation state of heavy metals before and after thermal hydrolysis and anaerobic digestion.

After thermal hydrolysis, the content of oxidation state Pb, Cr, and As decreased, whereas content of oxidation state for the other five kinds of heavy metals increased remarkably (Fig. 4). In addition, the overall speciation of heavy metals tended to be stabilized after anaerobic digestion.

The elevated content of sulfide and organic binding state (oxidation state) indicated that the sulfur compounds (such as sulfate) in sludge can be transformed intoS^{2-} by sulfate reducing bacteria during anaerobic digestion [19]. If various speciations of heavy metals coexist in sludge, the S^{2-} can be effectively removed in the form of insoluble metal sulfide precipitation through a variety of interaction between S^{2-} and different sorts of heavy metals. Meanwhile, the stability of heavy metals in sludge increases. Consequently, the toxicity of heavy metals and S^{2-} will be greatly inhibited simultaneously through the thermal hydrolysis and anaerobic digestion processes, which is beneficial for sludge treatment.

3.5. *Recovery percentage*

Recovery percentage of the heavy metals studied ranges from 95.37 to 106.27%. Therefore, a modified BCR can be a useful tool to research the speciation distribution of heavy metals in the sludge from the Yu Liangzhou Municipal WWTP.

4. Conclusions

The speciation change of eight kinds of heavy metals before and after thermal hydrolysis and anaerobic digestion was investigated using the modified BCR sequential extraction process. The overall conclusions are:

(1) Thermal hydrolysis and anaerobic digestion process did not have a significant effect on reducing the content of heavy metals in sludge, and the content percentage of almost all the studied heavy metals in the tested samples increased slightly after the process as some organic matter consumed.
(2) Heavy metals can form stable state more easily in anaerobic digestion reaction after thermal hydrolysis, and the process of thermal hydrolysis played a crucial role in stabilizing heavy metals in sludge.
(3) The overall stability of heavy metals in sludge was extremely enhanced by thermal hydrolysis and anaerobic digestion. After the thermal hydrolysis, the content of Cd in the stable state was enhanced 76.43% and its increasing trend was the most obvious one among these studied heavy metals. At the same time, the stability of Hg, Cr, Zn, and Ni slightly changed after anaerobic digestion, indicating that speciation of heavy metals trended to remain in stable state.
(4) The toxicity of H_2S and heavy metal ions can be greatly inhibited simultaneously by the interaction between heavy metals and H_2S, which is beneficial for excess sludge disposal.

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References

[1] M.H. Romdhana, D. Lecomte, B. Ladevie, C. Sablayrolles, Monitoring of pathogenic microorganisms contamination during heat drying process of sewage sludge, Process Saf. Environ. Prot. 87 (2009) 377–386.

[2] M. Koncewicz-Baran, K. Orłowska, K. Gondek, Characteristics of selected fractions of heavy metals in biologically and thermally transformed sewage sludge, Desalin. Water Treat. 52 (2014) 3783–3789.

[3] M.M. Marchioretto, H. Bruning, W. Rulkens, Heavy metals precipitation in sewage sludge, Sep. Sci. Technol. 40 (2005) 3393–3405.

[4] T.B. Chen, G.D. Zheng, D. Gao, H.T. Liu, W. Du, J. Yang, Key problems in municipal sludge composting and its industrialization process, Chin. Water Wastewater 25 (2009) 104–108.

[5] L. Dąbrowska, Speciation of heavy metals in non-volatile solids of sewage sludge, Desalin. Water Treat. 52 (2014) 3761–3766.

[6] R. Dewil, J. Baeyens, E. Neyens, Reducing the heavy metal content of sewage sludge by advanced sludge treatment methods, Environ. Eng. Sci. 23 (2006) 994–999.

[7] X.G. Liu, B. Dong, L.L. Dai, X.H. Dai, Analysis of chemical speciation and bioavailability of heavy metals in waste activated sludge during anaerobic digestion, J. Agro-Environ. Sci. 31 (2012) 1630–1638.

[8] A.M. Ure, P.H. Quevauviller, H. Muntau, Speciation of heavy metals in soils and sediments, an account of the improvement and harmonization of extraction techniques undertaken under the auspices of the BCR of the Commission of the European Communities, Int. J. Environ. Anal. Chem. 51 (1993) 135–151.

[9] M.D. Petit, M.I. Rucandio, Sequential extractions for determination of cadmium distribution in coal fly ash, soil and sediment samples, Anal. Chim. Acta 401 (1999) 283–291.

[10] C.M. Davidson, A.S. Hursthouse, D.M. Tognarelli, A.M. Ure, G.J. Urquhart, Should acid ammonium oxalate replace hydroxylammonium chloride in step 2 of the revised BCR sequential extraction protocol for soil and sediment?, Anal. Chim. Acta 508 (2004) 193–199.

[11] Ş. Tokalioğlu, Ş. Kartal, A. Gültekın, Investigation of heavy-metal uptake by vegetables growing in contaminated soils using the modified BCR sequential extraction method, Int. J. Environ. Anal. Chem. 86 (2006) 417–430.

[12] M. Pueyo, J. Mateu, A. Rigol, M. Vidal, J.F. López-Sánchez, G. Rauret, Use of the modified BCR three-step sequential extraction procedure for the study of trace element dynamics in contaminated soils, Environ. Pollut. 152 (2008) 330–341.

[13] S.P. Feng, S.T. Liu, W. Du, B. Guo, X.F. Zhao, H.B. Liu, Assessment of Cu, Zn, Fe, Mn species in different soil by modified BCR and Tessier extraction procedures, J. Instrum. Anal. 28 (2009) 297–300.

[14] J. Cao, Y.F. Tan, L. Xing, H.L. Zhang, Analysis of morphological distribution of heavy metal in sludge with anaerobic digestion process, Henan Chem. Ind. 6 (2003) 33–34.

[15] X.N. Shen, J.L. Xie, W.L. Kan, Z. Peng, F.H. Wang, J.Y. Song, Distribution of chemical form of heavy metal in anaerobically digested sludge, Chin. Water Wastewater 18 (2002) 51–52.

[16] C.M. Davidson, R.P. Thomas, S.E. McVey, R. Perala, D. Littlejohn, A.M. Ure, Evaluation of a sequential extraction procedure for the speciation of heavy metals in sediments, Anal. Chim. Acta 291 (1994) 277–286.

[17] X.P. Sun, A.T. Wang, X.H. Li, Z.H. He, Migration of heavy metals in sludge treatment by thermal hydrolysis process, Chin. Water Wastewater 26 (2010) 66–68.

[18] J. Jiang, G. Wang, L. Fang, Complexation between soil water-soluble organic matter and heavy metal, Soil Environ. Sci. 10 (2001) 67–71.

[19] J.L. Xie, Y.Q. Zhang, X.N. Shen, Effect of sulfate on chemical form of heavy metals during sludge anerobic digestion, Chin. Biogas 21 (2003) 22–25.

[20] D.G. Cirne, F.P. Van Der Zee, M. Fernandez-Polanco, F. Fernandez-Polanco, Control of sulphide during anaerobic treatment of S-containing wastewaters by adding limited amounts of oxygen or nitrate, Rev. Environ. Sci. Biotechnol. 7 (2008) 93–105.