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Bioleaching of heavy metals from pig manure with indigenous sulfur-oxidizing bacteria: effects of sulfur concentration

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Abstract

The purpose of this work was to study the sulfur concentration on bioleaching of heavy metals from pig manure employing indigenous sulfur-oxidizing bacteria. Also, the variations in physicochemical properties of pig manure before and after bioleaching were investigated. The results showed that sulfur concentration significantly affected the rate of acidification, sulfate production and metal solubilization during pig manure bioleaching process. A Michaelis–Menten type equation was utilized to interpret the relationships between sulfur concentration, sulfate production and metal solubilization in the bioleaching process. The rates of metal solubilization during pig manure bioleaching were well described by a first order kinetic equation related to time. After 12 days of bioleaching, 93%–97% of Zn, 96%–98% of Mn and 48%–94% of Cu were leached out from pig manure, respectively. The metals remaining in the pig manure residual were mainly existed in stable forms. In addition, elemental analysis showed that bioleaching process could significantly modify the dewaterbility and organic composition of pig manure. However, fertility analysis found that 9.0%–19.1% of nitrogen, 68.5%–71.0% of phosphorus, 76.5%–78.8% of potassium and

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47.5%−49.4% of the total organic carbon (TOC) were lost from pig manure in the bioleaching process. Therefore, bioleaching process used in this study could be applied to remove heavy metals effectively from the pig manure, but more detailed studies need to be done to decrease the nutrients loss from pig manure.

Keywords: Environmental science, Engineering, Biotechnology, Microbiology

1. Introduction

Intensive livestock breeding has caused a sharp increase of the yield of livestock and poultry manure both in China and many other countries worldwide (Buelna et al., 2008; Maccari et al., 2016; Quan et al., 2016; Tigini et al., 2016). Pig manure is an organic nutrient sources, which has been widely utilized as organic fertilizer in agriculture. However, the antibiotic residues and heavy metals in pig manure limit its direct land application (Lv et al., 2016; Wang et al., 2016). Trace metals, such as Cu, Zn and Cd, are used worldwide in intensive animal breeding for preventing infectious diseases and as growth promoters (Long et al., 2004; Mccarthy et al., 2013). These heavy metals can accumulate in soil, be uptaken by plants and affect the health of animals and people through food chain (Buelna et al., 2008; Qureshi et al., 2008).

Generally, compost and anaerobic digestion methods have been implemented for treating the pig manure containing heavy metals (Marcato et al., 2008; Lu et al., 2015; Quan et al., 2016; Tigini et al., 2016). However, they show some limitations such as the heavy metals can just be fixed and the total amount of heavy metals is not reduced (Lv et al., 2016). Bioleaching technology, because of its simplicity, cost effectiveness and low impact on the environment, has been developed as a promising method to remove heavy metals from sludge, sediments or soil (Rastegar et al., 2014; Yang et al., 2016; Zeng et al., 2016). The removal efficiency of most heavy metals is usually higher than 85% (Blais et al., 1993). Nevertheless, research on bioleaching of heavy metals from pig manure has been limited.

Compared to chemical leaching or iron-mediated bioleaching process, the bioleaching process inoculated with sulfur-oxidizing bacteria for metals leaching is more economical and efficient at low plant capacities and high solid concentrations (Sreekrishnan and Tyagi, 1996; Zhou et al., 2012). In the bioleaching process, the removal of heavy metals from manure is primarily achieved by the acidification and oxidation reactions caused by sulfur-oxidizing bacteria. Generally, the addition of exogenous microorganisms plays a critical role on achieving high leaching efficiency during the bioleaching process. Moreover, the enriched nature microbial communities, especially those enriched directly from the pollution source, show better structure stability of consortiums and better efficiency of metal solubilization in

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comparison to single strains and artificial microbial consortiums (Zeng et al., 2016). There have been studies on bioleaching with indigenous microorganisms for removal of heavy metals from sewage sludge and sediment (Chen and Lin, 2004a; Zhang et al., 2008). However, bioleaching of heavy metals from pig manure with acclimated indigenous microorganisms has not been reported to date.

The effectiveness of bioleaching process depends on various physical, chemical, and biological parameters in the system (Chen and Lin, 2004a; Seidel et al., 2006; Kumar and Nagendran, 2007). A thorough understanding of the parameters that control the bioleaching process is crucial to achieve the maximum efficiency of metal solubilization. In bioleaching process, elemental sulfur is extensively added as an energy source for growth of sulfur-oxidizing bacteria (Liu et al., 2008; Zhang et al., 2008). Acid production and pH variations are influenced by the amount of sulfur added. If the added elemental sulfur exceeds the requirement for the growth of sulfur-oxidizing bacteria and metals solubilization, the remaining sulfur will lead to the reacidification of treated pig manure during land application. Besides, the physicochemical properties of the bioleached pig manure are also important for its final disposal and literature related has not been reported. Therefore, a complete understanding of the optimum sulfur concentration for pig manure bioleaching and the effect of bioleaching on its physicochemical properties is important for effective use of the bioleaching processes.

In the present study, indigenous sulfur-oxidizing bacteria was enriched as an inoculums for bioleaching of pig manure. The effects of sulfur concentration on heavy metals solubilization and transformation of binding forms were investigated. Moreover, the fertility and elemental analysis of pig manure before and after bioleaching were conducted to evaluate the effect of bioleaching on pig manure.

2. Materials and methods

2.1. Samples

Fresh pig manure used in this study was taken from a local pig farm in Tianjin (China). Pig manure sample was collected in plastic drum and stored at 4 °C. To ensure the accuracy of the results, every batch of pig manure sample was characterized before its use in bioleaching experiment. Selected physicochemical properties of the tested swine manure is presented in Table 1.

2.2. Microorganism

Fresh pig manure obtained from the pig farm was used as seed inoculum to enrich and culture the indigenous sulfur-oxidizing bacteria. Fresh pig manure (solid content: 23.6%) was diluted to solid content of 2% (w/v) with distilled water. About

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Table 1. Measured characteristics of the pig manure.

Parameters	Value (dry weight)	Parameters	Value (dry weight)
pH	8.22 ± 0.11	Zn (mg/kg)	1283.3 ± 44.9
Total solid (%)	23.57 ± 1.31	Cu (mg/kg)	286 ± 8.7
TOC (mg/kg)	589.52 ± 28.43	Mn (mg/kg)	534.5 ± 11.5
Total N (%)	3.47 ± 0.12	Pb (mg/kg)	21.44 ± 1.56
Total P (%)	3.01 ± 0.07	As (mg/kg)	14.37 ± 0.35
Total K (%)	1.41 ± 0.02	Ni (mg/kg)	15.75 ± 0.74

100 mL of diluted pig manure (solid content: 2%) and 10 g/L of tyndallized elemental sulfur were added to a 250 mL Erlenmeyer flask and agitated on a gyratory shaker (ZQLY-180F, China) at 180 r/min and 28 °C. The pH of the pig slurry was monitored. When the pH dropped below 2.0, 10 mL of the acidified sludge was then transferred to 100 mL of fresh pig slurry with 10 g/L of elemental sulfur and the process was repeated under the same conditions. After four such transfers, the indigenous sulfur-oxidizing bacteria in pig slurry achieved the highest acidification rate. The inoculum for the bioleaching experiment was obtained.

In order to analysis of the sulfur-oxidizing bacteria in inoculum, a salt medium was utilized to enrich sulfur-oxidizing bacteria. The components of the medium are as follows: 0.4 g/L $(NH_4)_2SO_4^{2-}$, 3.0 g/L KH_2PO_4, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.25 g/L $CaCl_2 \cdot 2H_2O$. The initial pH of the medium was adjusted to 4.0 utilizing 1:1 H_2SO_4. 10 g/L of elemental sulfur was added as energy source. The incubation was conducted at 28 °C and 180 r/min on a gyrator shaker (ZQLY-180F, China). The enriched bacterial community was clarified from the analysis of data based on 16S rRNA gene clone library analysis (Wei et al., 2018). All clones sequenced had same sequences and had a 98% sequence similarity to *Acidithiobacillus thiooxidans*.

2.3. Bioleaching experiments

The bioleaching experiments were carried out in 500 mL Erlenmeyer flask with 200 mL pig manure sample (2% solid content, w/v). With an addition of 5% (v/v) previously activated indigenous sulfur-oxidizing bacteria, the pig slurry was agitated at 180 r/min and 28 °C on a gyrator shaker (ZQLY-180F, China). For all experiments, the pig slurry solid content was 2% (w/v). Various content (2, 5, 8 and 10 g/L) of elemental sulfur were then added to the flasks. The changes during each bioleaching experiment were monitored by periodic sampling and analyzing the pig slurry for pH, ORP, sulfate and soluble heavy metals (Cu, Zn, Mn). During bioleaching process the water loss via evaporation was compensated by adding distilled water based on weight loss. All experiments were done in triplicate.

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2.4. Analytical methods

The total solid (TS) content of pig manure was measured by oven-drying at 105 °C till constant weight. Organic matter content of dried manure was measured by a TOC analyzer (Multi N/C 300, Analytik Jena, Germany). The pH of pig manure (1:10 w/v) was measured in deionized water by a pH meter (HANNA HI 8424, Italy). The total nitrogen (TN) of pig manure before and after bioleaching was measured by a LEEMAN CHNS elemental analyzer. The total phosphorus (TP) and total potassium (TK) in pig manure before and after bioleaching were determined according to Standard Methods (APAH, 1995).

During the bioleaching experiment, pH and ORP values were measured using Ag/AgCl electrode (HANNA HI 8424, Italy). Five binding fractions (exchangeable, carbonates-bound, Fe/Mn oxides, organic/sulfates and residual) of heavy metals (Cu, Zn, Mn) in the pig manure before and after bioleaching were measured using the sequential extraction procedure based on Tessier et al. (1979), as shown in Table 2. The pig slurry sample taken from the flasks at two-days interval was centrifuged at a speed of 12000 r/min for 15 min and filtered through a 0.45 μm filter membrane. The filtrate was analyzed for its sulfate concentrations according to Standard Methods (APAH, 1995). Heavy metal analysis was carried out using a flame/graphite atomic absorption spectrophotometer (TAS-990, China). Ultimate analysis of pig manure was carried out with a LEEMAN EA300 elemental determinator.

Table 2. Sequential selective extraction procedure.

Extracted metal form	Reagent	Extraction procedure
Exchangable	1 mol/L $MgCl_2$, pH 7.0	The ratio of solution to pig manure was 8:1, 2 h oscillation at 25 °C, centrifugal separation
Carbonates-bound	NaOAc (1 mol/L)-HOAc, pH 5.0	The ratio of solution to pig manure was 8:1, 3 h oscillation at 25 °C, centrifugal separation.
Fe/Mn oxides	$NH_2OH \cdot HCl$ (0.04 mol/L)-HOAc (25%)	The ratio of solution to pig manure was 10:1, digested at 96 °C for 3 h, centrifugal separation.
Organic/sulfides	0.04 mol/L HNO_3, 30% H_2O_2, CH_3COONH_4 (3.2 mol/L)-HNO_3 (20%)	Added HNO_3 one time and H_2O_2 two times, the ratio of solution to pig manure was 3:1 and 5:1, respectively, digested at 85 °C twice (1.5 h each time); added CH_3COONH_4 (3.2 mol/L)-HNO_3 (20%), the ratio of solution to pig manure was 5:1, 3 h oscillation at 25 °C, centrifugal separation.
Residual	Aqua regia (HNO_3:HCl = 1:3), $HClO_4$, 0.5 mol/L HNO_3	Heated the aqua regia on the hot plate until it dried, added $HClO_4$ and heated to white and dissolved using 0.5 mol/L HNO_3, centrifugal separation.

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3. Results and discussion

3.1. Variations of pH and ORP in bioleaching

During bioleaching, elemental sulfur was oxidized to sulfuric acid by indigenous sulfur-oxidizing bacteria which resulted in a decrease in pH and solubilization of metals. Fig. 1a shows variations of pH for different sulfur concentrations. It is

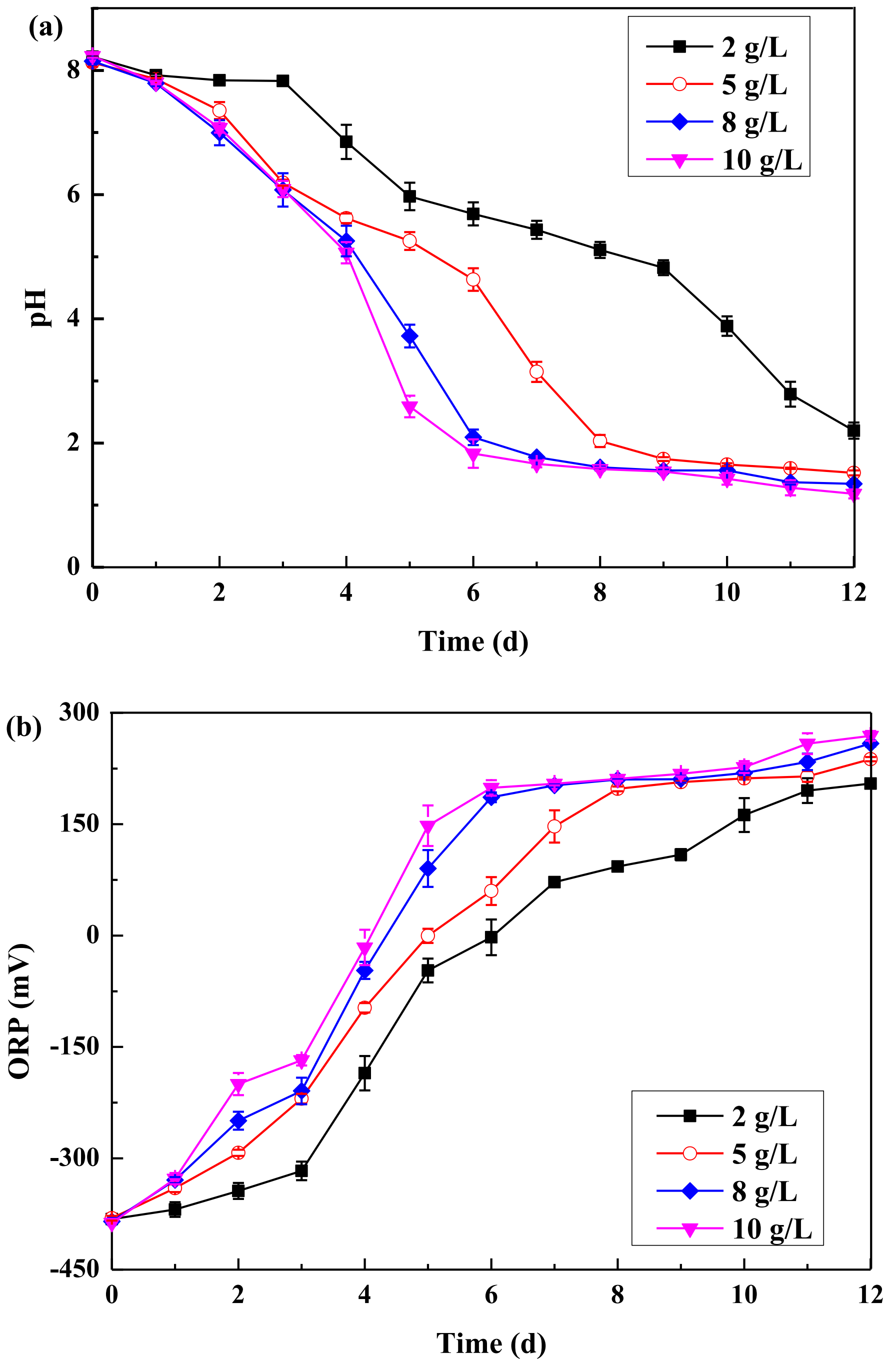

Fig. 1. Changes of pH (a) and ORP (b) in bioleaching process for different sulfur concentration.

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evident that the acidification rate of pig slurry increases as the sulfur concentration increases. The pH value of pig slurry decreased from 8.2 to about 2.0 in 6, 6, 8 and 12 days for sulfur concentration of 10, 8, 5 and 2 g/L, respectively. According to Chen and Lin (2001), higher sulfur concentration means higher availability of total surface area of sulfur particles. Therefore, the increase of sulfur concentration led to an improvement of the bacteria adsorption onto sulfur particles. Adsorption was followed by oxidation by indigenous sulfur-oxidizing bacteria, which enhanced the rate of pH reduction. However, adding 10 g/L of elemental sulfur into flask only had a slightly increase in comparison with the other elemental sulfur increases.

During the bioleaching process, sulfur oxidization by sulfur-oxidizing bacteria led not only to pH decrease of pig slurry but also to an increase in ORP. Fig. 1b displays the variation of ORP during bioleaching for different sulfur concentrations. The oxidization of sulfur led to a rapid increase of ORP between 2 days and 8 days. Higher sulfur concentration led to higher rates of ORP increase. With 2, 5, 8 and 10 g/L sulfur addition, it took about 6, 6, 8 and 11 days to achieve the ORP higher than 200 mV vs Ag/AgCl. After 12 days of bioleaching, the ORP values recorded in systems were 205, 238, 259 and 269 mV for sulfur concentration of 2, 5, 8 and 10 g/L, respectively. High ORP is considered as an indicator for the presence of sulfur-oxidizing bacteria (Kumar and Nagendran, 2007). Thus, the higher ORP achieved for 8 and 10 g/L sulfur concentration indicated a faster growth of sulfur-oxidizing bacteria. In addition, related studies have shown that decreasing the pH of pig slurry alone did not lead to conversion of metals from organic/sulfides forms into ion exchangeable states (Zhou et al., 2012). Therefore, when solubilizing heavy metals from pig manure, care needs to be taken to regulate both pH and ORP.

3.2. Sulfate production in bioleaching

Fig. 2 shows the variations of sulfate for different sulfur concentrations during bioleaching. The results show that after 12 days of bioleaching the sulfate production increased to 5473, 7038, 8976 and 9468 mg/L at sulfur concentration of 2, 5, 8 and 10 g/L, respectively. During the bioleaching process, the rate of sulfate production increased with the increase of sulfur concentration. This may be attributed to the higher growth rate of sulfur-oxidizing bacteria at higher sulfur concentration. The results also display that the sulfate was produced linearly with time. The rate of sulfate production can be determined based on the following equation (Zeng et al., 2016):

$$V_{sulfate} = \frac{d\left[SO_4^{2-}\right]}{dt} \tag{1}$$

where $V_{sulfate}$ is the rate constant of sulfate production (mg/L/d). The rate constants of sulfate production at different sulfur concentrations are listed in Table 3.

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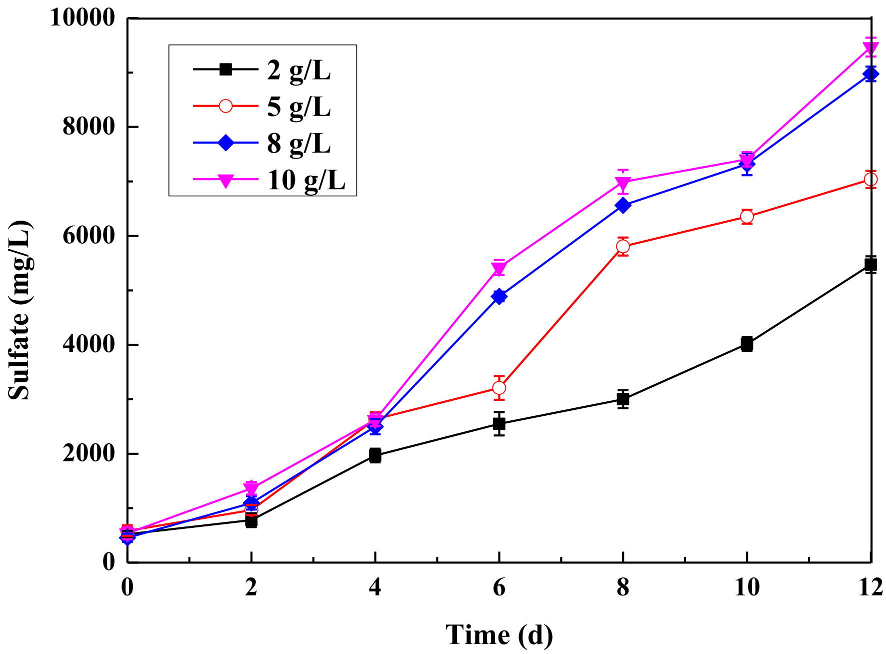

Fig. 2. The variations of sulfate in bioleaching process for different sulfur concentrations.

The correlation coefficients (R^2) obtained by Eq. (1) were all greater than 0.96, indicating that the changes of sulfate concentration during bioleaching were finely consistent with the linear equation. The observed dependence of sulfate production rates on sulfur concentrations is exhibited in Fig. 3. A Michealis-Menten type of equation is used to describe the effect of sulfur concentrations on the sulfate production rate:

$$\frac{d\left[SO_4^{2-}\right]}{dt} = V_{sulfate} = \frac{V_s^{max} S}{K_s + S} \quad (2)$$

where K_s is the saturation constant (g/L), S and V_s^{max} represent the sulfur concentration (g/L) and the maximum sulfate production rate (mg/L/d), respectively. As shown in Fig. 3, the values of V_s^{max} and K_s were 1037.6 mg/L/d and 3.4 g/L,

Table 3. Predicted sulfate production rate during bioleaching for different sulfur concentrations.

Sulfur (g/L)	$V_{sulfate}$ (mg/L)	R^2
2	399.3	0.96
5	594.8	0.96
8	751.2	0.98
10	771.2	0.97

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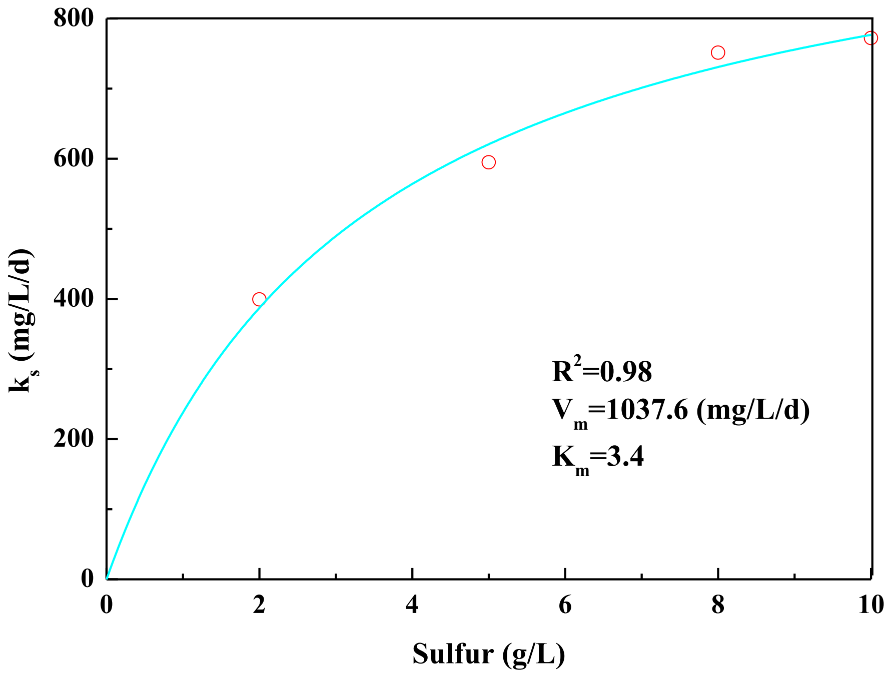

Fig. 3. Sulfate production rate at different sulfur concentrations during pig manure bioleaching process (the solid line represents the fit to Michaelis-Menten type of equation).

respectively. These values were obtained by fitting Eq. (2) to the data from the various sulfur concentrations and consistent with the experimental data.

Besides, according to the results in Fig. 3, it is worth to note that after 12 days of bioleaching the utilization ratio of sulfur was 82.6%, 47.4 %, 37.4% and 31.6% for sulfur concentration of 2, 5, 8 and 10 g/L, respectively. A drastic decrease of sulfur utilization ratio was observed when increasing the sulfur concentration from 2 to 10 g/L. The results indicated that increase the addition of sulfur could increase the sulfate production and accelerate the acidification during bioleaching process, but the sulfur remained in the pig manure also increased. The residual sulfur powder was required to be removed or recycled from the bioleached pig manure before its land application. However, the removal and recycle of sulfur powder was not easy to implement in pig manure bioleaching. Actually, some recoverable forms of sulfur was reported to be reusable and helpful in sediment bioleaching (Chen et al., 2003). These recoverable forms of sulfur can also be applied to pig manure bioleaching process.

3.3. Metal solubilization in bioleaching

Fig. 4 shows the metal solubilization for different sulfur concentrations during bioleaching process. There were lag phases that existed while bioleaching heavy metals from the pig manure. The lower sulfur concentration had a longer lag phases. It is

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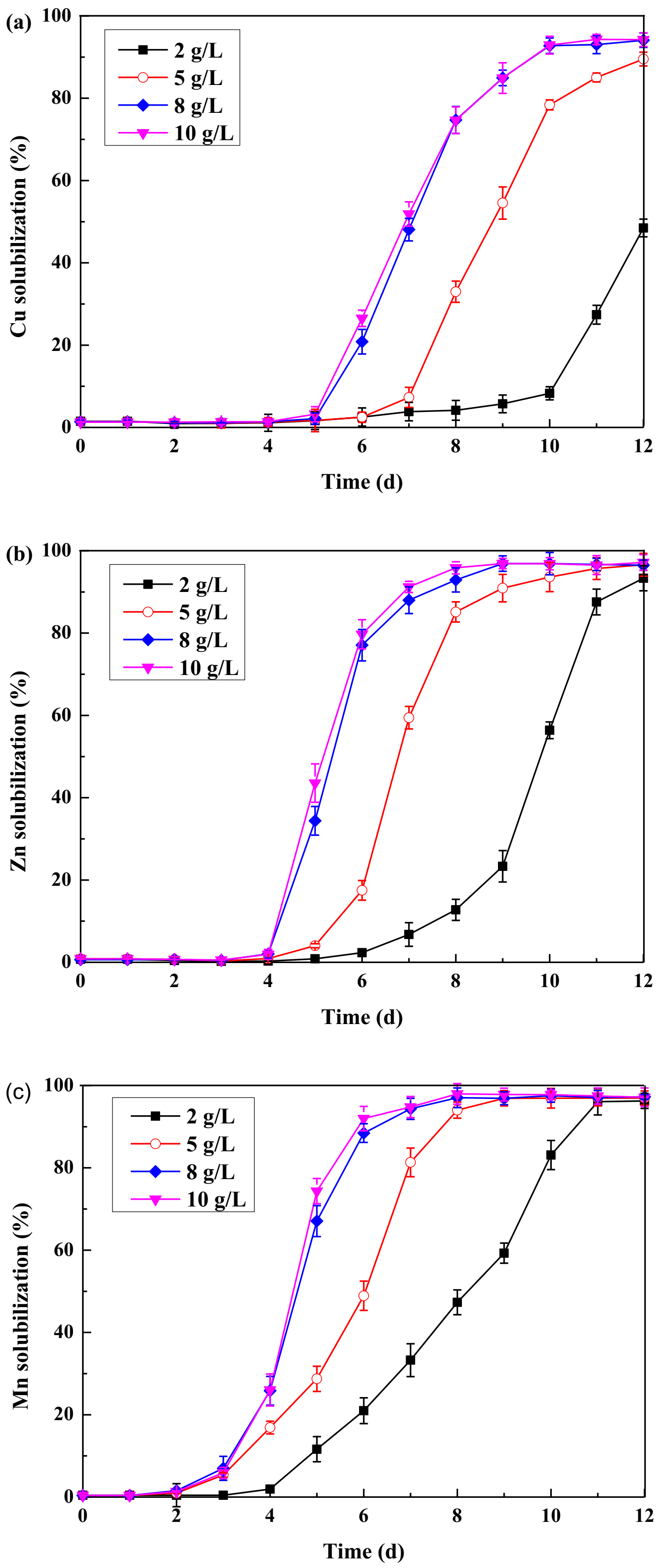

Fig. 4. The metal solubilization at different sulfur concentrations (a) Cu, (b) Zn, (c) Mn during the bioleaching process.

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likely that the lower sulfur as electron donor led to lower rate of sulfur-oxidizing bacteria growth and sulfate production, and further resulted in a lower acidification rate and a longer time to achieve the pH required for metals leaching during pig manure bioleaching (Chen and Lin, 2001; Mario et al., 2013). As Cu (Fig. 4a) for example, the lag phase with 8 and 10 g/L sulfur addition only lasted 5 days, much shorter than that with 2 g/L (10 days) and 5 g/L (7 days) sulfur addition. With addition of 5, 8 and 10 g/L sulfur, 12, 9 and 9 days were required to achieve the solubilization efficiency of Cu higher than 90%. While the Cu solubilization with 2 g/L sulfur addition was reached only 48.5% after 12 days of bioleaching. The trends of solubilization of Zn (Fig. 4b) and Mn (Fig. 4c) were similar to Cu but with shorter lag phase and faster leaching rate, which may be attributed to their binding forms in pig manure (Zhou et al., 2012). With 8 and 10 g/L sulfur addition, it took about 7 days for Zn and 6 days for Mn to achieve the solubilization efficiency higher than 90%, which was 4 and 2 days short for both Zn and Mn with 2 and 5 g/L sulfur addition, respectively. After 12 days of bioleaching, the solubilization efficiency measured for Zn was highest at 93.3%−97.2% for different sulfur concentrations, and the solubilization of Mn was 96.3%−97.8%. These results indicated that the increase of sulfur concentration from 2 to 8 g/L significantly accelerated heavy metals solubilization, but continuing to increase sulfur concentration from 8 to 10 g/L did not obviously benefit the removal of heavy metals.

3.4. Kinetic study

A kinetic model (Bayat and Sari, 2010) was utilized to further evaluate the effect of sulfur concentration on metal solubilization. The equations governing the kinetic models are presented below:

$$-\frac{dM}{dt} = k(M_s - M) \tag{3}$$

and

$$\ln\left[\frac{M_s}{M_s - M}\right] = kt \tag{4}$$

where k is the rate constant for metal solubilization (d^{-1}), M_s and M represent initial mass (mg) of metal in pig manure and mass (mg) in the aqueous phase, respectively, and t is the bioleaching time. Table 4 shows the rates constants of metal solubilization obtained from Eq. (4) in the bioleaching process. As can be seen from Fig. 5, the rate of metal solubilization increased markedly as the sulfur concentration increased from 2 to 8 g/L, while it increased only slightly when sulfur concentration was further increased to 10 g/L. This can be attribute to that the sulfur was the primary limiting factor for the growth of sulfur-oxidizing bacteria only when the growth rate did not reach its equilibrium under the certain reaction system.

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Table 4. Rate constant of metal solubilization from pig manure in bioleaching process for different sulfur concentrations.

Sulfur concentration (mg/L)	Cu		Zn		Mn	
	k (d^{-1})	R^2	k (d^{-1})	R^2	k (d^{-1})	R^2
2	0.222	0.961	0.456	0.783	0.433	0.820
5	0.410	0.956	0.569	0.964	0.582	0.88
8	0.498	0.968	0.702	0.987	0.643	0.934
10	0.511	0.982	0.742	0.977	0.702	0.935

The sulfur enhanced the activities of sulfur-oxidizing bacteria which finally enhanced the production of acid. The rate of metal solubilization is highly correlated to the rate of pH reduction (Tyagi et al., 1993). Accordingly, it is understood that the trends of rate of metal solubilization in the pig manure bioleaching process followed the Michealis−Menton type of model (Chen and Lin, 2004a):

$$V_{metal} = \frac{V_m^{max} S}{K_m + S} \tag{5}$$

where K_m is a saturation constant (g/L), V_m^{max} and S are the maximum rate of metal solubilization (d^{-1}) and the sulfur concentration (g/L), respectively. The estimate values of parameters (V_m^{max}, K_m and R^2) from Eq. (5) are exhibited in Table 5.

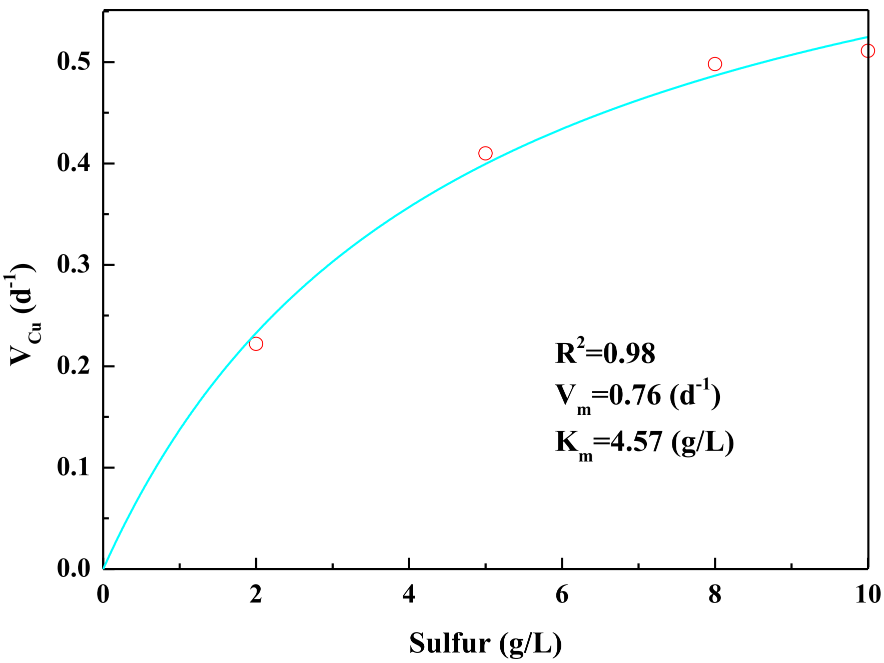

Fig. 5. Relationship between rate of Cu solubilization and sulfur concentration (the solid line represents the fit to Michealis−Menton type of equation).

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Table 5. Parameters of Michealis−Menton equation for rate of metal solubilization from pig mnure.

Metal	V_m (d^{-1})	K_m (g/L)	R^2
Zn	0.87	2.02	0.90
Mn	0.8	1.78	0.97
Cu	0.76	4.57	0.98

The correlation coefficients (R^2) obtained from Eq. (5) are greater than 0.90 for Cu, Zn and Mn. The maximum rate of metal solubilization from pig manure for the three metals in decreasing order were Zn > Mn > Cu. The rate of metal solubilization (Tables 1 and 5) was correlated to the initial contents of metal in the pig manure. These results are similar to the results reported by Chen and Lin (2004b).

3.5. Changes in physicochemical properties of pig manure

3.5.1. Changes in binding forms of heavy metals

The different binding forms of heavy metal in pig manure are caused by different energy states. The binding forms determine not only the efficiency of metal solubilization but also the bioavailability of heavy metals in pig manure during bioleaching (Zhang et al., 2008). Metals in exchangeable, carbonate-bound, and Fe/Mn oxide-bound fractions are mobile, dangerous and bioavailable, while the organic matter/sulfide-bound and residual metals are stable and non-bioavailable (Liu et al., 2008). Therefore, bioleaching mechanism of metals solubilization in pig manure can be explained by transformation of binding forms of heavy metals.

The partitioning of heavy metals before and after bioleaching were shown in Fig. 6. Fig. 6a shows that Zn (65.6%) and Mn (72.7%) mainly existed in mobile forms. Cu (73%) was predominantly found in stable forms, of which 64.8% of Cu was associated with sulfides/organic matter. Even in a stable form, Cu could be efficiently solubilized by sulfur-oxidizing bacteria (Fig. 4a). These results explained the high solubilization efficiency of Cu, Zn and Mn achieved in this study and indicated that bioleaching is a promising technology in removing heavy metals from pig manure. After the bioleaching process, metals remaining in pig slurry had relatively low concentrations and mainly existed in stable forms (Fig. 6b). Low levels of metal remaining in mostly stable forms lead to the conclusion that the treated pig manure produced through the bioleaching process is safer for use as a fertilizer.

3.5.2. Changes of pig manure composition

Table 6 displays elemental analysis results of pig manure samples at different sulfur concentrations. Compared with the raw pig manure, the H/C and N/C ratios in pig

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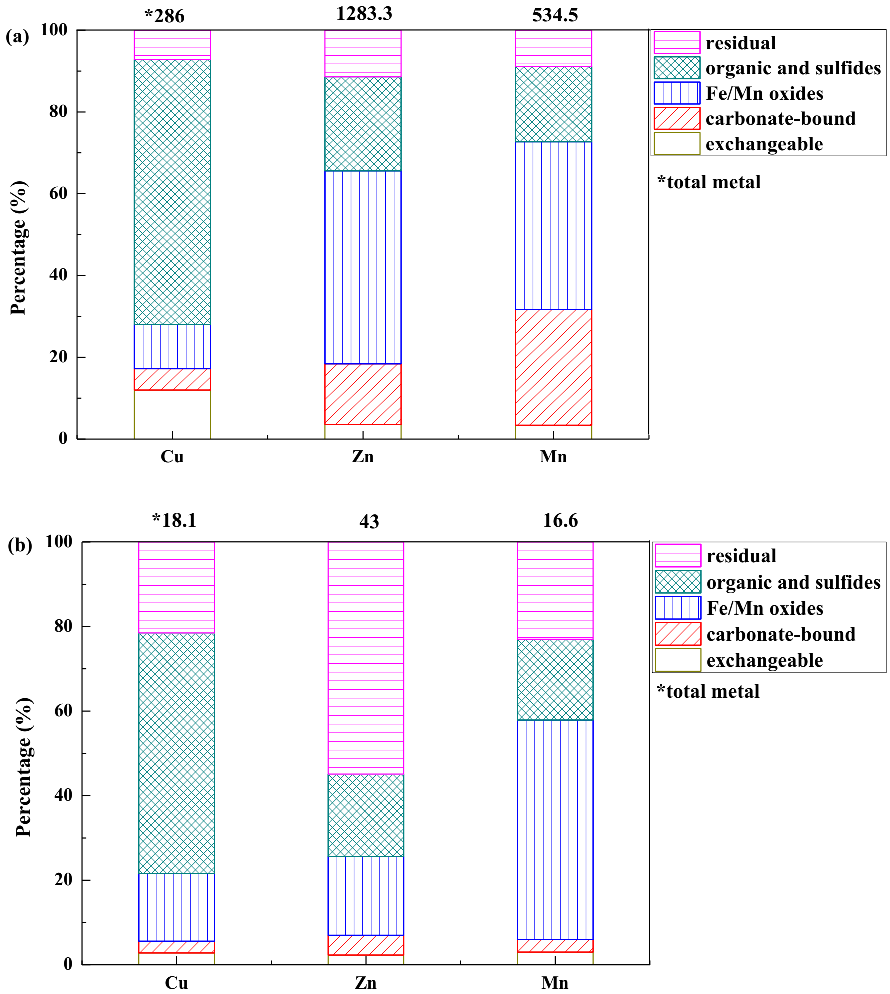

Fig. 6. Metal speciation in pig manure before (a) and after (b) the bioleaching process.

manure both decrease after 12 days of bioleaching. The N/C ratio is inversely related to the polymerization degree of organic matter in pig manure. The N/C ratio decreased with the increase of sulfur concentration during bioleaching process, from 0.08 (raw pig manure) to 0.05 (sulfur = 10 g/L). Organic matter containing

Table 6. Ultimate analysis of pig manure samples.

Sulfur concentration (g/L)	C (wt%)	H (wt%)	N (wt%)	N/C[a]	H/C[a]
Raw pig manure	39.57 ± 2.96	3.96 ± 0.18	3.47 ± 0.11	0.08	1.2
2	49.53 ± 0.5	4.87 ± 0.16	3.16 ± 0.25	0.06	1.18
5	52.76 ± 1.80	5.02 ± 0.12	2.81 ± 0.08	0.05	1.14
8	52.89 ± 1.37	4.79 ± 0.05	3.00 ± 0.02	0.05	1.09
10	53.13 ± 1.74	4.79 ± 0.19	3.08 ± 0.27	0.05	1.08

[a] Molar ratio.

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more nitrogenous functional groups would be less polymerized (Gascó et al., 2005; Chen et al., 2015). Therefore, the bioleaching treatment changed the polymerization degree of pig manure, and the increasing of sulfur concentration during bioleaching slightly increased the polymerization degree of pig manure and further suggests the increase of its dewatering capacity. Similar results were also observed in variations of H/C ratio. The variations of N/C and H/C suggest that the organic matter composition of pig manure has been changed during bioleaching process. This result also demonstrates that the bioleached pig manure contains more aromatic groups than raw pig manure. It could be attributed to the solubilization of light organic compounds during the bioleaching process (Gascó et al., 2005; Gascó and Lobo, 2007). These findings indicate that despite the higher efficiency for removal of heavy metals from pig manure, the higher sulfur concentration lowered the performance for the presence of elevated aliphatic carbon and long chains (with CH_2 groups) (Gascó et al., 2005; Zhang et al., 2011; Chen et al., 2015). These changes in composition of organic compound could be further revealed in the variations of nutrients of pig manure.

3.5.3. *Changes in nutrients of pig manure*

One major concern about bioleaching was the potential loss nutrients including N, P, K and organic matter, which in return reduced its value as soil fertilizer or conditioner. Fig. 7 illustrates the concentrations of total nitrogen (TN), total phosphate

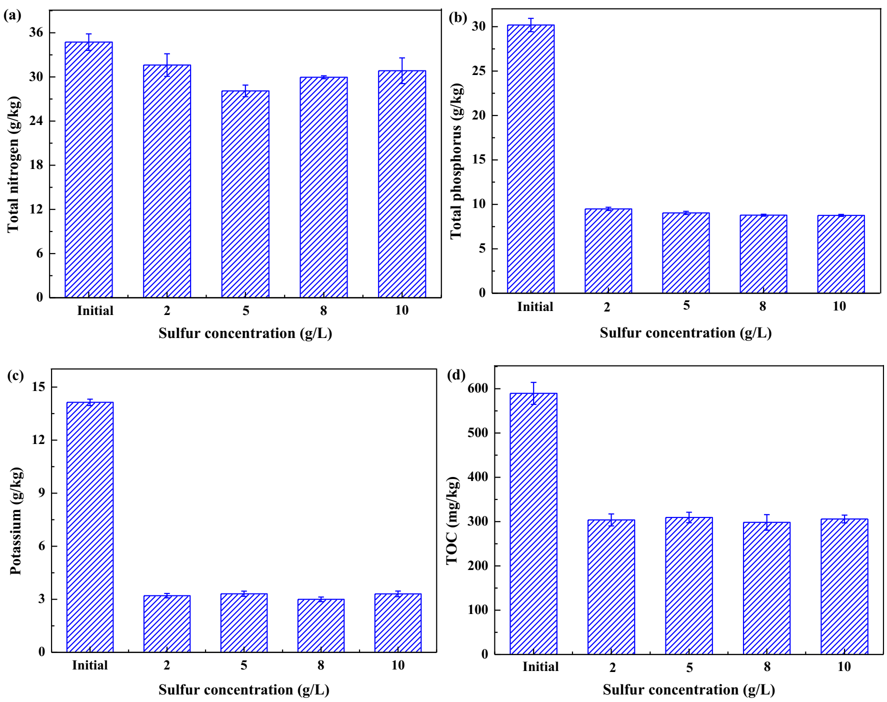

Fig. 7. The solubilization of total nitrate (a), total phosphate (b), potassium(c) and total organic carbon (d) in pig manure during bioleaching process.

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(TP), total potassium (TK) and total organic carbon (TOC) at different sulfur concentrations before and after bioleaching process. The results show that the percentage of TN, TP, TK and TOC released from the pig slurry after 12 days of bioleaching were 9.0%–19.1%, 68.5%–71.0%, 76.5%–78.8% and 47.5%–49.4%, respectively. The bioleaching treatment led to undesirable loss of essential plant nutrients, but the sulfur concentration did not significantly affect the nutrients loss after 12 days of bioleaching. The loss of nutrients could be caused by the highly oxidizing and acidifying conditions during the bioleaching process resulting in an environment suitable for dissolving heavy metals and also enhancing the decomposition of organic matters in pig manure (Zhou et al., 2006; Nareshkumar and Nagendran, 2008).

4. Conclusion

Bioleaching of heavy metals from pig manure was conducted employing indigenous sulfur-oxidizing bacteria. Results show that sulfur concentration had a significant influence on the performance and efficiency of the pig manure bioleaching process. Increasing the sulfur concentration from 2 to 10 g/L enhanced the rate of pig manure acidification, sulfate production and metal solubilization during bioleaching. The influence of sulfur concentration on both sulfate production and metal solubilization were well described by the Michealis–Menton type equation. After the bioleaching process, heavy metals remaining in pig manure had relatively low concentrations and mainly existed in stable forms. The composition analysis of pig manure before and after bioleaching revealed that the physicochemical properties of pig manure was to some extent modified. The fertility analysis showed that large amount of nutrients was lost in bioleaching process. These results indicate that bioleaching was an efficient alternative for removing the heavy metals from pig manure, but more detailed studies need to be done to further evaluate the suitability of the technique for field application. Studies on minimizing the loss of nutrients from pig manure during bioleaching process are under progress in our laboratory.

Declarations

Author contribution statement

Xiaocheng Wei: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Dongfang Liu, Lirui Liao, Zhendong Wang, Wenjiao Li, Wenli Huang: Performed the experiments; Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

APAH, 1995. Standard Methods for the Examination of Water and Wastewater, nineteenth ed. American Public Health Association, Washington, DC.

Bayat, B., Sari, B., 2010. Comparative evaluation of microbial and chemical leaching processes for heavy metal removal from dewatered metal plating sludge. J. Hazard. Mater. 174, 763−769.

Blais, J.F., Tyagi, R.D., Auclair, J.C., 1993. Bioleaching of metals from sewage sludge: microorganisms and growth kinetics. Water Res. 27, 101−110.

Buelna, G., Dubé, R., Turgeon, N., 2008. Pig manure treatment by organic bed biofiltration. Desalination 231, 297−304.

Chen, S.Y., Chiu, Y.C., Chang, P.L., Lin, J.G., 2003. Assessment of recoverable forms of sulfur particles used in bioleaching of contaminated sediments. Water Res. 37, 450−458.

Chen, S.Y., Lin, J.G., 2004a. Bioleaching of heavy metals from contaminated sediment by indigenous sulfur-oxidizing bacteria in an air-lift bioreactor: effects of sulfur concentration. Water Res. 38, 3205−3214.

Chen, S.Y., Lin, J.G., 2004b. Bioleaching of heavy metals from livestock sludge by indigenous sulfur-oxidizing bacteria: effects of sludge solids concentration. Chemosphere 54, 283−289.

Chen, S.Y., Lin, J.G., 2001. Effect of substrate concentration on bioleaching of metal-contaminated sediment. J. Hazard. Mater. 82, 77−89.

Chen, Z., Hu, M., Cui, B., Liu, S., Guo, D., Xiao, B., 2015. The effect of bioleaching on sewage sludge pyrolysis. Waste Manag. 48, 383−388.

 https://doi.org/10.1016/j.heliyon.2018.e00778

Gascó, G., Blanco, C.G., Guerrero, F., Lázaro, A.M.M., 2005. The influence of organic matter on sewage sludge pyrolysis. J. Anal. Appl. Pyrol. 74, 413–420.

Gascó, G., Lobo, M.C., 2007. Composition of a Spanish sewage sludge and effects on treated soil and olive trees. Waste Manag. 27, 1494–1500.

Kumar, R.N., Nagendran, R., 2007. Influence of initial pH on bioleaching of heavy metals from contaminated soil employing indigenous *Acidithiobacillus thiooxidans*. Chemosphere 66, 1775–1781.

Liu, Y.G., Zhou, M., Zeng, G.M., Wang, X., Li, X., Fan, T., Xu, W.H., 2008. Bioleaching of heavy metals from mine tailings by indigenous sulfur-oxidizing bacteria: effects of substrate concentration. Bioresour. Technol. 99, 4124–4129.

Long, C., Yu-jun, W., Dong-mei, Z., Yuan-hua, D., 2004. Heavy metals pollution in poultry and livestock feeds and manures under intensive farming in Jiangsu Province, China. J. Environ. Sci. 16, 371–374.

Lu, X.M., Lu, P.Z., Chen, J.J., Zhang, H., Fu, J., 2015. Effect of passivator on Cu form transformation in pig manure aerobic composting and application in soil. Environ. Sci. Pollut. Res. 22, 14727–14737.

Lv, B., Xing, M., Yang, J., 2016. Speciation and transformation of heavy metals during vermicomposting of animal manure. Bioresour. Technol. 209, 397–401.

Maccari, A.P., Baretta, D., Paiano, D., Leston, S., Freitas, A., Ramos, F., Sousa, J.P., Klauberg-Filho, O., 2016. Ecotoxicological effects of pig manure on Folsomia candida in subtropical Brazilian soils. J. Hazard. Mater. 314, 113–120.

Marcato, C.E., Pinelli, E., Pouech, P., Winterton, P., Guiresse, M., 2008. Particle size and metal distributions in anaerobically digested pig slurry. Bioresour. Technol. 99, 2340–2348.

Mario, V., Axel, S., Wolfgang, S., 2013. Progress in bioleaching: fundamentals and mechanisms of bacterial metal sulfide oxidation-part A. Appl. Microbiol. Biotechnol. 97, 7529–7541.

Mccarthy, G., Lawlor, P.G., Gutierrez, M., Gardiner, G.E., 2013. Assessing the biosafety risks of pig manure for use as a feedstock for composting. Sci. Total Environ. 463–464, 712–719.

Nareshkumar, R., Nagendran, R., 2008. Changes in nutrient profile of soil subjected to bioleaching for removal of heavy metals using *Acidithiobacillus thiooxidans*. J. Hazard. Mater. 156, 102–107.

Quan, W., Zhen, W., Awasthi, M.K., Jiang, Y., Li, R., Ren, X., Zhao, J., Feng, S., Wang, M., Zhang, Z., 2016. Evaluation of medical stone amendment for the reduction of nitrogen loss and bioavailability of heavy metals during pig manure composting. Bioresour. Technol. 220, 297–304.

Qureshi, A., Lo, K.V., Liao, P.H., Mavinic, D.S., 2008. Real-time treatment of dairy manure: implications of oxidation reduction potential regimes to nutrient management strategies. Bioresour. Technol. 99, 1169–1176.

Rastegar, S.O., Mousavi, S.M., Shojaosadati, S.A., 2014. Cr and Ni recovery during bioleaching of dewatered metal-plating sludge using Acidithiobacillus ferrooxidans. Bioresour. Technol. 167, 61–68.

Seidel, H., Wennrich, R., Hoffmann, P., Löser, C., 2006. Effect of different types of elemental sulfur on bioleaching of heavy metals from contaminated sediments. Chemosphere 62, 1444–1453.

Sreekrishnan, T.R., Tyagi, R.D., 1996. A comparative study of the cost of leaching out heavy metals from sewage sludges. Proc. Biochem. 31, 31–41.

Tessier, A., Campbell, P.G.C., Bisson, M., 1979. Sequential extraction procedure for the speciation of particulate trace metals. Anal. Chem. 51, 844–851.

Tigini, V., Franchino, M., Bona, F., Varese, G.C., 2016. Is digestate safe? A study on its ecotoxicity and environmental risk on a pig manure. Sci. Total Environ. 551–552, 127–132.

Tyagi, R.D., Sreekrishnan, T.R., Campbell, P.G.C., Blais, J.F., 1993. Kinetics of heavy metal bioleaching from sewage sludge—II. Mathematical model. Water Res. 27, 1653–1661.

Wang, L., Chen, G., Owens, G., Zhang, J., 2016. Enhanced antibiotic removal by the addition of bamboo charcoal during pig manure composting. RSC Adv. 6, 27575–27583.

Wei, X.C., Liu, D.F., Li, W.J., Liao, L.R., Wang, Z.D., Huang, W.W., Huang, W.L., 2018. Biochar addition for accelerating bioleaching of heavy metals from swine manure and reserving the nutrients. Sci. Total Environ. 631–632, 1553–1559.

Yang, Z., Zhang, Z., Chai, L., Wang, Y., Liu, Y., Xiao, R., 2016. Bioleaching remediation of heavy metal-contaminated soils using *Burkholderia* sp. Z-90. J. Hazard. Mater. 301, 145–152.

Zeng, J., Gou, M., Tang, Y.Q., Li, G.Y., Sun, Z.Y., Kida, K., 2016. Effective bioleaching of chromium in tannery sludge with an enriched sulfur-oxidizing bacterial community. Bioresour. Technol. 218, 859–866.

 https://doi.org/10.1016/j.heliyon.2018.e00778

Zhang, B., Xiong, S., Xiao, B., Yu, D., Jia, X., 2011. Mechanism of wet sewage sludge pyrolysis in a tubular furnace. Int. J. Hydrogen Energy 36, 355–363.

Zhang, P., Yi, Z., Zhang, G., Zou, S., Zeng, G., Zhen, W., 2008. Sewage sludge bioleaching by indigenous sulfur-oxidizing bacteria: effects of ratio of substrate dosage to solid content. Bioresour. Technol. 100, 1394–1398.

Zhou, J., Zhou, L., Liu, F., Zheng, C., Deng, W., 2012. Transformation of heavy metals and the formation of secondary iron minerals during pig manure bioleaching by the co-inoculation acidophilic thiobacillus. Environ. Technol. 33, 2553–2560.

Zhou, S.G., Zhou, L.X., Wang, S.M., Fang, D., 2006. Removal of Cr from tannery sludge by bioleaching method. J. Environ. Sci. 18, 885–890.

https://doi.org/10.1016/j.heliyon.2018.e00778