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Article

Effects of Solid Content and Substrate Concentration on Bioleaching of Heavy Metals from Sewage Sludge Using *Aspergillus niger*

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Abstract: The presence of heavy metals in sewage sludge not only affects the performance of sludge anaerobic digestion process but also restricts the land application of treated sewage sludge. Therefore, a fungi-mediated bioleaching process for simultaneous metal leaching and sludge digestion by *Aspergillus niger* was developed to treat the sewage sludge containing heavy metals in this study. The effects of two important parameters, sludge solid content and substrate (sucrose) concentration, on the performance of fungal bioleaching were investigated in this study. The results showed that the rate of pH reduction increased with increasing sludge solid contents and sucrose concentrations. In this study, the efficiency of metal removal decreases in the order of Mn > Zn > Ni > Pb. The efficiencies of metal leaching and solid degradation (SS and VSS) were found to be decreased with an increase of sludge solid content and a decrease of sucrose concentration. At 2 days of reaction time, the maximum efficiency of metal solubilization was 95, 56, 21 and 13% for Mn, Zn, Ni and Pb, respectively.

Keywords: *Aspergillus niger*; bioleaching; heavy metal; sludge digestion; solid content; sucrose

1. Introduction

Due to running out of landfill space, landfill disposal of huge quantity of wasted sludge generated from sewage treatment plants are becoming more limited in Taiwan. In addition, sludge incineration causes the emissions of harmful air pollutants and is no longer acceptable in most countries. The management of wasted sewage sludge will be a major environmental challenge faced by Taiwan in the foreseeable future. Land application of wasted sewage sludge is being considered as a cost-effective alternative of sludge disposal because large amounts of nutrients for plant growth are contained in the wasted sewage sludge. However, the presence of heavy metals is often one of the major obstacles to use wasted sewage sludge as a soil conditioner [1]. Generally, several physico-chemical methods are commonly used to remove heavy metals from wasted sewage sludge, such as chemical extraction, supercritical fluid extraction, electrokinetic, electrodialysis and ultrasonication [2–6]. However, due to high chemical or energy consumption, high operation cost and low efficiency, the application of these physico-chemical methods are still restricted [7]. Therefore, it is crucial to develop an effective method for removing heavy metals from sewage sludge prior to its land application. To this end, the bioleaching process has been introduced as a promising, cost-effective and environmentally friendly approach for removing heavy metals from sludge, soil and sediment [8–12].

The bioleaching process has been defined as the solubilization of metals from solids either directly by the metabolism of leaching microorganisms or indirectly by the products of metabolism [13]. In the bioleaching process, the removal of heavy metals from metal containing sludge, soil or sediment is performed by various groups of microorganisms. Commonly, chemolith-autotrophic bacteria, including *Acidithiobacilus* and *Thiobacillus* species, are of great importance and are used for the bioleaching process [14]. Additionally, heterotrophic fungi like *Aspergillus* and *Penicillium* species have been indicated to play key roles in the bioleaching process due to their high ability to excrete organic acids for metal leaching [15–18]. Generally, the conventional bioleaching process with either sulfur-oxidizing bacteria or iron-oxidizing bacteria was not suitable for the solid particles containing high proportion of nonsulfidic minerals (carbonates, oxides and silicates). However, the heavy metals from carbonates, oxides and silicates could be chelated by the organic acids produced in the fungal bioleaching process [19]. Besides, the presence of a high concentration of organic matters or organic acids was found to be inhibitory to the growth of *Acidithiobacillus ferrooxidans* and subsequent metal solubilization from the sludge in the bioleaching process [20]. Compared with bacterial bioleaching, the fungal bioleaching usually has a faster leaching rate because fungi are able to grow at high pH and to tolerate toxic materials and have a shorter lag phase as well [21]. Due to the above advantages over bacterial bioleaching, the fungal bioleaching shows great potential to be an alternative method for the removal of heavy metals from sewage sludge.

The main mechanisms of metal solubilization involving in the fungal bioleaching process include: (i) acidolysis; (ii) complexolysis; (iii) redoxolysis and (iv) bioaccumulation [21]. The excretion of organic acids by genus *Aspergillus*, such as *Aspergillus niger*, firstly causes the acidolysis and subsequent metal solubilization in the fungal bioleaching process. The large amounts of organic acids (critic acid, oxalic acid, malic acid and gluconic acid) produced by *Aspergillus niger* also have strong abilities to chelate metal from solid particles and then keep them in solution at higher pH values, at which the metals would otherwise precipitate [22]. Meanwhile, the excreted organic acids by fungi are able to initiate the redoxolysis reaction that can cause the solubilization of metals from solid particles during the fungal bioleaching process [17]. On the other hand, when the bioleaching process was applied for treating waste sewage sludge, organic matters were also degraded simultaneously by the inoculated and indigenous microorganisms in the sludge besides the metal solubilization. The simultaneous metal leaching and sludge digestion have been demonstrated in the previous studies [8,22–24]. The related reactions between the different organic acids and metal ions are listed as below [25]:

$$n[C_6H_{11}O_7^-] + M^{n+} \rightarrow M[C_6H_{11}O_7]_n \text{ (Gluconic metallic complex)} \quad (1)$$

$$n[C_2HO_4^-] + M^{n+} \rightarrow M[C_2HO_4]_n \text{ (Oxalic metallic complex)} \quad (2)$$

$$n[C_6H_7O_7^-] + M^{n+} \rightarrow M[C_6H_7O_7]_n \text{ (Critic metallic complex)} \quad (3)$$

$$n[C_6H_6O_7^{2-}] + 2M^{n+} \rightarrow M_2[C_6H_6O_7]_n \text{ (Critic metallic complex)} \quad (4)$$

$$n[C_6H_5O_7^{3-}] + 3M^{n+} \rightarrow M_3[C_6H_5O_7]_n \text{ (Critic metallic complex)} \quad (5)$$

where M^{n+} represents the metal ions with certain valence. The performance of fungal bioleaching is commonly affected by various parameters, such as solid content, substrate concentration, fungus species, initial pH, inoculum size, temperature and particle size [18,19,26–29]. A thorough understanding of these parameters is very important for scale-up, reactor design and practical application of bioleaching technology. Usually, the solid content (loading) is the key parameter in the bioleaching process for evaluating bioreactor size and reaction time. Although an increase in solid content led to an increase in solid loading rate, a decrease in metal removal efficiency and a long reaction time were obtained in the bioleaching process. On the contrary, the high metal removal efficiency was reached at a low solid content but a large size of bioreactor was required for the bioleaching process [24]. Also, it was demonstrated that the profitability was highly related to the solid loading which significantly affecting the size (cost) of batch bioreactor during the techno-economic analysis for scale-up of bioleaching process [30]. Deng et al. [15] investigated the bioleaching of heavy metals in the mixture of contaminated soil and slag by using *Penicillium chrysogenum*. It was observed that higher removal percentages of heavy metals were obtained in the fungal bioleaching experiment with lower solid content; however, increased solid content resulted in declined heavy metal removal and increased reaction time. This observation was also found in the studies of Urik et al. [16], Mirazimi et al. [19] and Amin et al. [27]. On the other hand, Gharehbagheri et al. [26] revealed that the production of organic acids and the efficiency of metal leaching increased with increasing sucrose (substrate) concentration in the bioleaching of uranium ores by *A. niger*. Ghazala et al. [31] stated that increase in citric acid production and metal leaching efficiency was observed when the sucrose concentration increased from 15 g/L to 60 g/L. Due to the over growth of the mycelium which resulted in high viscosity of the medium, citric acid production and metal leaching efficiency decreased at sucrose concentration higher than 70 g/L. Therefore, it is important to determine the optimum conditions for microorganism growth and metal solubilization during the fungal bioleaching. The purposes of this study were to investigate the effects of two important parameters, sludge solid content and substrate (sucrose) concentration, on the efficiency of metal solubilization and solid degradation in the fungal bioleaching process.

2. Materials and Methods

2.1. Sludge Sampling

The wasted activated sludge (WAS) was collected from a municipal wastewater treatment plant located in the City of Tainan, Taiwan. The sludge was screened through a 20-mesh (0.84 mm) sieve to remove the debris, desiccated to reduce the moisture content and then was stored at 4 °C before the experiment. The total solids (TS) of WAS was determined using Standard Methods [32] and the pH value was measured by way of the procedures recommended by USEPA (U.S. Environmental Protection Agency, Washington, DC, USA) [33]. The heavy metal contents of WAS were determined by HNO_3-HCl microwave-assisted acid digestion [34]. The WAS contained 2.2% TS with an average pH of 6.9. The total metal content (on a dry weight base) in the WAS was 3430, 2020, 100 and 260 mg/kg for Mn, Zn, Ni and Pb, respectively.

2.2. Fungal Strains and Inoculum Preparation

The strain of fungus used in this study was *Aspergillus niger* (BCRC 32073) obtained from the Bioresource Collection and Research Center (BCRC), Hsinchu, Taiwan. The strain was cultured with a liquid medium composed of 20 g/L malt extract and was incubated in a rotary shaker at 150 rpm and 25 °C for 7 days. Prior to bioleaching experiments, the fungal strain was acclimated to WAS for a prolonged adaption period. In the acclimation period, 25 mL of spore suspension and 2.5 g (dry weight) of WAS were added to a 1000 mL plastic Erlenmeyer flask containing 250 mL of sucrose medium. The medium composed of sucrose (100 g/L), $NaNO_3$ (1.5 g/L), KH_2PO_4 (0.5 g/L), $MgSO_4{\cdot}7H_2O$ (0.025 g/L), KCl (0.025 g/L) and yeast extract (1.6 g/L). The flask was agitated in a shaking incubator at 150 rpm and 25 °C. In this study, the sucrose was added as the sole energy source for growth of *A. niger*. The sucrose was then metabolized to form various organic acids (oxalic acid, citric acid and gluconic acid), resulting in the decrease of pH in the acclimation experiments. The production of organic acids positively correlated with the growth of *A. niger*. Therefore, the growth of *A. niger* was monitored by measuring the sludge pH during the acclimation [14,19]. The acclimation procedure was completed when the sludge pH dropped to approximately 2.5. Afterwards, 25 mL of the pre-acclimated sludge solution and 2.5 g (dry weight) of WAS were transferred to another flask containing 250 mL of fresh sucrose medium. The aforementioned acclimation procedure was repeated at least three times. The acclimated sludge solution was then used as an inoculum for the subsequent fungal bioleaching experiments.

2.3. Fungal Bioleaching Experiments

The fungal bioleaching experiments were conducted in 1000 mL plastic Erlenmeyer flasks containing 400 mL sucrose media (10–100 g sucrose/L) and 40 mL of inoculum. Then different amount of concentrated sludge was added into the flasks with media to obtain the sludge solid content of 1–10% (*w*/*v*). The flasks were incubated for 14 days in a shaking incubator at 150 rpm and 25 °C. Table 1 listed the experimental conditions to examine the effects of sludge solid content and substrate concentration on the performance of fungal bioleaching process. During the bioleaching experiments, the pH of sludge was monitored daily and sludge samples were daily taken from the flask for chemical analyses. The suspended solids (SS) and volatile suspended solids (VSS) of the sludge samples were measured according to the Standard Methods [33]. The biomass concentration in the sludge sample was also determined based on the Bradford method of determining protein content [35]. In addition, the sludge samples were centrifuged and filtered to determine the amounts of soluble organic acids (oxalic acid, citric acid and gluconic acid) and heavy metals (Mn, Zn, Ni and Pb). The concentration of soluble heavy metals was measured with an atomic absorption spectrophotometer (Hitachi ZA2000, Tokyo, Japan). The analysis of oxalic acid, citric acid and gluconic acid was carried out by high performance liquid chromatography (HPLC) (Shimazu LC20AT, Kyoto, Japan) equipped with a TSKgel C18 column (ODS-100V, 4.6 mm × 250 mm, Sigma–Aldrich, Inc., Darmstadt, Germany), a TSKgel cation-exchange resin column (SCX(H+), 7.8 mm × 300 mm, Sigma-Aldrich, Inc., Darmstadt, Germany) and a variable wavelength detector (VWD) at 210 nm. The injection volume was 20 µL and all samples and standards were injected in triplicate. The mobile phase used for analysis of oxalic acid and citric acid was of 0.1% phosphoric acid at a flowrate of 0.5 mL/min. The mobile phase for gluconic acid analysis was composed of 15% acetonitrile and 85% phosphoric acid (2 mM) at a flowrate of 0.5 mL/min.

Table 1. Design of experimental conditions in this study.

Affecting Parameter	Sludge Solid Content (%)	Sucrose Concentration (g/L)
Sludge Solid Content	1	55
	5.5 [1]	55
	10	55
Sucrose Concentration	5.5	10
	5.5	55
	5.5	100

[1] Triplicate.

3. Results and Discussion

3.1. Variation of pH

The variations of pH during the fungal bioleaching experiments are shown in Figure 1. In general, the pH mainly decreased with time due to the production of organic acids by *Aspergillus niger* [18,19]. As revealed in Figure 1a, after 2 days of reaction time, the pH decreased drastically to the value lower than 5.0 for solid contents of 1, 5.5 and 10% (*w*/*v*). After the complete utilization of sucrose, the organic acid was not accumulated and then the pH increased with the decreasing solid content. However, the sewage sludge contained abundant hydrocarbons and nutrients [36,37] which were favorable for the organic acid production by *Aspergillus niger*. Subsequently, the higher sludge solid content the more organic acids produced after 2 days of bioleaching. The pH values escalated to 7.8, 7.6 and 7.5 for solid contents of 1, 5.5 and 10% (*w*/*v*), respectively, after 14 days of reaction time.

Figure 1b shows the variation of pH during the fungal bioleaching experiment with different sucrose concentrations. Because the sucrose concentration of 10 g/L was insufficient for *Aspergillus niger* to produce organic acids for decreasing the pH of sludge, it was observed that the pH remained between 7.2 and 7.7 during the fungal bioleaching experiment. In addition, the pH decreased to approximately 4.5 at sucrose concentrations of 55 and 100 g/L after 2 days of reaction. Similarly, due to complete utilization of sucrose, the pH increased on day 3 and 9 at sucrose concentration of 55 g/L and 100 g/L, respectively. After 14 days, the final pH values were 7.2, 7.4 and 7.5 at sucrose concentration of 10, 55 and 100 g/L, respectively.

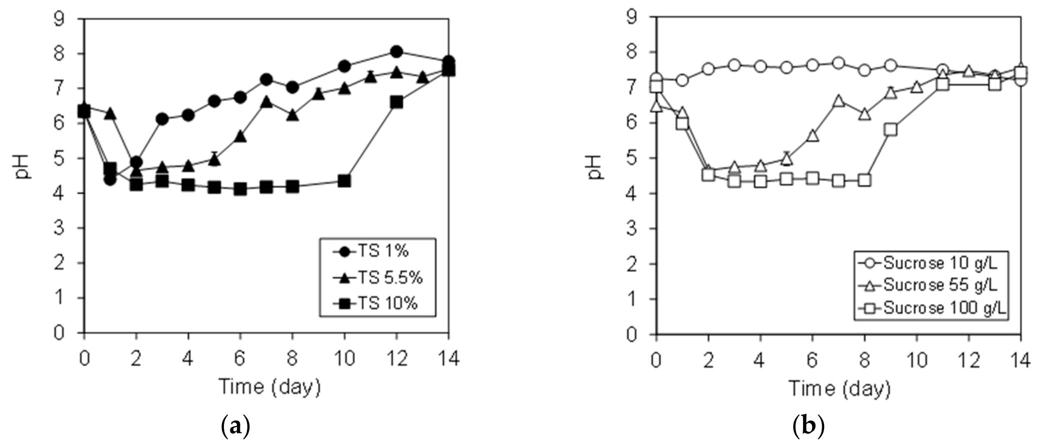

Figure 1. Variations of pH during the fungal bioleaching experiments (**a**) effects of sludge solid content, (**b**) effects of sucrose concentration.

3.2. Variation of Organic Acids

Figure 2a–c indicate the variation of organic acids during the fungal bioleaching experiments with different sludge solid contents. Das et al. [38] revealed that due to the activity of invertase enzyme, complete hydrolysis of sucrose added in the fungal bioleaching experiment was observed after 1 day of the incubation period. Also, the rate of sugar consumption was found to be higher after 2 days of incubation period, which indicated that, *Aspergillus* strains were in active growth phase after 2 days. Therefore, the organic acids produced with the consumption of sucrose during the fungal bioleaching experiment. It was found that the concentration of oxalic acid increased with time and the final oxalic concentration increased with increasing sludge solid content (Figure 2a).

As shown in Figure 2b, the concentration of citric acid drastically increased to the highest values in 2 days. It was observed that the level of citric acid reduced to zero after 14 days of operation time. The explanation could be that the citric acid either completely transformed to other byproducts or chelated with heavy metals [39]. The similar results were also found in the variations of gluconic acid during the fungal bioleaching process (Figure 2c). Because the pH value depends on the variations of organic acids, the increase in organic acids leads to a decrease in pH value. The pH gradually increased with the decrease in organic acids after the second day of the fungal bioleaching experiment (Figure 1). During the fungal bioleaching process, the concentration and kind of secreted organic acids by *Aspergillus niger* are significantly affected by the pH in the culture medium [40]. Generally, because of the induction of enzyme oxaloacetate hydrolase by biosynthesis, the pH greater than 7 is preferable for production of oxalic acid [41]. Therefore, the concentration of oxalic acid produced in the fungal bioleaching experiment of this study was higher than those of citric and gluconic acids (Figure 2). On the other hand, the low pH of the culture medium is a pivotal parameter for the production of citric acid by *Aspergillus niger* [42]. Due to the increase of pH, the critic acid decreased after 2 days of bioleaching time. The level of citric acid was the lowest compared to other two types of organic acids produced during the fungal bioleaching experiment. These results are in agreement with those reported in the study by Horeh et al. [17]. Figure 2d–f show the variation of organic acids during the fungal bioleaching experiment with different sucrose concentrations. The trends of organic acid production were similar to those in Figure 2a–c. The concentrations of organic acid increased as the sucrose concentration increased in the fungal bioleaching experiment. Amiri et al. [43] reported that the decrease in the fungus growth rate caused the consumption of organic acids in the late stage of the bioleaching experiment, resulting in a less organic acid production (Figure 2).

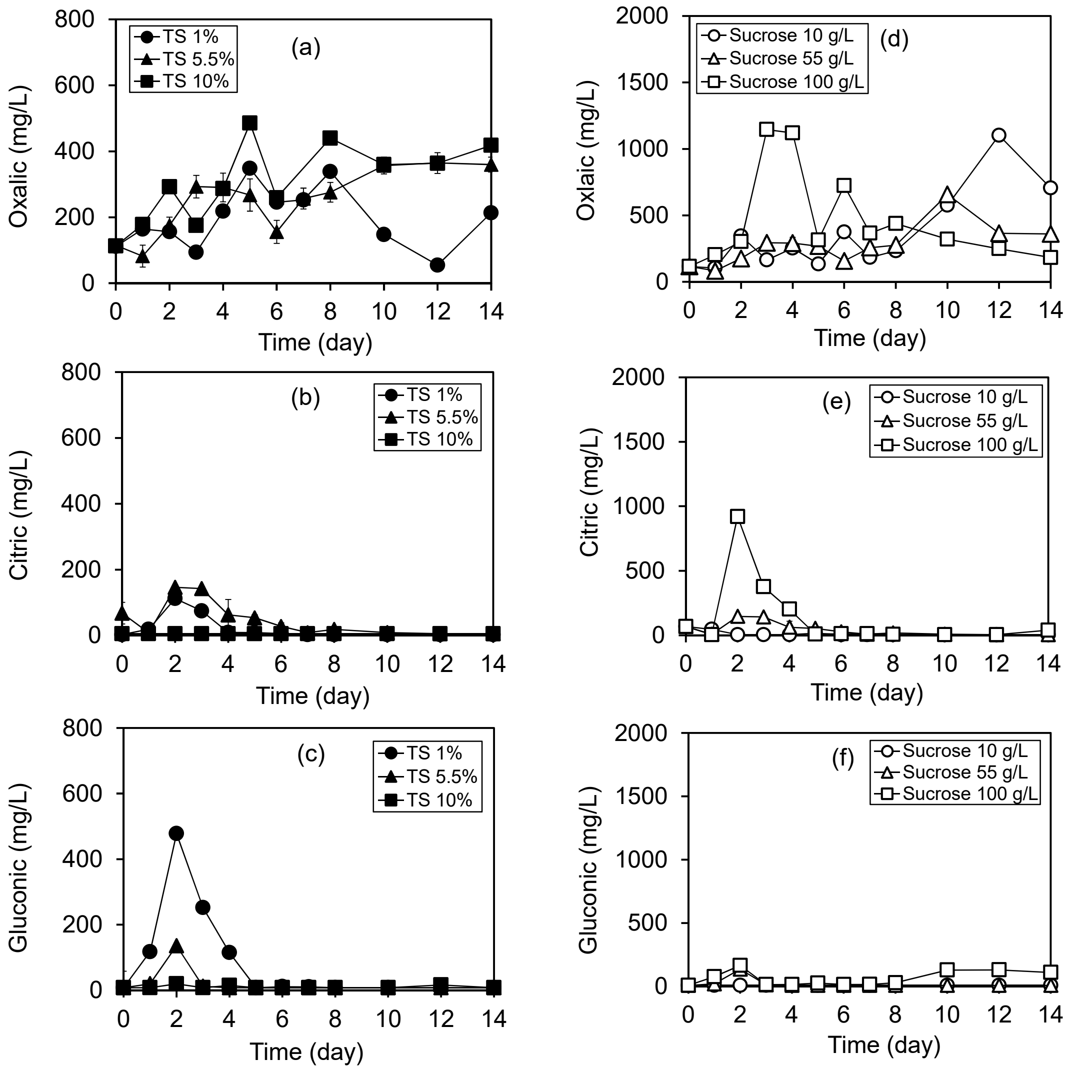

Figure 2. Production of organic acids during the fungal bioleaching experiments (**a**–**c**) effects of sludge solid content, (**d**–**f**) effects of sucrose concentration.

3.3. Removal of Heavy Metals

Figure 3 illustrates the effect of solid content on the removal of heavy metals from sewage sludge. It is well known that the removal efficiency of heavy metal correlated with the value of pH during the bioleaching process [44]. Due to the lowest solid content and the lowest pH value observed at the first day in the fungal bioleaching experiment with solid content of 1%, it was found that the removal of heavy metal increased to the high efficiency of 96, 95, 21 and 29% for Mn, Zn, Ni and Pb, respectively. For the solid contents of 5.5 and 10%, the efficiency of metal removal attained to the highest value during the first 2–3 days of the bioleaching experiment. Subsequently, the efficiency of metal removal decreased gradually in the end of the bioleaching experiment. It was evident that the efficiency of metal removal negatively correlated with the pH in the fungal bioleaching experiment (Figure 1). Meanwhile, it was found that the increase of sludge solid content resulted in the decrease of the metal removal efficiency. Overall, the efficiency of metal removal from sewage sludge in the first 7 days of fungal bioleaching experiment was obviously in the order of Mn > Zn > Ni > Pb. It is noticed that the increase in the efficiency of metal removal was related to the increase of the organic acid concentrations, indicating that the biogenically produced organic acids played a direct and important role in the fungal bioleaching process. Therefore, the depletion of bioleaching agents could be one reason in decreasing the efficiency of metal removal [43].

Figure 4 shows the effect of sucrose concentrations on the removal of heavy metals during the fungal bioleaching experiment. As indicated in Figure 4, the maximum removal efficiencies were 75, 48 and 24% for Mn, Zn and Ni, respectively, after 8 days of bioleaching at sucrose concentration of 100 g/L. Then a decrease trend was also observed in the efficiency of metal removal in the end of bioleaching experiment. This may be attributed by the same reasons as discussed previously. However, the decreased sucrose concentration apparently resulted in the decline of the metal removal efficiency. It was found that heavy metals were not removed from the sludge at sucrose concentration of 10 g/L, indicating that the fungi did not grow well during the bioleaching experiment at low sucrose concentration. It was evident that higher metal removal efficiency was observed with an increasing sucrose concentration. Del Mundo Dacera and Babel [45] investigated Zn and Ni leaching from contaminated sewage sludge by *A. niger* fermented raw liquid (containing 5340 mg/L of citric acid) from pineapple wastes. It was observed that after 11 days of reaction time, the highest leaching efficiencies of Zn and Ni were 95% and 94%, respectively, at solid content of 5% and initial pH of 3.73. Also, Xu and Feng [46] examined the feasibility of sewage sludge bioleached by *A. niger* at 1% of solid content and 400 g/L of sucrose concentration for 12 days, which mentioned that the highest removal percentages Zn and Pb after 12 days were 88% and 82%, respectively. Due to higher sucrose (or organic acid) concentration added in the fungal bioleaching experiments, the metal solubilization efficiencies obtained from above studies were higher than those from this study. Table 2 shows the matrix of correlation coefficients between metal removal efficiency and organic acids produced in the first 7 days of fungal bioleaching experiments. It was found that pH value negatively correlated with metal (Mn and Zn) removal efficiency in statistical significance ($p < 0.05$). Since organic acids played a significant role in the fungal bioleaching process, the oxalic acid, gluconic acid and total organic acids highly correlated with metal removal efficiency.

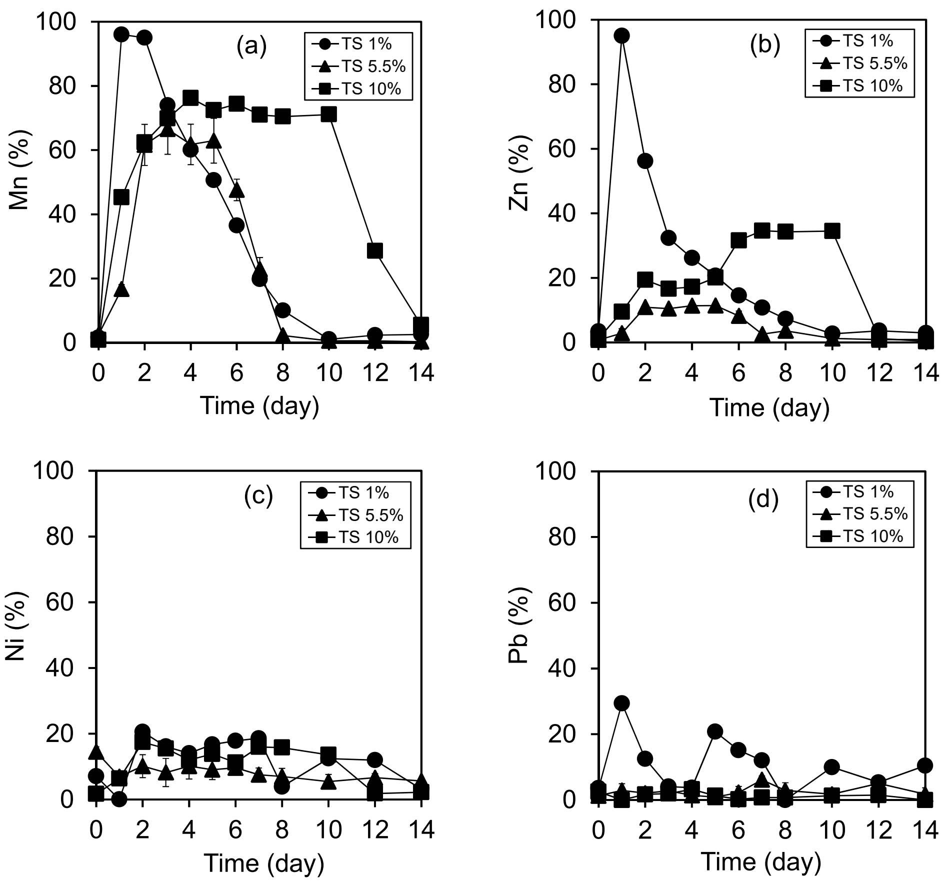

Figure 3. Effects of sludge solid content on the metal solubilization during the fungal bioleaching experiments (**a**) Mn, (**b**) Zn, (**c**) Ni and (**d**) Pb.

The significant effects of the lower solid content and higher sucrose concentration on metal removal in the fungal bioleaching process were in agreement with previous studies. Urik et al. [16] reported that the solid content of 10 g/L (1%) was optimal for aluminum removal in the bioleaching by *Aspergillus niger*. However, at higher solid content, the metal removal efficiency were inhibited by the organic acids produced by fungi due to high concentration of solubilized metal in the bioleaching experiment. For the influence of high substrate concentration, Gharehbagheri et al. [26] found that the maximum efficiency of vanadium removal from uranium ore residue was at 100 g/L of sucrose. Generally, higher sucrose (substrate) concentration was favorable to the production of organic acids and removal of heavy metals in the fungal bioleaching experiment. However, because sucrose is a food industry product and expensive, the use of inexpensive or waste carbon sources as the alternative substrate would be a great advantage to the real application and cost reduction of fungal bioleaching process. Several food and agricultural wastes, such as molasses, potato peels and straw infusion, were evaluated as potential alternatives of sucrose for the fungal bioleaching process [28,47]. A further research about waste carbon sources used as the alternative substrate for fungal bioleaching of heavy metals from sewage sludge will be carried out in the future. Moreover, Bayat and Sari [48] and Qu et al. [49] indicated that the bioleaching process achieves a higher removal efficiency of heavy metals from waste sludge than the chemical leaching method, though chemical leaching has the shortest process time. Considering the enormous amount of wasted sludge and subsequent high cost of chemicals, the bioleaching process was suggested to be an alternative or adjunct to conventional physico-chemical techniques for removing heavy metals from wasted sludge.

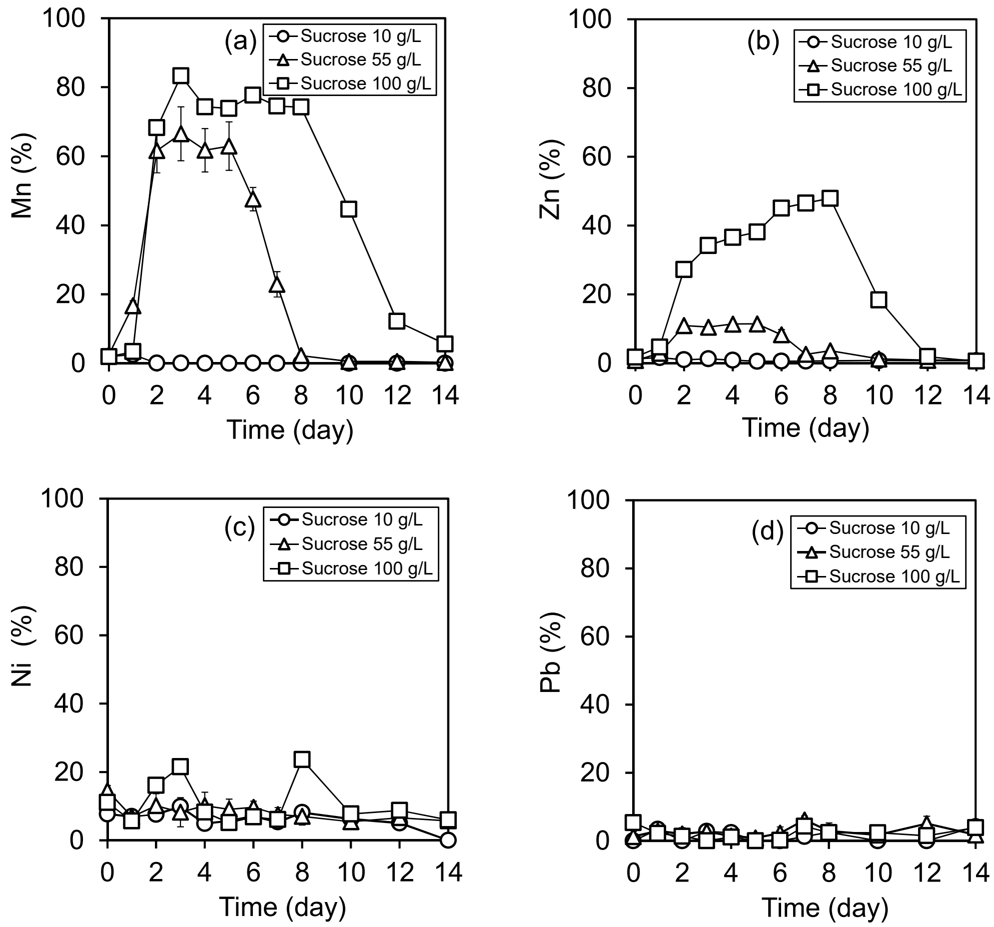

Figure 4. Effects of sucrose concentration on the metal solubilization during the fungal bioleaching experiments (**a**) Mn, (**b**) Zn, (**c**) Ni and (**d**) Pb.

Table 2. Correlation coefficients between metal removal efficiency and organic acids.

Factor	pH	Oxalic Acid	Critic Acid	Gluconic Acid	Organic Acids
Organic Acids	−0.45 [1]	0.80 [1]	0.76 [1]	0.28	-
Mn	−0.87 [1]	0.40 [1]	0.26	0.41 [1]	0.52 [1]
Zn	−0.59 [1]	0.31	0.17	0.50 [1]	0.43 [1]
Ni	−0.23	0.22	0.34	0.37 [1]	0.41 [1]
Pb	0.06	−0.16	−0.11	0.31	−0.09

[1] Correlation is significant at the 0.05 level (2−tailed).

3.4. Variation of Biomass Concentration

Figure 5 shows the variations of biomass concentration during the fungal bioleaching experiment. As shown in Figure 5a, the biomass concentration initially increased to the maximum of 1700, 2260 and 3630 mg protein/L for solid contents of 1, 5.5 and 10% (*w*/*v*), respectively, in the first 2 days of bioleaching. This elucidates that fungi had entered the active growth period. The same results were also reported by Amiri et al. [43] and Aung and Ting [50]. Thereafter, the biomass concentration decreased gradually after the second day of bioleaching, which is because of the inhibition of secreted primary and secondary metabolites for fungi growth [17,42]. The biomass concentration also increased to the maximum in the first two days and subsequently decreased with time for sucrose concentrations of 55 and 100 g/L (Figure 5b). It was evident that fungi did not grow at sucrose concentration of 10 g/L. It implies that fungi ceased to grow in the bioleaching process at the low sucrose concentration. Meanwhile, the biomass increased when the sucrose (substrate) concentration was increased in the fungal bioleaching experiment. A similar finding has also been observed by Carstro et al. [51].

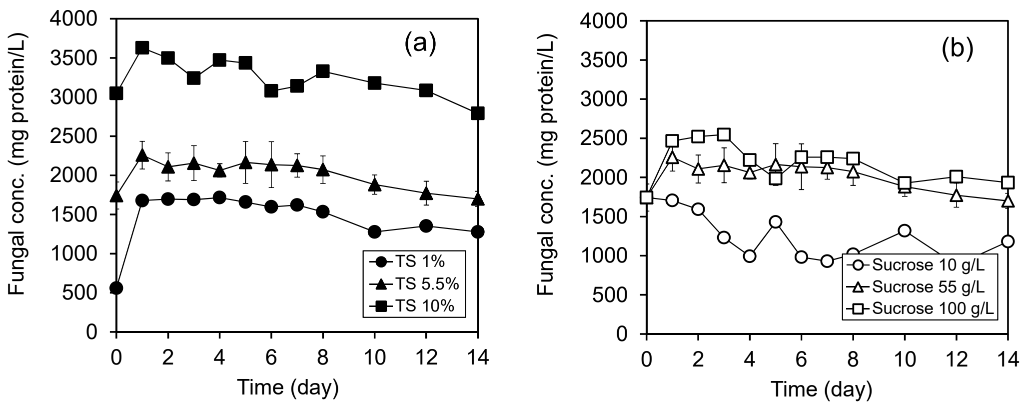

Figure 5. Variations of biomass concentration during the fungal bioleaching experiments (**a**) effects of sludge solid content, (**b**) effects of sucrose concentration.

3.5. Degradation of Solids

Figure 6a,b show the solid degradation during the fungal bioleaching experiments with different sludge solid content. Previous studies found that the degradation of the sludge occurs simultaneously with the metal leaching in the bioleaching process inoculated with the acclimated indigenous microorganisms [8,24]. After 14 days of bioleaching, it was found that 0, 20 and 32% of SS were destructed at solid contents of 1, 5.5 and 10% (*w*/*v*), respectively. Meanwhile, 0, 24 and 33% of VSS were degraded at solid contents of 1, 5.5 and 10% (*w*/*v*), respectively. It was found that the efficiency of solid degradation increased with the increasing sludge solid content. Apparently, because the yield of biomass was more than the reduction of the sludge, no decrease of SS and VSS was found at the solid content of 1% (*w*/*v*). Figure 6c,d illustrate the solid degradation during the fungal bioleaching experiments with different sucrose concentrations. It was found that either 4 to 20% of SS or 3 to 23% of VSS were degraded in the fungal bioleaching experimental experiments at sucrose concentration of 10 to 100 g/L after 14 days of reaction time. When increased the sucrose concentration to 100 g/L, the efficiency of solid degradation drastically decreased. Similarly, the explanation was that more biomass was produced than the degradation of the sludge at 100 g/L of sucrose concentration.

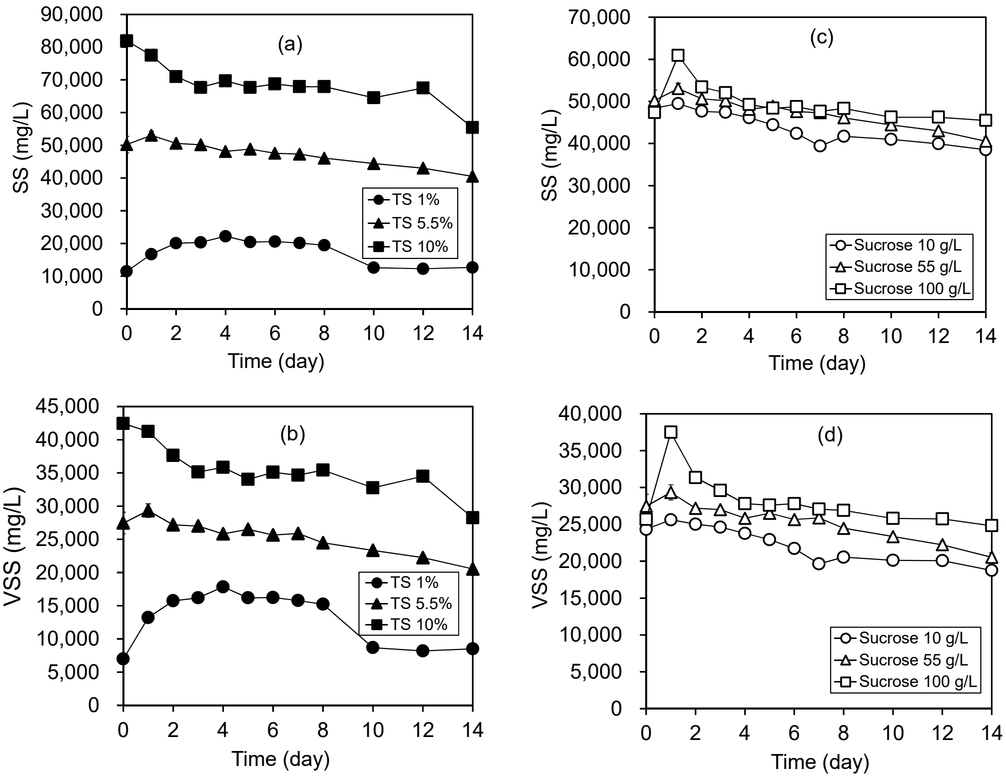

Figure 6. Degradation of solids during the fungal bioleaching experiments (**a**,**b**) effects of sludge solid content, (**c**,**d**) effects of sucrose concentration.

4. Conclusions

The fungal bioleaching process developed in this study could simultaneously achieve metal removal and solids degradation of sewage sludge. As the sludge solid content and sucrose concentration increased, the rate of pH decline increased. Both metal leaching efficiency and solid degradation tended to decrease with sludge solid content. However, the increase of sucrose concentration let to the increase in efficiencies of metal leaching and solid degradation. In the fungal bioleaching process, removal efficiency of Mn was found to be the highest whereas Pb was the lowest. At 2 days of reaction time, the maximum efficiency of metal removal from the sludge was 95, 56, 21 and 13% for Mn, Zn, Ni and Pb, respectively.

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Conflicts of Interest: The authors declare no conflict of interest.

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