Influence of different iron sources on Sb(III) removal from water by active iron-oxidizing bacteria and its mechanism

Yongchao Li a,b,*, Jialing Liu a, Zhonggeng Mo b and Liyuan Li a

a Key Laboratory of Recycling and Eco-treatment of Waste Biomass of Zhejiang Province, Zhejiang University of Science and Technology, Hangzhou 310023, P.R. China

b School of Civil Engineering, Hunan University of Science and Technology, Xiangtan 411201, P.R. China

*Corresponding author. E-mail: nkliyongchao@163.com

YL, 0000-0001-8973-3013; JL, 0000-0002-0605-7097; LL, 0000-0002-2496-7326

ABSTRACT

Iron-oxidizing bacteria played an important role in the treatment of Sb-containing wastewater. In this study, effect of different iron sources on Sb(III) removal ability by isolated iron-oxidizing bacteria (named as IOB-L) was conducted systemically in batch experiment. Moreover, ferrous lactate and zero-valent iron were chosen as iron sources for IOB-L. The results showed that after inoculation of 2% volume of IOB-L, Sb(III) concentration in water decreased from initial 18 mg/L to 4.1 mg/L at optimal pH of 7.0. There was no reaction between Sb(III) and ferrous lactate, whereas corrosion product of iron can adsorb certain amount of Sb. When active IOB-L cultivated in ferrous lactate, a better removal rate of Sb(III) can be reached with a longer stagnate phase for bacteria. However, Sb(III) removal ability of IOB-L using zero-valent iron as iron source was lower. SEM-EDS, FTIR, and XPS analysis further indicated that ferrous lactate was oxidized by IOB-L and precipitated as biogenic iron oxides which had strong adsorption ability towards Sb(III), whereas zero-valent iron was not a good iron source.

Key words: ferrous lactate, influence mechanism, iron-oxidizing bacteria, Sb(III) removal, zero-valent iron

HIGHLIGHTS

- IOB-L biomass had a little adsorption ability towards Sb(III).
- Biogenic iron oxides was generated and played an important role in Sb removal from water.
- Although ferrous lactate could be used as iron source for IOB-L, a longer adaptation period was necessary.
- Ferrous ions from zero-valent iron corrosion was hard to be oxidized directly by IOB-L.
- Oxidation of Sb(III) by biogenic iron oxides was not observed.
Antimony (Sb) is classed as metalloids or semimetals as arsenic. Now, Sb and its compounds have been widely used in catalyst, flame retardant, and semi-conductor materials industries (Rakshit et al. 2011). Due to its high toxicity, Sb is included as priority pollutant by United States Environmental Protection Agency. In natural water, Sb mainly exists in the form of Sb(III) and Sb(V). National drinking water standard of China also required that the maximum contaminant level of total Sb should be lower than 5 μg/L (He et al. 2012). However, due to improper human activity Sb was released to various water bodies, especially the ones that near to mining and smelter areas. It was reported that the dissolved Sb in a stream flowing through tailing piles in Sb mine area was in the range of 4.58–29.4 mg/L (Zhu et al. 2009). Therefore, treatment of Sb-containing wastewater was important for the water environment.

Compared with coagulation, ion exchange, and electrochemical technique, adsorption method is commonly used for Sb removal from wastewater due to its cheap price and easy realization, many different kinds of absorbents have been developed (Saleh et al. 2017; Sharma et al. 2021). Recently, it was widely demonstrated that Sb adsorption capability of chemosynthetic iron-based oxides was better than that of many other adsorbents, on account of the large specific surface area, electrostatic attraction, and complexation (Qi & Pichler 2016). While, due to highly diverse and abundant iron-oxidizing bacteria (IOB) which can oxidize Fe(II) ions to Fe(III) and form iron hydroxides extracellularly, biogenic iron oxides minerals commonly existed in natural systems (Williams et al. 2016). For example, a live Pseudogulbenkiania sp. strain 2002 could oxidize 3 mM of Fe(II), and the generated ferrihydrite-goethite efficiently immobilized arsenic (As) from groundwater (Xiu et al. 2019). It was also found that biogenic iron (oxyhydr)oxides were more effective at adsorbing Cu, Pb, and Zn from water than their synthetic counterparts (Whitaker & Duckworth 2018).

A strain of heterotrophic iron-oxidizing bacteria named as IOB-L was isolated and identified in our laboratory (Li et al. 2020). It can be assigned to genus Klebsiella sp. and used for Sb(V) removal from wastewater. Batch experiment demonstrated that 62% of 20 mg/L Sb(V) was significantly removed by Klebsiella sp. in presence of zero-valent iron after reaction for 168 hours. Characterization analysis of reaction products indicated that amorphous Fe₂O₃ and FeOOH were generated in biotic systems, which then had better adsorption ability towards Sb(V). Although thermodynamic equilibrium considerations indicated that Sb(III) mainly existed in anoxic conditions, significant amounts of Sb(III) was observed in oxic waters (Takayanag & Cossa 1997). Moreover, Sb(III) species was much more toxic than Sb(V) (Tschan et al. 2010). But, researches on Sb(III) removal ability of Klebsiella sp. are still limited.

More importantly, iron source was very important for the generation of biological iron oxides, and most of biogenic iron oxides minerals in lab experiments were formed by bio-oxidation of ferrous sulfate, ferric sulfate and ammonium ferric citrate...
(Pathak et al. 2009; Khan et al. 2010). Whereas, the effect of other iron-containing compounds on Sb(III) removal ability of iron-oxidizing bacteria was not well studied. Recently, a few studies found that aerobic corrosion of zero-valent iron can be influenced by iron-oxidizing bacteria, resulting in production of green rust (Liu et al. 2017; Li et al. 2020). Whether it can be used as iron source for active iron-oxidizing bacteria during Sb(III) removal was not clear. So, it was urgent to study the influence of different iron sources on Sb(III) removal by iron-oxidizing bacteria and its mechanism.

The main objectives of this study were to: (1) investigate Sb(III) removal performance of active IOB-L at different solution pH; (2) evaluate Sb(III) removal ability of added iron sources separately; (3) study the influence of organic iron compound and zero-valent iron on Sb(III) uptake ability of IOB-L; and (4) clarify the effect mechanism of Sb(III) removal by iron-oxidizing bacteria through the characterization of reaction product.

**METHODS**

**Chemicals and IOB-L culture**

The detailed isolation and identification process of obtained IOB-L strain was described in our previous research (Li et al. 2020). Sb(III) stock solution was obtained by dissolving antimony potassium tartrate (K(SbO)C4H4O6·½H2O) in deionized water. A medium with following composition was used for cultivation of IOB-L: ammonium ferric citrate (10 g/L), ammonium sulfate (0.5 g/L), magnesium sulfate heptahydrate (0.5 g/L), sodium nitrate (0.5 g/L), dipotassium phosphate (0.5 g/L), calcium chloride hexahydrate (0.5 g/L), calcium chloride hexahydrate (0.5 g/L).

**Sb(III) adsorption reaction of IOB-L biomass**

Before the adsorption experiment, IOB-L strain was cultured in a fluid nutrient medium which contained ammonium citrate instead of ammonium ferric citrate. 10 mL of IOB-L suspension was extracted after about 24 hours. In the adsorption experiments, IOB-L biomass was added to flasks containing 490 mL Sb(III) solution with initial pH of 4.0, 5.0, 6.0, 7.0, and 8.0, respectively. Then the flasks were put in rotary shaker at 30 °C. During the following time, the residue Sb content in solution was analyzed separately. Moreover, optical density of bacteria biomass was analyzed using ultraviolet–visible spectrophotometer (756MC, Jinghua Technologies Ltd, China) at 500 nm.

**Removal performance of Sb(III) from water by active IOB-L**

Removal of Sb(III) by active IOB-L was studied as follows. First, IOB-L strain was cultured for about 24 hours in fluid nutrient medium as described in Section Chemicals and culture of IOB-L. Then 10 mL of IOB suspension was added to 490 mL Sb(III) solution after its pH was adjusted to 4.0, 5.0, 6.0, 7.0, and 8.0, respectively. The conical flasks were then placed on a rotary shaker at 150 rev/min. After reaction for 1, 3, 6, 12, 24, 48, 72, 96, and 120 hours, 3 mL supernate was separately extracted, and filtered through 0.45 μm membrane filters. The residual Sb in water was monitored using a flame atomic absorption spectrophotometer (AA-7001, East & West Analytical Instruments, Inc., China).

**Reaction between individual iron sources and Sb(III) in water**

Cheap and available ferrous lactate and zero-valent iron (2 mm in diameter) were chosen as the alternative iron source for IOB-L. The experiments were carried out by adding certain amount of zero-valent iron and ferrous lactate to Sb(III) solution, respectively. The reaction process was same as described above and residual Sb in solution was analyzed. In addition, the influence of solution pH on Sb(III) removal by zero-valent iron and ferrous lactate was also investigated.

**Influence of different iron sources on Sb(III) uptake ability of IOB-L**

The effect of ferrous lactate on Sb(III) removal by IOB-L was conducted as follows. First, fluid nutrient medium containing ferrous lactate instead of ammonium ferric citrate were prepared for the cultivation of IOB-L strain. Initial ferrous lactate concentration was 2, 6, 10, and 16 g/L, respectively, and the other constituents in nutrient were same as that in Section Chemicals and IOB-L culture. After IOB-L was cultivated in nutrient which contained different amount of ferrous lactate for about 24 hours, 10 mL of IOB suspension was then added to conical flasks filled with 490 mL Sb(III) solution with initial pH of 7.0. As the reaction processed, residual Sb in the solution was monitored.

The effect of zero-valent iron on Sb(III) removal ability of IOB-L was conducted as follows. First, IOB-L was cultivated in fluid nutrient medium containing ammonium citrate instead of ammonium ferric citrate. Then 10 mL of IOB suspension in logarithmic growth phase was inoculated to 490 mL Sb(III) solution with initial pH of 7.0. Next, 0.03, 0.05, 0.09 and 0.15 g of
zero-valent iron was added to flasks separately, which were then placed on a rotary shaker at 150 rev/min. Dissolved Sb in water was monitored on a predetermined time interval as outlined above.

**Characterization of reaction product**

In order to explore the effect mechanism of iron source on Sb(III) removal by IOB-L, material composition, active groups, morphology and valence of elements on the surface of precipitates collected from different reaction condition were studied. Surface morphology of the samples was analyzed using scanning electron microscope (SEM, JSM-7800F Prime, JEOL, Japan). Energy dispersive spectra (EDS) was taken using an energy dispersive X-ray spectrometer (NORAN System 7, Thermo Scientific, USA) to test elemental composition on the product surface. Functional groups and covalent bonding information of present compounds were detected by Fourier transform infrared spectroscopy (FTIR, Nicolet 670, ThermoFisher Scientific, USA). An X-ray photoelectron spectrooscope (XPS, Escalab 250Xi, Thermo Scientific, USA) was also used to determine the oxidation states of elements.

**RESULTS AND DISCUSSION**

**Sb(III) adsorption ability of IOB-L biomass**

Sb(III) removal from water by IOB-L cultivated in nutrient without ammonium ferric citrate was investigated. As the reaction processed ivory flocs were formed gradually. The optical density of suspension increased slowly during the first 12 hours, and reached to 0.51 at the 24th hours. It demonstrated that without addition of iron source, IOB-L can still survive. However, no dark-colored precipitates were found during the whole process, indicating no generation of biogenic iron oxides. As shown in Figure 1(a), Sb concentration decreased slowly to 15.70–16.28 mg/L after reaction for 120 hours, which meant that IOB-L biomass itself had a little adsorption ability towards Sb(III). When the initial solution pH ranged from 4.0 to 8.0, dissolved Sb content did not vary significantly.

**Sb(III) removal ability of active IOB-L**

Sb(III) removal ability of active IOB-L cultivated in nutrient containing ammonium ferric citrate is shown in Figure 1(b). It demonstrated that the whole process can be divided into three stages. In the first stage, dissolved Sb decreased slowly from initial 18 mg/L to about 17 mg/L at the 24th hours. In the second stage, Sb in solution decreased obviously to 7.71–9.37 mg/L at the 48th hours. In the third stage, Sb concentration began to decrease slowly, and was in the range of 4.10–7.05 mg/L in the end. According to our previous research (Li et al. 2020), it took 24 hours for IOB-L to adjust a new environment after inoculation, then IOB-L was in logarithmic phase during the next 24 hours, and later it could reach stagnate phase. In this study, reaction solution became turbid after receiving the inoculation of IOB-L, and 24 hours later reddish-brown precipitates was obviously found in flasks, proving the generation of biogenic iron oxides (Jones & Renaut 2007). It was found that Sb(III) removal ability of active IOB-L in the first stage was weak and similar to the adsorption ability of IOB-L biomass. However, Sb(III) removal ability was high in the second stage, which was probably due to the massive biogenetic iron oxides that had strong adsorption ability towards Sb(III). Then Sb(III) removal rate slowed down in the third stage, possibly because adsorption capacity of iron oxides reached saturation (Saleh et al. 2017).

From Figure 1, it also can be seen that although changes of residual Sb in water during the whole reaction process did present the same trend under different pH conditions, solution pH still had effect on Sb(III) removal rate. When initial solution pH was 4.0, 5.0, 6.0, 7.0 and 8.0, the final Sb concentration in solution was 6.49, 4.94, 4.71, 4.1, and 7.05 mg/L respectively. It showed that Sb(III) removal rate under pH of 7.0 was faster than the other pHs. This was probably because the activity of iron-oxidizing bacteria was commonly better in neutral condition (Kato et al. 2013), and more amount of biogenic iron oxides might be produced. More importantly, through the comparison between Sb(III) removal performance of active IOB-L and Sb(III) adsorption of IOB-L biomass, it could be inferred that iron sources in nutrient had important effluence on the generation of biogenic iron oxides.

**Zero-valent iron and ferrous lactate removal characteristic towards Sb(III), separately**

Sb(III) removal from water by nanoscale or micron-sized zero-valent iron had been investigated in other studies. It showed that fresh iron (hydr)oxides resulting from corrosion of Fe⁰ played the main role in removing Sb(III) via adsorption or precipitation/co-precipitation (Zhao et al. 2014; Zeng et al. 2020). However, particle diameter of zero-valent iron (about 2 mm)
used in our study was significantly larger than that of nanoscale or micron-sized zero-valent iron, implying less amount of iron oxides would be generated.

Sb(III) removal performance by zero-valent iron is shown in Figure 2(a). As the reaction processed, residual Sb decreased in the first 24 hours, which is probably due to the high accessibility of free or active adsorbent sites. In the following 96 hours, it did not change seriously due to the partial saturation of accessible active sites (Sharma et al. 2022). When 0.03, 0.05, 0.09, and 0.15 g iron was added, dissolved Sb in solution at the end of experiment was 15.33, 13.39, 12.02 and 10.41 mg/L, respectively. It can be seen that most of the Sb(III) was reduced during the first 24 hours, which was significantly different from Sb(III) removal performance of active IOB-L. Moreover, compared with nanoscale or micron-sized zero-valent iron, Sb(III) removal ability of zero-valent iron in our study was not high.

Effect of solution pH on Sb(III) removal ability by zero-valent iron is presented in Figure 2(b) and was studied at different pH values such as 4.0, 5.0, 6.0, 7.0, 8.0. Results showed that Sb(III) removal rate was 25.44%, 26.11%, 25.56%, 25.61%, 16.78%, respectively. It demonstrated that Sb(III) removal ability of zero-valent iron under solution pH of 4.0–7.0 did not changed seriously, which was because Sb(III) removal was mainly controlled by chemisorption (Zeng et al. 2020).

Sb(III) removal ability of ferrous lactate was first conducted under solution pH of 7.0. When ferrous lactate dose was 0.02, 0.06, 0.1, and 0.16 g, after reaction for 120 hours residual Sb in solution was 17.89, 17.78, 17.83, and 17.69 mg/L, respectively.

Figure 1 | Sb(III) removal ability of IOB-L cultivated in nutrient (a) without and (b) with adding iron source.

Figure 2 | (a) Sb(III) removal ability of zero-valent iron and (b) effect of solution pH on Sb(III) removal rate.
Moreover, residual Sb had always maintained at 17.6–17.8 mg/L even though the solution pH changed from 4.0 to 8.0. It implied that Sb(III) removal ability of pure ferrous lactate could be ignored.

Effect of different iron sources on Sb(III) removal performance of active IOB-L

Zero-valent iron and ferrous lactate were used as iron source for IOB-L instead of ammonium ferric citrate in culture. Sb(III) removal ability by IOB-L with adding different dose of iron sources was surveyed, and the results are shown in Figure 3. Meanwhile, Sb(III) removal from water by active IOB-L cultivated in ammonium ferric citrate culture was indicated as control.

During the reaction process, brown precipitates were found in bottles, indicating the generation of biogenic iron oxides. As demonstrated in Figure 3(a), when 2–16 g/L ferrous lactate was adopted, residual Sb decreased to approximately 17 mg/L at the 24th hours, and then it decreased seriously to about 8 mg/L at the 72th hours. The final Sb content in water was in the range of 3.65–6.17 mg/L. Although the whole degradation trend of Sb(III) by IOB-L using ferrous lactate as iron source was similar to that in the control experiment, at the 48th hours Sb(III) concentration was seriously higher than that in the control experiment. It was proved that lactate can be used as carbon source for several kinds of bacteria, such as sulfate-reducing bacteria (Desulfovibrio spp.) and marine phototrophic ferrous-iron-oxidizing bacteria (Rhodovulurn iodosurn sp.) (Straub et al. 1999; Xia et al. 2019). In this study, although a long period was needed for IOB-L to utilize ferrous lactate efficiently, the decrease of residual Sb demonstrated that ferrous lactate could not only be used as the carbon source but also iron source for active IOB-L. Therefore, except ammonium ferric citrate, ferrous lactate can be adopted as another iron source for IOB-L, and a better removal rate of Sb(III) can still be reached with a longer stagnate phase.

Whether zero-valent iron can be used as the iron source for IOB-L was also studied. As shown in Figure 3(b), when 0.03, 0.05, 0.09, 0.15 g of iron was added to 18 mg/L Sb(III) solution inoculated with IOB-L suspension, Sb concentration was decreased to 17.78, 15.94, 14.18, and 12.01 mg/L after reaction for 24 hours, respectively. The final residual Sb was 14.16, 12.58, 10.55, and 9.43 mg/L, respectively. Moreover, the whole removal trend of Sb(III) was totally different from that in the control experiment, but it was similar to Sb(III) removal performance of zero-valent iron as demonstrated in Figure 2. This implied that Fe⁰ played the major role in Sb(III) removal. The consequence was different from our previous study which indicated that large amount of biotic iron oxides was generated quickly during Sb(V) removal in iron + IOB system (Li et al. 2020). The most possible reason may be that compared with Sb(V), Sb(III) was more toxic to IOB-L which then needed longer time to grow prosperously, whereas, the generated Fe²⁺ from the corrosion of zero-valent iron was mostly oxidized by oxygen and precipitated as iron (hydr)oxides during this period (Ferris 2005), leading to less Fe²⁺ can be used for the active IOB-L. Besides, it was found that the final Sb(III) removal ability of IOB-L in the presence of zero-valent iron was a bit higher than that of Sb(III) reduction by pure zero-valent iron. This was probably because IOB-L strain still survived and grew.

![Figure 3](http://iwaponline.com/wst/article-pdf/doi/10.2166/wst.2022.069/1009405/wst2022069.pdf)

*Figure 3* | Sb(III) removal ability of active IOB-L using (a) ferrous lactate and (b) zero-valent iron as iron source. Initial pH of Sb(III) solution was 7.0.
in this situation, and bacterial cell had certain adsorption ability towards Sb(III) as indicated in Figure 1. So, it could be inferred that zero-valent iron was hard to be used as iron source for the isolated IOB-L in the process of Sb(III) removal.

**Characterization of precipitates after reaction**

**SEM-EDS analysis**

In order to investigate the influence mechanism of different iron source on IOB-L removal ability towards Sb(III), SEM-EDS, FTIR, and XPS techniques were used to characterize the reaction precipitates.

From Figure 4 it can be seen that SEM images in two systems were different, which implied that constituent and crystalline state of products were not the same. When ferrous lactate was used as iron source for IOB-L, irregular blocks made of smaller nanoparticles were observed in Figure 4(a) and 4(b). Different from the rod-shape of isolated IOB-L, these agglomerated clusters with rough surfaces were probably caused by the generation of biogenic iron oxides. Whereas, when zero-valent iron was added to reaction solution, there were no agglomerated clusters after reaction (Figure 4(c) and 4(d)), indicating no production of biogenic iron oxides.

EDS was used to conduct the elemental analysis of precipitates yielded in two systems. As shown in Figure 5(a), when IOB-L was cultivated in nutrient containing ferrous lactate, element O, Fe and Sb accounted for 28.65%, 42.09%, and 18.77% of the reaction precipitates’ mass. The presence of Fe and O elements were due to the generation of iron oxides. The high content of Sb in precipitates proved that Sb(III) was removed from the water and fixed onto solid-state product. A small quantity of C, N, Si and P elements were also found, which was probably owing to the organic material in cells of bacteria.

![Figure 4](image_url) **Figure 4** | SEM images of precipitates from Sb(III) removal reaction by IOB using (a, b) ferrous lactate and (c, d) zero-valent iron as iron source.
As shown in Figure 5(b), when zero-valent iron was used as iron source for IOB-L, reaction product consisted major elements C, O, N, and a small amount of Na, Cl, Mg, S, K. The presence of these elements in precipitates might come from the IOB-L cell or culture medium for microorganisms. Although large-grained zero-valent iron was added to Sb(III) solution, it was strange that element Fe only accounted for 0.21% of the precipitates' mass. This was probably because the surface of iron was mostly covered by IOB-L biofilm or nutrient culture (Zhang et al. 2015). It once again indicated that biogenic iron oxides were barely produced by IOB-L in the presence of zero-valent iron. Moreover, the quantity of element Sb in the product was just 1.61%, showing a lower Sb(III) removal ability of IOB-L using zero-valent iron as iron source.

SEM-EDS analysis results demonstrated that IOB-L can grow with adding of different iron sources. Different from zero-valent iron, ferrous lactate was oxidized by IOB-L, and the generated biogenic iron oxides had better adsorption ability towards Sb(III), which was also proved by the removal rate demonstrated in Figure 3.

FTIR analysis

The reaction products were also characterized by FTIR. As illustrated in Figure 6(a) and 6(b), there were some similar results of FTIR spectra between two samples. For example, the broad absorption peaks observed at 3,347 cm\(^{-1}\)–3,291 cm\(^{-1}\) in two samples were assigned to the bond stretching of O–H in adsorbed water. The peak at 1,645 cm\(^{-1}\)–1,623 cm\(^{-1}\) was assigned to C=O stretching. The absorption band ranged from 1,404 cm\(^{-1}\) to 1,391 cm\(^{-1}\) could be assigned to C–O bending from carboxylate ions (Bartošová et al. 2011). The band in the region from 1,100 cm\(^{-1}\) to 1,000 cm\(^{-1}\) in two samples might be characteristic of C–O stretching of carbohydrates (Zhao et al. 2014). Although the position of absorption peaks of C=O and C–O group in two products were not exactly the same, their presence suggested surviving of IOB-L biomass during the reaction.
There was still some difference between the FTIR spectrum of two products. As shown in Figure 6(a), when ferrous lactate was used as iron source for IOB-L, the peak at 1,391 cm\(^{-1}\) may be attributed to C–H bending vibrations (Zhao et al. 2014). While, when zero-valent iron was used as iron source for IOB-L, the absorption band appeared at 2,929 cm\(^{-1}\) (Figure 6(b)) was closely related to CH\(_2\) asymmetric stretching vibration (Morent et al. 2008). This indicated that the structure and ingredient of generated organics in two systems changed with the addition of different iron source.

More importantly, the peaks ranging from 700 cm\(^{-1}\) to 400 cm\(^{-1}\) observed in two samples were totally different. On one hand, a band at 489 cm\(^{-1}\) was found in Figure 6(a). It was known that an absorption band of iron oxides can be commonly observed around 460 cm\(^{-1}\) due to the stretching vibrations of Fe–O (Lan et al. 2016). So, the apparent frequency shift of Fe–O band in Figure 6(a) was possibly due to the occurrence of interactions between Fe–O bonds in biogenic iron oxides with Sb(III) ions. On the other hand, the characteristic peak of Fe–O was not found in Figure 6(b). Instead, a weak band around 563 cm\(^{-1}\) appeared, which might be assigned to Sb–O–Sb symmetric stretching (Sudarsan et al. 2002). In a word, the result of FTIR analysis was basically consistent with that of SEM-EDS.

XPS analysis

Surface elemental compositions of the products were further determined by XPS technique. Figure 7(a) showed that when ferrous lactate was used in nutrient for IOB-L, the principal elements of product were C, O, N, P, Fe and Sb. It was
known that C, O, P and N peaks resulted from the IOB-L biomass. And the presence of Fe and Sb proved the formation of biogenic oxides and adsorption of Sb. Figure 7(b) demonstrated that when zero-valent iron was used as iron source for IOB-L, the main elements on product surface were C, O, N, P and Sb.

Figure 8(a) and 8(b) show the high-resolution XPS spectra of Fe2p and O1s + Sb3d in the product collected from system which used ferrous lactate as iron source. The photoelectron peaks for Fe 2p3/2 and 2p1/2 centered at 711.7 eV and 724.5 eV, separately, which were indicative for Fe (III) specie in iron oxides (Gerber & Erasmus 2018). Although the overlapping of Sb 3d and O 1s photo-electron peaks, deconvolution of O1s + Sb 3d5/2 spectra yielded two peaks: peak I (529.6 eV) and peak II (530.8 eV). The spectra of peak I was assigned to Sb(III) species (Kameda et al. 2015), and peak II might be assigned to oxygen atoms in iron oxides (Basly et al. 2013). Therefore, it can be inferred that when ferrous lactate was used as iron source for IOB-L, most of Sb(III) was adsorbed to generated biogenic iron oxides, and did not oxidized to Sb(V).

Figure 8(c) and d display Fe2p and O1s + Sb3d spectra of the products collected from system which used zero-valent iron as iron source. It can be seen that the characteristic peak of Fe 2p in Figure 8(c) was not distinct, which did agree with that of EDS analysis. This phenomenon indicated again that there was no massive generation of biogenic iron oxides, or product surface was covered by the IOB-L biofilm. The curve fitting of O1s + Sb 3d5/2 in Figure 8(d) showed two peaks at 529.6 eV (peak I) and 531.2 eV (peak II), which were attributed to Sb(III) and oxygen atom in C = O group, respectively (Kameda et al. 2015). Moreover, the intensity of Sb(III) peaks in Figure 8(d) was much weaker, demonstrating a lower binding affinity of Sb(III) to the precipitates.

![Figure 8](http://iwaponline.com/wst/article-pdf/doi/10.2166/wst.2022.069/1009405/wst2022069.pdf)
CONCLUSIONS
In our study, a strain of isolated heterotrophic iron-oxidizing bacteria (named as IOB-L), which belonged to *Klebsiella* sp., was used for water treatment. The effect of iron sources on its removal ability towards Sb(III) was further investigated in batch experiment. The results showed that IOB-L biomass had a little adsorption ability towards Sb(III). There was no reaction between Sb(III) and ferrous lactate, whereas corrosion product of zero-valent iron can adsorb certain amount of Sb(III). Although ferrous lactate was able to be used as iron source for active IOB-L, a longer adaptation period was necessary. When zero-valent iron was added to Sb(III) solution inoculated with IOB-L, Sb(III) removal trend was similar to that in pure iron system. Compared with the organic iron, zero-valent iron was hard to be oxidized by IOB-L directly, which probably due to the faster chemical oxidation of Fe^{2+} in water. Moreover, SEM-EDS, FTIR, and XPS analysis of precipitates collected from different systems were consistent. Interactions between Sb(III) and ferrous lactate, whereas corrosion product of zero-valent iron can adsorb certain amount of Sb(III).

ACKNOWLEDGEMENTS
This work is sponsored by the National Natural Science Foundation of China (No. 42177380 and 51504094).

DATA AVAILABILITY STATEMENT
All relevant data are included in the paper or its Supplementary Information.

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First received 14 September 2021; accepted in revised form 10 February 2022. Available online 24 February 2022