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Deep removal of arsenic from regenerated products of spent V_2O_5-WO_3/TiO_2 SCR catalysts and its concurrent activation by bioleaching through a novel mechanism

Tianqi Niu[a], Jia Wang[b], Huichao Chu[a], Can Qian[a], Ning Duan[a], Geoffrey Michael Gadd[c], Wen Shi[a], Baoping Xin*[a]

[a]School of Materials, Beijing Institute of Technology, Beijing 100081, P. R. China

[b]College of Environmental and Energy Engineering, Beijing University of Civil Engineering and Architecture, Beijing 100080, P. R. China

[c]Geomicrobiology Group, School of Life Sciences, University of Dundee, Dundee, DD1 5EH, Scotland, UK

Abstract: Selective catalytic reaction (SCR) is widely used in flue gas denitrification to convert NO_x air pollutants into N_2 and H_2O in the presence of SCR catalyst, but arsenic poisoning causes serious deactivation of SCR catalyst and produces a huge amount of spent SCR catalyst worldwide. Therefore, the regeneration and recycling of spent SCR catalysts are of great necessity for the sustainable flue gas denitrification due to the very high cost of replacing commercial catalysts. Nowadays, a combination of alkali treatment and acid washing is usually used to regenerate spent SCR catalysts. However, high residue of As strongly inhibits the catalytic activity of the regenerated products. In this study, an active bioleaching liquor produced by *Acidithiobacillus thiooxidans* as indirect bioleaching was attempted to deeply remove As from the

* E-mail address: xinbaoping@bit.edu.cn (B.P. Xin)

regenerated product for the first time. The results showed that bioleaching resulted in maximal As removal of 53.1% even at a high pulp density of 10% to harvest a minimum As residue of 174 mg/kg below the limiting value of 200 mg/kg; whereas H_2SO_4 leaching attained 6.7% As removal and as high as 345 mg/kg of As residue. A stronger activation of the regenerated products simultaneously occurred with the bioleaching. A great amount of cysteine in the bioleaching liquor secreted by the microbe accounts for both the enhancement of As removal and strong activation of the product. A novel mechanism is proposed to explain the excellent As removal by the indirect bioleaching based on nucleophilic attack of the sulfhydryl groups in cysteine.

Keywords: arsenic removal; spent catalyst; regenerated product; bioleaching; endogenous cysteine

1. Introduction

A huge amount of denitrification catalysts are widely applied in coal-fired power plants, metallurgical industries, cement manufacture, solid waste incineration and coal-fired heating systems to selectively convert NO_x air pollutants into N_2 and H_2O by using NH_3 as a reducing agent in a selective catalytic reaction (SCR) [1,2]. The most common commercial SCR catalyst is V_2O_5-WO_3/TiO_2 with high selectivity, activity and SO_2 resistance [3], in which TiO_2 is the carrier, V_2O_5 is the active component and WO_3 acts to promote thermostability and SO_2 resistance. Because of the high price of three valuable metals V, W and Ti, V_2O_5-WO_3/TiO_2 catalysts are very expensive (3500-4000 $/ton), and can make up 30-50% of the total cost of the air pollution control system [4]. In addition, SCR catalysts suffer from deactivation during commercial applications due

to various reasons including sintering, blockage, poisoning, attrition, crushing, loss of vanadium or its speciation changes [4-7]. In particular, the extremely complex components of coal-fired flue gas accelerate SCR catalyst deactivation by adsorbing or depositing alkali, alkaline earth, metalloid and heavy metals (e.g., K, Na, Ca, Mg, As, Hg and Pb) [8-10]. On average, SCR catalysts have a service lifetime of ~3-5 years, resulting in a large amount of obsolete SCR catalyst materials [11].

Although spent SCR catalyst contains multiple valuable metals such as V, W and Ti, it is recognized as a hazardous waste due to the presence of toxic elements such as As, Hg and Pb. Thus, its improper disposal will inevitably cause severe environmental pollution and is a huge waste of valuable resources [4]. As an alternative, a regeneration and recycling process is highly desirable for making better use of spent SCR catalyst especially when considering the very high cost of replacing commercial catalyst [11,12]. Regeneration of abandoned SCR catalyst can be achieved through decontaminating the poisoning metals by using water washing, alkali or acid leaching [13,14]; the detoxified leached residue containing V, W and Ti as recycled products are adopted to refabricate fresh catalyst by impregnating the active components V and W [4,11]. However, although water washing is efficient for the removal of alkali metals, there is no effect on toxic elements such as As [11]. Washing by strong acid is adequate for efficient removal of alkali/alkaline earth metals, but leaves a significant amount of remaining As^{5+} [15]. Washing by alkalis can almost completely remove both As^{3+} and As^{5+}, but usually results in a massive loss of V and W [4]. Therefore, a combination of alkali treatment and acid washing is usually used to regenerate spent SCR catalyst in

commercial process, and the remanufactured catalyst has comparable activity to commercial fresh catalyst after replenishing active components [11,16].

Due to the extremely high coal consumption of more than 3.5 billion tons per year and the ultra-low emission standards for flue gas (50 mg/m^3 for NO_x), approximate 1.0 million tons of SCR catalyst is currently in use in China, resulting in an annual production of 300,000 tons of spent SCR catalyst. However, the rather high arsenic content in raw coal (average dosage of 3.79 mg/kg) causes significant deposition and accumulation of the toxic element As on catalyst surfaces and inside catalyst channels in the forms of As_2O_3 and As_2O_5 which will prevent NO from reaching catalyst active sites and destroy the catalyst reactive sites by reacting with vanadium [17,18,19]. Hence, arsenic poisoning is considered to be the most common and severe cause of deactivation of SCR catalyst, and so the regeneration of As-enriched spent SCR catalyst is of great importance in China and around the world [11,15,16].

Currently, a combination of alkali treatment and acid washing is the mainstream commercial approach to simultaneously remove As and alkali metals and acquire the regenerated powder products containing Ti, W and V. However, a certain proportion of As always remains in the regenerated products despite the high performance of the combined process. As^{3+} and As^{5+} are adsorbed on both active sites (mainly V_2O_5) and non-active sites (TiO_2 support), leading to the replacement of hydroxyl groups by As hydroxyls which inhibits the adsorption of CO or NH_3 on the Lewis acid sites of the remanufactured catalyst [20]. Because V_2O_5 exists in an extremely small dosage in the commercial catalyst, even small amounts of residual As can cause drastic effects on the

activity of the remanufactured catalyst [21]. For example, Peng et al. [22] reported that a maximum As removal of 50% was achieved from spent SCR catalyst containing 0.48 wt.% of As, but the NO conversion efficiency of the regenerated catalyst was only 50-60%. In order to ensure no activity degradation of the remanufactured catalyst, the Chinese Ministry of Science and Technology lately unveiled an initiative "Solid Wastes Recycling" to require that the regenerated products of SCR catalysts should have an As residue concentration below 200 mg/kg [23]. Now, the limit concentration of 200 mg/kg for As residue has been served as the industrial standard in the sector of spent catalyst regeneration. In many cases, however, the residual As concentration exceeds the maximum permissible concentration of 200 mg/kg when the content of As in spent SCR catalyst is above 0.3 wt.%. Therefore, a deep removal of As from the regenerated products of spent SCR catalysts grows an urgent issue both in China and worldwide, which is crucial for sustainable development of the SCR industry.

Bioleaching represents an eco-friendly and economical technique to liberate target metals from solid materials such as low-grade sulfide ores and solid wastes by certain microorganisms at ambient temperature and constant pressure [24]. The sulfur- and/or Fe^{2+}-oxidizing acidophilic chemoautotrophic bacteria *Acidithiobacillus thiooxidans*, *Acidithiobacillus ferrooxidans*, *Leptospirillum ferriphilum* are the most widely used bioleaching cells to release the valuable metals through direct (contact) and/or indirect (non-contact) mechanisms [25]. Bioleaching is first exploited to extract different valuable metals such as Cu, Ni, Co and Zn from a variety of low-grade sulfide ores. In this case, the autotrophic bacteria get energy from biooxidation of the low-valence

sulfur/iron in sulfide ores, in which an extracellular polymeric substance (EPS)-mediated adhesive contact between bacteria and metal sulfides is necessary as direct mechanism [26]. In recent years, bioleaching has been widely applied to release valuable metals from a variety of solid wastes such as spent batteries, obsolete refinery catalysts, smelting slag and electronic waste [27-30]. Because the solid wastes are basically oxides or hydroxides, the cells do not gain any energy from the non-sulfides matters. Thus, inorganic energy sources such as sulfur and pyrite are required in the bioleaching of solid wastes. In this case, the release of valuable metals is due to the acidic solution and/or oxidation/reduction reactions between the solid wastes and the biogenic metabolites such as H^+, Fe^{3+} and Fe^{2+} which is gained from a series of biological and chemical reactions of sulfur and pyrite [29]. As a result, no contact is absolutely necessary between bacteria and solid wastes as indirect mechanisms in the bioleaching of solid wastes [31].

Although there are a number of studies in regard to bioleaching of a wide range of solid waste, no report is available about deep removal of residual As from regenerated products of obsolete SCR catalysts by bioleaching, for which both alkali washing and acid leaching are no longer competent. In this study, bioleaching was used to deeply decontaminate highly toxic As in the SCR catalyst regenerated product to ensure that the final residual concentration of As is below the permitted limit value of 200 mg/kg at a high pulp density of 10% (w/v). For this purpose, a highly active bioleaching liquor produced by *Acidithiobacillus thiooxidans* (*A. thiooxidans*) was used to extract As from the regenerated product in an indirect manner to prevent the resulting product from

being contaminated by both bacterial cells and solid sulfur. Along with the deep removal of As, activation of the product occurred for a better catalysis performance, which was verified by thermal, surface and adsorptive analysis. The unknown active molecule in the leaching liquor which enhanced the extraction efficiency for As and activated the product was recognized as cysteine via metabolic pathway analysis and metabolite identification. The detailed As release mechanism mediated by cysteine was elucidated via monitoring and comparing the transformed As products in different leaching systems. Thereby a novel bioleaching mechanism involving cysteine for efficient release of the toxic As and concurrent activation of the regenerated product was expounded.

2. Materials and methods

2.1. The regenerated powder product of spent SCR catalyst

The regenerated product from spent SCR catalyst was kindly supplied by Anhui SCR Environmental Science and Technology Company, Ltd., Chuzhou city, Anhui Province, Eastern China. The regenerated product is a white powder with D_{90} ~ 5.0 μm and specific surface area ~ 60 m^2/g. It was obtained through treating the spent SCR catalyst with a sequential procedure including water washing, ultrasonic washing, coarse crushing, arsenic removal (by a mixed solution of NaOH and H_2O_2), silicon removal (by a mixed solution of H_2SO_4 and HF), drying and grinding. The content of metals in the regenerated powder product was analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES) after digestion using the HNO_3-HF-HCl method (USEPA 3052, 1996), revealing 75.46 wt.% for Ti, 7.23 wt.% for W, 0.27 wt.% for V,

0.037 wt.% for As. Although the concentration of residual As in the regenerated product significantly decreased from more than 3000 mg/kg to approximately 370 mg/kg, it is still higher than the limit content of 200 mg/kg which is mandatory for the qualified regenerated product [23]. Therefore, because of its high toxicity on the SCR catalytic activity, deep removal of As from the regenerated product is necessary for the cyclic reuse of spent SCR catalysts and the sustainable development of the SCR industry.

2.2. Preparation of the highly active bioleaching liquor

In this work, the acidophilic bacterium *Acidithiobacillus thiooxidans* (ATCC19377) was utilized to manufacture the highly active bioleaching liquor. The logarithmic phase bacterial cells were inoculated at an inoculation density of 5.0% (v/v) into modified inorganic media consisting of the following substances: 1.0 g $(NH_4)_2SO_4$, 1.0 g KNO_3, 1.0 g KH_2PO_4, 1.0 g Na_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.25 g $CaCl_2$, 20.0 g elemental sulfur (purity≥99%, size≤100 mesh) as an energy source, 1000 mL distilled water, pH 2.0-2.5. The inoculated media was then incubated in a shaker (32 ± 1.0℃, 120 rpm) for the production of highly active bioleaching liquor. When the pH of the media decreased to 0.8 after 8-10 days incubation, the media were centrifuged at 7012×*g* for 10 min to remove the cells and elemental sulfur, then the resulting supernatant was collected as the active bioleaching liquor. Detailed information about the source, preservation and culture of *A. thiooxidans* is available in previous works [27,29].

2.3. Removal of As from the regenerated product by bioleaching

In order to prevent the regenerated product from being contaminated by bacterial cells and elemental sulfur, the active bioleaching liquor (pH 0.8) produced by the

acidophilic cells was used to extract As from the regenerated product, which had a high As residue level, by an indirect bioleaching manner [24]. A dilute H_2SO_4 solution at pH 0.8 was also used to release As from the regenerated product as a control.

Bioleaching experiments were conducted in 250 mL Erlenmeyer flasks. The regenerated powder product was supplemented into 250 mL Erlenmeyer flasks containing 100 mL of active bioleaching liquor at pulp densities ranging from 5.0% to 10% (w/v). The Erlenmeyer flasks were then shake-incubated at 150 rpm under different temperatures (35℃, 50℃, 65℃, 80℃) to initiate bioleaching separately. 2 mL samples were taken at regular intervals over the whole duration of 24 h and used to measure the extraction concentrations and release efficiencies of As, Ti, W and V as well as the residual concentrations of As in the regenerated powder product after deep removal of As. All experiments, including the controls, were carried out in triplicate, and the actual value was a mathematic average of the three measured values.

2.4. Activation characterization of the bioleached regenerated product

After 24 h contact with the bioleaching liquor or dilute H_2SO_4 (pH 0.8) at a pulp density of 5.0% under incubation temperature of 35℃, the regenerated product was harvested by centrifugation (7012×*g*, 10 min), washed three times with distilled water, dried at an oven at 60℃ for 12 h, and ground lightly to a powder (size≤100 mesh). The bioleached, H_2SO_4-leached and raw sample were characterized to probe changes in thermal, surface and adsorptive properties.

Phase transformation of the regenerated product was analyzed using an X-ray diffractometer (XRD, Hitachi Rigaku-D/Max-2550 PC, Tokyo, Japan) with CuK_α

radiation. Morphology of the particles was examined by scanning electron microscopy (SEM, Hitachi S-4800, Tokyo, Japan), and elemental composition and distribution were assayed using Energy Dispersive X-ray analysis (EDX, Oxford Instruments Ltd., Bucks, UK). Thermal stability and decomposition of the regenerated products were determined using a thermal gravimetric analyzer (NETZSCH TG 209 F1 Libra, Selb, Germany). The Raman images of various regenerated products were characterized with a Thermo Fisher DXR2xi (Thermo Fisher Scientific, Waltham, MA, USA) instrument to detect changes in the chemical bonds of oxides. The hydrophilicity of different regenerated products was assayed by measuring the contact angle (Dataphy OCA 15Pro, Dataphysics Instruments GmbH, Filderstadt, Germany). The N_2 adsorption-desorption isotherms and BET surface area were measured using an ASAP-2460 Analyzer (Micromeritics Instrument Corporation, Norcross, USA). Temperature-Programmed Desorption (TPD) of NH_3 was carried out in a Micromeritics Autochem II 2920 analyzer (Micromeritics Instrument Corporation, Norcross, USA). 0.1 g of sample was packed in a quartz tube, heated at 400℃ for 30 min under an Ar atmosphere and cooled to 50℃; pure NH_3 was then adsorbed on the surface of the product for 10 min, finally, the samples were heated to 600℃ at a rate of 10℃/min to desorb the NH_3.

2.5. Identification of active molecules to enhance As release and product activation

In order to uncover the reasons why bioleaching exhibited much higher As removal and better activation of the regenerated product than chemical leaching by H_2SO_4, a specific metabolic pathway, involving both cysteine and sulfur, that occurs in *Acidithiobacillus caldus* SM-1 which belongs to the same genus with *Acidithiobacillus*

thiooxidans, was drawn via deeply mining and analyzing the KEGG database (https://www.genome.jp/kegg/pathway.html), because it was discovered that cysteine-rich low-molecular weight proteins such as metallothioneins (MTs) display high binding capacity of As owing to their interaction with the sulfhydryl groups (-S-H) in cysteine [32,33].

A drop of active bioleaching liquor produced by *A. thiooxidans* was placed on a glass slide and then air dried for a Fourier transform infrared spectroscopy (FT-IR; Model Nicolet iS50, Thermo Fisher Scientific, Waltham, MA, USA). Similarly, a mixed aqueous solution of 100 mg/L cysteine (analytical grade, Beijing Tongguang Fine Chemicals Company) and H_2SO_4 at pH 0.8 was also subjected to the same procedure as controls. The active bioleaching liquor was further assayed by reversed phase high performance liquid chromatography (RP-HPLC). RP-HPLC system was comprised of Agilent 1200 series instrument (Agilent Technologies, Pittsburgh, PA, USA) equipped with an Agilent TC-C18 column (250 mm × 4.6 mm, 5 μm) to separate cysteine and measure its concentration using pure cysteine as an internal standard. Operational details were as follows: 60% (v/v) acetonitrile used as mobile phase A; 0.1% H_3PO_4 used as mobile phase B (pH was adjusted to 1.0 with concentrated HCl); a mixed solution of 60% acetonitrile and 0.1% H_3PO_4 at an initial ratio of 80: 20 (v/v); a flow rate of 1.0 mL/min; column temperature 30℃; detection wavelength 210 nm. Three-dimensional(3D) fluorescence spectrometry (Fluorescence Spectrophotometer F-7000, Hitachi, Tokyo, Japan) was used to analyze organic components and change in the active bioleaching liquor at various incubation time, and the consumption and

transformation of organic matter before and after bioleaching at different temperatures from 35℃ to 80℃ [34].

2.6. Mechanism of cysteine enhanced As bioleaching from regenerated products

In order to expound the cysteine-enhanced mechanisms for As bioleaching from the regenerated product, three sets of leaching experiments (chem-leaching, bioleaching, enhanced bioleaching with addition of cysteine at 100 ppm) were conducted at a pulp density of 10% (w/v) at 65℃ to compare the variation of the total concentration of As, As^{3+} dosage and As^{5+} dosage in the solutions. Total released concentrations of As were measured using ICP-OES, As^{3+} and As^{5+} were assayed using high-performance liquid chromatography coupled with hydride generation-atomic fluorescence spectrometry (HPLC-HG-AFS; Haiguang LC-AFS6500, Beijing, China). The different residues after leaching were washed three times with distilled water, dried at an oven at 60℃ for 12 h, and ground lightly to a powder (size≤100 mesh), and then analyzed by using X-ray photoelectron spectroscopy (XPS, ESCALAB, Thermo-VG Scientific 250, Waltham, MA, USA) to characterize variation in distribution of both As^{3+} and As^{5+} on the surface of regenerated products.

3. Results and discussion

3.1. Removal of As from recycled product by bioleaching

Fig.1a-1c and **Table S1** show the removal efficiency of As from the regenerated powder product under varying pulp densities at different temperatures by the *A. thiooxidans*-derived bioleaching liquor, together with the chem-leaching performance by H_2SO_4 at pH 0.8 as controls. It was found that H_2SO_4-based chemical leaching

displayed a much lower As removal capacity than the bioleaching liquor. Final As removal was 30.5%-37.5% over a temperature range of 35℃-80℃ by chem-leaching at a pulp density of 5.0%; whereas the bioleaching liquor showed a higher As removal of 67.6%-88.7% over the temperature range of 35℃-80℃ (**Fig. 1a**). Further, it was found that with the increase of pulp density from 5% to 10%, As removal efficiency evidently decreased; on the other hand, As removal efficiency was recovered when the temperature elevated from 35℃ to 80℃ (**Fig. 1a-1c and Table S1**). However, there was a great difference between bioleaching and chem-leaching in both pulp density-responsive drop and temperature-responsive lift in As removal efficiency.

In the case of chem-leaching, a slight lift in As removal efficiency occurred when the temperature rose from 35℃ to 80℃, with 7.6% from 12.2% to 19.8% at 7.5% of pulp density and 3.2% from 3.5% to 6.7% at 10% of pulp density, respectively (**Fig. 1b,1c, Table S1**). The results suggested that for chem-leaching, the temperature-responsive lift in As removal efficiency becomes less noticeable with the increase in pulp density. In the case of bioleaching, however, a great increase in As removal occurred when the temperature lifted over the same temperature range, with 31.9% from 34.2% to 66.1% at 7.5% of pulp density and 36.7% from 16.4% to 53.1% at 10% of pulp density, respectively (**Fig. 1b, 1c**,**Table S1**). The results indicated that for bioleaching, the temperature-responsive elevate in As removal becomes more noticeable with the lift in pulp density. There are two conclusions. First, bioleaching possesses a much higher deep-removal ability for As than chem-leaching by H_2SO_4. Second, although As removal efficiency declines with the increase of pulp density in

both bioleaching and chem-leaching, the elevate of temperature can more greatly improve the poor performance of As removal at high pulp density by bioleaching than by chem-leaching. These advantages endow *A. thiooxidans*-derived bioleaching liquor with a greater potential for deep removal of As from the regenerated products of the spent SCR catalyst.

The limit concentration of 200 mg/kg for As residue in the regenerated products of spent SCR catalyst was suggested by the Chinese Ministry of Science and Technology to guarantee no activity degradation of the remanufactured catalyst [23]. Obviously, such a high As residue of 370 mg/kg in the regenerated products is much greater than the limit concentration of 200 mg/kg. Hence, a deep removal of As from the regenerated products is an urgent demand for the spent SCR catalyst regeneration industry. **Fig. 1d and Table S2** present a detailed comparison of the As residue contents after deep removal at varied pulp densities under different temperatures between chem-leaching and bioleaching. It was observed the As residue concentration by chem-leaching ranged from 257 to 231 mg/kg over the temperature range from 35℃ to 80℃ at a pulp density of 5.0%, which was higher than the concentration limit of 200 mg/kg. In contrast, bioleaching gained a low As residue of 120-41.8 mg/kg over the temperature range of 35℃-80℃, which was much lower than the desired concentration limit. It was concluded chem-leaching was completely inadequate for deep removal of As even at a low pulp density of 5.0%; whereas bioleaching was fully qualified for the hard work.

In the case of higher pulp density of 7.5% or above, an extremely high As residue concentration occurred by chem-leaching, respectively being 325-297 mg/kg at 7.5%

of pulp density and 357-345 mg/kg at 10% of pulp density over the temperature range of 35℃-80℃ (**Fig. 1d,Table S2**), which displayed that chem-leaching is incapable of efficiently deep removal of As. Peng et al. adopted a combined chem-leaching process of 0.2M H_2SO_4 and 4M H_2O_2 to remove As from the poisoned SCR catalyst with 0.48 wt.% As to gain around 50% of As removal and as high as 2200 mg/kg of As residue, further demonstrating the incompetence of chem-leaching techniques in As removal [22]. However, there was an entirely different situation with bioleaching. Although an unacceptable As residue concentration (>200 mg/kg) was noticed at higher pulp density of 7.5% or above at 50℃ or below, 149 and 174 mg/kg of As residue concentration were gained at pulp densities of 7.5% and 10% when the temperature climbed to 65℃ and 80℃, respectively. This showed that bioleaching is still adequate for deep removal of As from the As-rich regenerated products even at higher pulp densities of 7.5%-10% by increasing the reaction temperature (**Fig. 1d and Table S2**). Concomitant with the efficient removal of As by bioleaching, only a small amount of V (8.8%-13.6%) was lost, with no loss for both Ti and W (**Fig. 1e**). In the present study, indirect bioleaching was used to deeply remove As from high As-residue regenerated product for the first time. The results reveal the indirect bioleaching has incomparable advantages over conventional chem-leaching in deep removal of As to ensure that the regenerated products reach the standard of As residue <200 mg/kg.

3.2. Activation of the regenerated product by bioleaching

Apart from high removal of As, efficient activation of the regenerated product is of importance for maintaining high catalytic activity of the remanufactured catalyst, such

as increasing the number of active sites, boosting the surface area/inner hole area and elevating the adsorption capacity of reactants. Therefore, to understand the activation process and mechanisms, the bioleached product, H_2SO_4-leached product and crude regenerated product were analyzed using SEM-EDX, XRD, Raman spectroscopy, TG, contact angels test, BET and NH_3-TPD to compare the variations in microtopography, elemental distribution, crystal structure, bond types, heat stability, hydrophilicity, surface area, inner hole area and NH_3-adsorption capacity between the different treatments.

As shown by SEM-EDX (**Fig. 2**), there were no obvious changes in the surface morphology and As, Ti, W and V distribution between the raw sample (**Fig. 2a**) and H_2SO_4-leached product (**Fig. 2b**), suggesting H_2SO_4-leaching has a low modification capacity and As removal efficiency for the regenerated product. However, compared with the crude and H_2SO_4-leached product, the bioleached product (**Fig. 2c**) exhibited a smoother appearance and a much lower distribution density of As on the surface, indicating that bioleaching has a strong modification ability and high As removal efficiency. There were also no differences detected between the H_2SO_4-leached product and crude sample for both the XRD analysis (**Fig. 3a**) and Raman spectra (**Fig. 3b**). However, evident variations occurred with the bioleached product. A new and obvious peak at $2\theta=23.8°$ corresponding to titanyl sulfate ($TiOSO_4$) (PDF#86-0647) appeared in the XRD image due to unique reactions in the bioleaching, which enhances the Lewis acidity for NH_3 adsorption [35]. At the same time, in the Raman spectrum the peak representing As-O stretching (778 cm^{-1}) disappeared with the bioleached product,

showing that bioleaching simultaneously achieves activation of the regenerated product and efficient removal of As [36].

The weight loss over the low range of 30-160℃ in the thermogravimetric (TG) curves (**Fig. 4a**) gradually increased in the order: bioleached sample > H_2SO_4-leached sample > raw sample, suggesting that removal of As increased the number of polar active sites in the regenerated product to adsorb more water molecules and that bioleaching activated more polar active sites than H_2SO_4 leaching [37]. In agreement with TG analysis, the contact angle analysis (**Fig. 5**) also exhibited that both the polarity and surface energy increased in the same order: bioleached sample (**Fig. 5c,5f**) > H_2SO_4-leached sample (**Fig. 5b,5e**) > raw sample (**Fig. 5a,5d**), again demonstrating that bioleaching was superior over H_2SO_4-leaching in activating/recovering polar active sites. On the other hand, the derivative thermogravimetric (DTG) analysis **(Fig. 4b)** showed that the bioleached sample had a higher sintering range than the H_2SO_4-leached and raw samples, with the weight loss of the bioleached sample at the extremely high temperature of 1100℃ was lower than that of the H_2SO_4-leached sample and raw sample, indicating the bioleached product had higher thermal stability than the others due in part to the formation of $TiOSO_4$ (**Fig. 4c-4h**) [38].

Nevertheless, as presented in **Fig. 6a**, the BET assay further unveiled that the surface area declined in the order of bioleached sample < H_2SO_4-leached sample < raw sample, due to the removal of As deposits at the surface of the regenerated product. In contrast, the pore volume obviously increased in the sequence of bioleached sample > H_2SO_4-leached sample > raw sample, showing that the inner pores as a major SCR reaction

zone, were extensively cleared to remove the As deposits. As shown in **Fig. 6b**, the NH_3-TPD data directly demonstrated that the bioleached product exhibited a much higher NH_3 adsorption activity than the H_2SO_4-leached sample and raw sample over the entire range covering weak acid site (100-230°C), medium acid site (230-380°C) and strong acid site (>380°C), which may also be associated with the generation of $TiOSO_4$. In contrast, the H_2SO_4-leached sample only showed slightly higher NH_3 adsorption than the raw sample in the weak and the strong acid regions except for the medium acid region [39]. It can be concluded that bioleaching is advantageous over H_2SO_4 leaching in activating the regenerated product for a better SCR reaction. Furthermore, concomitant with the efficient removal of As, bioleaching resulted in the formation of $TiOSO_4$, improvement of heat stability and decrease of surface area, as well as a marked increase in polar active sites, surface energy, pore volume and NH_3 adsorptive capacity. A notable activation of the regenerated product occurred with bioleaching, which is of great importance to assure a high catalytic activity of the regenerated SCR. This work first showed that bioleaching is capable of both efficient removal of arsenic and strong activation of the regenerated product, which H_2SO_4 leaching failed to do. Such advantages of bioleaching over H_2SO_4 leaching was found to be associated with certain special active metabolites in the bioleaching liquor produced by *A. thiooxidans*, and is described in following sections.

3.3. Identification of active molecules to enhance As release and product activation

Identification of the mysterious active matter beyond H^+ in the bioleaching liquor derived from *A. thiooxidans*, which both markedly enhance extraction of As and

evidently activate the regenerated product, is of importance for both theoretical and practical development of bioleaching technology [24,29,30]. Inspired by the discovery that cysteine-rich proteins such as metallothioneins exhibit a high binding capacity of As due to the interaction between As and -SH groups in cysteine [32,33], we mapped a metabolic pathway involving both cysteine and sulfur through deeply mining and analyzing the KEGG database (https://www.genome.jp/kegg/pathway.html) (**Fig. S1**), which was found in *Acidithiobacillus caldus* SM-1 belonging to the same genus with *A. thiooxidans*. Such metabolic pathway mapping indicated the production of cysteine by *A. thiooxidans* in the presence of elemental sulfur as energy substance is highly possible to occur.

To confirm that cysteine was synthesized by *A. thiooxidans*, fresh bioleaching liquor was analyzed using FT-IR spectroscopy and compared with analytically pure cysteine as a reference (**Fig. 7a**). The band at 836 cm^{-1} indicated the symmetrical stretching of C=O, the band at 1730 cm^{-1} represented the stretching vibration of C-O, and the band at 3407 cm^{-1} corresponded to the stretching modes of O-H, showing that carboxyl groups were present in the bioleaching liquor[40-42]. The bands at 928 and 1056 cm^{-1} are linked with C-C and C-N stretching vibration, C-H vibration was observed at 1240, 1449, 2371, 2308 and 2925 cm^{-1}, and two other broad adsorption peaks at 2066 and 3277 cm^{-1} were attributed to the stretching modes of N-H in NH_2 groups [43-47]. Especially, the sulfur-hydrogen (S-H) stretching was observed evidently at 2550 cm^{-1} [43]. The FT-IR spectrum for the bioleaching liquor was almost identical to that of the reference cysteine, confirming that the bioleaching liquor contained cysteine as a major

component. This was further confirmed by RP-HPLC analysis according to the same retention time with the reference cysteine (**Fig. 7b**).

In order to more directly reflect the transformation of the major active cysteine and other unidentified organic substances at various temperatures before and after bioleaching, Three-dimensional fluorescence spectrometry as an advanced and visual assay means was utilized for this purpose (**Fig. 8**). Regions I and II represent aromatic proteins; region III is related to fulvic acid-like substances; region IV represents soluble amino acid cysteine; and region V is linked to humic acid-like organics [34]. It was clear that the organic components appeared in regions I, II and IV, these results demonstrated that the active bioleaching liquor mainly contained unidentified aromatic proteins and cysteine. With an increase in culture incubation from 5 to 12 days (**Fig. 8b-8d**); **Fig. 8a** shows pure cysteine as control), both the peak areas grew larger and the color markedly darkened in regions I, II and particularly IV, indicating that aromatic proteins and cysteine, especially cysteine significantly accumulated in the bioleaching liquor. With an increase in reaction temperature from 35℃ to 80℃ (**Fig. 8e-8h**), however, the peak areas decreased and the color became lighter because of dissociation of functional groups, such as -SH, -COOH and -NH_2, in the aromatic proteins and cysteine at high temperature resulting in thermal fluorescence quenching [48]. However, the dissociated proteins and cysteine possess a stronger activity to attack As, leading to higher As removal from the regenerated product. After bioleaching, the peak areas grew smaller and the color became fairly lighter in regions I, II and IV, suggesting that cysteine and proteins were greatly consumed in the bioleaching process (**Fig. 8e vs. 8i;**

8f vs. 8j; 8g vs. 8k; 8h vs.8l).

All the data support the conclusion that the active substance was recognized as cysteine which enhances release of As and activation of the regenerated product. It was the first time to definitely discern cysteine as an active small molecular species being extensively produced by *A. thiooxidans*. Both efficient removal of As and strong activation of the regenerated product by the *A. thiooxidans*-derived cysteine indicate that bioleaching has a unique advantage and promising application potential in the deep removal of As from regenerated product, recycling of spent SCR catalyst and sustainable treatment of hazardous NO_x worldwide [49].

3.4. As bioleaching mechanism from regenerated products enhanced by cysteine

To understand the role of cysteine in the efficient bioleaching of As, three sets of experiments (H_2SO_4 leaching, bioleaching by *A. thiooxidans*, bioleaching enhanced by adding cysteine at 100 ppm) were carried out to compare difference in released As concentrations and efficiencies for both As^{3+} and As^{5+} as a function of contact time (**Fig. 9**). It was found that in H_2SO_4 leaching, As^{5+} was slowly released up to 5.6% after 18h of contact, and no dissolution occurred with As^{3+}, showing that H_2SO_4 leaching was inefficient in extracting As^{5+} and absolutely incapable of removing As^{3+} to attain very low As removal (**Fig. 9a**). The difference in extraction performance between As^{5+} and As^{3+} implied that As^{3+} was more stable than As^{5+} [49]. In comparison with H_2SO_4 leaching, however, bioleaching achieved a much higher As^{5+} extraction of 41.5% and a slightly higher As^{3+} release of 2.1%, amounting to a total As removal of 43.6% (**Fig. 9b**). It is positive that the *A. thiooxidans*-derived cysteine greatly improves the release

of both As^{5+} and As^{3+}. Because the *A. thiooxidans*-derived cysteine is not enough, which is preferentially consumed to free As^{5+}, very low release occurred with As^{3+}. As expected, as shown in **Fig. 9c**, an enhanced bioleaching by the addition of 100 ppm cysteine showed almost complete release of both As^{5+} and As^{3+} resulting in 99.2% As removal. Furthermore, the exogenous cysteine further reduced released As^{5+} into As^{3+}, resulting in high As^{3+} accumulation (88.4%) and low As^{5+} accumulation (10.8%) in solution after 18h of contact.

It is known that cysteine binds As^{3+} through sulfhydryl (-S-H) groups, and the As^{3+} appeared at the surface or inside the pores of the regenerated product in the form of O-As^{3+} [33,49]. Hence, it is concluded that the electronegative $-S^-$ in the -S-H group attacks the electropositive As^{3+} in O-As^{3+}, activating the O-As^{3+} bond and releasing As^{3+} together with the formation of OH at acidic conditions. A rapid release occurred with As^{5+}, which exists in the form of O-As^{5+}, due to the stronger interaction between $-S^-$ and As^{5+} with greater electropositive. As a result, As^{5+} has much higher extraction efficiency than As^{3+} by bioleaching. However, excessive cysteine triggered a strong reduction of As^{5+} into As^{3+}, causing the accumulation of highly toxic As^{3+} in solution.

In order to probe the chemical states of remaining As^{5+} and As^{3+} in the regenerated products after process, the raw regenerated product, bioleached residues and cysteine-enhanced bioleached residues were further characterized by using XPS (**Fig. 10**). It was evident that both As^{5+} and As^{3+} existed in the raw samples (**Fig. 10b**), with the content of both As^{5+} and As^{3+} decreasing in the bioleached residues owing to the partial removal of As^{5+} and As^{3+} (**Fig. 10c**). Both As^{5+} and As^{3+} disappeared in the cysteine-enhanced

bioleached residues because of the complete removal of As (**Fig. 10d**). The addition of cysteine at 100 ppm markedly enhanced the removal of both As^{5+} and As^{3+} from the regenerated product, but the strong reduction of As^{5+} to more toxic As^{3+} by the added cysteine represents a negative effect of the application of the active cysteine.

In recent years, the metal resistance mechanisms of another important bioleaching microbe *Acidithiobacillus ferrooxidans* (*A. ferrooxidans*) have been explored based on gene expression, enzyme activities and metabolic profile analysis [50,51]. In the presence of high concentrations of Cd^{2+} or Zn^{2+}, *A. ferrooxidans* produced nearly double the control content of intracellular cysteine and glutathione (GSH) to chelate and sequestrate the toxic metals, while the content of cysteine and GSH declined in the presence of even rather low concentrations of Pb^{2+}. Different from *A. ferrooxidans*, the current study has first demonstrated that *A. thiooxidans* generates high amounts of cysteine as a major metabolite and secretes it extracellularly into the bioleaching liquor in the absence of toxic metals. This suggests that *A. thiooxidans* and *A. ferrooxidans* may have differing regulation of sulfur metabolic pathways which requires further study.

Because of the high activity of sulfhydryl groups, exogenous cysteine has been examined previously to improve the bioleaching performance for sulfide minerals by acidophilic bacteria [52,53]. A speculated mechanism involving the formation of disulphide Cys-S-S-minerals was proposed [53], but widely different effects even complexly opposite effects occurred with different sulfide minerals. For instance, the addition of cysteine promoted bioleaching of Ni-Cu sulfide, whereas strong inhibition of bioleaching occurred with marmatite by *A. caldus* in the presence of cysteine [52].

The effects of cysteine strongly depended on the presence of ferric sulfate [54]. In the absence of $Fe_2(SO_4)_3$ cysteine inhibited the dissolution of $CuFeS_2$ in a H_2SO_4 solution due to adsorption on the surface of the electrode. In the presence of $Fe_2(SO_4)_3$ cysteine was oxidized to cystine, whose reduction on the surface of $CuFeS_2$ electrode resulted in increased dissolution of $CuFeS_2$. To date, a convincing explanation is not available about the mechanisms of cysteine-mediated bioleaching of sulfide minerals. In this work, however, the highly active bioleaching liquor, which contained a large amount of excreted cysteine, was used to release As from the regenerated product as indirect bioleaching for the first time. A previously unreported mechanism involving cysteine enhancement of As extraction and activation of the regenerated product was proposed based on the nucleophilic attack of both O-As^{3+} and O-As^{5+} by the highly reactive -S-H groups in cysteine in the absence of $Fe_2(SO_4)_3$. The addition of cysteine further improved the removal efficiency for As, although, a strong reduction of As^{5+} to As^{3+} lead to more generation of highly toxic As^{3+}.

4. Conclusions

The eco-friendly and economical bioleaching was first used to deeply remove the highly toxic As from the SCR catalyst regenerated product in order to ensure that the final residual concentration of As is below the permitted limit value of 200 mg/kg. The indirect bioleaching by *A. thiooxidans* displayed a higher removal efficiency for As and stronger activation of the regenerated product than abiotic H_2SO_4 leaching. As low as 120 mg/kg of As residue from an initial dosage of 370 mg/kg was achieved by bioleaching at 35℃ at a pulp density of 5.0%, and 174 mg/kg As residue was still

attained even at a pulp density of 10% by elevating the leaching temperature to 80℃, below the accepted limiting dosage of 200 mg/kg [23]. In contrast, H_2SO_4 leaching resulted in 257 and 345 mg/kg As in the residue at pulp densities of 5.0% and 10%, respectively. In addition, an evident increase in polar active sites, surface energy, pore volume and NH_3 adsorptive capacity occurred with bioleaching, leading to marked activation of the resulting regenerated product which ensures a high catalytic activity.

It was the first time to demonstrate that *A. thiooxidans* generates high amounts of cysteine as a major metabolite and secretes it extracellularly in the absence of toxic metals. The *A. thiooxidans*-derived high concentration of cysteine in the bioleaching liquor is responsible for the high removal of As and strong activation of the regenerated product. A novel mechanism has been proposed to explain the high bioleaching efficiency for As based on nucleophilic attack of S in the sulfhydryl groups of the cysteine. The electronegative $-S^-$ attacks the electropositive As^{3+}/As^{5+} in O-As bonds, activating the O-As and releasing As^{3+}/As^{5+} together with the formation of OH under acidic conditions. Because of greater electropositivity, As^{5+} possesses much higher extraction efficiency than As^{3+}.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Conflicts of interest

There are no conflicts to declare.

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Captions

Fig. 1. The removal efficiency of toxic element As (a, b, c), residual concentration of As (d) and loss efficiency of valuable metals W, V and Ti (e) by bioleaching under different pulp densities from 5.0% to 10% at altered temperatures from 35℃ to 80℃.

Fig. 2. The SEM-EDX images of the raw sample (a), chemical-leached sample (b) and bioleached sample (c) under a pulp density of 5.0% at 35℃ after 24h of contact.

Fig. 3. The XRD image (a) and Raman spectra (b) of the raw sample, chemical-leached sample and bioleached sample at a pulp density of 5.0% at 35℃ after 24h of contact.

Fig. 4. The TG (a) and DTG (b) curves of the raw sample, chemical-leached sample and bioleached sample at a pulp density of 5.0% at 35℃ after 24h of contact, and the SEM images (c, d, e, f, g, h) of the raw sample by calcination under different high temperatures from 200℃ to 1000℃.

Fig. 5. The contact angels and surface chemical properties of the raw sample (a, d), chemical-leached sample (b, e) and bioleached sample (c, f) by the water test (a, b, c) and the ethylene glycol test (d, e, f) under a pulp density of 5.0% at 35℃ after 24h of contact.

Fig. 6. The BET (a) and NH_3-TPD (b) curves of the raw sample, chemical-leached sample and bioleached sample at a pulp density of 5.0% at 35℃ after 24h of contact.

Fig. 7. The FT-IR spectra (a) and HPLC image (b) of the cysteine (100 ppm) and the active bioleaching liquor derived from *A. thiooxidans*.

Fig. 8. Three-dimensional (3D) fluorescence spectrometry of the cysteine (100ppm) (a), and the active bioleaching liquor under different incubation time of 5, 9 and 12 days (b, c, d), and the change of 3D-fluorescence spectrometry before and after bioleaching at a pulp density of 10% (e/i, f/j, g/k, h/l).

Fig. 9. The concentration change of As^{5+} and As^{3+} in the solution during leaching of the regenerated products by H_2SO_4-leaching (a), bioleaching (b) and cysteine-added (100ppm) enhanced bioleaching (c) as a function of contact time.

Fig. 10. The XPS spectra of different kinds of catalyst samples, full spectra (a), As 3d of raw sample (b), As 3d of bioleached sample (c) and As 3d of cysteine-enhanced (100ppm) bioleached sample (d).