A novel green approach for fabricating visible, light sensitive nano-broccoli-like antimony trisulfide by marine Sb(v)-reducing bacteria: Revealing potential self-purification in coastal zones

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\begin{abstract}
Antimony trisulfide (Sb\textsubscript{2}S\textsubscript{3}) is industrially important for processes ranging from a semiconductor dopant through batteries to a flame retardant. Approaches for fabricating Sb\textsubscript{2}S\textsubscript{3} nanostructures or thin films are by chemical or physicochemical methods, while there have been no report focused on the biological synthesis of nano Sb\textsubscript{2}S\textsubscript{3}. In the present study, we fabricated nano-broccoli-like Sb\textsubscript{2}S\textsubscript{3} using Sb(V) reducing bacteria. Thirty four marine and terrestrial strains are capable of fabricating Sb\textsubscript{2}S\textsubscript{3} after 1–5 days of incubation in different selective media. The nano-broccoli-like bio-Sb\textsubscript{2}S\textsubscript{3} was light sensitive between 400–550 nm, acting as a photo-catalyst with the bandgap energy of 1.84 eV. Moreover, kinetic and mechanism studies demonstrated that a k value of \(0.27\) h\(^{-1}\) with an \(R^2 = 0.99\). The bio-Sb\textsubscript{2}S\textsubscript{3} supplemented system exhibited approximately 18.4 times higher photocatalytic activity for degrading methyl orange (MO) to SO\textsubscript{4}\textsuperscript{2-}, CO\textsubscript{2} and H\textsubscript{2}O compared with that of control system, which had a k value of \(0.015\) h\(^{-1}\) (\(R^2 = 0.99\)) under visible light. Bacterial community shift analyses showed that the addition of S or Fe species to the media significantly changed the bacterial communities driven by antimony stress. From this work it appears Clostridia, Bacilli and Gammaproteobacteria from marine sediment are potentially ideal candidates for fabricating bio-Sb\textsubscript{2}S\textsubscript{3} due to their excellent electron transfer capability. Based on the above results, we propose a potential visible light bacterially catalyzed self-purification of both heavy metal and persistent organic contamination polluted coastal waters.
\end{abstract}

1. Introduction

Antimony trisulfide (Sb\textsubscript{2}S\textsubscript{3}) is industrially important for processes ranging from a semiconductor dopant through batteries to a flame retardant due to its unique properties, including convenient band gap tuning, the capacity for multiple excitations, high absorption coefficient, intrinsic large dipole moment, and solution processability [1–3]. Accordingly, a lot of studies have been conducted for preparing Sb\textsubscript{2}S\textsubscript{3} with various morphologies including nanoparticles [4], nanowires [5], nanotube [6], dendrites [7] and etc. So far, approaches for fabricating Sb\textsubscript{2}S\textsubscript{3} nanostructures or thin films are by chemical or physicochemical methods, while there have been no report focused on the biological synthesis of nano Sb\textsubscript{2}S\textsubscript{3} [1,2,4–8].

Among all reported chemical approaches, solvothermal and hydrothermal methods were the most investigated processes due to their advantages of simple step and high efficiency [4,6–9]. For example, Lu et al. had realized the large scale formation of uniform Sb\textsubscript{2}S\textsubscript{3} nanorod-bundles via a simple and mild hydrothermal approach [9]. As previously reported, chemical syntheses usually offer commendable control over material structure and properties, resulting in excellent performance [4–8]. However, the green fabrication of metal chalcogenides (MChs, including Sb\textsubscript{2}S\textsubscript{3}) using microbes is gaining increased attention because of its low cost and eco-friendly [10]. Various bacteria, including Escherichia coli [11], Shewanella [12], Klebsiella [13], Desulfovibrio [14], Clostridiaceae [15] and Idiomarina [16], have been harnessed for the synthesis of a broad range of MChs [10–16]. However, there has been no report focused on the biosynthesis of Sb\textsubscript{2}S\textsubscript{3}.

Actually, there is a theoretical possibility to biologically produce Sb\textsubscript{2}S\textsubscript{3} by synchronous reduction of S(VI, IV, II) and Sb(V) since some bacteria, including sulfur reducing bacteria (SRB) and iron reducing bacteria (IRB) are capable of reducing sulfate, sulfite, thiosulfate or element sulfur into H\textsubscript{2}S [17–19]. Then Sb\textsubscript{2}S\textsubscript{3} is one of the probably reduction products...
if some of the above-mentioned microbes can also reduce Sb(V) to Sb (III). If not, in consideration of only three pure cultured bacteria that are capable of reducing Sb(V) to Sb(III) were isolated so far [20–22], the SRB and IRB are of particular concern in fabricating biosynthetic antimony trisulfide (bio-Sb2S3) in the presence of Sb(III). On the basis of these hypotheses, we conducted the present study with the aim of (i) developing a novel biological approach to fabricate Sb2S3; (ii) characterizing the as-prepared bio-Sb2S3; (iii) discussing the key population with the ability of fabricating Sb2S3.

In this study, the directional selection of bacterial resources that are capable of fabricating Sb2S3 was firstly conducted by using different selective media. Then, the preparation process of Sb2S3 (a pure cultured bacteria (Fig. S1). and the as-prepared bio-Sb2S3 was further confirmed through multiple characterizations. In addition, the catalytic performance of bio-Sb2S3 was also investigated via related photocatalytic experiments. Finally, the key microbiologic population with the ability of fabricating Sb2S3 was underlined from the perspective of bacterial community shift. Based on the above results, we propose a potential visible light bacterially catalyzed self-purification of both heavy metal and persistent organic contamination polluted coastal waters.

2. Materials and methods

2.1. Chemicals and materials

All commercially available chemicals were prepared from China National Pharmaceutical Group Co., Ltd (China) and Shanghai Macklin Biochemical Co., Ltd (China). All reagents used in the present study are of the highest analytical grade. The samples were taken from both sea and land. The marine sediment (1–5 cm) was taken from the deep-water region of Bohai Straits (Fig. S1), where was reported as one of the areas with high Sb concentrations in the Bohai Strait [22]. In addition, the terrestrial top soil (1–5 cm) was taken from a gold ore tailings disposal area in Zhaoyuan in view of the abundance of metal resistant bacteria (Fig. S1).

2.2. Media and cultivation

The enrichment media for screening Sb(V) reducing bacteria (Sb-RB), SRB (SRB-M) and IRB (IRB-M), LB medium and the modified 2216E medium (M2216EM) used in the present study are listed in the electronic supplemental information (SI, please see “materials and methods”). In addition, a selective medium (SM) used in this study contained 1.0 g/L NH4Cl, 0.8 g/L Na2HPO4, 0.2 g/L KH2PO4, 0.2 g/L MgCl2, 0.01 g/L CaCl2·2H2O, 2.0 g/L sodium lactate, 6 mM S2O32−, 1 mM Sb(V) and 100 mL distilled water (for terrestrial microbes) or 100 mL water region of Bohai Straits (Fig. S1), where was reported as one of the areas with high Sb concentrations in the Bohai Strait [22]. In addition, the terrestrial top soil (1–5 cm) was taken from a gold ore tailings disposal area in Zhaoyuan in view of the abundance of metal resistant bacteria (Fig. S1).

2.3. Isolation and identification of bacteria

The spread plate method was used to isolate individual bacterial cells from the enriched culture, first, the single colonies were picked and inoculated to the different liquid enrichment culture media. Then, the purified isolates with different 16S rRNA gene sequences were stored in related medium with 15 % glycerol at −80 °C. Finally, all terrestrial and marine isolates were aerobiocically cultured in LB or M2216E overnight, collected by centrifugation (10,000 rpm, 5 min), and further identified by the SM in a 130 mL autoclaved serum bottle after treating with 99 % N2 for at least 15 min. If the color of SM changed to orange-red (due to the formation of Sb2S3) after incubation, the strain was considered as potential candidate for fabricating bio-Sb2S3. The identification and classification of all candidates were further conducted based on 16S rRNA gene sequencing and homology analyses. After comprehensive comparisons, strain X3 was selected in the following studies, and it was further characterized by scanning electron microscope (SEM) and 16S rRNA gene sequencing analyses.

2.4. Preparation of bio-Sb2S3

The selected strain X3 was first cultured aerobiocically overnight in 100 mL M2216E (1 %, v/v) in a rotary incubator shaker at 30 °C (150 rpm). The cells were harvested by centrifugation (5 min, 10,000 rpm) and washed twice with a HEPES solution (20 mM, pH 7.2 ± 0.2). Then, 0.5 g cell/L X3 cells (dry weight) were re-suspended with SM and held in an anaerobic chamber. The experiment was conducted in a 130 mL autoclaved serum bottle supplemented with 100 mL SM (treated with 99 % N2 for at least 15 min) in dark for ~1–5 days. Don't stop the reaction until the color changed to deep orange-red. Then, the reaction mixture (centrifuged at 10,000 rpm, 5 min) was treated with 1 % sodium dodecyl sulfate (SDS) detergent (95 °C, 30 min) [24]. The new mixture was treated with ultrasonic processing for 30 min, washed twice with deionized water and harvested by centrifugation (11,000 rpm, 10 min). Finally, the obtained precipitate was dried at ~40 °C for 24 h in a vacuum freeze drier. The obtained dry powder is bio-Sb2S3.

2.5. Characterizations of bio-Sb2S3

Scanning electron microscope-energy dispersive X-ray (SEM-EDX, Hitachi S-4800, Japan), transmission electron microscope (TEM, JEM 1400 Plus, Japan), ultraviolet-visible diffuse reflection spectrum (UV-vis DRS, Jasco V560, Japan), Fourier transform infrared spectroscopy (FTIR, Jasco FT/IR-4100, Japan), X-ray photoelectron spectroscopy (XPS, ESCALAB 250Xi, England) and X-ray diffraction patterns (XRD, BRUKER D8 ADVANCE, Germany) were employed to identify the material surface, morphology, photoelectric properties, chemical group changes, valence state of each element and crystalline phase of the bio-Sb2S3.

2.6. Catalytic performance on photo-degradation of methyl orange

In a typical procedure, 50 mL solution containing 10 mg/L methyl orange (MO) was prepared. After adding 30 mg/L obtained bio-Sb2S3 to the solution, the resulted mixture was mixed and stirred for 1 h at ambient temperature in dark to achieve adsorption equilibrium. Then, irradiate visible light (λ max = 400 − 700 nm) was obtained using a 500 W long-arc xenon lamp (Shanghai Jiguang Special Lighting Appliance Factory, China; 35°1 cm) with a lamp cover (approximately 50 cm above the reaction systems and equipped with cooling water). Aqueous samples (2 mL) were taken and the supernatant liquid was obtained by centrifugation (2 min, 12,000 rpm) for analysis at selected time intervals. Each experiment was conducted in triplicate.

2.7. Analytical methods

The concentrations of Sb species and the dissolved sulfide were analyzed as described in our previous study [22]. The products of
photo-catalytic reaction were analyzed using mass spectrum fitted with Sapphire C18 column (4.6 mm × 200 mm). The mobile phase consisted of methanol and water (70:30, v/v) at 1.0 mL/min. A modified zero-order model and a pseudo-first-order model were applied to describe the kinetics of MO degradation. The zero-order constant and first-order rate constant $k$ (h$^{-1}$) and $k_1$ (h$^{-1}$) are determined according to the following Eq. (1) and (2):

$$1 - C_t/C_0 = k_0 t$$  \hspace{1cm} (1)

$$\ln\left(\frac{C_t}{C_0}\right) = -k_1 t$$  \hspace{1cm} (2)

where $t$ (h), $C_0$ (mg/L) and $C_t$ (mg/L) are the reaction time, the initial and residual MO at time zero and t, respectively. The energy band gaps ($E_g$) of the as-prepared bio-Sb$_2$S$_3$ were calculated according to the Eq. (3):

$$\alpha h \nu = A (h \nu - E_g)^{1/2}$$  \hspace{1cm} (3)

where $\alpha$, $h$, $\nu$, $A$ and $E_g$ represent absorption coefficient, Planck constant, light frequency, proportionality constant and band gap, respectively.

2.8. DNA extraction and 16S rRNA gene sequencing

Culture samples were collected at the beginning and end of each experimental system as described in section 2.2. Total genomic DNA was extracted from each sample by using PowerSoil DNA Isolation Kit (MoBio, Carlsbad, CA, USA), according to the manufacturer's instructions. DNA concentration and quality were measured using a NanoDrop spectrophotometer (Thermo Scientific, Inc., USA). PCR amplification of 16S rRNA gene (V4 region) using universal primers 515F and 806R was performed under the following conditions: 95 °C, 3 min; 32×(95 °C, 30 s; 55 °C, 30 s; 72 °C, 30 s); 72 °C, 10 min. Then, the amplicon library was built followed by sequencing using an Illumina Miseq platform from Novogene Science & Technology (Tianjin) Co., Ltd.

2.9. Data processing and statistical analysis

Sequences with > 97% similarity were assigned to the same operational taxonomic unit (OTU) and the abundance of OTUs was normalized before subsequent analyses. Alpha diversities including Observed-species, Shannon, Simpson, Chao1, ACE, Goods coverage and PD whole tree and beta diversities on weighted/unweighted unifrac distances and Bary-Curtis were calculated by using QIIME software (Version 1.9.1). Principal component analysis (PCA) and principal coordinate analysis (PCoA) were analyzed using R software (Version 2.15.3). Unweighted pair-group method with arithmetic means (UPGMA) clustering was executed to illustrate the weighted and un-weighted unifrac matrix using average linkage and run by QIIME software (Version 1.9.1). In addition, Tukey's test and wilcox's test were used to test statistical differences of alpha diversity and beta diversity between groups (p < 0.05).

3. Results and discussion

3.1. The directional selection of bacterial resources for Sb$_2$S$_3$ biosynthesis

In order to facilitate the practical application in the future, all candidates selected for Sb$_2$S$_3$ biosynthesis in the present study are facultative anaerobic bacteria that can proliferated rapidly under aerobic conditions. First, Sb(V) reducing bacteria (Sr-RB) were enriched from terrestrial soil and marine sediment, respectively. The enrichment culture was transferred repeatedly to the new medium 7 times during 42 days of cultivation. As a result, 5 marine Sr-RB and 6 terrestrial Sr-RB were isolated from marine sediment and terrestrial soil, respectively (Fig. 1 and Table S1). Further studies showed that most marine Sr-RB were more efficient than the terrestrial Sr-RB in reducing thiosulfate (data not shown) and fabricating Sb$_2$S$_3$ (Table S1), which might due to the relatively rich concentration of sulfate in marine environment. On the basis of these result, marine sediment was further selected for the enrichment of SRB and IRB that capable of reducing Sb(V) to Sb(III).

Then, 12 SRB and 11 IRB were eventually isolated as shown in Fig.1 and Table S1. All these 23 strains were able to fabricate Sb$_2$S$_3$ when thiosulfate was used as the sole S source. However, those stains fail to fabricate Sb$_2$S$_3$ when sulfate was used as the sole S source due to that they can't reduce sulfate directly (Table S1). According to the 16S rRNA data, the phylogenetic tree of stains selected from different media was shown in Fig.1. As can be seen, Bacillus, Halomonas, Acinetobacter, Sporosarcina, Stenotrophomonas are the dominant bacteria that can efficiently fabricate Sb$_2$S$_3$ (Fig.1 and Table S1). Through a comprehensive consideration of their novelty, growth rate, Sb(V) and thiosulfate re-ducing efficiencies, Halomonas sp. × 3 was thus selected for preparing bio-Sb$_2$S$_3$ in the following experiments.

Fig. S2 showed the morphology of colony, SEM image, and phylogenetic tree of Halomonas sp. × 3. When strain X3 was grown on an M2216E agar plate under aerobic conditions, its colony was round, opaque and yellowish-brown (Fig. S2a). The SEM analysis showed that the morphology of strain X3 is a rod-shaped bacterium with dimensions of 1.8 × 0.4 μm$^2$ (Fig. S1b). On the basis of the 16S rRNA gene sequences, the homology between strain X3 (GenBank accession number MN428805) and Halomonas biodiversity LC1 (GenBank accession number NZ_JH393258.1) is ∼ 97 %. Thus, it can be concluded that strain X3 belongs to the genus Halomonas. The phylogenetic tree of strain X3 was shown in Fig. S2c. To the best of our knowledge, strain X3 is the first reported marine Halomonas sp. that capable of fabricating bio-Sb$_2$S$_3$ suggesting it has potential applications in Sb$_2$S$_3$-related industry.

So far, the preparation of Sr-B$_2$S$_3$ using biological resources in bio-chemical manner has been not investigated yet. In the present study, the feasibility of biological preparation of Sr-B$_2$S$_3$ using bacterial resources was first studied. Sb(V) and S$_2$O$_3^{2-}$ were selected for Sr-B$_2$S$_3$ biosynthesis, since Sr(III) is more toxic to microbes than Sb(V) [25], and S$^{2-}$ could damage the cell membrane of bacteria due to its permeation in the undisassociated form [26]. Compared to the detailed data on microbial reduction of S species, however, the knowledge of microbial Sb(V) reduction is much limited [27]. To the best of our knowledge, there are only three reported pure culture bacteria that capable of reducing Sb(V) to Sb(III) [20-22]. Recently, more and more studies have focused on the reduction of high valence metal ions with marine microorganisms, due to their strong adaptability to bad environment as they get exposure to such unfavorable conditions naturally [28]. Besides, marine ecosystems are thought to be excellent resource of metal-tolerant microbes due to continuously release of metals via human or natural activity (Such as urban sewage and volcanoes). Accordingly, marine sediment was thus selected as bacterial source. Based on the result of our macroscopic experiment, we found that SRB-M was the most efficient medium for isolating Sr-RB bacteria under laboratory conditions.

3.2. Characteristics of bio-Sb$_2$S$_3$

In the present study, we fist examined the feasibility of strain X3 in fabricating bio-Sb$_2$S$_3$. As shown in Fig. S3, the color of reaction systems changed from white to orange-red after 5 days of static incubation in dark, indicating the formation of Sb$_2$S$_3$. The suspension was treated according to the steps described in Section 2.4, and the orange-red powder is considered as bio-Sb$_2$S$_3$ (insert photo in Fig. S3b). However, the UV-vis spectra between C-Sb$_2$S$_3$ and Bio-Sb$_2$S$_3$ are very different. A possible explanation is that they have different crystal phases.

The SEM results of bio-Sb$_2$S$_3$ were shown in Fig. 2a and b. As can be seen, the as-prepared bio-Sb$_2$S$_3$ displayed a regular aggregate morphology, which consisted of a great quantity of broccolli-like particles with nanoscale size and rough surfaces (Fig. 2b). The TEM images of the as-prepared bio-Sb$_2$S$_3$ were shown in Fig. 2c and d. It is observed that the morphology and size of biosynthetic Sb$_2$S$_3$ are accord with the
results of SEM observation. EDX analysis of bio-Sb$_2$S$_3$ showed that Sb and S peaks were appeared and the atomic ratio of Sb/S is approximately 2/3 (Fig. 2e), indicating the formation of Sb$_2$S$_3$ (confirmed by subsequent XPS analysis). In addition, it is noticeable that the morphology and composition of bio-Sb$_2$S$_3$ are much different from that without SDS and ultrasonic treatments (Figs. 2 and S4). Compared with the bio-Sb$_2$S$_3$ without SDS and ultrasonic treatments, the morphology of bio-Sb$_2$S$_3$ treated with SDS (in hot water bath) and sonication is more regular. Meanwhile, the treated bio-Sb$_2$S$_3$ has less C/O-contained compounds (FigS. 2e and S4c), indicating the removal of cell fragments and extracellular polymeric substances.

XPS analysis was employed for evidencing the chemical state information and electronic properties of biologically prepared Sb$_2$S$_3$. As a matter of fact, strain X3 had proved to be capable of reducing S$_2$O$_3^{2−}$ and Sb$^{5+}$ since S$_2^{−}$ and Sb$^{3+}$ could be detected in the above-mentioned SM. In this part, XPS results provide more hard evidence for the reduction of S$_2$O$_3^{2−}$ and Sb$^{5+}$ (Fig. 3a and b). The Sb 3d$_{3/2}$ and Sb 3d$_{5/2}$ peaks is representative for Sb species. As shown in Fig. 3a, the two apparent symmetrical characteristic spin-orbit splitting of Sb 3d peaks at 529.2 eV and 535.5 eV were assigned to Sb 3d$_{3/2}$ and Sb 3d$_{5/2}$, suggesting most Sb(V) is reduced to Sb(III). In addition, the distance between Sb 3d$_{5/2}$ and Sb 3d$_{3/2}$ peaks is approximately 9.3 eV, which is in line with previous study [8]. As can be seen in Fig. 3b, the two characteristic peaks for S 2p$_{1/2}$ and S 2p$_{3/2}$ in bio-Sb$_2$S$_3$ located at 162.3 eV and 161.2 eV indicate the coexistence of bonded S$_2^{−}$ (Sb$_2$S$_3$) and free S$_2^{−}$.

To characterise the crystal structure of bio-Sb$_2$S$_3$, XRD analysis was thus performed for both bio-Sb$_2$S$_3$ and a commercial Sb$_2$S$_3$ (C-Sb$_2$S$_3$, Prepared by chemical method and purchased from Aladdin Industrial Corporation). As shown in Fig. 3c, the XRD patterns of commercial C-Sb$_2$S$_3$ could be well indexed as the orthorhombic phase (JCPDS No. 42-1393). For the XRD pattern of bio-Sb$_2$S$_3$, some obvious characteristic peaks were appeared, including the (020) peak, (240) peak and (440) peak. However, the intensity of these peaks varied, indicating that the exposed surfaces of commercial Sb$_2$S$_3$ and bio-Sb$_2$S$_3$ were much different. A possible explanation is that the presence of strain X3 affects the crystallinity of Sb$_2$S$_3$, and simultaneously, the residual cell fragments or extracellular polymeric substances adsorbed on the surface of bio-Sb$_2$S$_3$ may have a negative effect on XRD pattern.

FTIR spectra can reveal possible physical and chemical interactions between Sb$_2$S$_3$ and chemical groups on the cell surface of X3, we further investigated the Sb$_2$S$_3$ before and after treating with SDS (in hot water bath) and sonication with FTIR analyses. As can be seen in Fig. 3d, some peaks decreased significantly after above-mentioned treatment. These changes are center at 1060−1729 cm$^{-1}$, attributed to characteristic absorption bands of C−O−C, −COOH, −CONH, and etc. These amides and carboxyl groups mainly belong to cell fragments or extracellular secretion (glycolipids, proteins, and etc.) of strain X3. Besides, the
characteristic absorption peaks around $\sim 624 \text{ cm}^{-1}$ are related to $S$-contained groups. These findings imply that the SDS and ultrasonic treatments removes a large amount of surface attachments on the as-prepared bio-$\text{Sb}_2\text{S}_3$, which is consistent with our previous SEM results.

3.3. Catalytic performance of bio-$\text{Sb}_2\text{S}_3$ on MO photo-degradation

Before study the catalytic performance of bio-$\text{Sb}_2\text{S}_3$ on MO photo-degradation, the optical adsorption property of bio-$\text{Sb}_2\text{S}_3$ was first analyzed. As shown in Fig. 4a, both the C-$\text{Sb}_2\text{S}_3$ and bio-$\text{Sb}_2\text{S}_3$ samples exhibited intense absorption bands in the visible-light regions. One main concern is that the absorption edge of bio-$\text{Sb}_2\text{S}_3$ (400−550 nm) showed a blue-shift compared to that of C-$\text{Sb}_2\text{S}_3$ (400−750 nm). This phenomenon may attributed to the difference between their degrees of crystallinity, since Li et al. also found that the absorption edge of amorphous $\text{Sb}_2\text{S}_3$ is lower than that of crystal $\text{Sb}_2\text{S}_3$ [8]. In addition, the bandgap energies of C-$\text{Sb}_2\text{S}_3$ and bio-$\text{Sb}_2\text{S}_3$ were determined to be 1.51 and 1.84 eV, respectively (Fig. 4b). Surprisingly, bio-$\text{Sb}_2\text{S}_3$ has a wider bandgap than C-$\text{Sb}_2\text{S}_3$. As indicated in previous studies, development of large bandgap (1.80−1.85 eV $E_g$) semiconductor materials is crucial for photo-catalysts, as they could prevent the excessive combination of carriers and thus facilitate their application [29]. Thus, bio-$\text{Sb}_2\text{S}_3$ maybe has potential application in the preparation of nano-sized photocatalysts.

To demonstrate the photocatalytic activity of bio-$\text{Sb}_2\text{S}_3$, the photo-degradation of MO was investigated in the presence of bio-$\text{Sb}_2\text{S}_3$. The bio-$\text{Sb}_2\text{S}_3$-mediated photo-degradation curves of MO were illustrated in Fig. S5a. In the absence of a photocatalyst, less than 5 % MO decomposition could be observed within 3 h under visible light irradiation. In the presence of bio-$\text{Sb}_2\text{S}_3$, more than 85 % MO decomposition could be achieved after 3 h of photo-catalytic reaction. On the basis of these data, the kinetic analyses were further carried out by using the modified zero-order model and pseudo-first-order model (Fig. 4c and d). As can be seen, the data could be well described by zero-order equation ($R^2 = 0.99$, Fig. 4c). The $k_0$ values of bio-$\text{Sb}_2\text{S}_3$ supplemented system and control system are 0.2712 h$^{-1}$ and 0.01473 h$^{-1}$, respectively. For the pseudo-first-order model, the $k_1$ value for above two systems are 0.4244 h$^{-1}$ ($R^2 = 0.94$) and 0.00724 h$^{-1}$ ($R^2 = 0.99$), respectively (Fig. 4d). Besides, according to the data obtained in the bio-$\text{Sb}_2\text{S}_3$ supplemented system, MO degradation could be divided into a two-step reaction with different $k$ values ($k_a 0.3514$, $R^2 0.98$; $k_b 1.0005$, $R^2 0.95$; $k_a$ and $k_b$ are k value of the first and second step, respectively; Fig. 4d).

We speculate that the enhanced reaction rate is due to gradual shedding of substances adsorbed on the surface of bio-$\text{Sb}_2\text{S}_3$ and more active sites.
are exposed, which could enhance the separation and migration efficiency of the electron-hole pairs [8]. In addition, the k values (h⁻¹) of bio-Sb₂S₃-mediated MO degradation and that of two chemical synthetic Sb₂S₃-mediated MO degradation were compared (the related data were 0.87 h⁻¹ and 2.14 h⁻¹, respectively). The results showed that the catalytic activity of biological synthetic Sb₂S₃ was not as good as the two chemical synthetic Sb₂S₃. However, the k value of the second-step (k₂) of bio-Sb₂S₃-mediated system was at similar level compared to that of some chemical synthetic Sb₂S₃-mediated system.

As shown in Fig. S5b, the variation in UV–vis absorption spectra of MO was measured at different reaction time point for bio-Sb₂S₃ supplemented systems. The intensity of MO’s characteristic peak (∼463 nm) gradually disappeared within 3 h irradiation time. To investigate the degradation products, mass spectrometry was employed and the results were shown in Fig. 4e and f. After 0.5 h of reaction, ∼11 possible intermediates (marked as M1 ∼ M7 and M2’ ∼ M4’, illustrated in Fig. S6) could be observed in Fig. 4e. When the reaction went on, the concentration of M1 (MO without Na+) decreased sharply, and M5 (SO₄²⁻) became final product with the highest concentration (Fig. 4f).

On the basis of these detected intermediates, two possible degradation pathways of MO in the presence of bio-Sb₂S₃ under visible light irradiation were proposed: (i) the first path was started with oxidation nearby the azo group and (ii) the second path was started with demethylation (Fig. S6). Moreover, what these two paths have in common is that they all end up with an accumulation of sulfate, which is consistent with the results of product characterization.

3.4. Bioinformatic analyses

Although the feasibility of biological preparation of visible-light sensitive Sb₂S₃ had been verified through above experiments, we need to know more about which types of microbe were potential candidates for bio-Sb₂S₃ synthesis. Thus, we further investigated the change of bacterial communities in different cultivation systems by illumina sequencing based on 16S rRNA genes. After 42 days of microcosm cultivation, the main alpha diversity index (Observed species, Shannon, Simpson, Chao1, ACE and PD whole tree) of bacteria community in different cultivation systems decreased obviously (Table 1), indicating the decrease of microbial community and diversity. These results also suggested that all the selective media used in the present study have helped reshape the bacterial community.

As a matter of fact, the presence of S and Fe species were beneficial to sort efficient bacterial resources for Sb₂S₃ biosynthesis. According to
our results, the order of four media for the purpose of directional selection is MSRB > MFe > MSb > LSb (Fig. 1 and Table S1). As shown in Fig. 5b, the dominant phyla in all samples were Proteobacteria and Firmicutes after 42 days of directional selection. Moreover, Firmicutes were likely the most sensitive phylum to Sb species, as they increased from 0.25 % (M0) and 0.71 % (L0) to 12.21 % (MSb), 56.60 % (MSRB), 86.01 % (MFe) and 70.13 % (LSb), respectively (Fig. 5b).

From the class level, Clostridia (Firmicutes), Alpha/Gamma-proteobacteria (Proteobacteria) and Bacilli (Proteobacteria) were major bacterial communities for marine samples, while Negativicutes increased from 0.25 % (M0) and 0.71 % (L0) to 12.21 % (MSb), 56.60 % (MSRB), 86.01 % (MFe) and 70.13 % (LSb), respectively (Fig. 5b).

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**Table 1**

Summary of the alpha diversity index of bacteria community in different cultivation systems.

| Sample name | Observed species | Shannon | Simpson | Chao1 | ACE | PD whole tree | Goods coverage |
|-------------|------------------|---------|---------|-------|-----|--------------|----------------|
| M0          | 803              | 7.738   | 0.986   | 862.702 | 844.598 | 64.699       | 0.997          |
| L0          | 751              | 7.779   | 0.989   | 797.929 | 787.366 | 57.444       | 0.997          |
| LSb         | 403              | 3.794   | 0.76    | 467.667 | 479.277 | 38.764       | 0.996          |
| MSRB        | 392              | 4.911   | 0.926   | 522.245 | 518.693 | 37.565       | 0.996          |
| MFe         | 497              | 4.883   | 0.926   | 669.068 | 683.421 | 45.262       | 0.994          |
| MSb         | 372              | 3.93    | 0.773   | 468.119 | 480.024 | 38.046       | 0.996          |

Fig. 4. The UV–vis diffuse reflectance spectra (a) and Plots of $\alpha h\nu^2$ versus photon energy ($hv$) for the band gap energies of biosynthetic Sb$_2$S$_3$; $1-(C_t/C_0)$ (c) and $-\ln(C_t/C_0)$ (d) versus reaction time of MO degradation in the presence of bio-Sb$_2$S$_3$; The mass spectrum of degradation product at 0.5 h (e) and 3 h (f), respectively.
(Firmicutes) and Clostridia (Firmicutes) for terrigenous sample (Fig. S7).

As previous studies indicated, Proteobacteria and Firmicutes were widely reported as special types of bacteria that related to extracellular electron transfer \[^{30–36}\]. For instance, the Clostridium of Firmicutes and Halomonas of Proteobacteria could be used as biocatalysts to realize reduction of V(V) \[^{31}\], U(VI) \[^{32}\], Fe(III) \[^{33}\], NO\(_3^–\) \[^{34}\], Cr(VI) \[^{35}\] and to transfer electrons for microbial electricity generation \[^{36}\]. Moreover, three reported bacteria that can reduce Sb(V) to Sb(III) belong to Proteobacteria (Sinorhizobium and Shewanella) and Firmicutes (Bacillales) as well \[^{20,20}\]. Based on the macroscopic and microscopic experimental results, we declare that bacteria belonging to Clostridia, Bacilli and Gammaproteobacteria in marine sediment are ideal potential candidates for fabricating bio-Sb\(_2\)S\(_3\).

### 3.5. Implications

In the natural environment, antimony (heavy metal, HM) and MO (persistent organic pollutant, POP) are two kinds of typical environmental pollutants as they are harmful to living things. Their combined pollution may not only cause diseases to human beings but also significantly alter microbiota and thus hardly threat the ecological

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**Fig. 5.** UPGMA clustering based on (a) unweighted unifrac distance and (b) weighted unifrac distance followed by the structure of microbial communities at phylum level. L0: untreated terrigenous soil; M0: untreated marine sediment; LSb: the terrigenous soil treated with TSb-M for 45 days; MSb: the marine sediment treated with MSb-M for 45 days; MSRB: the marine sediment treated with SRB-M for 45 days; MFe: the marine sediment treated with IRB-M for 45 days.

**Fig. 6.** Principal component analysis (PCA) based on Euclidean distance (a) and Principal coordinate analysis (PCoA) based on weighted Unifrac distance (b) showing the overall distribution pattern of bacterial communities under different selective conditions. L0: untreated terrigenous soil; M0: untreated marine sediment; LSb: the terrigenous soil treated with TSb-M for 45 days; MSb: the marine sediment treated with MSb-M for 45 days; MSRB: the marine sediment treated with SRB-M for 45 days; MFe: the marine sediment treated with IRB-M for 45 days.
environment [37]. The coastal zone serves as a link connecting land and sea, the increasing industrialization and extensive agricultural activities in this area contribute to the elevated levels of HMs and POPs (including Sb(V) and MO) in seawater bodies [38,39]. In the present study, we found that some marine bacteria are capable of fabricating Sb2S3 by synchronous reduction of Sb(V) and S2O3−2, and the as-prepared bio-Sb2S3 could be used as catalyst for fully photo-degradation of MO. In consideration of sunlight and the widely presence of S-contained salts in shallow sea, we proposed a potential visible light bacterially catalyzed self-purification driven by special bacteria in coastal zones that may suffer from combined pollutions of Sb(V) and azo dye MO (Fig. 7). Moreover, various MChs that were reported as photocatalyst on transformation of multiple pollutants are all possibly synthesized by these mentioned bacteria [18]. Therefore, these photochemical transformation processes of environmental pollutants driven by special functional microbes may be a kind of self-purification that exists extensively in coastal zones.

4. Conclusions

In conclusion, we developed a novel green approach for fabricating visible, light sensitive nano-brocilli-like Sb2S3 by various Sb(V)-reducing bacteria. The as-prepared bio-Sb2S3 could be used as photo-catalyst for degrading MO under visible light. In addition, the addition of S or Fe species can help reshape the bacterial communities and thus enrich more ideal potential candidates (Clostridia, Bacilli and Gammaproteobacteria) for fabricating bio-Sb2S3. At last, we speculate a potential visible light bacterially catalyzed self-purification in HMs and POPs polluted coastal waters.

Supporting information

Supporting Information associated with this article can be found in the online version, at doi: https://doi.org/10.1016/j.enzmictec.2020.109514.

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Appendix A. Supplementary data

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