Effects of CdS NPs on Sulfate Bioreduction and Oxidative Stress to *Desulfovibrio Desulfuricans*

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Title: Effects of CdS NPs on sulfate bioreduction and Oxidative Stress to *Desulfovibrio desulfuricans*

Graphical abstract

Highlight

(1) The efficiency of bioreduction of sulfate and the amount of biomass could be increased by CdS NPs under light conditions.

(2) Exogenous addition of EPS and polysaccharides could increase the sulfate reduction efficiency of RSB.

(3) EPS had a significant contribution to the increase of photocurrent intensity.

(4) External addition of HA could alleviate the oxidative stress induced by CdS NPs to SRB .

1 ABSTRACT

2 Sulfate-containing wastewater has a serious threat to the environment and human
3 health. Microbial technology has great potential for the treatment of sulfate-containing
4 wastewater. It was found that nano-photocatalysts could be used as extracellular
5 electron donors to promote the growth and metabolic activity of non-photosynthetic
6 microorganisms. However, nano-photocatalysts could also induce oxidative stress and
7 damage cells. In this paper, the mechanism and regulation strategy of cadmium sulfide
8 nanoparticles (CdS NPs) on the growth of sulfate reducing bacteria and the sulfate
9 reduction process were investigated. The results shows that the sulfate reduction
10 efficiency could be increased by 6.43% through CdS NPs under light conditions.
11 However, the growth of C09 was seriously inhibited by 55.00% due to the oxidative
12 stress induced by CdS NPs on cells. The biomass and sulfate reduction efficiency
13 could be enhanced by 6.84% and 5.85%, respectively, through external addition of
14 humic acid (HA). At the same time, the mechanism of the CdS NPs strengthening the
15 sulfate reduction process by sulfate bacteria was also studied. Which can provide
16 important theoretical guidance and technical support for the development of microbial
17 technology combined with extracellular electron transfer (EET) for the treatment of
18 sulfate-containing wastewater.

19 **Keywords:** *Desulfovibrio desulfuricans*; cadmium sulfide nanoparticles; sulfate
20 reduction; EPS; oxidative stress

21 1. INTRODUCTION

22 Large amounts of industrial wastewater containing sulfate have led to
23 deterioration of water bodies and soils, which not only pollutes the natural
24 environment, but also affects human health (Radić et al. 2014). Chemical, physical,
25 and biological techniques are commonly used in the treatment of sulfate-containing
26 wastewater. Among them, biotechnology is considered to be the most promising
27 method. Commonly, it mainly used sulfate reducing bacteria to reduce sulfate to S^{2-}
28 through dissimilatory action. The carbohydrate is the electron donor, and the sulfate
29 ion is the electron acceptor.

1 Recently, it was found that microorganisms could carry out extracellular
2 respiration energy metabolism (Lovley et al. 2008), which could consume the
3 extracellular electron to promote the growth of microbial and the transference of
4 energy (Xiao et al. 2017). Microbial could achieve extracellular electron transfer
5 (EET) through the interaction of cytochromes, electronic shuttles, and others
6 conductive materials in the out membrane (Li et al. 2012; Li et al. 2019). Previous
7 studies have found that extracellular polymeric substances (EPS) could play a very
8 important role in the process of EET (Xiao et al. 2017; Sathishkumar et al. 2020). For
9 example, results of proteomic analysis of *Shewanella oneidensis* HRCR-1 showed
10 that there was a large number of proteins in extracellular polymeric substances (EPS),
11 among which c-type cytochromes and riboflavin redox substances MtrC and OmcA
12 were related to EET (Cao et al. 2011). The EPS of *S.oneidensis* MR-1 included
13 cytochromes and riboflavin redox substances (Xiao et al. 2017). The EPS of
14 *S.oneidensis* and *Pseudomonas putida* contained heme-binding protein related to EET
15 (Li et al. 2016). The quinone in humic acid could act as an electron shuttle to
16 accelerate the reduction of dissimilated ferric ion (Wolf et al. 2009). Therefore, some
17 substances in EPS could mediate EET of microorganisms.

18 As a photocatalyst, CdS NPs had the characteristics of low band gap and high
19 photosensitivity, which could generate photo-excited electrons under light conditions
20 (Prabhu et al. 2005). It was found that these electrons could be consumed to enhance
21 certain metabolic capabilities of microbial (Dong et al. 2020). Wang et al (Wang et al.
22 2017) found that hydrogen production of *Escherichia coil* was increased when the cell
23 surface precipitated CdS NPs under visible light illumination. CdS NPs could provide
24 photo-excited electrons for *Rhodopseudomonas palustris* to enhance nitrate reduction.
25 In addition, CdS NPs could be excited to generate photo-electrons and holes under
26 light condition. Then cysteine was used as an electron sacrificial agent, and the
27 electrons combine with H^+ to form [H] for *Moorella thermoacetica* to reduce CO_2 to
28 acetic acid (Gai et al. 2020). Enhanced reduction efficiency of sulfate under
29 sulfate-reducing condition was obtained by synergism between EPS and graphene

oxide (Yan et al. 2020). Therefore, the photosensitive nanomaterials could indeed provide energy for bacteria in the form of EET.

However, it was found that the hydroxyl free radicals could be generated by oxidizing water by CdS NPs under light conditions. The increased intracellular reactive oxygen species (ROS) content will cause the oxidative stress effect in the microbial cells. Chandran (Chandran et al. 2014) studied the oxidative stress effect of CdS NPs on *E.coli* (ATCC 13534), *E.coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923), respectively. It was found that more than 90% growth of biomass were severely inhibited when the cells treated with CdS NPs of 1 µg/mL. Behera (Behera et al. 2019) found that NiO NPs could inhibit the activity of *Bacillus subtilis* and *E.coli* due to the damage of ROS produced by NiO NPs. Fortunately, it has been found that many natural organic macromolecular substances could alleviate the damage of oxidative stress to microorganisms, such as humic acid (HA). Shang (Shang et al. 2017) showed that HA could reduce the distribution of CdS NPs in solution, thereby decrease the amount of the production of $\cdot\text{OH}$ and $^1\text{O}_2$. Deng (Deng et al. 2016) studied the photocatalytic activity of graphene-cadmium sulfide (G-CdS) nanocomposites on the bactericidal effect of *E. coli*, they found that the addition of HA could react with ROS to greatly reduce the toxicity of G-CdS to *E. coli*. In summary, the external addition of HA could be used as an ROS scavenger to reduce the amount of ROS generated by nanoparticles.

On the one hand, some photo-nano-catalysts can act as extracellular electron donors to promote the growth and metabolic activity of non-photosynthetic microorganisms; On the other hand, photo-nano-catalysts can also produce cytotoxicity, such as generating oxidative free radicals, which can induce oxidative stress and damage cells. Similar problems were still existed in the process using CdS NPs to promote the activity of sulfate-reducing bacteria to treat sulfate-containing wastewater, but there were no detailed public reports.

In this paper, a self-screened sulfate reducing bacteria (SRB) combined with the self-synthesized CdS NPs were adopted to explore the process mechanism of sulfate

reduction by sulfate reducing bacteria strengthened by CdS NPs. Moreover, the effect of oxidative stress on SRB induced by CdS NPs and the corresponding control strategies were also studied. The results can provide important theoretical guidance and technical support for the development of microbial technology combined with EET for the treatment of sulfate-containing wastewater.

2. MATERIALS AND METHODS

2.1 Strain isolation and identification

Sulfate reducing bacteria (SRB) was isolated from river sludge, Shanghai, China. The sludge samples containing SRBs were collected in sealing bag and stored at 4°C. The bacteria were enriched in medium containing Fe^{2+} and isolated under anaerobic incubator at 35°C. The characteristics of SRB were investigated.

2.2 Cultured medium and condition

The SRB was cultured using the Postgate medium. It was ($\text{pH } 7.0 \pm 0.2$) consisted of 0.5 g/L K_2HPO_4 , 1.0 g/L NH_4Cl , 0.1 g/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 2.0 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 2.0 g/L (60% w/v) DL-Na-lactate, 1.0 g/L yeast extract, 0.1 g/L ascorbic acid, 0.15 g/L $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$, which were obtained from Shanghai Aladdin Reagent Company (Shanghai, China). All chemical were of analytical reagent grade.

The isolated strain were incubated in the sterile medium and cultured in a 250 mL serum bottle with working volume of 100 mL at 35°C at 100 rpm in a shaker.

To provide anaerobic condition, serum bottles were flushed with N_2 (99.99%) for at least 20 min.

2.3 Synthesis of CdS nanoparticles

The CdS NPs were prepared via the hydrothermal synthesis method reported with some modification (Aboulaich et al. 2012).

The $\text{CdCl}_2 \cdot 2.5 \text{H}_2\text{O}$ aqueous solution of 0.175 mM was prepared (about 0.0390 g cadmium chloride hydrate in 20 mL aqueous solution). The pH of solution was adjusted to about 10 through NaOH aqueous solution. The thiourea aqueous solution of 0.289 mM was prepared (about 0.0219 g thiourea in 20 mL aqueous solution). Then

cadmium chloride solution and thiourea solution were mixed and stirred for 20 min. The $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ aqueous solution of 0.6337 mM (about 0.1522 g Sodium sulfide nonhydrate in 20 mL aqueous solution) was slowly dropped into the aforementioned solution under the continuous stirring at room temperature for 30 min. The mixture was transferred to a 100 mL Teflon-line stainless steel autoclave, which was sealed and placed in a 160°C oven for 8 h. After the reaction, the autoclave was cooled to room temperature naturally. Finally, the yellow precipitate was collected by high-speed centrifugation from the reaction solution. The precipitate was rinsed three times with ethanol and distilled water, and then freeze-dried for use.

2.4 Characterization

The absorption spectrum of CdS were measured using a UV-vis, Spectrophotometer (Varian, USA) in the wavelength range of 200 - 800 nm.

The crystal structure of the CdS were characterized by X-Ray diffraction (Rigaku, Japan) at voltage of 40 kV using monochromatic $\text{CuK}\alpha$ radiation.

2.5 Sulfate and cell concentration

The sulfate concentration were measured by the barium sulfate turbidimetric method. One mililiter of culture solution was centrifuged at 12000 rpm for 10 min, and 0.5 mL of supernatant was taken in a test tube. Adding 0.5 mL of distilled water, 1 mL of 0.5 M hydrochloric acid, 2 mL of polyvinyl alcohol solution, and 2 mL of saturated barium chloride solution into the tube. After mixing, the absorbance of the sample at 420 nm was determined by an ultraviolet-visible spectrophotometer in a 1 cm cuvette (UV-2102C, Unico Shanghai Instruments Co., Ltd., Shanghai, China) in three minutes later. Microscopic counting method was used to determine the amount of bacteria.

2.6 EPS extraction, purification and identification

Desulfovibrio desulfurican C09 cells after growth for 24 h were collected by centrifugation at 8000 rpm for 10 min for EPS extraction. Thermal extraction method (Dai et al. 2016) was used to extract EPS of C09. The dissolved structural EPS was dialyzed for 24 h against demineralized water in dialysis tubing with a molecular

weight cut off 3500 Da, frozen at -80°C and freeze-dried. The EPS were used for quantification and identification. Three-dimensional excitation emission matrix (EEM) fluorescence spectroscopy (Perkin Elmer, USA) and Fourier transform infrared spectroscopy (FT-IR, Nicolet, USA) were employed to identify EPS. The liquid EPS was used for three-dimensional EEM analysis. The EPS of freeze - dried were mixed with KBr in a wave number range of 4000-500 cm⁻¹ for FT-IR analysis. In order to compare the effects of EPS components on the instantaneous photo-current of CdS NPs, EPS was treated with protease (2.5 µL/mL, Proserpina K 10 mg/mL) and polysaccharase (10.0 µL/mL, Pullulanase 1000 U/mL) (Draper et al. 2020).

2.7 Analysis of photo-current

The photo-current responses of CdS NPs in the presence or absence of EPS were determined using amperometric I-t curves for 300 s in a three-electrode chamber containing a working electrode (ITO conductive glass, 1×1 cm²), a counter electrode (a platinum-wire electrode), and a reference electrode (Ag/AgCl electrode with saturated KCl solution). The 0.001 g CdS NPs and 20 µL Nafion aqueous solution were mixed in 2 mL ethanol solution and 0.1 M Na₂SO₄ aqueous solution. The photo-current change was determined by 0.3 V constant potential.

2.8 Reactive oxygen species (ROS), ·OH and Malondialdehyde (MDA) content

The bacterial intracellular ROS content was detected using an ROS assay Kit. The cells that had been cultured for 8 h were collected by centrifugation at 8000 rpm for 8 min. And then the pellet was rinsed three times with 0.1 M phosphate balanced solution (PBS), and resuspended in 1 mL 0.1 M PBS to determine OD₆₀₀. One microliter of 2', 7' - dichlorofluorescein diacetate (DCFH - DA) probe was then added to 1 mL of the cell resuspension solution. After that, it was kept away from light at 37°C for 20 min in a water bath incubator. After centrifugation at 16,000 g for 8 min, the cell pellet was washed with 1 mL of sterile water. This washing and centrifuging process was repeated for three times. The cell pellet was resuspended again in 200 µL of sterile water, shaken well, and then transferred to a non-transparent 96 - well plate. Then, it was read under a micro-plate reader with excitation and emission

wavelengths of 488 and 525 nm, respectively. The fluorescence (FL) value is positively correlated with the ROS content. OD600 was used to represent the amount of cells in a re-suspension solution. The ratio of the FL value to the OD600 (FL/OD600) was defined as the intracellular ROS content per unit cell.

The bacterial extracellular $\cdot\text{OH}$ content was detected using an $\cdot\text{OH}$ assay Kit (Nanjing Jiancheng, China) according to the manufacturer's instructions. The content of MDA was measured from the productions of MDA and thiobarbituric acid (TBA) following Draper and Hadley. (Draper et al. 1990) All test was run in triplicate.

3. RESULTS

3.1 Screening and identification of sulfate-reducing bacteria

In this study, sulfate-reducing bacteria were screened from sludge in our lab as model bacterium. After the primary and second isolation, the partial results of sulfate reduction efficiency of sulfate-reducing bacteria were shown in Fig.1. The sulfate reduction efficiency of No. 9 was the highest than those of others. The results of physiological and biochemical characteristics of No. 9 were shown in Table 1, which were consistent with those of *Desulfovibrio desulfuricans*. The results of sequence of 16S rDNA of No. 9 indicated that there were 99.7% homology similarity with that of (NR_104990.1) *Desulfovibrio desulfuricans* strain Essex 6. The phylogenetic tree of No. 9 based on 16S rDNA sequences was shown in Fig. 2. According to above results, the target strain No. 9 was identified as genus *Desulfovibrio* and named as *Desulfovibrio desulfuricans* C09.

3.2 Characterization of CdS NPs

The CdS NPs were synthesized using hydrothermal methods. The UV absorption spectra, XRD, and SEM of CdS NPs were shown in Fig. 3 a, 3 b and 3 c, respectively.

As shown in Fig. 3 a, the UV absorption wavelength of CdS NPs was scanned in the range of 200 nm-800 nm. The absorption wavelengths were in the range of 200 nm-600 nm. The maximum absorption wavelength was 506 nm.

$$E_g = 1240/\lambda g(\text{eV}) \quad (1)$$

According to the formula (1), the band gap of CdS NPs was calculated as 2.4 eV, indicating that CdS NPs had a low band gap (Alipour et al. 2021). It also had absorption characteristics in both the ultraviolet and visible regions.

The XRD pattern of CdS NPs were shown in Fig. 3 b. The characteristic diffraction peaks of CdS NPs were appeared at 2 theta of 26.5°, 43.3°, and 52.1°, respectively. They were sharp and independent of each other, indicating the high purity and cubic crystals of the CdS NPs synthesized by hydrothermal method (Riaz et al. 2020), which had better photo-catalytic effect than the hexagonal crystal system (Lang et al. 2014). CdS NPs were mainly spherical with the size between 50 nm and 150 nm, as shown in Fig.3 c.

3.3 Effect of CdS NPs on growth and sulfate reduction process of C09

When CdS NPs were combined with C09, the surface of C09 was studied by scanning electron microscopy (SEM) and energy dispersive spectrometer (EDS) with results shown in Fig. 4.

Fig. 4 a and 4 b were the images of the cells combined without and with CdS NPs, respectively. It could be clearly seen that a large number of solid particles was attached to the surface of the C09 (Fig. 4 b). Analysis results by EDS scanning revealed that Cd and S were distributed on the surface of the bacteria (Fig. 4 c). Therefore, it could be determined that there were CdS NPs attached to the surface of the C09.

The effects of 1 mM CdS NPs on the activities of growth and sulfate reduction of C09 were explored in this study with results shown in Fig. 5. It was observed that the sulfate concentration decreased with time due to the sulfate reduction of C09. The sulfate reduction efficiencies of the groups without CdS NPs and with 1 mM CdS NPs in dark were similar because of no light-excited electron generation from CdS NPs. However, the efficiencies of the group without CdS NPs and with 1 mM CdS NPs under light conditions were 46.89% and 53.32%, respectively (Fig. 5 a). This means that the reduction efficiency of C09 to sulfate could indeed be improved by CdS NPs under light conditions.

In addition, the biomass increased with time under different conditions with results shown in Fig. 5 b. Under light conditions, the biomass of the group with 1 mM CdS NPs was significantly higher than that of the other groups. While the amounts of biomass were similar between the groups with CdS NPs under dark conditions and without CdS NPs (Fig. 5 b). This means that the growth of C09 could not be affected by CdS NPs in dark conditions. Zhu et al. (Zhu et al. 2018) studied the effect of CdS NPs on the nitrate reduction efficiency of attached microorganisms under light conditions and the results showed that the addition of CdS NPs increased the removal of nitrate by 1.5 times. Wang et al. (Wang et al. 2019) combined CdS NPs on the surface of the light-energy heterotrophic bacterium *Rhodopseudomonas palustris*, which could not only enhance the nitrogen fixation capacity of the bacteria, but also increase the biomass under light conditions. Therefore, the growth and sulfate reduction efficiency of C09 could indeed be improved by CdS NPs under light conditions.

3.4 Effects of EPS on the enhanced activity of C09 in CdS NPs

It was found that microbial EPS could play a very important role in the process of CdS NPs to enhance bacterial metabolism and growth activity. Therefore, this study investigated the influence of CdS NPs on the growth of C09 and the sulfate reduction process in the presence or absence of EPS.

The experimental group without EPS (R-EPS) was added with 1 mM CdS NPs. The control group with EPS (C-EPS) was not added with CdS NPs. Regardless of whether CdS NPs were added, the sulfate concentration of all the groups decreased with time, as shown in Fig. 6 a. The sulfate reduction efficiencies of the C-EPS group and R-EPS group added with CdS NPs were 58.52% and 43.84%, respectively, while the sulphate reduction efficiencies of the C-EPS group and R-EPS group without CdS NPs were 50.19% and 48.26%, respectively as shown in Fig. 6 a. This result shown that the experimental group with EPS was retained, and the presence of CdS NPs caused the biomass of C09 to be significantly higher than that of the other groups shown in Fig. 6 b.

1 It was well known that most of microorganisms were wrapped by EPS, which
2 contains polysaccharides, proteins, humic acid, and other substances (Flemming et al.
3 2007). EPS could not only serve as a space barrier to protect microbial cells, but also
4 participate in electron transfer during cell metabolism. Zhu et al.(Zhu et al. 2018)
5 found that the reduction of nitrate was inhibited after the removal of EPS. Thus, when
6 CdS NPs were added, the sulfate reduction rate and the biomass of C09 without
7 extracellular EPS were lower than other groups, which indicating that EPS may
8 mediate the transfer of electrons produced by CdS NPs to C09 to participate in sulfate
9 reduction and bacterial growth process.

10 **3.5 The influence of CdS NPs on EPS components**

11 The effect of CdS NPs on EPS synthesis of C09 under the condition of 8.1
12 mW·cm⁻² light intensity was studied. Compared with the control group, the
13 polysaccharides, proteins, and nucleic acids in the 1 mM CdS NPs group increased by
14 142.5%, 208.88%, and 63.95%, respectively. Among them, the increase of protein was
15 the most obvious among all experimental groups (Fig. 7 a). Previous research results
16 shown that the addition of conductive nanomaterials could promote the expression of
17 redox-active proteins, such as cytochromes (Jing et al. 2017). Ren et al. (Ren et al.
18 2012) used carbon nanotubes to bind the active center of horseradish peroxidase and
19 observed EET. On the limited cell surface area, the higher the protein content in EPS,
20 the more likely it is to facilitate the electron transfer between cells and CdS NPs. It
21 was possible to realize EET between CdS NPs and microorganisms by using CdS NPs
22 as electron donors to transfer electrons to electroactive proteins (Xiao et al. 2017; Li
23 et al. 2016). Therefore, the increased proteins content in EPS of C09 might belong to
24 EET related proteins.

25 In this study, the changes of EPS of C09 were analyzed by ATR-IR with/without
26 CdS NPs with results shown in Fig. 7 b. The N-H and C=O absorption peaks in
27 proteins were appeared at 3370.0 cm⁻¹ and 1611.0 cm⁻¹, respectively (Sun et al. 2012).
28 The absorption peak of C-H was appeared at 2998.1 cm⁻¹. The absorption peak of
29 -COOH in esters was at 1393.4 cm⁻¹. The absorption peak of C-O-C in

polysaccharides (Gómez-Ordóñez et al. 2011) was at 1091.2 cm^{-1} . There were no obvious shift between the peaks at 3370.0 cm^{-1} and 1611.0 cm^{-1} with/without CdS NPs, indicating that CdS NPs did not affect the protein structure in EPS.

In contrast, there were obviously shifted between the peaks at 2998.1 cm^{-1} and 1091.2 cm^{-1} with/without CdS NPs, indicating that polysaccharides could interact with CdS NPs. Wang et al. (Wang et al. 2016) found that polysaccharides in EPS could adsorb SiO_2 and ZnO NPs through FITR. Thus, the ability of CdS NPs to attach to the surface of bacteria was probably related to the polysaccharides in EPS.

The EPS composition analysis with/without CdS NPs were carried out by three-dimensional fluorescence spectroscopy with results shown in Fig. 8 a and Fig. 8 b. Ex / Em 200-250 / 250-380 nm peaks in region (1) were tyrosine proteins. Ex / Em 200-230 / 380-480 nm peaks in region (2) were humic acids substance (Li et al. 2016). Ex / Em 250-290/290-400 nm peaks in region (3) were soluble microbial metabolites. The signal intensities of the CdS NPs-free and CdS NPs groups increased by 122.15%, 9.00% and 27.73% in regions (1), (2) and (3), respectively, indicating that the content of protein, humic acid, and the soluble metabolites in EPS could be increased by CdS NPs.

3.6 The effect of EPS components on sulfate reduction and photo-current

The purified EPS was treated with proteinase K, polysaccharidase, and the two enzymes mixtures to investigate the effects of key components of EPS on the sulfate reduction process, respectively. The results were shown in Fig. 9.

EPS often contained a variety of components, usually including polysaccharides, proteins, nucleic acids, and humic acids. These substances had redox properties or electrical activity, and could carry out electron transfer inside and outside the cell. The effect of different components of EPS on sulfate reduction process were investigated. The protein and nucleic acid in EPS can be digested by proteinase K and polysaccharase. The sulfate reduction efficiencies of C09 of the proteinase K group, the polysaccharase group, the combined proteinase and polysaccharase group and the no enzyme group were 58.80%, 56.79%, 57.05% and 61.16%, respectively. The

1 results showed that the experimental group without any enzymes had the best sulfate
2 reduction efficiency, and the addition of enzymes would decrease the sulfate reduction
3 efficiency. Among them, the polysaccharide in the EPS component could significantly
4 promote the reduction of sulfate. It was consistent with the research results of Yan
5 (Yan et al. 2020).

6 In order to investigate the influence of the composition of EPS on the electron
7 transfer ability. The effect of different EPS components on the transient photo-current
8 intensity was investigated under conditions of the same amount of EPS components,
9 electrolyte solution concentration, and the light intensity. The results were shown in
10 Fig. 10.

11 The results of the instantaneous photo-current produced by CdS NPs showed that
12 the photo-current intensity of both the group of added EPS and the group of EPS with
13 proteinase K treatment were stronger than those of groups with polysaccharidase
14 alone, combination of polysaccharidase and proteinase K, and no EPS. Zhu et al (Zhu
15 et al. 2018). found that externally added EPS had greater photocurrent intensity than
16 that without EPS. It was indicated that some components of extracellular EPS,
17 especially polysaccharide, contributed to the separation of electrons from holes and
18 thus might be mainly responsible for the enhanced electron utilization and increased
19 sulfate reduction efficiency of C09 (Yan et al. 2020). After the removal of EPS, CdS
20 NPs cannot be adsorbed on the surface of C09, so the electrons generated by CdS NPs
21 could not be used by C09 to promote the reduction of sulfate and bacterial growth
22 process.

23 It was found that the polysaccharide components of EPS might be the electron
24 sacrificial agents to help the separation of electrons and holes of CdS NPs, thereby
25 enhancing the utilization of electrons by C09.

26 **3.7 Effects of CdS NPs on sulfate reduction, biomass, extracellular $\cdot\text{OH}$,** 27 **intracellular ROS and MDA content under light**

1 The effects of CdS NPs on the growth of C09 and the sulfate reduction process
2 were investigated with results shown in Fig. 4 a. The sulfate concentration and C09
3 biomass in Fig. 4 b were compared and analyzed with results shown in Fig. 11 at 8 h
4 and 24 h.

5 The sulfate concentration in the $8.1 \text{ mW}\cdot\text{cm}^{-2}$ group was 7.63% lower than that
6 of the $0 \text{ mW}\cdot\text{cm}^{-2}$ group (control), and the biomass decreased by 55.00% compared to
7 the control at 8 h. While at 24 h, the biomass increased by 16.39% compared with
8 control. Shang (Shang et al. 2017) investigated the effect of 5 mg/L of CdS NPs for
9 *E.coli* under the UV irradiation with results shown that only 3.3% of *E.coli* were
10 survived. However, Zhu (Zhu et al. 2017) found that CdS NPs could increase the
11 abundance of bacteria under light condition. Therefore, there is no clear conclusion
12 about the influence of nanoparticles on the growth of bacteria.

13 However, CdS NPs could catalyze water to produce $\cdot\text{OH}$ under anaerobic and
14 light conditions. It was found that $\cdot\text{OH}$ could oxidize proteins and the cell membrane
15 lipids (Dutta et al. 2012). Therefore, the oxidation products of cell membranes are
16 used as important indicators for $\cdot\text{OH}$ oxidative damage. In view of the characteristics
17 embodied in the early stage of culture, the extracellular $\cdot\text{OH}$ and intracellular ROS in
18 the early stage of culture were measured. In the experiment, 1 mM CdS NPs were
19 added to the culture medium of C09 at light intensity of $0 \text{ mW}\cdot\text{cm}^{-2}$ (Control) and 8.1
20 $\text{mW}\cdot\text{cm}^{-2}$ under the anaerobic condition for 8 h. The extracellular $\cdot\text{OH}$, intracellular
21 ROS, and MDA content were determined with results shown in Fig.12 a, b and c.

22 The contents of extracellular $\cdot\text{OH}$, intracellular ROS and MDA in the solution
23 increased by 15.36%, 26.07%, and 966.99%, respectively, compared with the control
24 under $8.1 \text{ mW}\cdot\text{cm}^{-2}$. It was indicated that the content of extracellular $\cdot\text{OH}$ and
25 intracellular ROS, and the cellular peroxidation could be increased by CdS NPs under
26 the light. ZnO NPs would increase the intracellular ROS content of *E.coli* and
27 *S.aureus* under light condition (Akhil et al. 2017). Lin (Lin et al. 2006) found that the
28 ROS and MDA contents of A549 cells induced by CeO_2 NPs had increased. Therefore,

1 the increase in the content of extracellular $\cdot\text{OH}$ and intracellular ROS could affect the
2 growth and metabolism of C09.

3 Accordingly, CdS NPs could generate cavitation oxidized water to form $\cdot\text{OH}$
4 under light condition, the content of extracellular $\cdot\text{OH}$ increases, oxidizes cell
5 membrane lipids, thereby inhibiting the function of cell membrane. However, in the
6 middle and late stages of culture, the inhibitory effect does not seem to be obvious. It
7 was assumed that oxidation radicals could be consumed by sulfate reduction products.

8 In order to verify the effect of reducing products of this sulfate bioreduction
9 process, such as S^{2-} , on the removal of $\cdot\text{OH}$, a non-biological experiment was
10 designed. At first, the $\cdot\text{OH}$ content in the uninoculated solution were measured every
11 8 h, and then 100 μL of 0.1 M $\text{Na}_2\text{S}\cdot 9\text{H}_2\text{O}$ solution was added to the medium at 24 h.
12 The experimental results was shown in Fig. 13.

13 As shown in Fig. 13, the content of $\cdot\text{OH}$ in the solution of the CdS NPs group
14 and the CdS NPs-free group decreased by 65.49% and 21.87% at 24 h, respectively,
15 compared with 16 h, which indicates that S^{2-} could consume the $\cdot\text{OH}$. Therefore, the
16 amount of the products of sulfate reduction in the early stage of C09 growth was little,
17 and there was a lack of reducing substances to neutralize the ROS produced from CdS
18 NPs. However, a large amount of sulfate was reduced in 24 h to produce more
19 reducing substances, such as S^{2-} , HS^- , H_2S , which could alleviate the oxidative stress
20 of CdS NPs. Therefore, the oxidative stress produced by CdS NPs in the early growth
21 stage of C09 cannot be ignored.

22 However, as an antioxidant, HA is also an important component of EPS.
23 Therefore, it is necessary to investigate the influence of HA on the research process.

24 **3.8 Effect of HA on the contents of $\cdot\text{OH}$, ROS and MDA induced by CdS NPs**

25 In order to verify that HA functions as an antioxidant, the contents of
26 extracellular $\cdot\text{OH}$, intracellular ROS and MDA at 8 h of culture were measured after
27 the exogenous addition of HA with results shown in Fig. 14 a, b and c.

28 It was shown that the content of extracellular $\cdot\text{OH}$, intracellular ROS and MDA
29 in the experimental group with HA was lower than that of the control. After eight

1 hours of culture, the content of extracellular $\cdot\text{OH}$, intracellular ROS and MDA in the
2 solution of the HA experimental group was reduced by 32.86%, 18.77%, and 20.53%,
3 respectively under the light intensity of $8.1\text{ mW}\cdot\text{cm}^{-2}$ compared with the control.
4 Thereby the damage of extracellular $\cdot\text{OH}$ and intracellular ROS to cell membranes
5 could be reduced. Deng (Deng et al. 2016) found that the addition of HA could reduce
6 the toxicity of G-CdS. The reason was that HA could reduce the content of MDA and
7 ROS, increase the activity of CAT and SOD, thereby reducing the oxidative stress
8 induced by G-CdS, and then reduce lipid, protein and nucleic acid damage, and even
9 cell death.

10 Therefore, in order to solve the problem of oxidative stress in the initial growth
11 phase of C09, one strategy of external adding HA in the initial growth phase was
12 proposed for the process of CdS NPs strengthening C09 sulfate reduction. And the
13 reduction products such as S^{2-} could effectively alleviate the oxidative stress caused
14 by the photosensitivity of CdS NPs on C09 at the middle and later stage of culture.
15 Previous studies had shown that humic acid was also present in EPS, so that the
16 problem could be solved by strengthening the synthesis of EPS of C09. There were
17 two reasons for this. The first reason was that HA could adsorb nanoparticles to
18 reduce their light absorption characteristics (Akhil et al. 2017), The second reason
19 was that HA as a natural antioxidant that could undergo redox reactions with holes
20 and avoid the generation of holes (Deng et al. 2016).

21 In the experiment, the effect of HA on the growth of C09 and the process of
22 sulfate reduction was investigated, under the conditions of lighting and 1 mM CdS
23 NPs, by adding 10 mg/L of HA at the initial growth phase. The result of sulfate
24 concentration gradually decreases with time, which was shown in Fig. 15 a. The
25 addition of HA at initial growth phase could significantly improve the sulfate
26 reduction efficiency. Among them, the reduction efficiency of sulfate increased by
27 5.85%. It can be seen from Fig. 15 b that the biomass of C09 gradually increased with
28 time. The biomass of C09 increased by 6.84% at 8 h after the addition of HA. Deng
29 (Deng et al. 2016) studied the bactericidal effect of G-CdS nanocomposites on *E. coli*

1 by the photocatalytic activity of G-CdS nanocomposites. It was found that the
2 addition of HA through exogenous sources would reduce the toxicity of G-CdS to *E.*
3 *coli*. In summary, the addition of HA could alleviate the inhibitory effect of CdS NPs
4 on the initial growth phase of C09, which is of great significance for promoting the
5 reduction of sulfate.

6 **3.9 The possible mechanism of C09 sulfate reduction strengthened by CdS NPs**

7 In summary, a possible mechanism for CdS NPs was proposed to enhance the
8 sulfate reduction process of *D. desulfuricans* C09. Under light conditions, the
9 photoelectrons generated by CdS NPs could pass through the electron receptors (such
10 as cytochrome c, etc.) bound to the extracellular EPS of *D. desulfuricans* C09, and
11 then enter the intracellular electron transport chain to couple with the sulfate reduction
12 process (Kornienko et al. 2016; Deng et al. 2020). The polysaccharide in the
13 extracellular EPS of C09 could adsorb CdS NPs on the surface of the bacteria, and it
14 could possibly acts as an electron sacrificial agent to promote the separation of
15 electrons and holes. Therefore, C09 gains energy to promote its growth and sulfate
16 reduction efficiency. However, nanoparticles could also generate oxidative free
17 radicals, which had certain negative effects on the initial growth phase of C09, but
18 humic acid in EPS or external addition could eliminate its negative effects. The
19 mechanism of CdS NPs enhancing the sulfate reduction of *D. desulfuricans* C09,
20 which was shown in Fig. 16, should be further studied to develop more effective
21 sulfate reduction technology.

22 **4. Conclusion**

23 A strain with sulfate reduction function was selected from sludge, and identified
24 as *Desulfovibrio desulfuricans* C09. The synthesis of CdS NPs with photosensitivity
25 could increase the efficiency of *Desulfovibrio desulfuricans* C09 to reduce sulfate by
26 6.43% and significantly increase the biomass under light conditions. CdS NPs could
27 also increase the synthesis and secretion of EPS. Compared with the control group,
28 exogenous addition of EPS and polysaccharides could increase the sulfate reduction

1 efficiency by 3.43% and 5.78%, respectively. Furthermore, it was found that EPS had
2 a significant contribution to the increase of photocurrent intensity. The strategy of
3 external addition of HA in the initial growth phase could effectively alleviate the
4 effect of oxidative stress induced by photosensitive nanomaterials CdS NPs.

5 **Availability of data and materials**

6 All data supporting the findings of this study are available in the article,
7 supporting information, or upon request from the corresponding author.

8 **Abbreviations**

9 CdS NPs: cadmium sulfide nanoparticles

10 HA: humic acid

11 EET: extracellular electron transfer

12 EPS: extracellular polymeric substances

13 ROS: reactive oxygen species

14 G-CdS: graphene-cadmium sulfide

15 SRB: sulfate reducing bacteria

16 EEM: excitation emission matrix

17 FT-IR: Fourier transform infrared spectroscopy

18 MDA: Malondialdehyde

19 PBS: phosphate balanced solution

20 FL: fluorescence

21 TBA: thiobarbituric acid

22 EDS: energy dispersive spectrometer

23 **Declarations**

24 **Ethics approval and consent to participate**

25 Not applicable

26 **Competing interests**

27 The authors declare that they have no competing interests

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Authors' contributions

GC, XZ, WT and MZ designed the experiments. GC and HD carried out the experiments. GC, XZ, HD and analyzed the experimental results. GC, XZ, HD and HS wrote the manuscript. All the authors edited the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Consent for publication

All authors have read and approved the final submitted manuscript.

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References

Aboulaich A, Billaud D, Abyan M, Balan L, Gaumet JJ, Medjadhi G, Ghanbaja J, Schneider R (2012) One-Pot Noninjection Route to CdS Quantum Dots via Hydrothermal Synthesis. *ACS Applied Materials & Interfaces* **4** (5): 2561-2569. DOI: 10.1021/am300232z

Akhil K and Sudheer Khan S (2017) Effect of humic acid on the toxicity of bare and capped ZnO nanoparticles on bacteria, algal and crustacean systems. *Journal of Photochemistry and Photobiology B: Biology* **167**: 136-149. DOI: 10.1016/j.jphotobiol.2016.12.010

Alipour A, Mansour Lakouraj M and Tashakkorian H (2021) Study of the effect of band gap and photoluminescence on biological properties of polyaniline/CdS QD

1 nanocomposites based on natural polymer. Sci Rep **11**: 1913. DOI:
2 <https://doi.org/10.1038/s41598-020-80038-1>

3 Behera N, Arakha M, Priyadarshinee M, Pattanayak BS, Soren S, Jha S, Mallick BC
4 (2019) Oxidative stress generated at nickel oxide nanoparticle interface results in
5 bacterial membrane damage leading to cell death. RSC Adv **9**: 24888-24894.
6 DOI: 10.1039/C9RA02082A

7 Cao B, Shi L, Brown RN, Xiong Y, Fredrickson JK, Romine MF, Marshall MJ, Lipton
8 MS and Beyenal H (2011) Extracellular polymeric substances from *Shewanella sp.*
9 HRCR - 1 biofilms: characterization by infrared spectroscopy and proteomics.
10 Environmental microbiology **13**: 1018-1031. DOI: [https://doi.org/10.1111/j.1462-](https://doi.org/10.1111/j.1462-2920.2010.02407.x)
11 [2920.2010.02407.x](https://doi.org/10.1111/j.1462-2920.2010.02407.x)

12 Chandran P, Kumari P, Khan SS (2014) Photocatalytic activation of CdS NPs under
13 visible light for environmental cleanup and disinfection. Solar Energy **105** (9):
14 542-547. DOI: 10.1016/j.solener.2014.04.028

15 Dai YF, Xiao Y, Zhang EH, Liu LD, Zhao F (2016) Effective methods for extracting
16 extracellular polymeric substances from *Shewanella oneidensis* MR-1. Water Science
17 and Technology **74** (12): 2987-2996. DOI: 10.2166/wst.2016.473

18 Deng CH, Gong JL, Zeng GM, Jiang Y, Zhang C, Liu HY, Huan SY (2016)
19 Graphene–CdS nanocomposite inactivation performance toward *Escherichia coli* in
20 the presence of humic acid under visible light irradiation. Chemical Engineering
21 Journal **284**: 41-53. DOI: 10.1016/j.cej.2015.08.106

22 Deng X, Dohmae N, Kaksonen AH, Okamoto A (2020) Biogenic Iron Sulfide
23 Nanoparticles to Enable Extracellular Electron Uptake in Sulfate-Reducing Bacteria.
24 Angewandte Chemie International Edition **59** (15): 5995-5999. DOI: 10.1002/anie.

1 202002154

2 Gdab D , Hw F , Zy A (2020) Cadmium sulfide nanoparticles-assisted intimate
3 coupling of microbial and photoelectrochemical processes: Mechanisms and
4 environmental applications. Science of The Total Environment **740**: 140080 . DOI:
5 10.1016/j.scitotenv. 2020. 140080

6 Draper HH, Hadley M (1990) Malondialdehyde determination as index of lipid
7 Peroxidation. Methods in Enzymology **186**: 421-431. DOI: [https://doi.org/10.1016/](https://doi.org/10.1016/0076-6879(90)86135-I)
8 0076-6879(90)86135-I

9 Dutta RK, Nenavathu BP, Gangishetty MK and Reddy AVR (2012) Studies on
10 antibacterial activity of ZnO nanoparticles by ROS induced lipid peroxidation.
11 Colloids and Surfaces B: Biointerfaces **94**: 143-150. DOI: 10.1016/j.colsurfb. 2012.
12 01.046

13 Flemming HC, Neu TR, Wozniak DJ (2007) The EPS matrix: The House of Biofilm
14 cells. Journal of Bacteriology **189**: 7945-7947. DOI: 10.1128/JB.00858-07

15 Gómez-Ordóñez E, Rupérez P (2011) FTIR-ATR spectroscopy as a tool for
16 polysaccharide identification in edible brown and red seaweeds. Food Hydrocolloids
17 **25 (6)**: 1514-1520. DOI: 10.1016/j.foodhyd.2011.02.009

18 Jia Y, Wy A, Liang X, Wang S, Xie J, Zhong K, Bao M, Yang J, Wen H, Li S, Chen Y,
19 Gu J-D, Zhang H (2020) Enhanced reduction of sulfate and chromium under
20 sulfate-reducing condition by synergism between extracellular polymeric substances
21 and graphene oxide. Environmental Research **183**: 109157. DOI: [https://doi.org/](https://doi.org/10.1016/j.envres.2020.109157)
22 10.1016/j.envres.2020.109157

1 Jing Y, Wan J, Angelidaki I, Zhang S, Luo G (2017) iTRAQ quantitative proteomic
2 analysis reveals the pathways for methanation of propionate facilitated by magnetite.
3 Water Research **108**: 212-221. DOI: 10.1016/j.watres.2016.10.077

4 Kornienko N, Sakimoto KK, Herlihy DM, Nguyen SC, Alivisatos AP, Harris CB,
5 Schwartzberg A, Yang P (2016) Spectroscopic elucidation of energy transfer in hybrid
6 inorganic–biological organisms for solar-to-chemical production. Proceedings of the
7 National Academy of Sciences **113** (42): 11750-11755. DOI: 10.1073/pnas.
8 1610554113

9 Lang D, Xiang Q, Qiu G, Feng X, Liu F (2014) Effects of crystalline phase and
10 morphology on the visible light photocatalytic H₂-production activity of CdS
11 nanocrystals. Dalton Transactions **43** (19): 7245-7253. DOI: 10.1039/c3dt53601g

12 Lei L, Ming L, Li Y, Ma X, Tang X, Li Z (2016) Changes in dissolved organic matter
13 composition and metabolic diversity of bacterial community during the degradation of
14 organic matter in swine effluent. Environmental Science and Pollution Research **23**
15 (13): 13498-13507. DOI: 10.1007/s11356-016-6536-1.

16 Li SW, Sheng GP, Cheng YY, Yu HQ (2016) Redox properties of extracellular
17 polymeric substances (EPS) from electroactive bacteria. Scientific Reports **6** (1):
18 39098. DOI: 10.1038/srep39098

19 Li Y, Luo Q, Li H, Chen Z, Shen L, Peng Y, Wang H, He N, Li Q, Wang Y(2019)
20 Application of 2-hydroxy-1,4-naphthoquinone- graphene oxide (HNQ-GO) composite
21 as recyclable catalyst to enhance Cr(VI) reduction by *Shewanella xiamenensis*.
22 Journal of Chemical Technology & Biotechnology **94**: 446-454. DOI: 10.1002/jctb.
23 5788

- 1 Lin W, Huang YW, Zhou XD, Ma Y (2006) Toxicity of Cerium Oxide Nanoparticles
2 in Human Lung Cancer Cells. *International Journal of Toxicology* **25 (6)**: 451-457.
3 DOI: 10.1080/10915810600959543
- 4 Lovley DR (2008) Extracellular electron transfer: wires, capacitors, iron lungs, and
5 more. *Geobiology* **6 (3)**: 225-231. DOI: 10.1111/j.1472-4669.2008.00148.x
- 6 Min S, Li WW, Mu ZX, Wang HL, Yu HQ, Li YY, Harada H (2012) Selection of
7 effective methods for extracting extracellular polymeric substances (EPS) from
8 *Bacillus megaterium* TF10. *Separation and Purification Technology* **95**: 216-221. DOI:
9 10.1016/j.seppur.2012.05.010
- 10 Prabhu RR, Khadar MA (2005) Characterization of chemically synthesized CdS
11 nanoparticles. *Pramana* **65 (5)**: 801-807. DOI: 10.1007/BF02704078
- 12 Radić S, Vujčić V, Cvetković Ž, Cvjetko P, Oreščanin V (2014) The efficiency of
13 combined CaO/electrochemical treatment in removal of acid mine drainage induced
14 toxicity and genotoxicity. *Science of The Total Environment* **466-467 (1)**: 84-89. DOI:
15 10.1016/j.scitotenv.2013.07.011
- 16 Ren L, Dong Y, Zhong W (2012) Enhanced enzyme activity through electron transfer
17 between single-walled carbon nanotubes and horseradish peroxidase. *Carbon* **50 (3)**:
18 1303-1310. DOI: 10.1016/j.carbon.2011.10.053
- 19 Riaz S, Raza ZA, Majeed MI (2019) Preparation of cadmium sulfide nanoparticles
20 and mediation thereof across poly(hydroxybutyrate) nanocomposite. *Polymer Bulletin*
21 **77**: 775-791. DOI: 10.1007/s00289-019-02775-2
- 22 Ks A, Yi LA, Es B (2020) Electrochemical behavior of biochar and its effects on
23 microbial nitrate reduction: Role of extracellular polymeric substances in extracellular

1 electron transfer. Chemical Engineering Journal **395**: 125077. DOI: 10.1016/j.cej.
2 2020.125077

3 Shang E, Niu J, Yang L, Zhou Y, Crittenden JC (2017) Comparative toxicity of Cd,
4 Mo, and W sulphide nanomaterials toward *E. coli* under UV irradiation.
5 Environmental Pollution **224**: 606-614. DOI: 10.1016/j.envpol.2017.02.044

6 Shang E, Li Y, Niu J, Zhou Y, Wang T, Crittenden JC(2017) Relative importance of
7 humic and fulvic acid on ROS generation, dissolution, and toxicity of sulfide
8 nanoparticles. Water Research **124**: 595-604. DOI: 10.1016/j.watres.2017.08.001

9 Wang B, Xiao K, Jiang Z, Wang J, Yu J-C, Wong P-K (2019) Biohybrid
10 photoheterotrophic metabolism for significant enhancement of biological nitrogen
11 fixation in pure microbial cultures. Energy & Environmental Science **12** (7):
12 2185-2191. DOI: 10.1039/C9EE00705A

13 Wang B, Zeng C, Chu KH, Wu D, Yip HY, Ye L, Wong PK (2017) Enhanced
14 Biological Hydrogen Production from *Escherichia coli* with Surface Precipitated
15 Cadmium Sulfide Nanoparticles. Advanced Energy Materials **7** (29): 1700611.1-
16 1700611.11. DOI: 10.1002/aenm.201700611

17 Wang Q, Kang F, Gao Y, Mao X, Hu X (2016) Sequestration of nanoparticles by an
18 EPS matrix reduces the particle-specific bactericidal activity. Scientific Reports **6** (1):
19 21379. DOI: 10.1038/srep21379

20 Wang S (2020) Solar - Powered Organic Semiconductor–Bacteria Biohybrids for CO₂
21 Reduction into Acetic Acid. Angewandte Chemie International Edition **59** (18):
22 7224-7229. DOI: 10.1002/anie.202001047

23 Wolf M, Kappler A, Jiang J, Meckenstock RU (2009) Effects of Humic Substances
24 and Quinones at Low Concentrations on Ferrihydrite Reduction by *Geobacter*

1 metallireducens. Environmental Science & Technology **43** (15): 5679-5685. DOI:
2 10.1021/es803647r

3 Xiao Y, Zhao F (2017) Electrochemical roles of extracellular polymeric substances in
4 biofilms. Current Opinion in Electrochemistry **4**: 206-211. DOI: 10.1016/j.coelec.
5 2017.09.016

6 Xiao Y, Zhang E, Zhang J, Dai Y, Yang Z, Christensen HEM, Ulstrup J and Zhao F
7 (2017) Extracellular polymeric substances are transient media for microbial
8 extracellular electron transfer. Science Advances **3** (7): e1700623. DOI: 10.1126/
9 sciadv. 1700623

10 Xin L, Liu H, Luo D, Li J, Huang Y, Li H, Fang Y, Xu Y, Zhu L (2012) Adsorption of
11 CO₂ on heterostructure CdS(Bi₂S₃)/TiO₂ nanotube photocatalysts and their
12 photocatalytic activities in the reduction of CO₂ to methanol under visible light
13 irradiation. Chemical Engineering Journal **180**: 151-158. DOI: 10.1016/j.cej. 2011.11.
14 029

15 Zhu N, Tang J, Tang C, Duan PF (2018) Combined CdS nanoparticles-assisted
16 photocatalysis and periphytic biological processes for nitrate removal. Chemical
17 Engineering Journal **353**: 237-245. DOI: 10.1016/j.cej.2018.07.121

Figures and Tables

Table 1. Results of the physiological and biochemical experiment of *Desulfovibrio desulfuricans* C09

Test name	Results
Gram staining	-
Indole test	+
Methyl red test	-
V-P	-
Gelatin	-
Hydrogen sulfide	+

Fig. 1. Sulfate reduction efficiency of part of isolated strains

Fig. 2. Phylogenetic tree of C09 based on 16S rDNA gene sequences

Fig. 3. The characteristic of CdS NPs
(a: UV of CdS NPs; b: XRD of CdS NPs; c: SEM of CdS NPs)

Fig. 4. The SEM image and its corresponding EDS spectrum of C09 after treatment with CdS NPs.

a: without CdS NPs; b: with CdS NPs; c: EDS of C09 with CdS NPs

Fig. 5. Effects of the CdS NPs on C09 sulfate reduction and growth process.

a: Sulfate concentration; b: Biomass

Fig. 6. Effects of CdS NPs on sulfate reduction, growth and EPS composition of strain C09 that removed/contained EPS

a: Sulfate concentration; b: Biomass

Fig. 7. ATR-IR analysis and composition of EPS

a: Composition of EPS; b: ATR-IR of EPS

Fig. 8. 3D-EEM of extracellular polymeric substance.

a: without CdS NPs; b: with CdS NPs

Fig. 9. Effects of EPS components on the efficiency of sulfate reduction of C09
a: rude EPS and CdS NPs; b: without EPS, but with CdS NPs; c: EPS with proteinase treatment; d: EPS with polysaccharidase treatment; e: EPS with both proteinase and polyaccharidase; f: EPS with CdS NPs

Fig. 10. Effect of EPS components on instantaneous photocurrent intensity.

A: EPS with proteinase treatment; B: EPS with both proteinase and polysaccharidase;

C: no-added EPS; D: EPS with polysaccharidase; E: added EPS;

Fig. 11. Effect of CdS NPs on growth of C09 and reduction of sulfate under light.

a: Sulfate concentration at 8 h and 24 h; b: Biomass at 8 h and 24 h

Fig. 12. The effects of light intensity on $\cdot\text{OH}$, intracellular ROS and MDA contents
(A: $\cdot\text{OH}$ concentration; B: Intracellular ROS content; C: MDA concentration)

Fig. 13. The effect of adding S^{2-} on the content of $\cdot OH$ in medium.

Fig. 14. Effects of HA on ·OH, intracellular ROS and MDA contents for C09.

a: ·OH concentration; b: Intracellular ROS content; c: MDA concentration

Fig. 15. Effects of HA on sulfate reduction and growth of C09

a: Sulfate concentration; b: Biomass

Fig. 16. Mechanism of sulfate reduction process of CdS NPs binding to *Desulfovrio desulfuricans* C09

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