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Exploration of the reduction mechanism of Cr(VI) in anaerobic hydrogen fermenter☆

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ABSTRACT

The bio-reduction of hexavalent chromium (Cr(VI)) by anaerobic fermentation is considered as a promising, low-cost and environment-friendly way. However, it is unclear for the reduction mechanisms of Cr(VI) in an anaerobic hydrogen fermenter, such as reduction kinetics, related electron donors, migration and transformation, reduction site and key components, and related microorganisms. To clarify these issues, a hydrogen fermenter was designed to reduce Cr(VI) at 55 °C with glucose as initial substrate. Results show that 100 mg/L Cr(VI) can be completely reduced (99.5%) to trivalent chromium (Cr(III) through chemical and biological reactions. Bio-reduction dominates Cr(VI) removal in a first-order exponential decay mode with both glucose and its metabolites (volatile fatty acids) as electron donors. Moreover, volatile fatty acids are more suitable as electron donors for Cr(VI) bio-reduction than glucose. *Bacilli*, *Clostridia* and *Thermotogae* in the fermenter dominated the reduction of Cr(VI) by regulating the production and composition of extracellular polymers (EPSs), in which carboxyl and hydroxyl groups play an important role for Cr(VI) reduction by coordination. The results can guide us to regulate the bio-reduction of Cr(VI), and provide reference for the development of bio-reduction technology of Cr(VI).

1. Introduction

More and more chromium (Cr) is discharged largely into water environment from electroplating, leather, printing and dyeing factories (Sandana Mala et al., 2015). Chromium that has not been properly disposed in water environment not only poses ecological risks to animals, plants and microorganisms, but also endangers human health because of its carcinogenic, teratogenic and mutagenic properties (Ali et al., 2013; Gifford et al., 2018). The valence of chromium has a great influence on its toxicity levels. For example, the hexavalent Cr(VI) is more toxic than trivalent Cr(III). Furthermore, the mobility of Cr(VI) is higher than Cr(III) in water environment (Hu et al., 2018a). Thus, various effective methods for the reduction of Cr(VI) were developed to reduce the toxicity levels of Cr (Jia et al., 2017).

Various techniques and methods have been extensively studied to reduce the harm of heavy metal chromium, which are mainly divided into chemical reduction and biological reduction (Ko et al., 2018; Zhong et al., 2017). Bio-reduction is more concerned than chemical reduction due to its high efficiency, low cost and low secondary pollution (Shi et al., 2019; Zhong et al., 2017). Among these methods, anaerobic digestion is one of the most promising alternatives to the reduction of Cr(VI) to Cr(III) (Lu et al., 2016; Wang et al., 2018). On the other hand, heavy metal chromium pollution creates high redox potential and extremely harsh living conditions, which is detrimental to the growth and reproduction of anaerobic microorganisms. Under these conditions, hydrogen-producing bacteria have higher environmental adaptability and impact resistance than methanogens. The reasons were as follows: (1) the microbial extracellular polymers (EPSs) has a small size and a high density under acidic hydrogen fermentation process, which not only facilitates the dispersion and mass transfer of the substance but also enhances the microbial impact resistance; (2) more compressed and dense structure of EPSs can be formed in acidic

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anaerobic condition, which can offer much complex acidic sites for the adsorption surface (Naja et al., 2005; Wang et al., 2012). Therefore, research on the reduction of chromium by hydrogen anaerobic fermentation is valuable and necessary. As one of the metabolites of microorganisms, EPSs play an important role in the reduction of chromium. The composition of EPSs not only affects the reduction efficiency of chromium, but also affects its reduction mechanism (Lai et al., 2018; Sheng et al., 2013). On the other hand, Cr(VI) also affects the content and composition of EPSs in anaerobic digestion process. Therefore, elucidation of the interaction between Cr(VI) and EPSs is the key factor in revealing the mechanism of Cr(VI) reduction.

A large number of studies have shown that organic matter and its metabolites (volatile acids and hydrogen) act as electron donors in the anaerobic biological reduction of Cr(VI) (Jiang et al., 2018; Lloyd, 2003). Cr (VI) bio-reduction with acetic acid, glucose and hydrogen as electron donors are shown as equations (1)–(3):

$$3C_2H_4O_2 + 8CrO^{2-}{}_4 + 14H_2O = 8Cr(OH)_3 + 10OH^- + 6HCO_3^- \quad (1)$$

$$C_6H_{12}O_6 + 8CrO^{2-}{}_4 + 14H_2O = 8Cr(OH)_3 + 10OH^- + 6HCO_3^- \quad (2)$$

$$3H_2 + 2CrO^{2-}{}_4 + 4H^+ = 2Cr(OH)_3 + 2H_2O \quad (3)$$

However, in different reduction systems, the role of these electronic donors has not yet been elucidated. The reduction performance of each electron donor in same complex systems also needs to be further explored.

Sulfate-reducing bacterium, enterobacter cloacae, trichococcus, aceticlastic methanogens, thermophilic methanogen were reported for the reduction of Cr(VI) under anaerobic condition (Sandana Mala et al., 2015). The Cr(VI) reduction mechanism and the Cr(III) location are different due to the different bacterial strains and environmental factors (temperature, electron donor, and pH) (Chen et al., 2017; Kantar and Bulbul, 2016). For example, *Streptomyces* sp. MC1-induced reduction of Cr(VI) occurs within cells, while *Pseudochrobactrum. saccharolyticum* causes Cr(VI) reduction outside the cells (EPSs) (Abdel-Mageed et al., 2013). However, in complex multi-strain systems, Cr(VI) reduction may occur both intracellularly and extracellularly, or involves multiple mechanisms. Therefore, it is necessary to clarify which microbes play a key role in the reduction of Cr(VI) during the acidic anaerobic hydrogen fermentation process and the related reduction mechanism.

In this study, anaerobic hydrogen fermentation was used to reduce Cr(VI). The Cr(VI) reduction and removal kinetics, related electron donors, migration and transformation, reduction site and key components, biological or chemical reduction and related microorganisms were elucidated and analyzed by UV–Vis spectroscopy, three-dimensional fluorescence spectroscopy, X-ray photoelectron spectroscopy (XPS) and 16S rRNA gene sequencing. We found that I) Cr(VI) was bio-reduced in a first-order exponential decay mode; II)metabolites (VFAs) are more suitable as electron donors for Cr(VI) bioreduction than glucose; III) *Bacilli, Clostridia* and *Thermotogae* in the fermenter dominate Cr(VI) reduction by regulating the production and composition of EPSs.

2. Material and methods

2.1. Materials

Potassium dichromate ($K_2Cr_2O_7$), glucose, acetic acid, hydrochloric acid, sodium hydroxide (NaOH), sodium carbonate (Na_2CO_3) nitric acid (HNO_3), magnesium chloride ($MgCl_2$), diphenylcarbazide and sodium chloride (NaCl) were analytical grade and purchased from Shanghai Guoyao Pharmaceutical Group Co., Ltd. Anaerobic sludge (total solids: 32.77 g L^{-1}, volatile solids: 27.63 g L^{-1}) was collected from an anaerobic wastewater treatment plant of a citric acid factory (Rizhao, China). The initial pH of the anaerobic sludge was adjusted to 5.8 with hydrochloric acid and NaOH solution. Then, the sludge was cultured at 55 °C in several 500 mL serum bottles with glucose as initial substrate.

2.2. Cr(VI) reduction experiment

2.2.1. Reduction of Cr(VI)

Anaerobic hydrogen fermenters (500 ml) were filled with 100 mg/l of Cr(VI), 1 g/l of glucose and 10% of anaerobic sludge, and then adjusted to a volume of 450 ml with deionized water. The bacterial species at the phylum, class and genus level were introduced in Fig. 5. Then, the pH of the system was adjusted to 5.8 with hydrochloric acid and sodium hydroxide. After capping, the air in the system was removed with nitrogen. Finally, it was cultured in a water bath at 55 °C. The performance of anaerobic hydrogen fermenter was introduced in supporting information. According to the results of the preliminary experiments, the concentration of Cr(VI), the yield of volatile fatty acid and hydrogen, and the change of EPSs were sampled and analyzed at different time intervals according the reduction rate of Cr(VI). In initial stage the time interval of sampling is 2–4 h, and 5–10 h in later stage. The same amount of Cr(VI) was added to the fermenter after the Cr(VI) was almost reduced after 72 h, 42 h and 85 h using glucose as the initial electron donor, and after 9 h, 18 h and 27 h with acetic acid as the initial electron donor. Sludge of 5 ml was sampled under the condition of full shaking, and then was centrifuged (10,000 rpm, 10 min) and filtered with water-based filter membrane (0.45 μm). The filter residue is suspended with 10 ml ultra-pure water and transferred into 100 ml digestion tube. Then the centrifugal tube was washed twice with 10 ml ultra-pure water, and washing solution was also transferred into the digestion tube. $MgCl_2$ of 0.2 g, phosphoric acid buffer (pH = 7) of 0.5 ml and digestion solution of 30 ml were added into the digestion tube, and then digested at 90 °C for 2 h in a water-bath oscillation. The digestion solution (pH > 11.5) was prepared by dissolving 20 g NaOH and 30 g Na_2CO_3 into 1000 ml ultrapure water. After cooling to room temperature, the solution was filtered with a 0.45 μm water-based filter membrane. The digestion tube was washed with 10 ml ultra-pure water for 2 times. The filtrate and the digestion tube flushing solution were transferred to a 200 ml conical bottle. HNO_3 solution (5 mol/l) was used to adjust the pH of the solution to 7.5, and then transferred to a capacity bottle of 100 ml. The content of Cr(VI) was determined by diphenylcarbazide spectrophotometry. The amount of Cr(VI) in fermenter is the sum of Cr(VI) in filtrate and filter residue.

2.2.2. Determination of biological reduction or chemical reduction

Hexavalent chromium exhibits strong oxidability under acidic conditions, while glucose shows reducibility. Therefore, it is necessary to explore the contribution of chemical reduction and biological reduction to Cr(VI) reduction in an acidic anaerobic hydrogen fermentation system. On the basis of the above-mentioned reduction experiment design of Cr(VI), two other sets of comparative experiments were designed. The first group of experiments contained inoculated sludge (10%), and the second group of experiments showed no inoculation of sludge, and the other conditions were the same. The blank group experiment only contained sludge (10%) without any substrate.

2.2.3. Determination of electron donor for Cr(VI) reduction

In order to determine the electron donor during the acidic anaerobic hydrogen fermentation, the four fermenters were tested with glucose, acetic acid, hydrogen saturated aqueous solution as

electron donors under the same culture conditions. Three replicates were set for all experiments and the final result was the average of three replicate experiments. Specific experimental settings refer to support information.

2.3. Analysis of sludge components and microbial metabolites

Soluble chemical oxygen demand (SCOD), ammonia nitrogen (NH_3—N), total solids (TS), volatile solids (VS), Cr(VI), total chromium (T-Cr) were measured using UV-2600 (Shimadzu, Japan) according standard methods (Federation and Association, 2005). Biogas produced from the serum bottles was measured by glass injector. Volatile Fatty Acids (VFAs) were measured by liquid chromatography using a LC-2030 liquid chromatograph (Shimadzu, Japan). Mobile phase: 0.05% dilute phosphoric acid, flow rate 0.7 mL/min; Detector: UV detector, wavelength = 210 nm; Analytical column: Shim-pack GIST C18 chromatographic column (250 * 4.6 mm, 5 μm), column oven constant 55 °C; Sampling: auto sampler injection, injection volume 20 μL; Quantitative: An external standard method based on peak area. Sample Handling: Transfer to a brown auto sampler via a 0.22 μm aqueous filter.

2.4. Analysis of the interaction between EPSs and chromium

2.4.1. Extraction of EPSs

The extracellular polymeric substances (EPSs) of the sludge samples were extracted using a modified method of ultrasonic and centrifugation (Wong et al., 2015). Sludge of 10 mL was centrifuged at 4000 rpm for 15 min under 4 °C, the supernatant obtained was slime-EPSs. The residual sludge was resuspended to 10 mL with 0.1 mol/L NaCl solution, then the solution was placed in a centrifuge tube with glass beads. The suspension was sonicated (40 kHz, 100 W) for 1 min, shaken horizontally for 10 min, and sonicated for another 1 min. Thereafter, loosely bound-extracellular polymeric substances (LB-EPS) was collected by centrifugation at 9000 rpm and 4 °C for 15 min. After that, the residual sludge was resuspended to 10 mL with 0.1 mol/L NaCl solution. Ultrasonic (40 kHz, 100 W) the suspension for 2 min, tightly bound-extracellular polymeric substances (TB-EPS) was collected by centrifugation at 10,000 rpm and 4 °C for 15 min. Then, all the supernatants were filtered with 0.45 μm filter membrane for further use.

2.4.2. Interaction between EPSs and chromium

Three-dimensional excitation-emission matrix (3DEEM) fluorescence spectroscopy combined with micro-titrimetry was used to quantitatively explore the metal-EPS association. Three kinds of EPSs and Cr(VI) were mixed at 25 °C, then were analyzed after 30 min, respectively. EEM fluorescence spectroscopy and synchronous fluorescence spectra 15 and 60 were used to characterize the EPSs using an F-4600 fluorescence spectrophotometer (Hitachi, Japan), fluorescence spectra was set as: excitation wavelength (Ex) 200—400 nm, emission wavelength (Em) 300—550 nm.

The functional groups and chemical composition of the EPSs was determined using X-ray photoelectron spectroscopy (ThermoFisher SCIENTIFIC, ESCALAB 250) with an Al K Alpha source. The analyser mode and energy step size are CAE (Pass Energy 20.0 eV) and 0.050 or 1.000 eV. The precipitates in the anaerobic fermenter were dried in a vacuum freeze-drying oven for 12 h and powdered for XPS analysis.

2.5. High-throughput 16S rRNA gene sequencing analysis

Using glucose as an initial carbon source, the sludge was sampled after 8 days of operation with and without addition of 100 mg/L Cr(VI). DNA were extracted and sequenced using previous reported method (Song et al., 2019), DNA inserts were double-end sequenced using target region amplification in conjunction with an IlluminaMiSeq sequencer. This part of work was conducted by Novogene Institute Allwegene Technology (Beijing, China).

3. Results and discussion

3.1. Reduction of chromium in the fermenter

XPS was used to determine the product in the reduction process of Cr(VI). As shown in Fig. 1A, two distinct peaks appeared around 578.5 eV and 588.5 eV, corresponding to Cr 2p3/2 and Cr 2p1/2, respectively. The result suggested Cr(VI) was reduced to Cr(III) and then precipitated to the bottom of the anaerobic hydrogen fermenter, which could be mainly $Cr(OH)_3$ or Cr_2O_3 reported previously (Shi et al., 2019).

Fig. 1B demonstrates the reduction and reduction kinetics of Cr(VI) in the anaerobic fermenter for 200 h. Glucose (1 g/l) and Cr(VI) (100 mg/l) were added into the reaction system at the initial of three stages. Almost all Cr(VI) (99.6%) is reduced in a hydrogen fermenter within 70 h in the first hydrogen fermentation stage (I) with glucose as initial substrate. The hydrogen fermenter exhibits better reduction performance than that of 96 h reported by Pan et al. in which planktonic cells of *B. subtilis* were used as reducing microorganisms (Pan et al., 2014). In the second stage, no substrate except 100 mg/l chromium was added into the fermenter, and 94% of Cr(VI) was reduced within 42 h. Better performance in this stage may be due to the redox of glucose during anaerobic hydrogen fermentation, which can promote the reduction of Cr(VI) owing to the production of VFAs (shown in Table S1). More time (about 85 h) was spent on the complete reduction of chromium (99.5%) in the third stage. The reason may be that the consumption of glucose and VFAs reduces the rate of Cr(VI) reduction. Although a large number of studies have shown that hydrogen and volatile fatty acids can be used as electron donors for the reduction of Cr(VI) (Hu et al., 2018b), whether they play the role of the electron donor and the degree of their contribution are still need further exploration.

From the analysis results of Fig. 1B and Table S2, Cr(VI) reduction is generally assumed to be a pseudo-first order reaction. Considering the limitation of chromium transfer from aqueous phase to solid phase, the residual Cr(VI) concentration is modified as Eq. (4).

$$C_{Cr(VI)} = C_{ultimate} + \left(C_{Cr(VI)0} - C_{ultimate}\right) \times a \times e^{-kt} \quad (4)$$

Where $C_{ultimate}$ is the ultimate residual Cr(VI) concentration for the hydrogen fermentation reduction process, α is the variation coefficient to the ideal first-order kinetic, k is the observed first-order reaction rate constant, t is the reaction time, $C_{Cr(VI)0}$ is the initial concentration of Cr(VI), and $C_{Cr(VI)}$ is the concentration of Cr(VI) at time t. $C_{ultimate}$, α and k can be determined by non-linear regression from Fig. 1B. The regression parameters and R^2 in Eq. (4) are summarized in Table S2.

3.2. Determination of chromium-reduced electron donors in the hydrogen fermenter

In order to clarify the role of biological and chemical effects on Cr(VI) reduction and to determine the electron donors in the hydrogen anaerobic digestion system, hydrogen, glucose and acetic acid (as a model of volatile acids) are used as reducing agents for Cr(VI) reduction in chemical and biological reduction experiments, respectively. As shown in Fig. 2, glucose significantly reduces chromium, while hydrogen and acetic acid do not exhibit their reduction of chromium in the chemical reduction experiment.

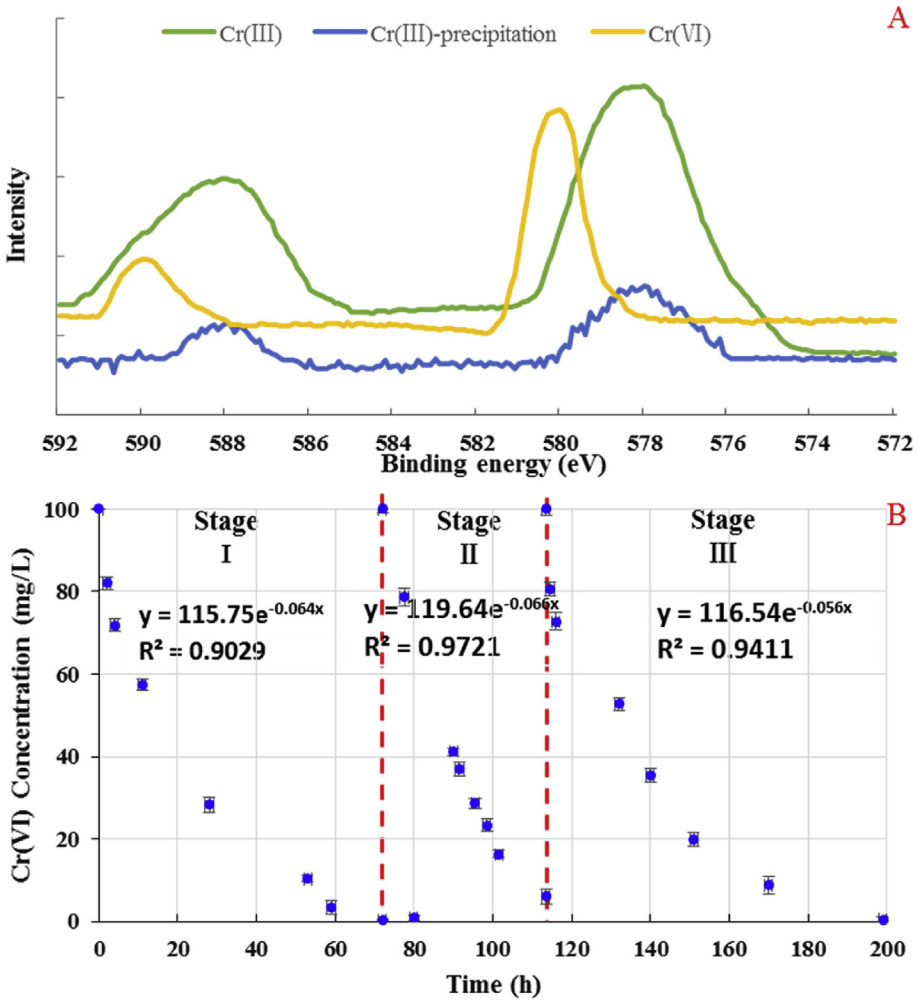

Fig. 1. XPS analyses of the chromium precipitate in the hydrogen anaerobic fermenter (A); Bio-removal of Cr(VI) in three stages with Cr(VI) of 100 mg/l addition at each stage and glucose as initial substrate (B). Condition: pH 5.8, 55 °C, Cr(VI) = 100 mg/l.

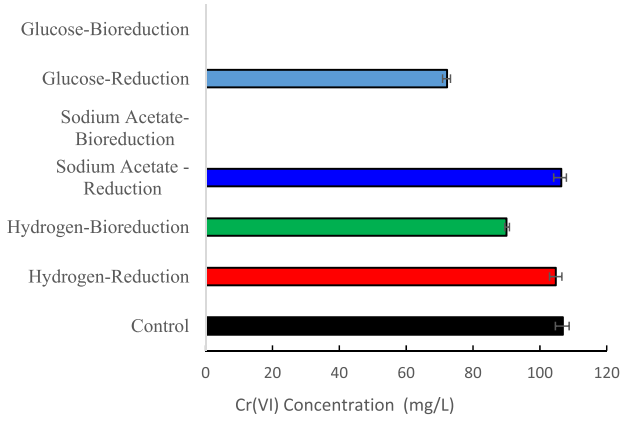

Fig. 2. Determination of electron donors for the reduction of Cr(VI) with various reducing substances (hydrogen, glucose, sodium acetate). Condition: pH 5.8, 55 °C, Cr(VI) = 100 mg/l.

Glucose and acetic acid exhibit a significantly better Cr(VI) reduction effect in the biological reduction system than that under abiotic conditions. In the biological reduction system using hydrogen as an electron donor, Cr(VI) is reduced to some extent, but its reducing ability is limit. It is also possible that the extracellular polymer substances of the strain (inocula) exhibit a reduction effect on Cr(VI) (Shou et al., 2018; Yan et al., 2017). In the biological reduction system in which acetic acid or glucose is used as a substrate, chromium is rapidly and sufficiently reduced. Although it was confirmed that glucose and its metabolites (VFAs) simultaneously played a role in the reduction of chromium, it is still unclear which of the substances exhibit a better reduction effect. It is related to whether hydrogen anaerobic fermentation technology can be applied for Cr(VI) reduction with other inexpensive and readily available wastes (such as straw) as substrate.

Fig. S1 presents the bio-reduction of Cr(VI) with acetic acid as substrate for 50 h, in which the same fermentation condition was set as the glucose-based hydrogen fermentation. Chromium (100 mg/L) is completely reduced within 9 h in first stage. The rate of Cr(VI) reduction is gradually slowing down in followed second (within 17 h) and third stage (within 19 h), probably because the gradual consumption of acetic acid reduces the rate of Cr(VI) reduction. Although Cr(VI) can be reduced by glucose under abiotic conditions (shown in Fig. 2), the reduction rate of Cr(VI) is still much slower in the glucose-based hydrogen fermenter than that in the acetic acid-based hydrogen fermenter. Therefore, it is concluded that acetic acid-based matrix anaerobic fermentation exhibits faster and better reduction of Cr(VI). Based on the good

results of hydrogen fermentation technology and acetic acid as the main metabolite of waste anaerobic fermentation, the technology can be applied to the treatment of actual chromium-containing wastewater.

3.3. Bio-reduction mechanism of chromium

Why reduction efficiency is very poor, as glucose and acetic acid react alone with chromium under abiotic condition, respectively; while the reduction efficiency increases sharply in the presence of microorganisms? Microorganisms can regulate the production and composition of EPSs to adapt to unfavorable circumstances, which affect chromium speciation and behavior (Shou et al., 2018). Therefore, it is inevitable to analyze the interaction between EPSs and chromium.

3.3.1. Distribution of chromium in EPSs

Although many investigations show up evidence of how the Cr(VI) is reduced, only considering the removal efficiency is inaccurate, since its reduction was not taken into account with respect to total Cr (Durán et al., 2018). Therefore, the total chromium in the EPSs were determined, shown as in Fig. 3A. Result shows the content of total Cr gradually decreases from the outside to the inside of EPSs. Coordination reaction between Cr(III) and organic matter functional groups (e.g., hydroxyl, carboxyl, amino and mercapto) can produce dissolved organo-Cr(III) complexes (Dogan et al., 2011; Gong et al., 2018), which explain to some extent the reason of the higher concentration in dissolved slime than that in undissolved LB-EPS and TB-EPS. However, the total concentration of Cr in EPSs is still much lower than the initial concentration of Cr, indicating that most of the reduced Cr(III) precipitated to the bottom of the reactor, or was associated with cells. Similar result was obtained by Chinnannan et al. who found Cr(III) attached to cells of Cellulosimicrobium *funkei* Strain AR8 (Karthik et al., 2017).

3.3.2. Metal coordination to EPSs by fluorescence spectroscopy

Fluorescence spectroscopy was used to characterize metal coordination to dissolved EPSs. Fig. 3B and C shows 3DEEM fluorescence spectroscopy of slime-EPSs, LB-EPSs, TB-EPSs before and after Cr(VI) exposure, in which the main fluorescent chromophores are humic acid-like organics, tryptophan-like and tyrosine-like organics, tryptophan-like organics, respectively. This result suggests that these fluorescent chromophores in EPSs can act as a natural fluorescence probe, which is critical to explore chromium-EPS coordination (Shou et al., 2018). The fluorescence was quenched significantly in slime and LB-EPSs and slightly quenched in TB-EPS, suggesting coordination reactions mainly occur in slime and LB-EPSs verifying the distribution of Cr in EPSs. This coordination reaction enhanced the reduction of chromium, owing to accepting electrons produced by microbial extracellular respiration.

Fig. 3D shows the synchronous fluorescence spectra 15 and 60 of EPSs. The intensity of synchronous fluorescence spectra 60 is higher than synchronous fluorescence spectra 15 of EPSs, suggesting more tryptophan-like organics in EPSs than that of tyrosine-like organics. In addition, the synchronous fluorescence spectra 15 and 60 of EPSs are quenched significantly, which reconfirms the tryptophan and tyrosine substances play an important

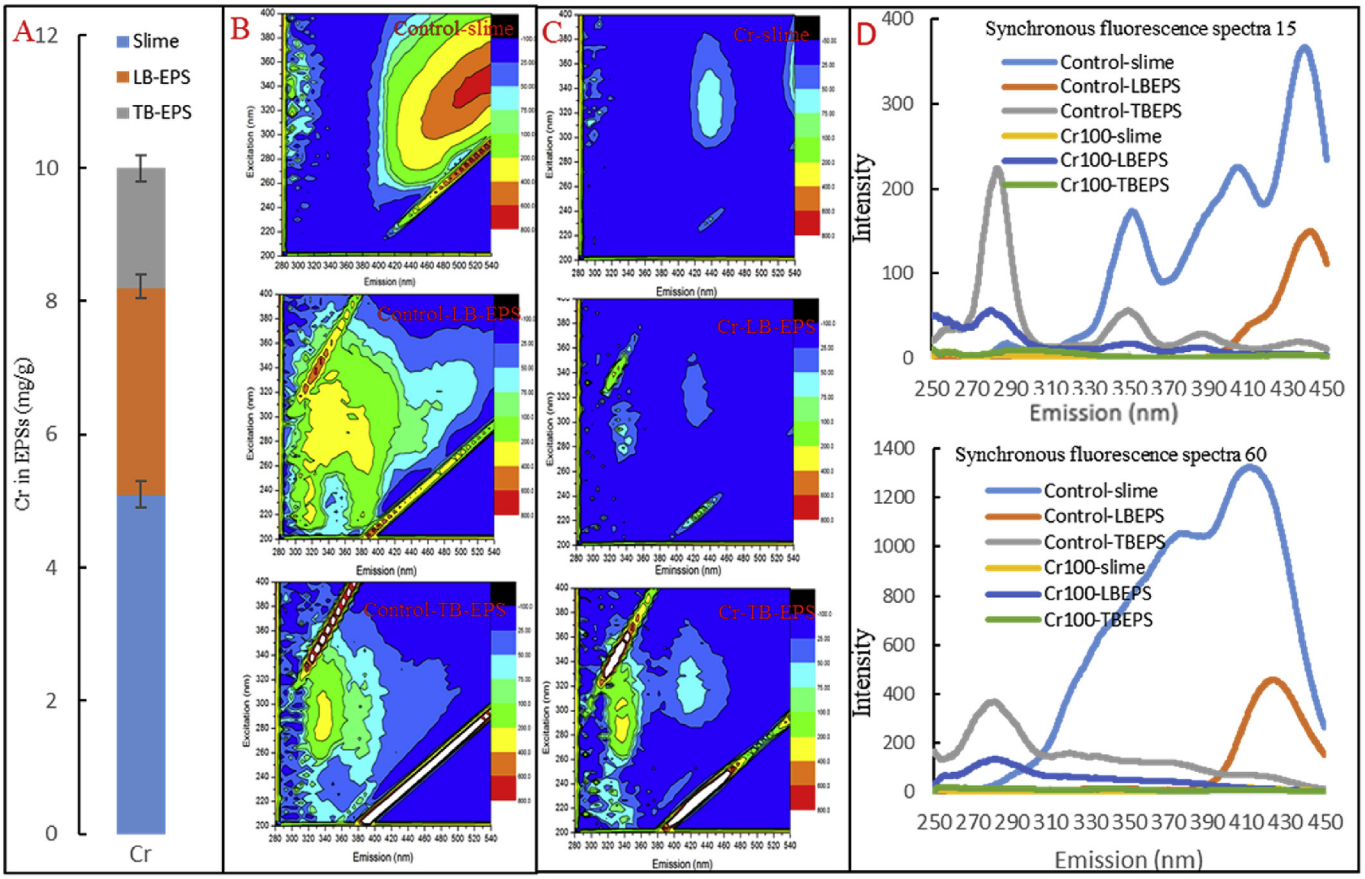

Fig. 3. Concentration of total Cr in the sludge EPSs (A), three-dimensional excitation-emission-matrix profiles of EPSs in the fermenters of control (B) and Cr(VI) exposure (C), synchronous fluorescence spectra ($\Delta\lambda = 15$ nm and $\Delta\lambda = 60$ nm) of EPSs (D).

role for chromium-EPSs coordination.

3.3.3. Interaction mechanism between chromium and EPSs by XPS analysis

As shown in Fig. 4, the oxygen and carbon valence-electron changes in EPSs before and after Cr(VI) exposure were characterized with XPS analysis to obtain more specific information on the chemical bonding states of C and O, and their specific functions in the process of chromium bio-reduction. Four component peaks were analyzed from C 1s peak in EPSs before and after Cr(VI) exposure: (i) 284.5 and 284.6 eV (C—C, mainly from hydrocarbons), (ii) 286 and 286.1 eV (C—N, mainly from proteinaceous substances), (iii) 286.6 and 286.8 eV (C—O, as in hydroxyl, ether-based substances), and (iv) 287.5 and 287.7 eV (C═O, as in carboxylate, carbonyl, amide, acetals). The O 1s peak was resolved into two peaks, which are at 531.1 and 531.3 eV (O═C as in carboxylate, carbonyl, amide) and 532.7 and 532.7 eV (O—C, as in hydroxide; acetals or ether-based substances). By comparison, it was found that the oxygen and carbon signals in EPSs shift after exposure to chromium. The increase of bonding energies certifies that carboxyl and hydroxyl groups play an important role for chromium coordination in EPSs (Shou et al., 2018), which also emphasizes the importance of the carboxyl and hydroxyl groups for Cr(VI) reduction validating colorimetric results.

3.4. Related functional microorganisms

In order to determine the effects of microorganisms on the reduction of Cr(VI) and the change of microbial community structure with Cr(VI) exposure, 16S rRNA gene sequencing was performed for samples from blank group and exposed group after 200 h cultivation. Microbial diversity increased at the phylum, class and genus level after Cr(VI) exposure. As shown in Fig. 5A, after 8 days' exposure to Cr(VI), the four main phyla are: *Firmicutes* 49.31%, *Bacteroidetes* 11.43%, *Chloroflexi* 11.41%, *Thermotogae* 7.82%. While the microbial composition content of the control group was 82.12%, 7.03%, 2.52%, and 0%, respectively. *Firmicutes* and *Chloroflexi* have been mentioned in previous reports those are relatively abundant strains with high Cr(VI) concentrations (Desai et al., 2009; Li et al., 2017). Although *Firmicutes* is the highest proportion of the experimental group, the content of it is significantly lower than that of the control group, which is different from the reduction of Cr(VI) in the methanogenic reactor (Hu et al., 2018b). In the process of reduction of Cr(VI) into Cr(III), *Chloroflexi* has changed from a non-dominant specie to a dominant species, K. Katsaveli et al. also reached a similar conclusion in the study of bacterial diversity in Cr(VI) and Cr(III)-contaminated industrial wastewaters (Katsaveli et al., 2012). The proportion of *Chloroflexi* increase may lead to a decrease of *Firmicutes*. At the class level, shown in Fig. 5B, the composition of *Firmicutes* phyla changed a lot. In the control group, 93% of the *Firmicutes* was *Clostridia*, while *Bacilli* accounted for only 6%. After 8 days' exposure to Cr(VI), the *Bacilli* content increased to 56% and the *Clostridia* content decreased to 43%. And at the genus level, shown in Fig. 5C, *Bacillus*, which plays an important role in the reduction of Cr(VI) (Jin et al., 2017), has the highest proportion. The emerging strains are mainly *Thermotogae*, which is *Mesotoga* at the genus level. *Thermotogae* is a kind of thermophilic microorganism, it can reduce Cr(VI) (Segretin and Donati, 2018). In this experiment, the fermentation temperature was controlled at around 55 °C, so it is speculated that *Mesotoga also* involves the reduction of Cr(VI).

3.5. Reduction mechanism and speculative reduction pathways of chromium in hydrogen anaerobic fermenter

By analyzing the efficiency of Cr(VI) reduction by different electron donors under biotic and abiotic conditions, the bio-reduction pathways and mechanisms of Cr(VI) in the anaerobic hydrogen fermenter can be determined. As shown in Fig. S2, the reduction of Cr(VI) could involve five reaction pathways including

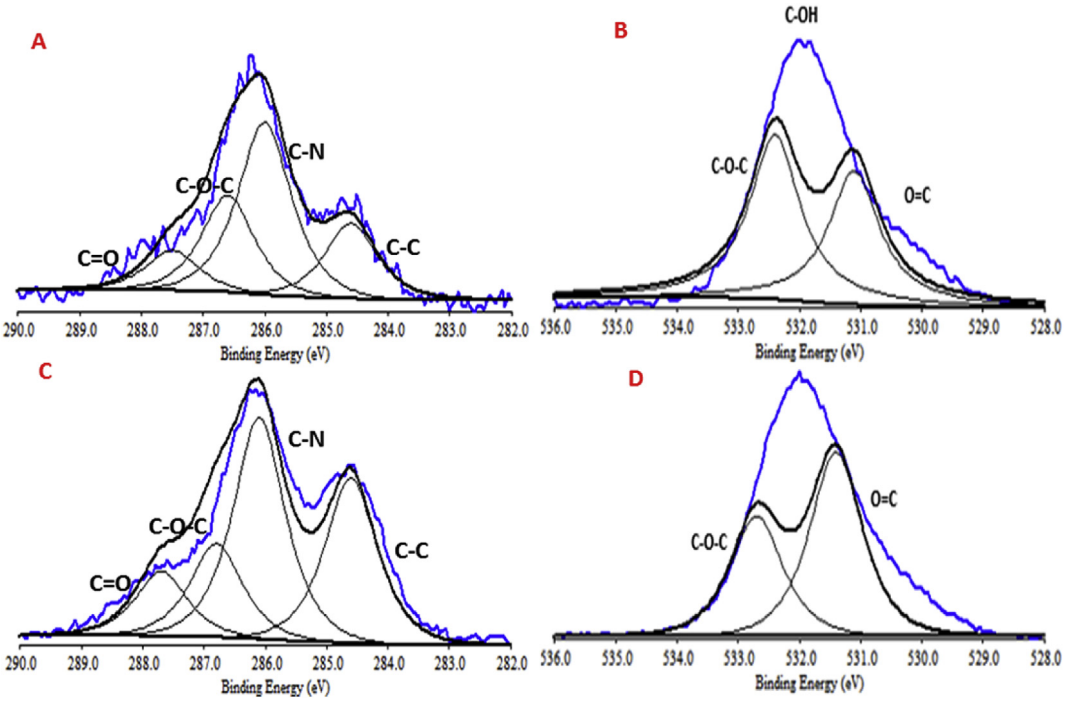

Fig. 4. Change in carbon and oxygen signals in the XPS spectra of EPSs before and after Cr(VI) exposure, Control-C 1s (A), Control-O 1s (B), Cr—C 1s (C), Cr—O 1s (D). Condition: pH 5.8, 55 °C, Cr(VI) = 100 mg/l.

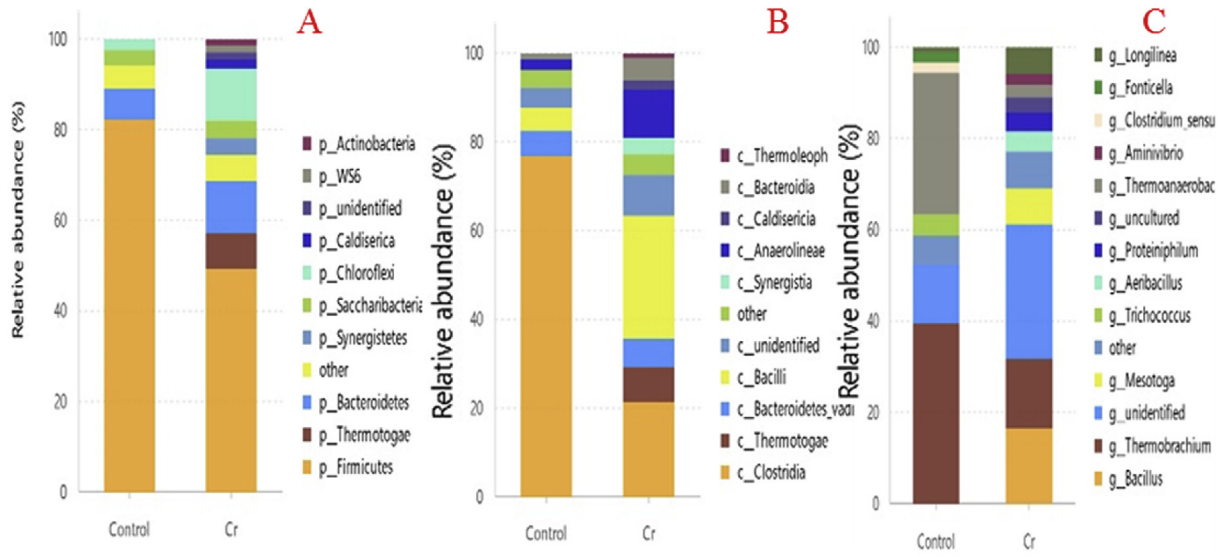

Fig. 5. Change of bacterial community structures at the phylum (B), class (C), genus (D) level in anaerobic hydrogen fermenter with Cr(VI) exposure. Condition: pH = 5.8, 55 °C, Cr(VI) = 100 mg/l.

chemical (1 and 3) and biological (2 and 4) reduction, and four of these reaction pathways have been confirmed (shown in Fig. 2). Extracellular and intracellular reactions are both involved in Cr(VI) reduction. Glucose and its metabolites (VFAs) both play an important role in the reduction process, but the bio-reduction efficiency of VFAs is significantly higher than that of glucose. Therefore, we can optimize the reduction of Cr(VI) by regulating the metabolism of anaerobic microorganisms, which can provide reference for the development of Cr(VI) bio-reduction technology.

4. Conclusion

Cr(VI) of 95% was completely reduced to trivalent chromium (Cr(III)) through chemical and biological reactions, involving in four pathways, in the anaerobic hydrogen fermenter. Bioreduction dominates Cr(VI) removal in a first-order exponential decay mode with glucose and its metabolites (VFAs) as electron donors. Moreover, VFAs are more suitable as electron donors for Cr(VI) bio-reduction than glucose. *Bacilli, Clostridia* and *Thermotogae* dominated the reduction of Cr(VI) by regulating the production and composition of EPSs, where −OH and −COOH play an important role for Cr(VI) reduction by coordination. Based on this experiment, the anaerobic hydrogen fermentation can be applied to the actual chromium-containing wastewater treatment. More attention should be paid to the regulation of microbial metabolites (e.g. volatile fatty acids) to further improve the reduction of Cr(VI).

Declaration of interests

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.

There is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2019.113042.

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