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Removal of Cr(VI) from Wastewater by *Lysinibacillus* sp. Immobilized Magnetite

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ABSTRACT

In order to prolong the usage life of magnetite in the reduction of Cr(VI) to Cr(III), a novel magnetite-*Lysinibacillus* sp. system was proposed in the study. The optimal condition of the combination system was optimized. The highest Cr(VI) removal rate was obtained with glucose of 8 g/L, yeast powder of 10 g/L and pH in a range of 8-10. As compared with the reaction of magnetite and *Lysinibacillus* sp. respectively, enhanced effect of biological-chemical system on Cr(VI) removal was observed, contributing to the synergetic effects between magnetite and the microorganism. With cyclic immobilization method, *Lysinibacillus* sp. was immobilized on the surface of magnetite successfully. The column experiment showed that the engineered system could remove 400 mg/L Cr(VI) from the solution for 24 h, which indicated that the immobilized magnetite is applicable for the practical treatment of Cr(VI)-containing wastewater.

INTRODUCTION

Chromium (Cr), an important raw material in industry, has been widely used in metallurgy, petroleum refining, dye production, electroplating and stainless steel manufacturing (Kimbrough et al. 1999, Ngah & Hanafiah 2008). Improper discharge of wastes from these factories results in Cr contamination. In China, about 6,00,000 tons of chromium slag is produced every year, accumulating about 6 million tons over the years, of which less than 17% is properly disposed or comprehensively utilized (Owlad et al. 2009). Chromium is predominantly observed in trivalent [Cr(III)] and hexavalent [Cr(VI)] forms in natural water (Lin et al. 2018). Cr(III) is relatively stable and more advantageous to form hydroxide precipitation, which is also an important micronutrient element in the human body (Cefalu et al. 2004). Whereas, Cr(VI) is highly mobile in aqueous solutions and almost 100 times more toxic than Cr(III)) and is known as a significant contaminant in the environment because of its carcinogenic, mutagenic and teratogenic properties (Miretzky & Cirelli 2010).

Recently, several technologies have been developed to remove Cr(VI) ions from wastewater, such as physical, chemical and biological processes (Mu et al. 2015). However, physical or chemical treatments are of high cost and may cause secondary pollution (Wanner et al. 2012). Remediation by biological method is an environmental friendly technology and an effective alternative to physicochemical techniques because of the high efficiency degradation performance, no secondary pollution and easy maintenance (Banerjee et al. 2017). Using microorganisms to remove Cr(VI) mainly contains three ways: biosorption, biodeposition and biotransformation (Humphires et al. 2005). As for biotransformation, studies have found that certain microorganisms can use Cr(VI) as an electron acceptor to reduce them to low valent ions and obtain energy from it to sustain growth (Humphires et al. 2005). Regarding the reaction mechanism, two main viewpoints are widely accepted: direct reduction and indirect reduction (Ozturk et al. 2012). Direct reduction is an enzymatic reaction catalysed by some reductases in microorganisms (Chen et al. 2016). Indirect reduction means that microorganisms participate in the reaction through their metabolites. While, low reduction rate hinders its application (Shaili & Indu 2006).

It is proposed that combination of chemical and biological methods could solve the problem caused by single approaches. Chemical method could promote the reaction rate, while microorganisms could prolong the service time. If we can find cheap chemical reducer and efficient microorganisms, the method will be more convenient and practical (Qiao et al. 2010).

Currently, attention has been paid to iron minerals, since Fe(II)-containing compounds can remove pollutants from water. Previous researches demonstrated that various iron minerals can be used to remove Cr(VI) from water, such as limonite, pyrite, siderite, magnetite and goethite (Huggins et al. 2016)

Accordingly, indirect reduction method, namely, biochemical combination method for treating Cr(VI) in wastewater has attracted tremendous interest. A variety of chromium reduction microorganisms, such as *Goebacter* and *Shewanella* have been verified to have the ability to reactivate the oxidized film by reducing the less soluble Fe(III) to moderately soluble Fe(II) (Cao et al. 2010). In practical applications, iron-reducing bacteria were used for remediating sulfamethoxazole contamination in soil via reduction of Fe(II) from Fe(III) (Mohatt et al. 2011). Xu et al. (2005) observed that *Cellulomonas flavigen* and Fe(III) could treat the chromium-containing wastewater. It should be noted that the addition of Fe(III) promotes the bioreduction process, improves the removal efficiency, and accelerates the removal rate of Cr(VI).

In this study, a combination system with magnetite and *Lysinibacillus* sp. was selected for reducing Cr(VI) from wastewater. The microorganism was immobilized on the magnetite, and the optimal environmental factors were evaluated. The mechanism of Cr(VI) reduction from aqueous solution and synergistic effect by iron stone with the assistance of immobilized bacteria was also studied.

MATERIALS AND METHODS

Strains: *Shewanella oneidensis* (ATCC 700550), *Shewanella decolorationis* (JCM 21555) and *Shewanella decolorationis* (MCCC 1A11454) were prepared by China Marine Microorganisms Collection. *Lysinibacillus* sp. VKM B-713, *Lysinibacillus* sp. JLT12, *Morganella morganii* subsp, *Bacterium* L9 and *Serratia marcescens* strain SW-4 were isolated from the anaerobic treatment plant in Shaoxing wastewater treatment plant (Shaoxing, Zhejiang, China), then preserved in Luria-Bertani (LB) bevel after identification. Strains were labelled as bacteria 1 to bacteria 8.

Reagents and reagents: Potassium dichromate ($K_2Cr_2O_7$) was obtained from Shanghai Jinlian Fine Chemical Co., Ltd. (China). Zerovalent iron (Fe^0) was provided by Shanghai Chemical Reagent Purchasing and Supply Station. Other reagents used in this study were purchased from Sino Pharm Chemical Reagent Co., Ltd. (Shanghai, China). The morphology of particle was analysed by a scanning electron microscope (SEM) (JSM-6360LV, JEOL), and the elemental analysis was performed by X-act energy spectrum (EDS) (Oxford, England). The total chromium (Cr) content in the solution was determined by inductively-coupled plasma atomic emission spectroscopy (ICP-AES) (Prodigy xp, Leeman), and the microbial community was identified with high-throughput sequencing in Shanghai Bioengineering Co. Ltd.

Selection of iron stone-microbial synergistic system: The mineral salt (MS) medium was prepared containing 50 mg/L $K_2Cr_2O_7$, then 2 mL of fresh seed solution and 0.2 g of haematite (magnetite, pyrite) were added into the 100 mL of MS under sterile conditions. After shaking up, 2 mL of the solution sample was withdrawn, centrifuged at 8000 rpm, and the concentration of Cr(VI) in the supernatant was determined via diphenylcarbazide colourimetric method by spectrophotometry. The cells were cultured at 30°C for 48 h under anaerobic, anoxic and aerobic conditions separately. Every 24 h, 2 mL solution sample was obtained, centrifuged at 8000 rpm, and the pH and Cr(VI) were determined. Afterward, the removal efficiency was calculated. Eventually, an iron ore-microbial synergistic system was selected. The Cr(VI) removal efficiency was calculated using Eq. 1 as follows:

$$\text{Removal efficiency (\%)} = (C_0\text{-}C_{48})/C_0\times100 \qquad ...(1)$$

Optimization of the removal conditions: With the basic salt medium, carbon source and concentration, nitrogen source and concentration and pH were optimized. Glucose, sodium acetate, sucrose and soluble starch were selected as carbon sources in the same amount, then the content of Cr(VI) was determined every 24 hours in order to obtain the best carbon source and the optimal concentration of best carbon source was evaluated later (2, 4, 6, 8, 10 g/L). The same method was used to select the optimal nitrogen source as well as the optimal concentration among yeast powder, peptone and ammonium chloride. The optimal pH was confirmed by adjusting pH of the basal medium to 4, 5, 6, 7, 8, 9, 10 and determining the residual content of Cr(VI) every 24 hours.

The enhancement of the removal of Cr(VI) by microorganisms: Optimal medium was prepared with $K_2Cr_2O_7$, and subsequently iron minerals were added. The selected strain was inoculated into the solution. The contents in Erlenmeyer flask were cultured at 30°C for 72 h in a thermostatic oscillation incubator. Meanwhile, samples were periodically taken out for the analysis of the content of Cr(VI) every 12 hours. The change of Cr(VI) in the solution was fitted to the first-order reaction kinetics (2) and second order reaction kinetics (3) equations respectively, and then k_1, k_2, R_1^2, R_2^2 were calculated.

$$C_t/C_0 = \exp(-k_1 t) \qquad ...(2)$$

$$C_t = C_0/(1+k_2 C_0 t) \qquad ...(3)$$

Immobilization of the microorganism on iron mineral: The LB liquid medium was prepared with target microorganism and inoculated for 24 hours for the preparation of suspension containing 10^7 strains. The granular iron stone

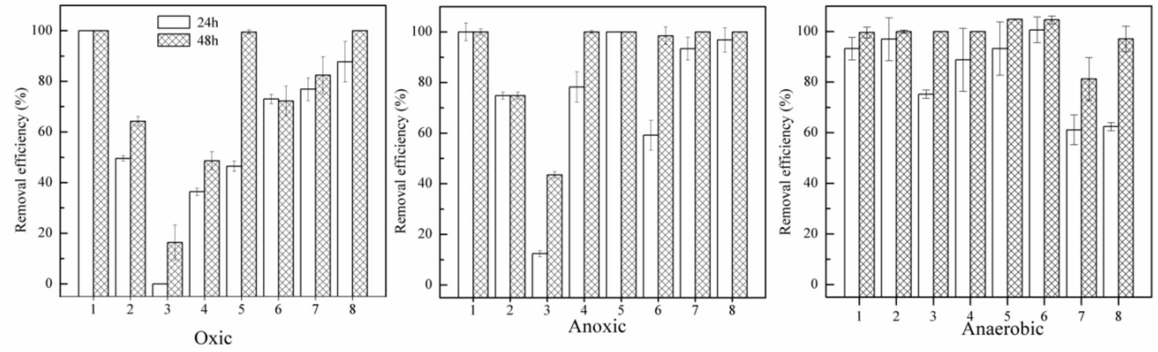

Fig.1: Removal of Cr (VI) with eight microorganisms and magnetite under different conditions.
1: *S. oneidensis*; 2: *S.decolorationis* (JCM 21555); 3: *S. decolorationis* (MCCC 1A11454); 4: *Lysinibacillus* sp. VKM B-713;
5: *Lysinibacillus* sp. JLT12; 6: *Morganella morganii* subsp; 7: *Bacterium* L9; 8: *Serratia marcescens* strain *S*W-4

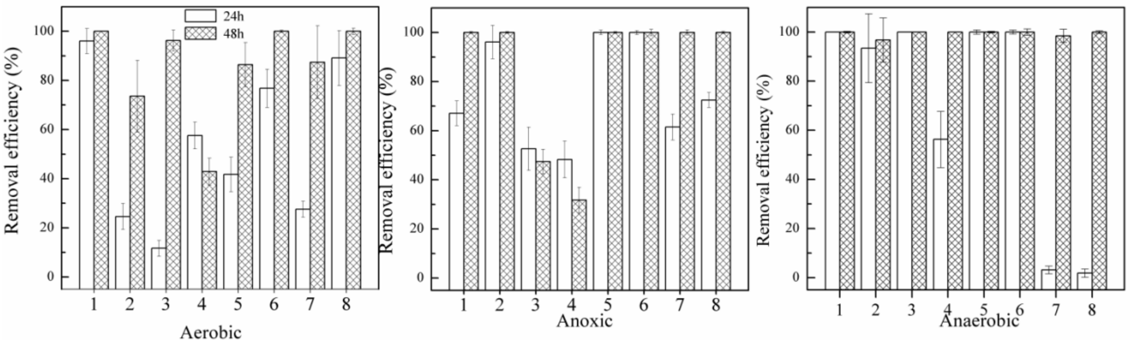

Fig. 2: Removal of Cr (VI) with eight microorganisms and pyrite under different conditions.
1: *S. oneidensis*; 2: *S.decolorationis* (JCM 21555); 3: *S. decolorationis* (MCCC 1A11454); 4: *Lysinibacillus* sp. VKM B-713;
5: *Lysinibacillus* sp. JLT12; 6: *Morganella morganii* subsp; 7: *Bacterium* L9; 8: *Serratia marcescens* strain *S*W-4

was used as the biofilm carrier. After immobilization, 2-3 g carrier was taken out, and the DNA was extracted for high-throughput sequencing analysis to determine whether the target strain was successfully immobilized on the vector.

Removal of Cr(VI) by the iron stone-immobilized microorganism: The material of immobilized iron mineral was placed in an adsorption column, and the nutrient solution containing 200 mg/L of Cr(VI) was passed through the adsorption column. The concentration of Cr(VI) was measured every 24 hours. Meanwhile, a new nutrient solution would be replaced when Cr(VI) was completely removed. After running for 14 days, 2-3 g of the carrier was taken out, and the DNA was extracted for high-throughput sequencing analysis which was performed to evaluate the loss of target species in the process of the treatment of Cr(VI)-containing wastewater.

RESULTS AND DISCUSSION

Screening of the iron mineral-microorganism system: The removal efficiency of Cr(VI) by bacterium 1-8 combined with haematite, magnetite and pyrite is displayed in Fig. 1 and Fig. 2 respectively. Due to the inefficiency in removal capacity, the results of haematite combination were not listed. By contrast, magnetite combined with bacterium 1 and bacterium 5 respectively, were of high efficiency than other combinations. Under the conditions of aerobic, anoxic and anaerobic, the removal efficiency of Cr(VI) all could reach 100% after 48 hours. Furthermore, bacteria 1 and 6 had superior efficiency in the synergistic system with pyrite compared to that of others. However, many studies have been focused on bacterium 1 (*S. oneidensis*) (Kaneko et al. 2002). From the perspective of developing new strains, magnetite + bacterium 5 and pyrite + bacterium 6 were chosen for research. Finally, magnetite + bacterium 5 was selected (*Lysinibacillus* sp. JLT12) as the target system by investigating the removal effect by increasing the initial concentration of Cr(VI).

Optimization of magnetite-*Lysinibacillus* sp. JLT12 synergistic system: The optimal reaction conditions for the removal of Cr(VI) of magnetite-*Lysinibacillus* sp. JLT12

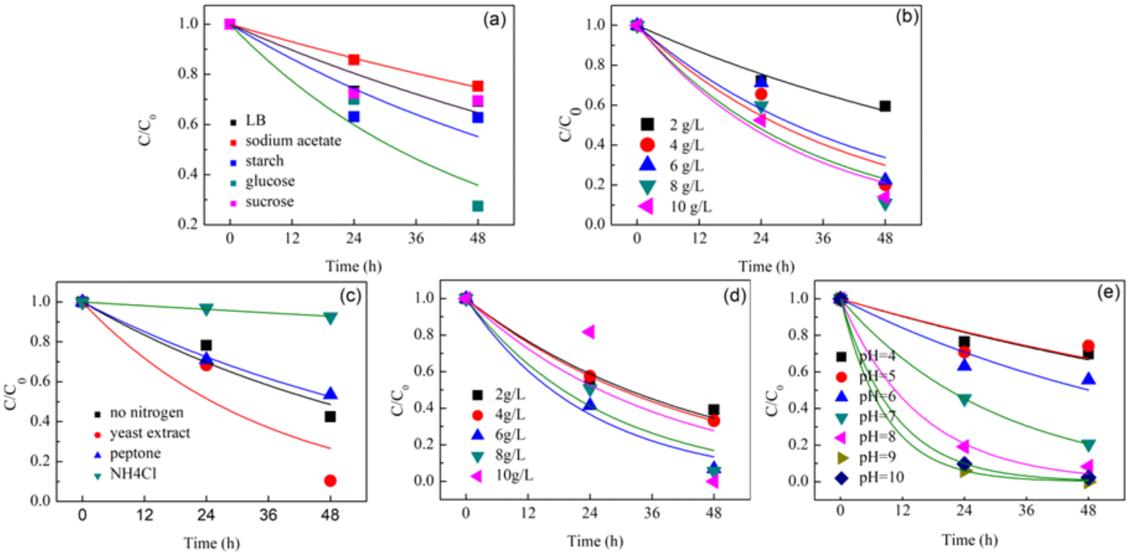

Fig. 3: Effects of carbon source, nitrogen source and pH on the removal of Cr(VI).

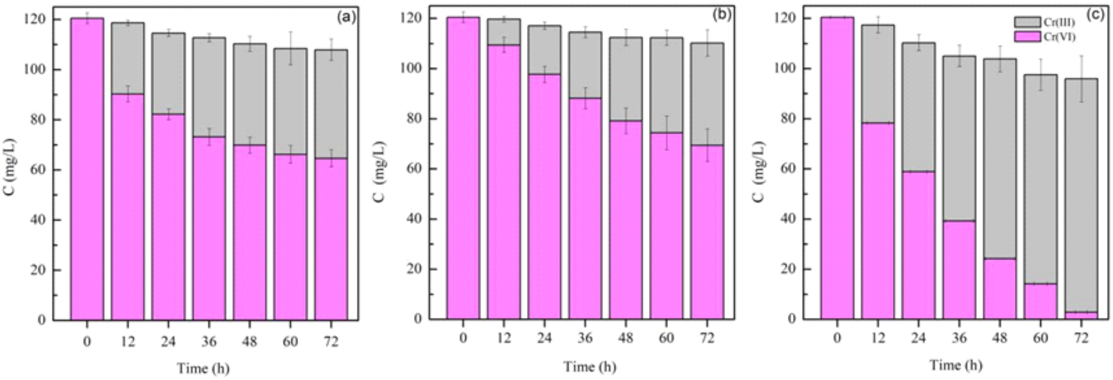

Fig. 4: Change of Cr(VI) and Cr(III) during the reduction process.
(a) Magnetite (b) *Lysinibacillus* sp. (c) *Lysinibacillus* sp.-magnetite

synergistic system were obtained by optimizing the type and concentration of carbon source and nitrogen source along with pH. Fig. 3 shows that the best carbon source was glucose of 8 g/L, the optimal nitrogen source was yeast powder of 6 g/L and the optimum pH was 8-10.

Mechanisms of magnetite-*Lysinibacillus* sp. JLT12 synergistic process: As illustrated in Fig. 4, the total chromium content decreased markedly during the reaction. The decrease is associated with the effect of Fe(II) in magnetite which can reduce part of Cr(VI) to Cr(III). Meanwhile, Fe(OH)*x* is formed which can coprecipitate with Cr in the removal process. To the best of our knowledge, under the reduction conditions, the decrease of total chromium content is caused by *Lysinibacillus* sp., including reductive reaction of Cr(VI) from enzyme and adsorption effect from extracellular polymer substances which could adsorb a part of Cr simultaneously. It must be pointed out that in the process of Cr(VI) removal by magnetite-*Lysinibacillus* sp. JLT12 synergistic system, the total chromium content was reduced, moreover, most of Cr(VI) was converted to Cr(III), so the toxicity was reduced visibly. The results confirm that *Lysinibacillus* sp. has an obvious effect on promoting magnetite on the reduction of Cr(VI).

The variations of Fe(II) and Fe(III) content in the magnetite system and the magnetite-*Lysinibacillus* sp. synergistic system are also studied and presented in Fig. 5. In the magnetite system, the content of Fe(II), Fe(III) and total Fe increased with the progress of the reaction. Unlike

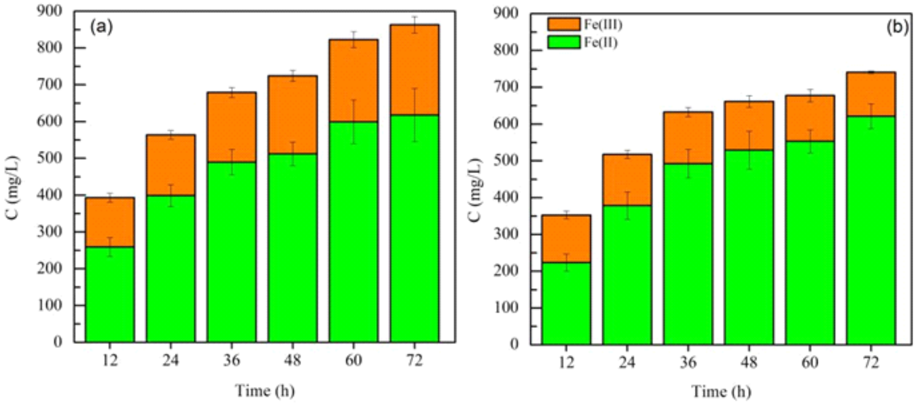

Fig. 5: Change of Fe(II) and Fe(III) during the reduction process.
(a) Magnetite (b) *Lysinibacillus* sp.-magnetite

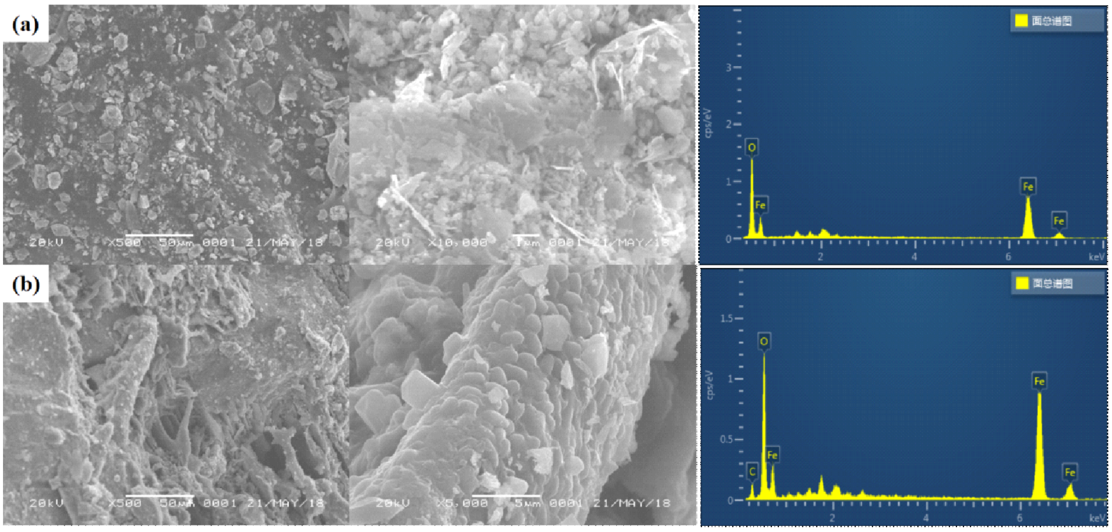

Fig. 6: SEM-EDS images of biochar before (a) and after (b) immobilized with *Lysinibacillus* sp.

the magnetite-*Lysinibacillus* sp. system, that the Fe(III) and total Fe content decreased relatively lower compared to those of the single system, which is mainly attributed to the adsorption of microbial extracellular polymer substances and the flocculation of Fe(OH)*x*. However, the content of Fe(II) decreased unconspicuously. It was assumed that *Lysinibacillus* sp. can reduce Fe(III) to Fe(II) under facultative conditions.

SEM-EDS analysis: It was reported that it is difficult for microorganisms to grow on the small specific surface area of iron ore. SEM-EDS analysis was carried out to confirm whether the microbes were immobilized on the surface of magnetite. As shown in Fig. 6, the magnetite demonstrates crystal structure, and the main elements involved are O and Fe before the film was deposited. Notably, after the film was hanged, the surface of the magnetite was covered with a white film. Through the high magnification phenomenon, we could find the microbes scale-like grew on the surface of the magnetite (Bai et al. 2017). On the other hand, the C element appeared on the surface of the magnetite after the membrane, as exhibited by elemental analysis, suggesting that the immobilization was successfully achieved. It may be related to the characteristics of *Lysinibacillus* sp., as the secreted extracellular polymer was more likely to adhere to the surface of magnetite (Feng et al. 2013).

To further confirm the results, 16S rDNA high-throughput sequencing was carried out to analyse the immobilized magnetite before and after the treatment with Cr(VI) nutri-

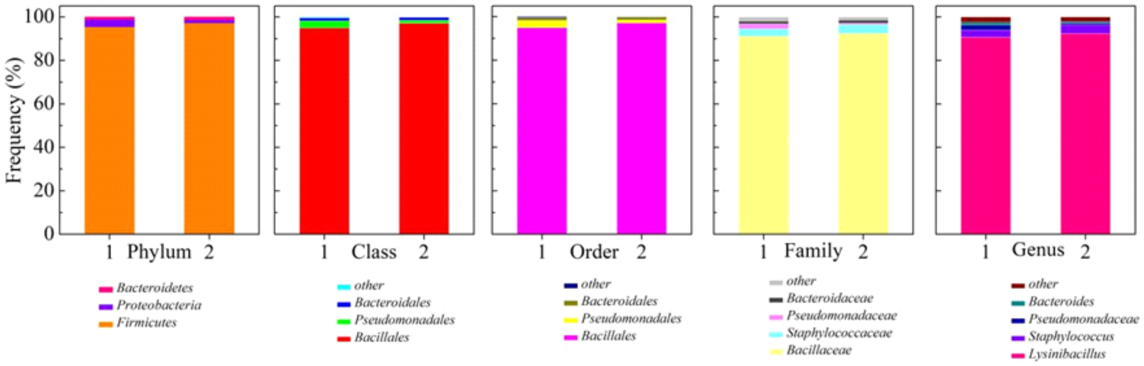

Fig. 7: Microbial communities in the immobilized magnetite before (1) and after treated with Cr(VI) (2).

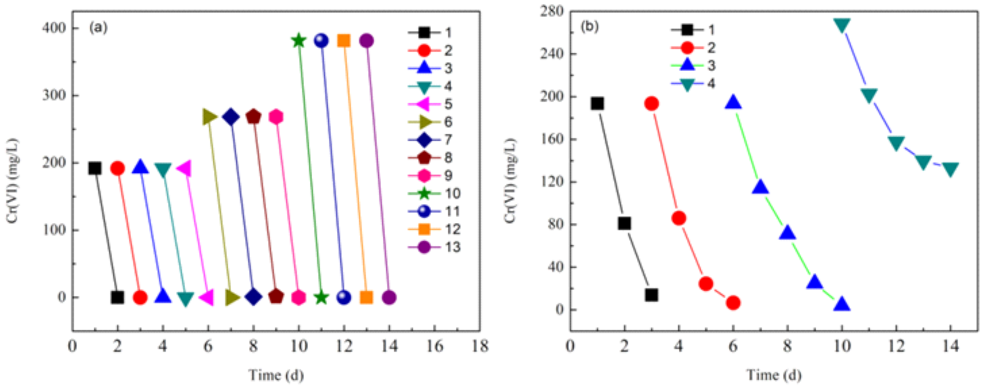

Fig.8: The removal efficiency of immobilized magnetite (a) and magnetite (b).

ent solution. The results can be seen in Fig. 7. It leads us to conclude that the dominant phylum on the magnetite is *Firmicutes*, the dominant class is *Bacillales*, the dominant order is *Bacillales*, the dominant family is *Bacillaceae*, and the dominant genus is *Lysinibacillus* which were completely consistent with the target microorganism *Lysinibacillus* sp. The result is consistent with the observation from SEM-EDS. Furthermore, after the treatment of Cr-containing wastewater, the dominant phylum, class, order, family, and genus did not demonstrate the significant difference, suggesting *Lysinibacillus* sp. could be the dominant bacteria in the treatment of Cr-containing wastewater (Owlad et al. 2009).

Performance of immobilized magnetite: Concerning to evaluate the removal ability of immobilized magnetite, 200 mg/L Cr(VI) was treated with immobilized magnetite and magnetite, respectively. It is noteworthy that after 24 hours of operation, the removal efficiency of Cr(VI) reached 100% in the immobilized system. Subsequently, although the concentration of Cr(VI) in influent was increased to 300 mg/L on the 7th day or even 400 mg/L on the 9th day, the removal rate of 24 h could still reach 100%. But in the initial operation of the control group, after 48 hours, the removal efficiency of Cr(VI) could reach 100%. What is more, it took 72 or even 96 h to completely remove Cr(VI) as the reaction proceeded. It can be seen that the immobilized technology could improve the Cr(VI) removal efficiency of magnetite, and the immobilized magnetite is a novel and effective material which can be used in the remediation of Cr(VI) contaminated water and soil (Fig. 8).

CONCLUSION

In this paper, a synergistic system of magnetite and *Lysinibacillus* sp., which can effectively remove Cr(VI) from water was successfully obtained. The optimal conditions for the system were glucose of 8 g/L, yeast powder of 6 g/L and pH 8-10. *Lysinibacillus* sp. was then successfully immobilized on magnetite via cyclic membrane method, in which magnetite was used as a carrier. The immobilized magnetite has a high removal efficiency for Cr(VI), which can completely remove 400 mg/L of Cr(VI) within 24 h after 14 days of operation. The immobilized magnetite exhibits excellent advantages over both chemical and biological methods, which assures a fast reaction rate from *Lysinibacillus* sp. and magnetite. Besides, Fe(III) produced from magnetite can be reduced to Fe(II) by *Lysinibacillus* sp., which prolong the usage life of magnetite. Due to the high efficiency, it is expected that the novel PRB material has potential applications in enhanced Cr(VI) removal from aqueous solutions.

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REFERENCES

Bai, Y.N., Lu, Y.Z., Shen, N., Lau, T.C. and Zeng, R.J. 2017. Investigation of Cr(VI) reduction potential and mechanism by *Caldicellulosiruptor saccharolyticus* under glucose fermentation condition. J.Hazard. Mater., 344: 585-592.

Banerjee, S., Joshi, S.R., Mandal, T. and Halder, G. 2017. Insight into Cr^{6+} reduction efficiency of *Rhodococcus erythropolis* isolated from coalmine wastewater. Chemosphere, 167: 269-281.

Cao, F., Li, F.B. and Liu, T.X. 2010. Effect of *Aeromonas hydrophila* on reductive dechlorination of DDTs by zero-valent iron. J. Agr. Food Chem., 58(23): 12366-12372.

Cefalu, W.T. and Hu, F.B. 2004. Role of chromium in human health and in diabetes. Diabetes Care, 27(11): 2741-2751.

Chen, C.Y., Chen, C.K., Hseih, M.C., Lin, S.T., Ho, K.Y., Li, J.W., Lin, C.P. and Chung, Y.C. 2016. Hexavalent chromium removal and bioelectricity generation by *Ochrobactrum* sp. YC211 under different oxygen conditions. J. Environ. Sci. Health A Tox. Hazard. Subst. Environ. Eng., 51: 502-508.

Feng, Y.L., Wang, L.J. and Li, H.R. 2013. Study on reduction of hematite by dissimilatory metal reducing bacteria. J. Central South University, 44(5): 1755-1762.

Huggins, T.M., Haeger, A., Biffinger, J.C. and Ren, Z.J.2016. Granular biochar compared with activated carbon for wastewater treatment and resource recovery. Water Res., 94: 225-232.

Humphires, A.C., Nott, K.P., Hall, L.D. and Macaskie, L.E.2005. Reduction of Cr (VI) by immobilized cells of *Desulfovibrio vulgaris* NCIMB 8303 and *Microbacterium* sp. NCIMB 13776. Biotechnol. Bioeng., 5: 589-596.

Kaneko, T., Sugita, S., Tamura, M., Shimasaki, K., Makino, E. and Silalahi, L.H. 2002. Highly active limonite catalysts for direct coal liquefaction. Fuel, 81(11-12): 1541-1549.

Kimbrough, D.E., Cohen, Y., Winer, A.M., Creelman, L. and Mabuni, C.1999. A Critical assessment of chromium in the environment. Crit. Rev. Env. Sci. Tec., 29(1): 1-46.

Lin, C., Luo, W.J., Luo, T.T., Zhou, Q., Li, H.F. and Jing, L.R. 2018. A study on adsorption of Cr(VI) by modified rice straw: Characteristics, performances and mechanism. J. Clean. Prod., 196: 626-634.

Miretzky, P. and Cirelli, A.F. 2010. Cr(VI) and Cr(III) removal from aqueous solution by raw and modified lignocellulosic materials: A review. J. Hazard.Mater., 180(1-3): 1-19.

Mohatt, J.L., Hu, L.H. and Finneran, K.T. 2011. Microbially mediated abiotic transformation of the antimicrobial agent sulfamethoxazole under iron-reducing soil conditions. Environ. Sci. Technol., 45(11): 4793-4801.

Mu, Y., Ai, Z., Zhang, L. and Song, F. 2015. Insight into core-shell dependent anoxic Cr(VI) removal with $Fe@Fe_2O_3$ nanowires: Indispensable role of surface bound Fe(II). ACS Appl. Mater. Inter., 7(3): 1997-2005.

Ngah, W.S.W. and Hanafiah, M.A.K.M. 2008. Removal of heavy metal ions from wastewater by chemically modified plant wastes as adsorbents: A review. Bioresour. Technol., 99(10): 3935-3948.

Owlad, M., Aroua, M. K. and Daud, W.A.W. 2009. Removal of hexavalent chromium-contaminated water and wastewater: A review. Water Air Soil Poll., 200(1-4): 59-77.

Ozturk, S., Kaya, T., Aslim, B. and Tan, S. 2012. Removal and reduction of chromium by *Pseudomonas* spp. and their correlation to rhamnolipid production. J. Hazard.Mater., 15: 64-69.

Qiao, L., Wen, D.H. and Wang, J.L. 2010. Biodegradation of pyridine by *Paracoccus* sp. KT-5 immobilized on bamboo-based activated carbon. Bioresour. Technol., 101(14): 5229-5234.

Shaili, S. and Indu, S.T. 2006. Evaluation of bioremediation and detoxification potentiality of *Aspergillus niger* for removal of hexavalent chromium in soil microcosm. Soil Biol. Biochem., 38: 1904-1911.

Wanner, C., Zink, S. and Eggenberger, U. 2012. Assessing the Cr(VI) reduction efficiency of a permeable reactive barrier using Cr isotope measurements and 2D reactive transport modeling. J. Contam. Hydrol., 131: 54-63.

Xu, W.H., Liu, Y.G., Zeng, G.M., Li, X., Tan, C.F. and Yuan, X.Z. 2005. Enhancing effect of iron on chromate reduction by *Cellulomonas flavigena*. J. Hazard. Mater., 126(1-3): 17-22.