

Research Article

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Production of a bioflocculant by using activated sludge and its application in Pb(II) removal from aqueous solution

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Abstract: In this study, the characteristics of a bioflocculant produced by using activated sludge as raw materials were investigated. The performance of this bioflocculant in the removal of Pb(II) from aqueous solution and the corresponding mechanisms were determined as well. After cultivating a bioflocculant-producing strain in an alkaline thermal pre-treatment sludge for 60 h, approximately 4.45 g of bioflocculant containing a protein backbone was harvested from 1 L of fermentation broth. This bioflocculant can remove 98.5% of Pb(II) from aqueous solutions under optimal conditions, which include a bioflocculant dosage of 6 mg/L and a CaCl₂ concentration of 70 mg/L at a pH of 6.5.

Keywords: Bioflocculant; Activated sludge; Pb(II).

1 Introduction

As economies develop, more and more heavy metal-containing wastewater is generated, and the release of these heavy metals cause serious environmental pollution and threaten human health [1]. Pb(II) is one of the most toxic heavy metals that contaminate the wastewater produced by plating, tanneries, oil refining, mining and other activities, and this ion causes many human diseases, including anemia, hepatitis, nephrite syndrome,

kidney failure, and diseases of the nervous system [2-4]. Therefore, removal of Pb(II) from wastewater is necessary and urgent for the protection of human health as well as the environment.

Various of methods have been developed for the removal Pb(II) from wastewater, and the most commonly used have been precipitation, adsorption, ion-exchange, filtration, electroplating, flocculation and coagulation [5,6]. There are some significant problem when these methods are applied to the removal of Pb(II) from wastewater, such as high energy consumption and a high yield of toxic sludge [7]. The development environment friendly technologies for the removal of Pb(II) is thus of both academic and practical interest.

Flocculation has attracted significant attention because of its advantages, which include effectiveness, efficiency, stability, and wide practicability [8,9]. However, widely used chemical flocculants have been shown to cause health problems and secondary environmental pollution [10,11], which greatly restrict the wide applications of these flocculants. These limitations have become especially important in light of increasingly stringent drainage rules that have gradually emerged. Bioflocculants, both active secreted by microorganisms and released upon cell lysis, are a kind of environmentally-friendly material with the advantages of non-toxicity and biodegradability, and they have been recognized as an alternative to the use of chemical flocculants in wastewater treatment [12]. Because of all of these factors, in recent years, the use of bioflocculants has been considered to be a potential solution to environmental pollution [13].

A high production cost has long been the main obstacle to the widespread use of bioflocculants [14]; thus, the search for low-cost substrates for the production of bioflocculants by cultivated microorganisms is of great practical significance. A potential source of such substrates is waste activated sludge, which is being generated in increasing amounts by wastewater treatment plants (WWTPs) all over the world [15]; for example, approximately 9.2 million tons of waste activated sludge

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were produced in China in 2017 [16]. The management of waste activated sludge is one of the most serious challenges for WWTPs, as it accounts for 60% of total operational expenses [17,18]. From the perspective of resource utilization, activated sludge contains macromolecular compounds, such as polysaccharides, proteins, and cellulose, which have been proposed as sources of bioflocculants. Thus, microorganisms that can effectively utilize these substrates in activated sludge to produce bioflocculants are of academic and practical interests.

The aim of the present study was to produce a bioflocculant by using activated sludge as a raw material and to investigate the performance of the bioflocculant in the removal of Pb(II) from aqueous solutions. In the production of bioflocculants, effects of the pre-treatment of activated sludge on bioflocculant yield were determined. In addition, the composition of the bioflocculant was analyzed, and a series of experiments were performed to investigate the effects of bioflocculant dosage, pH value and contact time on the removal of Pb(II).

2 Materials and Methods

2.1 Activated sludge

Activated sludge samples were obtained from Sanwayao sewage treatment plant, Sichuan province, China. This sewage treatment plant treats approximately 50,000 m³ of domestic sewage per day by using Anaerobic-Anoxic-Oxic (A²/O) technology. Samples of sludge were collected from secondary sedimentation tanks. Prior to bioflocculant production, raw sludge samples were subjected to sterilization (ST), alkaline-thermal (ALT) and acid-thermal (ACT) treatments. Sterilization (steam sterilization) was performed 30 min at 121°C according to [19]. ALT treatment was carried out by autoclaving at 121°C for 30 min after adjusting the pH value of the sludge to 10 with 1 M sodium hydroxide (NaOH). In ACT treatment, the pH value of the sludge was adjusted to 2 using 1 M hydrochloric acid (HCl) prior to autoclaving for 30 min at 121°C.

2.2 Bioflocculant production

The bioflocculant-producing strain used in the following experiments was *Saccharomyces cerevisiae*, purchased from the China Center for Type Culture Collection. To produce bioflocculant, the strain was inoculated in 100

mL of pre-treated activated sludge and incubated on a reciprocal shaker (SHA-A) at 150 rpm and 35°C. After 60 h of cultivation, the fermentation broth with the flocculating components was obtained. The fermentation broth was centrifuged at 5000 rpm for 30 min to remove cells, and the supernatant with the flocculating components initially remained in this liquid form. Cold ethanol (4°C) was added to the liquid at a volume ratio of 2:1. After precipitation for 24 h, the resulting precipitate was collected by centrifugation at 5000 rpm for 30 min. The pellet represented the crude bioflocculant [20]. The crude bioflocculant was dissolved in deionized water and purified by dialyzing overnight and was then lyophilized [21].

The total sugar of the bioflocculant was determined by the phenol-sulfuric acid method [22]. The protein of the bioflocculant was determined by the Bradford method [23]. The molecular weight of the bioflocculant was determined by gel permeation chromatography (GPC).

2.3 Pb(II) removal tests by the bioflocculant

Pb(NO₃)₂ (Hengxing Chemicals, China) was prepared by dilution of a 50 mg/L stock solution. Adsorption experiments were conducted to determine the effects of the bioflocculant dosage and solution pH in the presence of Ca²⁺ on the removal of Pb(II) from Pb(NO₃)₂ solutions. Before treatment, the pH of 300 mL of the above Pb(NO₃)₂ solution, as determined with a PHS-3C pH meter, was adjusted using 1 M NaOH or HCl. Subsequently, CaCl₂ and the bioflocculant (both prepared as 1 g/L solutions) were added into the beaker in turn. Next, the mixture was vigorously stirred (300 rpm) for 1 min and slowly stirred (80 rpm) for 4 min, and then allowed to stand for 10 min using a six-breaker jar tester. Finally, the supernatant was collected and the residual Pb(II) was determined. The removal efficiency (RE) and removal capacity of Pb(II) were calculated as follows:

$$RE(\%) = (C_0 - C_e) / C_0 \times 100 \quad (1)$$

$$\text{Removal capacity} = (C_0 - C_e) V / W_R \quad (2)$$

Where C_0 and C_e were the initial and equilibrium Pb(II) concentrations (mg/L), respectively. V was the volume of the Pb(NO₃)₂ solution (L), and W_R was the weight of the bioflocculant used (g).

All of the measurements in this study were carried out in triplicate.

Ethical approval: The conducted research is not related to either human or animal use.

Table 1: Thermal stability of the bioflocculant harvested from ALT sludge.

Temperature (°C)	10	20	30	40	50	60	70	80	90	100	110	120
Flocculating rate (%)	76.7	88.5	95.3	92.7	91.2	88.6	72.4	64.8	51.5	37.6	22.8	12.3

3 Results and discussion

3.1 Bioflocculant production

Although a single sample of sludge was used to produce the input for bioflocculant production by a single strain, various pre-treatments of the sludge led to distinct bioflocculant yields. As seen in Figure 1, in this study, 4.45, 2.58 and 0.76 g of purified bioflocculant were harvested per L of fermentation broth of ALT, ST and ACT sludge, respectively. All of these yields from pre-treated sludge were higher than that harvested from raw sludge (0.12 g/L). The variations in bioflocculant yields from different fermented broths were attributed to the specific chemical/physical changes induced by different treatments of the treated sludge [24].

Generally, treatment of sludge results in decomposition of organic components (including proteins and polysaccharides) of the material and the release of soluble carbon, and this soluble carbon is more suitable for utilization by microorganisms for bioflocculant production [25]. In this study, we found that bioflocculant yields following different pre-treatments were increased compared with that from the raw sludge, which might be explained by increases of soluble carbon levels that occurred regardless of the treatment method. The bioflocculant yield from the ALT sludge was the highest, meaning that the increase in soluble carbon likely was the highest in this case. Chen et al. [26] reported that under alkaline conditions, the soluble chemical oxygen demand and other low molecular weight soluble carbons were increased by sludge sterilization, and that these factors reached higher levels relative to acid treatment. Sun et al. [27] similarly concluded that sludge solubilization significantly increased with alkaline treatment and to a lesser extent by acid treatment. Aravinthan et al. [28] reported that mostly proteins of the sludge were solubilized by alkaline treatment whereas carbohydrate portions of the sludge preferentially solubilized by acid treatment. The carbon sources, nitrogenous organic materials, and nature of nutrients available in the medium change with the type of treatment and therefore can change bioflocculant secretion patterns and correspondingly the bioflocculant yields [29].

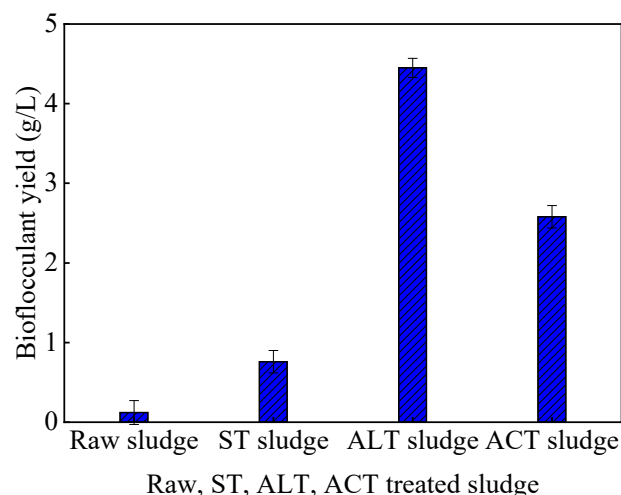


Figure 1: Effects of sludge pre-treatment on bioflocculant yield. [Error bars refer to standard deviation (3 replicates for each treatment)].

3.2 Characteristics of the bioflocculant

It has been reported that protein-dependent bioflocculants are sensitive to heat, while those whose active ingredient is polysaccharide are thermostable. These results might be explained by the fact that proteins can be denatured under hot conditions [25]. In the present work, the fermentation broth, which contains the flocculating components, from ALT-pretreated sludge was divided into 12 equal parts and separately treated at increasing temperatures over the range 10-120°C for 30 min, after which the flocculating activities towards a kaolin suspension (4 g/L) were measured according to the method reported by [19].

Results (Table 1) showed that more than 90% of the flocculating activities of the fermentation broth were retained when the temperature was lower than 60°C. However, when the temperature was adjusted to 90°C, the flocculating activity declined to about 50%, which was further decreased to approximately 10% upon pre-treatment at 120°C (Table 1). The poor heat stability indicated that the bioflocculant produced when ALT-pretreated sludge is used as a precursor may have protein as a primary component. These results are consistent with those found by [19,25].

Chemical analyses showed that the protein content of the bioflocculant was 94.7% (w/w), while the polysaccharide content was 5.2% (w/w). The molecular weight of the bioflocculant was 4.24×10^5 Da. Notably, other researchers have found that flocculant activity increases with increasing molecular weight [27].

3.3 Treatment of Pb(II) aqueous solution by the bioflocculant

Figure 2 depicts the effects of bioflocculant dosage on the removal of Pb(II) without adjustment of the pH of the solution. In the presence of 50 mg/L CaCl_2 , the removal efficiency of Pb(II) increased rapidly with increasing bioflocculant dosages from 0 to 6 mg/L. The maximum removal efficiency of 92.5% was obtained at the optimum bioflocculant dosage of 6 mg/L, and the corresponding removal capacity of Pb(II) by the bioflocculant was 77.1 mg/g. However, increasing the bioflocculant dosage above 6 mg/L had negligible effects on the increase in Pb(II) removal, which might be explained by the formation of aggregates at higher solid/liquid ratios or to precipitation of particles [4].

To determine the role of CaCl_2 in Pb(II) removal by the bioflocculant, the effect of CaCl_2 was investigated by adding different mass fractions of CaCl_2 and 6 mg/L of the bioflocculant into 1 L of 50 mg/L $\text{Pb}(\text{NO}_3)_2$ solutions without adjustment of the pH of the solutions. It can be seen from Figure 3 that the removal efficiency of Pb(II) was relatively low (36.3%) without the addition of CaCl_2 , but that the removal efficiency increased rapidly with increasing CaCl_2 . The maximum value of 97.6% appeared when the CaCl_2 concentration was 70 mg/L, and the corresponding removal capacity of Pb(II) by the bioflocculant under this condition was 81.3 mg/g. Feng et al. [4] reported that the Ca^{2+} might increase the initial adsorption capacity of a bioflocculant by decreasing its negative charge. However, excessive Ca^{2+} (>70 mg/L) had negative effects on the Pb(II) removal efficiency, which may be due to competition between the Pb(II) and excess Ca^{2+} for the key functional groups of the bioflocculant such as $-\text{OH}$ and $-\text{COO}^-$ [5].

The effects of solution pH value on the removal of Pb(II) by the bioflocculant were examined after 6 mg/L of bioflocculant and 70 mg/L of CaCl_2 were added into 1 L of $\text{Pb}(\text{NO}_3)_2$ solution. The pH values of the solutions were adjusted in the range of 2.5-7.5 to avoid the precipitation of Pb(II) in alkaline condition. As seen in Figure 4, at pH 6.5, the removal efficiency of Pb(II) increased gradually and peaked at 98.5%, and at this pH, the corresponding removal capacity of Pb(II) by the bioflocculant was 50

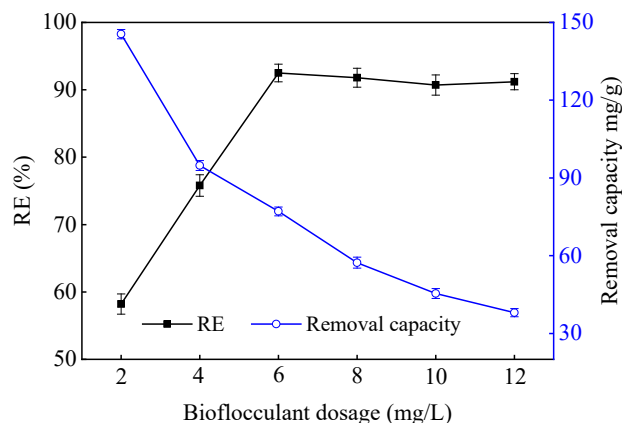


Figure 2: Effects of bioflocculant dosage on the removal of Pb(II) from aqueous solution. [Error bars refer to standard deviation (3 replicates for each treatment)].

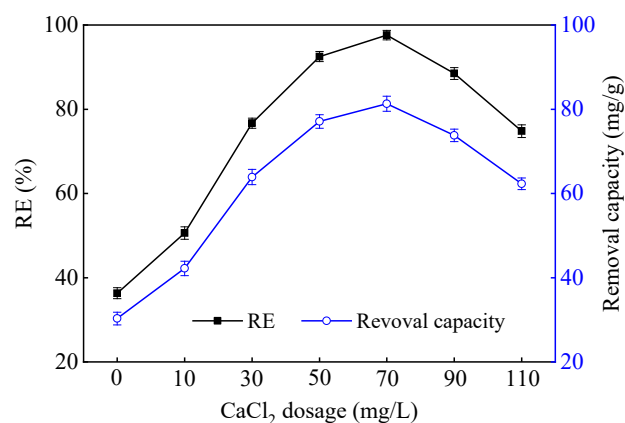


Figure 3: Effects of CaCl_2 dosage on the removal of Pb(II) from aqueous solution. [Error bars refer to standard deviation (3 replicates for each treatment)].

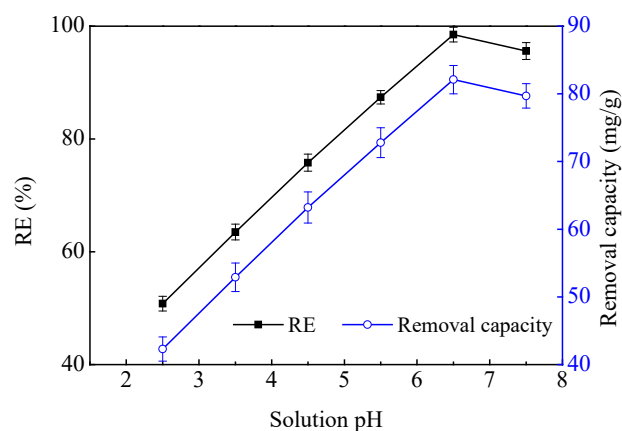


Figure 4: Effects of solution pH value on the removal of Pb(II) from aqueous solution. [Error bars refer to standard deviation (3 replicates for each treatment)].

mg/g. A similar optimal pH of 6 for Pb(II) removal has been reported by [5]. At low pH, the removal of Pb(II) was not optimal, likely because of competition for the functional groups of the bioflocculant between hydrogen ions (H^+) and Pb(II) [30].

4 Conclusions

This study demonstrates the successful production of a bioflocculant from activated sludge; after cultivation of the microorganism in ALT-sludge for 60 h, a relatively high yield of 4.45 g of protein-based bioflocculant was obtained per L of broth. This bioflocculant was most effective in the removal of Pb(II) from aqueous solution at pH 6.5, and the maximum removal efficiency of Pb(II) and the corresponding removal capacity were 98.5% and 82.1 mg/g. In conclusion, bioflocculants may be a feasible way to remove Pb(II) from wastewaters.

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Conflict of interest: Authors declare no conflict of interest.

References

- [1] Schneegurt MA, Jain JC, Menicucci JA, Brown SA, Kemner KM, Garofalo DF, et al. Biomass byproducts for the remediation of wastewaters contaminated with toxic metals. *Environ Sci Technol.* 2001; 35(8): 3786-91.
- [2] Li F, Zhang JD, Jiang W, Liu CY, Zhang ZM, Zhang CD, et al. Spatial health risk assessment and hierarchical risk management for mercury in soils from a typical contaminated site, China. *Environ Geochem Health.* 2017; 39: 923-34.
- [3] Li F, Zhang JD, Liu CY, Xiao M, Wu Z. Distribution, bioavailability and probabilistic integrated ecological risk assessment of heavy metals in sediments from Honghu Lake, China. *Process Saf Environ Prot.* 2018; 116: 169-79.
- [4] Feng J, Yang ZH, Zeng GM, Huang J, Xu HY, Zhang YY, et al. The adsorption behavior and mechanism investigation of Pb(II) removal by flocculation using microbial flocculant GA1. *Bioresour Technol.* 2013; 148: 414-21.
- [5] Guo JY, Yu J. Sorption characteristics and mechanisms of Pb(II) from aqueous solution by using bioflocculant MBFR10543. *App Microbiol Biotechnol.* 2014; 98: 6431-41.
- [6] Karatas M. Removal of Pb(II) from water by natural zeolitic tuff: kinetics and thermodynamics. *J Hazard Mater.* 2012; 199-200: 383-9.
- [7] Chakravarty S, Mohanty A, Sudha TN, Upadhyay AK, Konar J, Sircar JK, et al. Removal of Pb(II) ions from aqueous solution by adsorption using bael leaves (*Aegle marmelos*). *J Hazard Mater.* 2010; 173(1-3): 502-9.
- [8] Aljuboori AH, Idris A, Al-Joubory HH, Uemura Y, Ibn Abubakar BS. Flocculation behavior and mechanism of bioflocculant produced by *Aspergillus flavus*. *J Environ Manage.* 2015; 150: 466-71.
- [9] Guo JY, Chen C. Sludge conditioning using the composite of a bioflocculant and PAC for enhancement in dewaterability. *Chemosphere.* 2017; 185: 277-83.
- [10] Guo, JY, Chen C, Jiang SL, Zhou YL. Feasibility and mechanism of combined conditioning with coagulant and flocculant to enhance sludge dewatering. *ACS Sustain Chem Eng.* 2018; 6(8): 10758-65.
- [11] Okaiyeto K, Nwodo UU, Leonard VM, Arinze SO, Anthony IO. Characterization of a bioflocculant (MBF-UFH) produced by *Bacillus* sp. AEMREG7. *Int J Mol Sci.* 2015; 16(6): 12986-3003.
- [12] Cao G, Zhang Y. Production of a bioflocculant from methanol wastewater and its application in arsenite removal. *Chemosphere.* 2015; 141: 274-81.
- [13] Wang L, Ma F, Lee DJ, Wang AJ, Ren NQ. Bioflocculants from hydrolysates of corn stover using isolated strain *Ochrobactrum ciceri* W2. *Bioresour Technol.* 2013; 145: 259-63.
- [14] Giri SS, Harshiny M, Sen SS, Sukumaran V, Park SC. Production and characterization of a thermostable bioflocculant from *Bacillus subtilis* F9, isolated from wastewater sludge. *Ecotox Environ Safe.* 2015; 121: 45-50.
- [15] Guo JY, Jiang SL, Pang YJ. Rice straw biochar modified by aluminum chloride enhances the dewatering of the sludge from municipal sewage treatment plant. *Sci Total Environ.* 2019; 654: 338-44.
- [16] Liu CG. Enhancement of dewaterability and heavy metals solubilization of waste activated sludge conditioned by natural vanadium-titanium magnetite-activated peroxymonosulfate oxidation with rice husk. *Chem Eng J.* 2019; 359: 217-24.
- [17] Guo JY, Zhou YL. Transformation of heavy metals and dewaterability of waste activated sludge during the conditioning by Fe^{2+} -activated peroxymonosulfate oxidation combined with rice straw biochar as skeleton builder. <https://doi.org/10.1016/j.chemosphere.2019.124628>.
- [18] Liu CG, Lai LD, Yang X. Sewage sludge conditioning by $Fe(II)$ -activated persulphate oxidation combined with skeleton builders for enhancing dewaterability. *Water Environ J.* 2016; 30: 96-101.
- [19] Guo JY, Yang CP, Peng LY. Preparation and characteristics of bacterial polymer using pre-treated sludge from swine wastewater treatment plant. *Bioresour Technol.* 2014; 152: 490-8.
- [20] Pu SY, Qin LL, Che JP, Zhang BR, Xu M. Preparation and application of a novel bioflocculant by two strains of *Rhizopus* sp. using potato starch wastewater as nutrient. *Bioresour Technol.* 2014; 162: 184-91.
- [21] Liu WJ, Wang K, Li BZ, Yuan HL, Yang JS. Production and characterization of an intracellular bioflocculant by *Chryseobacterium daeguense* W6 cultured in low nutrition medium. *Bioresour Technol.* 2010; 101: 1044-8.
- [22] Chaplin MF, Kennedy JF. *Carbohydrate Analysis*, second Ed, Oxford University Press, New York. 1994.
- [23] Bradford MM. A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the

- principle of protein–dye binding. *Anal Biochem.* 1976; 72(1-2): 248-54.
- [24] More TT, Yan S, Hoang NV, Tyagi RD, Surampalli RY. Bacterial polymer production using pre-treated sludge as raw material and its flocculation and dewatering potential. *Bioresour Technol.* 2012; 121: 425-31.
- [25] Gong WX, Wang SG, Sun XF, Liu X.W, Yue QY, Gao BY. Bioflocculant production by culture of *serratia ficaria* and its application in wastewater treatment. *Bioresour Technol.* 2008; 99: 4668-74.
- [26] Chen Y, Jiang S, Yuan H, Zhou Q, Gu G. Hydrolysis and acidification of waste activated sludge at different pHs. *Water Res.* 2007; 41(3): 683-9.
- [27] Sun J, Zhang XH, Miao XJ, Zhou JT. Preparation and characteristics of bioflocculants from excess biological sludge. *Bioresour Technol.* 2012; 126: 362-6.
- [28] Aravinthan V, Mino T, Takizawa S, Satoh H, Matsuo T. Sludge hydrolysate as a carbon source for denitrification. *Water Sci Technol.* 2001; 43: 191-9.
- [29] Jorand F, Guicherd P, Urbain V, Manem J, Block JC. Hydrophobicity of activated sludge flocs and laboratory–grown bacteria. *Water Sci Technol.* 1994; 30(11): 211-8.
- [30] Huang W, Liu ZM. Biosorption of Cd(II)/Pb(II) from aqueous solution by biosurfactant-producing bacteria: Isotherm kinetic characteristic and mechanism studies. *Colloid Surface B.* 2013; 105: 113-9.