Bioflocculant BF-R1 production by *Bacillus* sp. R1 using wheat bran hydrolysate and its application in wastewater treatment

Rexiding Abuduaini[1,a], **Na-Ling Bai**[2,a], **Cai Hui**[1], **Sheng Wang**[1], **Hui Jiang**[1], **Yu-Hua Zhao**[1,*]

[1] Institute of Biochemistry, College of Life Sciences, Zhejiang University, Hangzhou 310058, P.R. China
[2] Eco-environmental Protection Research Institute, Shanghai Academy of Agricultural Sciences, Shanghai 201403, China
[a] Contributed equally to this work

Abstract: Bioflocculants are commonly used in wastewater treatment. In this study, an efficient bioflocculant-producing strain, *Bacillus* sp. R1, was isolated and identified; strain R1 could efficiently produce bioflocculant BF-R1 with wheat bran hydrolysate. The characteristics and flocculation mechanisms of BF-R1 were determined and it was then applied for the granular carbon particles treatment in wastewater. Notably, 3.71 g of BF-R1 were produced when 200 mL/L wheat bran hydrolysate was used as the sole carbon and nitrogen source. BF-R1 contained polysaccharides, proteins, and glycoproteins and showed a good flocculating efficiency of 91.00% for granular carbon particles in contaminated wastewater when 3.50 g/L BF-R1 was added, thus achieving successful recycling of fine particle-contaminated wastewater. Taken together, our findings for the first time demonstrate that BF-R1 fermented using WBH can probably be a promising candidate agent for wastewater management processes.

Keywords: Bioflocculant, *Bacillus* sp. R1, wheat bran hydrolysate, granular carbon particles, flocculation efficiency

1 Introduction

Granular carbon is an inexpensive material frequently serving as biocarrier or adsorbent for heavy metals and organic compounds in wastewater treatment process (Cornelissen et al., 2005). Granular activated carbon addition into the batch-mode anaerobic sludge digestion reactor could significantly accelerate substrate consumption and methane production (Yang et al., 2017). Biochar derived from rice straw can be applied to adsorb the endocrine disrupter nonylphenol (NP), and thus accelerating its biodegradation (Lou et al., 2015). However, granular carbon particles, particularly ultrafine particles (< 5 μ in diameter), create problems during screening and tend to float in water (Vijayalakshmi and Raichur, 2003). Moreover, it is difficult to separate or filter fine carbon particles from aquatic systems. For example, Wu et al. reported that granular activated carbon in the hybrid microfiltration process appeared to release micro- and nanoscaled fine carbon particles, which would form potential foulants (Wu et al., 2015). These fine carbon particles are cut down by hydrodynamic shear forces and form carbon-bacteria complexes (Zhao et al., 2015). Hence, fine particle grading, separation, and recycling, related to economic benefits and environmental effects, require great attention and in-depth study.

Fine carbon particles may act as a condensation nucleus of organic substances, therefore, flocculation or coagulation can alternatively be used to increase the particle sizes and then enhance the settling rates and separation process (Vijayalakshmi and Raichur, 2003). Flocculating agents are widely used in various industrial fields including wastewater treatment, dredging, and fermentation processes (Kurane et al., 1986). Flocculants synthesized by conventional chemical methods, such as aluminum sulfate and polyethylenimine, have many drawbacks (e.g., biotoxicity, narrow applicability, and lack of degradability). Whereas, bioflocculants, secreted by microbes during their growth, are usually biodegradable and environmentally friendly and are adaptable to pH variation (Salehizadeh and Shojaosadati, 2001). They have wide industrial applications in various wastewater treatment processes including sludge dewatering and swine wastewater pretreatment (Guo and Ma, 2015), heavy metal treatment (Abdel-Halim and Al-Deyab, 2011), and tannery wastewater containing organic materials (Yang et al., 2015). For example, it was reported that bioflocculant MBF-G22 produced by *Pseudomonas* sp. G22 showed high flocculating efficiency of microalgae (95.7%) at a dosage of 80 mg/L (Guo et al., 2018). Using bioflocculants for the aggregation and precipitation of granular carbon particles in wastewater could constitute a novel, cost-effective, and environmentally-friendly approach. Bioflocculants can induce particle-to-particle flocculation through the formation of bridges, resulting in the agglomeration and settling of

suspended fine particles.

Despite these advantages, the practical application of bioflocculants for the remediation of toxins in polluted aquatic environments has been proven challenging. Effective strains that can be cultivated in low-cost medium with maximum rates of bioflocculant production and auto-flocculating strains that can be harvested both are attractive research topics (Zhuang et al., 2012; Lee and Chang, 2018). The bioflocculant fermentation of *Rhodococcus* sp. R3 was optimized by active sludge alkaline-thermal treatment, with chemical oxygen demand and ammonium removal rates of 87.9% and 86%, respectively (Guo et al., 2013). Hydrolysates of other agricultural wastes, such as corn stover (Wang et al., 2013) and peanut hulls (Liu et al., 2016), have also been applied as inexpensive carbon sources for bioflocculants production. Guo et al. prepared a bioflocculant from hydrolysed rice stover using diluted sulfuric acid and hypothesized that the charge neutralization and inter-particle bridging led to the enhanced performance (Guo et al., 2015).

The increasing global demand for sustainable resources necessitates the effective utilization of agro-wastes. Wheat bran, an inexpensive substrate, is produced worldwide in large quantities (approximately > 150 million tons) as a by-product of white wheat flour milling for human consumption (Prückler et al., 2014; Bergmans et al., 1996). Moreover, wheat bran contains nutrients, such as non-starch polysaccharides, starch, proteins, and lignin (Maes and Delcour, 2001). *Clostridium beijerinckii* ATCC 55025 could produce 11.8 g/L ABE (acetone, butanol and ethanol) with the hydrolysate of wheat bran pretreated with dilute sulfuric acid as the substrate (Liu et al., 2010). Moreover, it was reported that alkaline pretreated wheat bran could be a great potential alternative feedstock with no further detoxification for polyhydroxybutyrate production (Annamalai and Sivakumar, 2016). However, there was little research focused on using wheat bran hydrolysate as an economical and environmental friendly way for bioflocculant production.

In this study, we isolated a novel bioflocculant-producing strain, *Bacillus* sp. R1, from activated swage sludge, determined the optimal fermentation conditions with wheat bran hydrolysate as substrate, evaluated the characteristics of the bioflocculant BF-R1, and subsequently examined its applications in granular biochar particles removal. According to our literature research, no previous report has investigated the use of wheat bran hydrolysate in bioflocculant production.

2 Materials and Methods

2.1 Materials

NP (CAS 84852-15-3, molecular weight: 220.35) was purchased from Aladdin Co. Ltd. (Shanghai, China). Wheat bran and granular carbon were purchased from Jinhua (Zhe-jiang, China). All other general compounds were of analytical grade.

2.2 Isolation and Identification of the Bioflocculant-Producing Strain

Activated sludge was collected from a wastewater treatment plant (Wenzhou, Zhejiang, China) and used to isolate strains capable of producing bioflocculants. Generally, one milliliter of sludge sample was serially diluted using sterilized distilled water and then spread onto 0.5×Luria-Bertani (LB) medium agar plates containing (per L) 5.0 g tryptone, 2.5 g yeast extract, 5.0 g sodium chloride, and 20 g agar. The formed single colonies were inoculated into 100 mL liquid flocculating strain screening (FSS) medium and incubated in a rotary shaker at 200 rpm and 30 °C for 24 h. The FSS medium was prepared as previously described (Yang et al., 2015) and contained (per L): 0.5 g glucose, 0.5 g starch, 0.5 g yeast extract, 0.5 g peptone, 0.5 g casein, 0.3 g K_2HPO_4, and 0.05 g $MgSO_4 \cdot 7H_2O$ (pH 7.0). Ultimately, four large and viscous colonies were chosen and stored at -80 °C in 20% glycerol.

The strain showing the highest flocculation efficiency, named R1, was selected for further investigation. The genomic DNA of R1 was extracted using a Genomic DNA Kit (Sangon Biotech Co. Ltd.) according to the manufacturer's instructions. Universal bacterial primers 27F (5'-AGAGTTTGATCATGGCTCAG-3') and 1492R (5'-TACGGTTACCTGTTACGACTT-3') were used to amplify the 16S rDNA gene with the following PCR program: pre-denaturation at 95 °C for 5 min; 30 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 90 s; and a final step at 72 °C for 5 min. After purification, the PCR product was sequenced (Sangon Biotech Co. Ltd., Shanghai, China). And the acquired sequence was blasted against the references sequences in the National Center for Biotechnology Information (NCBI) database. Multiple sequence alignments were conducted using CLUSTALX 2.0 software and a phylogenetic tree was then constructed using the neighbor-joining method with MEGA 6.0 software.

2.3 Flocculating Activity Assay

The flocculating activity was determined by measuring the flocculation efficiency in jar tests according to previous studies (Zhuang et al., 2012; Guo et al., 2015). 15 mg $CaCl_2$ and 2.0 mg bioflocculant were added to a 100 mL kaolin suspension (5.0 g/L) after the pH adjustion to 7.5 using NaOH or HCl. The reaction mixture was stirred thoroughly for 5 min and then allowed to settle for 5 min. following this, the optical density (OD) of the clarified solution was measured using a spectrophotometer (7320G; Shanghai, China) at 550 nm. A control experiment was conducted in the same manner without the bioflocculant addition. Flocculation activity was

calculated as described;

$$FE(\%) = \frac{(A_0 - A_t)}{A_0} \times 100 \qquad (1)$$

Where: A_0 and A_t are the OD_{550} values of control and the sample experiment, respectively; FE is the flocculation efficiency of the specified bioflocculant.

2.4 Preparation of the Wheat Bran Hydrolysate and Bioflocculant Production

Dried wheat bran was crushed into powder, sieved through 100-mesh screen, and stored in desiccators prior to hydrolysis by diluted sulfuric acid (1.7%, w/w) with a solid-liquid ratio of 1:10 (w/v) at 121 °C for 1.5 h. After cooling down, the slurry mixture was centrifuged at 10,000 rpm, 4 °C for 10 min. Then the supernatant was neutralized to 7.0 by $Ca(OH)_2$ and the generated precipitates was removed by centrifugation at 10,000 rpm, 4 °C for 5 min. Subsequently, the supernatant was stored at 4 °C and used as the sole carbon and nitrogen source for bioflocculant production.

Strain R1 cultured in 0.5×LB medium at 30 °C overnight was treated as the seed broth. Two milliliters of seed culture were then inoculated into 500 mL of fermentation medium, which was consisted of different concentrations of wheat bran hydrolysate in minimum salt medium (MSM) prepared according to a previous study (Bai et al., 2016). The fermentation product was centrifuged at 12,000 rpm, 4 °C for 5 min to remove the cells after 12h's incubation. Two volumes of pre-chilled ethanol were added into the supernatant to precipitate the bioflocculant. The resulting precipitate was washed with 75% ethanol and lyophilized to obtain the purified bioflocculant.

2.5 Characterization of the Bioflocculant

Saccharide, protein, and glycoprotein contents were identified according to the methods described by Zhao et al. (Zhao et al., 2016). Chemical characterization of the samples was analyzed by Fourier transform infrared spectroscopy (FT-IR; Nicolet AVatAR 370; Thermo Scientific, Waltham, MA, USA). And the sample spectrum was recorded on the spectrophotometer over a wave number range of 500–4000 cm^{-1}. Differential scanning calorimetry (DSC) analysis (Q2000 V24.11) was conducted to characterize the thermal behaviors of the bioflocculant under a nitrogen gas flow of 80 mL/min and a heating rate of 20 °C/min from 20 °C to 280 °C.

2.6 Fine Carbon Particles Removal in Wastewater

The dried carbon was crushed into powder and sieved through a 100-mesh screen. Then 150 mg/L granular carbon was poured into flasks containing 500 mg/L NP and cultured at 150 rpm and 25 °C for 30 min. Next, different amount of bioflocculant was added; the flasks were stirred at 150

rpm for 10 min and then allowed to settle for 30 min. The upper phase was then sampled in order to measure the residual amount of NP in the aqueous phase after absorption and flocculation by high performance liquid chromatography (HPLC) as previously described (Bai et al., 2017). All measurements were conducted in triplicate. Flocculation activity was observed by measuring the absorbance variation at 550 nm. Furthermore, the morphologies of BF-R1 and the flocculated/non-flocculated granular carbon were characterized using scanning electron microscopy (SEM) (JEOL JSM 5600 LV, Japan).

2.7 Statistical Analysis

Results were presented as mean value ± standard deviation (SD) of the three replicates. One way analysis of variance (ANOVA) followed by Least Significant Difference (LSD) test was performed using SPSS 16.0 software (IBM, Armonk, NY, USA) to determine significant differences in all the parameters. Values were considered to be statistically significant at $p < 0.05$. Figures were prepared using ORIGIN 8.0 (OriginLab Corporation, Northampton, MA, USA).

3 Results and Discussion

3.1 Isolation and Identification of Bioflocculant-Producing Strain

In this study, a bacterial strain, possessing relatively high flocculant productivity was isolated from activated sewage sludge (NCBI database accession number MH470262). After 16S rDNA alignment and BLAST, it showed that this strain was phylogenetically closely related to *Bacillus amyloliquefaciens* (Figure 1). Thus, we named this bacterium and its produced flocculant as *Bacillus* sp. R1 and BF-R1, respectively. Bacteria strains capable of fermenting bioflocculants, including *Rhodococcus erythropolis* (Guo and Ma, 2015), *Ochrobacterum ciceri* (Wang et al., 2013), *Paenibacillus jamilae* (Zhong et al., 2018), *Sphingomonas yabuuchiae* (Zhong et al., 2014), *Pseudomonas veronii* (Liu et al., 2016), and *Bacillus subtilis* (Wu and Ye, 2007), have been previously reported. Different fungi have also been shown to produce bioflocculants, such as *Levure casseeuse* (Poelmans and Swinnen, 2011) and *Aspergillus parasiticus* (Deng et al., 2005). Different types of bioflocculants can be applied in the disposal of heavy metal ions, drinking water, wastewater, and polluted soil. Guo et al. demonstrated that the bioflocculant with a main backone of polysaccharides efficiently removed 99.2% 2 mg/L arsenite (at 60 min) through bridging mechanisms (Guo and Chen, 2017).

3.2 The Effect of Wheat Bran Hydrolysate Concentration on BF-R1 Production

Bioflocculant characteristics usually depends on the bacterial internal features, the compositions of culture medium, and environmental conditions (Giri et al., 2015). Higher
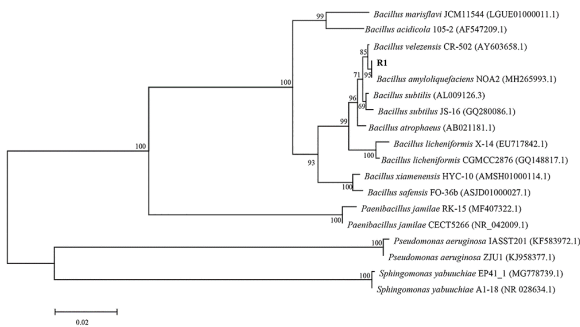
Figure 1. Neighbor-joining phylogenetic tree based on 16S rDNA gene sequences showing the evolutionary position of R1 using MEGA 6.0 software. Bootstrap values (percentages of 1000 replications) are shown at the branch points. The scale bar indicates 0.02 substitutions per nucleotide position (evolutionary distance).

costs related to expensive substrates or lower flocculation efficiency have limited microorganisms application for industrial processes (Yang et al., 2015). Strain R1 produced a maximum of 3.71 g/L bioflocculant when supplemented with 200 mL/L wheat bran hydrolysate (pH 7.0) (Figure 2A). As to our knowledge, this is the first report that wheat bran hydrolysate can be used as the sole carbon and nitrogen source for bioflocculant production. Notably, too high concentrations of wheat bran hydrolysate resulted in significant decrease of bioflocculant production, which may be due to inhibition of microbial activities by toxic by-products in the hydrolysate. It was found that the highest yield of bioflocculant (4.32 g/L) was achieved when 500 mL/L peanut hull hydrolysate and 3 g/L yeast extract were added to the fermentation medium (Liu et al., 2016); while the bioflocculant yield was only 1.25 g/L when the hydrolysate was reduced to 200 mL/L. Swine wastewater was used as raw materials and yielded 3.11 g/L bioflocculant after fermentation for 60 h at 30 °C and 150 rpm (Guo and Chen, 2017). Thus this procedure can be a novel way to reduce the material cost of bioflocculant production.
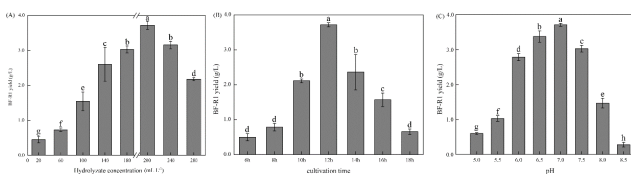

Figure 2. Effects of wheat bran hydrolysate supplement concentration (A), cultivation time (B), and initial pH (C) on bioflocculant yield of strain R1.

3.3 The Effects of Initial pH and Cultivation Time on BF-R1 Production

Agricultural waste is rich in lignocelluloses and its hydrolysate generally contained reducing sugars, such as glucose and xylose (Liu et al., 2016). However, bacterial fermentation can be inhibited by compounds formed during

agricultural waste pretreatment (Liu et al., 2016; Field et al., 2015). Microorganisms generally seemed to require more time for bioflocculant production from agriculture waste than chemical compounds. In our present study, the fermentation time of strain R1 was only 12 h (Figure 2B), slightly shorter than the findings of Zhuang et al. demonstrating that when *Bacillus licheniformis* utilized 30 g/L molasses as the sole carbon source fermentation time was reduced to 13 h, which was also obviously shorter than other reported fermentation times ranging from 13 to 30 h (Zhuang et al., 2012; Wang et al., 2013; Guo et al., 2015). Along with cultivation time, polymer quantity monotonically decreased to 0.65 g/L at 18 h, possibly due to the consumption as substitute for food during later bacterial growth stages (Wang et al., 2013).

The effect of pH on flocculant production was evaluated and presented in Figure 2C. *Bacillus* sp. R1 produced BF-R1 at a pH range of 5.0–8.5, with the highest flocculant production (3.71 g/L) at neutral pH. Cell growth may be inhibited under acidic or alkaline conditions, thus resulting in reduced concentrations of the extracellular flocculant. *Pseudomonas veronii* L918 exhibits the highest flocculating activity (91.67%) at pH 7.0 (Liu et al., 2016). The optimal conditions for *Sphingomonas yabuuchiae* SW-2 bilflocculant fermentation were 425 mg/L CODCr of chromotropic acid wastewater, an initial pH of 6.9, and inoculum size of 7.74% (Zhong et al., 2014). The salt-tolerant, alkaliphilic strain *Bacillus agaradhaerens* C9 exhibited the highest bioflocculant activity at pH 10.2 (Liu et al., 2015). Though different strains produced bioflocculant at specific pHs, too acidic or alkaline conditions may cause cellular stress, consequently leading to inhibition of bioflocculant production. Therefore, cultivation of bioflocculant-producing strain R1 using wheat bran hydrolysate had both economic and environmental benefits.
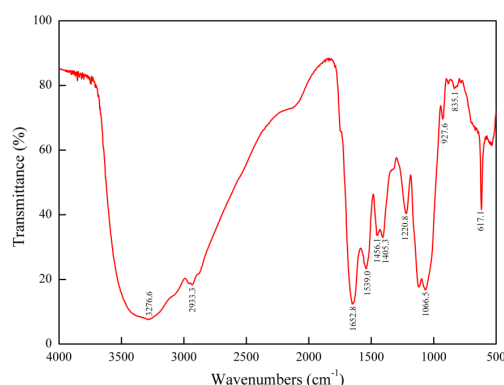
3.4 Characterization of Bioflocculant BF-R1

The Molisch's test showed the purple ring between the concentrated H_2SO_4 layer and the bioflocculant layer in the test tube, indicating saccharides existence in BF-R1 (Table 1) (Yang et al., 2015). Saccharides generate furfural or its derivatives via dehydration by concentrated H_2SO_4; the derivatives would correspondingly react with α-naphthol and generate purple substances. Saccharides are macromolecules and therefore their presence in the bioflocculant may help the bioflocculant bind with pollutants, such as granular carbon, in wastewater (Zhao et al., 2016). Many researches pointed that the bioflocculant main backbone was polysaccharides or proteins. For example, the bioflocculant fermented by *Bacillus* megaterium G04 with swine wastewater was composed of 85.5% polysaccharides, 14.3% proteins, and 0.2% sugars (Guo and Chen, 2017). Similarly, in this study, BF-R1 contained saccharides, proteins, and glycoproteins.

FTIR analysis (Figure 3) revealed a broad stretching peak near wavelength 3276.6 cm^{-1}, indicating the presence of pri-
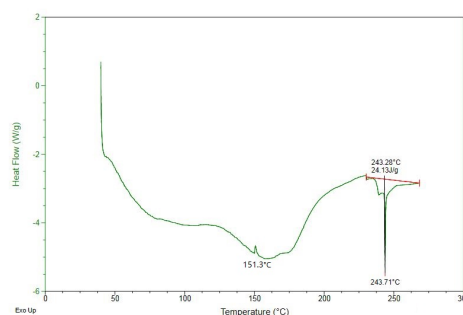
Table 1. List of possible functional groups from fungal, wood, and wood-immobilized biomass involved in Pb(II) sorption.

Identification experiment	Observed phenomenon	Interpretation
Molisch reaction	Positive	Contained saccharides
Biuret reaction	Positive	Contained proteins
Anthrone reaction	Positive	Contained glycoproteins

Figure 4. FTIR spectra of sorbents: WP (A) (B) dried fungi (F), and (C) dried wood immobilized with fungi (WP+F).

mary amino groups (–NH–), one of the main protein groups. Furthermore, the bending vibration peak for the primary amino group (–NH–) at wavelength 835.1 cm^{-1} indicated the presence of protein in the bioflocculant (Zhao et al., 2016). There was a peak of asymmetrical stretching vibration for –C=O– at 1652.8 cm^{-1}, a peak of stretching vibration for –C–O– at 1066.5 cm^{-1}, and a peak of bending vibration for –O–H– at 927.6 cm^{-1} (Guo et al., 2015; Zhao et al., 2016; Zhong et al., 2014; Ugbenyen et al., 2015). The peak at 1539.0 cm^{-1} for N-O bound indicated nitro compounds in BF-R1. An additional peak of stretching vibration for methyl and methylene at 2933.3 cm^{-1} and a peak of bending vibration for –C–H– around 1456.1 cm^{-1} were also detected (Zhao et al., 2016). In conclusion, FTIR analysis indicated the possible presence of proteins, saccharides, and lipids in the BF-R1 of *Bacillus* sp. R1 (El-Salam et al., 2017) and that the functional groups induced high binding capacity.
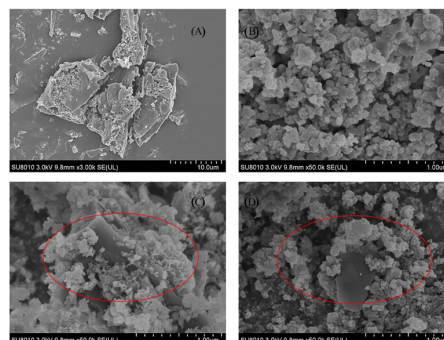
3.5 Application of BF-R1 for the Flocculation of Granular Carbon Particles

The micrographs of the morphological structure of granular carbon particles, purified BF-R1, and flocculated granular carbon particles covered by BF-R1 were analyzed using SEM (Figure 5). The pure BF-R1 appeared as relatively loose granular structure (Figure 5B), the interaction between BF-R1 and the granular carbon particles resulted into compacted floc formation (Figure 5C-D). Granular carbon particles were attached on the binding site of BF-R1, aggregated by the flocculant, and then precipitated into agglomeration of larger flocs.

Figure 3. Fourier transform infrared spectrum (FTIR) analysis of the BF-R1 bioflocculant fermented by Bacilllus sp. R1.

Figure 5. FTIR spectra of WP before (A) and after (B) Pb(II) treatment, and WP+F before (C) and after (D) Pb(II) treatment.

Thermal-stable bioflocculants are preferred for industrial applications (Shahadat et al., 2017). Thus, DSC analysis was performed to examine the thermal characteristics of BF-R1. DSC thermal analysis demonstrated a characteristic endothermic transition at 151.3 °C, which also indicated the melting point of the bioflocculant. Degradation began above 243.71 °C, indicating that the bioflocculant had good thermal stability, which enables an advantageous bioflocculant under under various thermal conditions. The melting point of the bioflocculant from *B. licheniformis* WSF-1 was determined to be 200–207.5°C, suggesting that BF-R1 was more stable than previously reported bioflocculants (Jenny et al., 2018).

BF-R1 was added to the reaction system after granular carbon absorbing NP in MSM for specific time periods. Both absorption and flocculation efficiency of granular carbon and the bioflocculant were measured. After 30–40 min, NP was almost completely cleared (99.85%) from the sample flasks (Figure 6). In terms of NP removal, there was a synergistic effect between BF-R1 and the granular carbon particles, as no flocculation on NP was observed with only BF-R1 (data not shown). The flocculant aggregated and precipitated the granular carbon particles and the carbon fines to a three-dimensional structure with a better settling capacity, thus accelerating NP absorption efficiency. The interaction of phenolic compounds with

activated carbon surface was site definite and monolayer adsorption (Hamdaoui and Naffrechoux, 2007); therefore, the addition of BF-R1 may facilitate granular particle flocculation and increase the surface area of the complex, thus promoting NP adsorption. Alternatively, the change of the net charge of the bioflocculant-carbon particle complex may also contribute to NP removal (Liu et al., 2016).

Nearly all of the fine particles (91.00%) were separated from the water at the dosage of 3.5 g/L BF-R1. Notably, the carbon fines were also significantly precipitated. Further increase of BF-R1 addition led to a gently decrease of flocculation activity (Figure S1), which may be caused by the increased electrostatic repulsion between the excessive bioflocculant chains. Additionally, the formerly formed flocs could be deflocculated due to the stronger repulsion force (Guo and Chen, 2017). Granular carbon particles are effective in adsorbing a wide range of organic compounds; meanwhile, carbon fines would reduce water quality because of turbidity and bacterial colonization (Zhao et al., 2015; Campe et al., 1986). Therefore, BF-R1 is capable of controlling the risk posed by granular carbon particles and carbon fines.
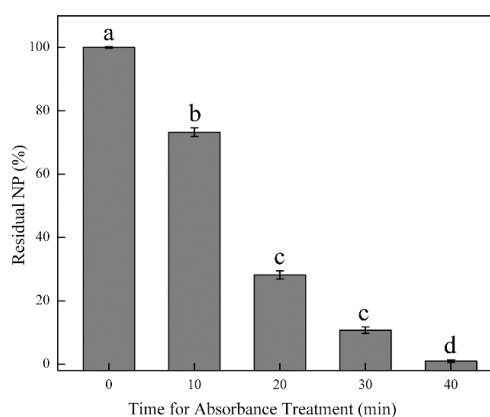

Figure 6. Residual Pb(II) ion concentration of biosorbents (WP, WP+F) in contact with 1000 (A), 2000 (B), and 4000 μg/mL Pb(II) (C) for 30 minutes in assembled CF system. Comparison of residual Pb(II) ion concentration of control (D), WP (E), and WP+F (F) in 1000, 2000, and 4000 μg/mL Pb(II) concentrations. Means that share at least one similar letter are not significantly different at Tukey's multiple comparisons test (P < 0.05). Bars indicate standard deviation of at least three replicates.

3.6 Fourier Transform Infrared Spectroscopy

Possible functional groups of fungal, wood, and wood-immobilized biomass applied in Pb(II) sorption were assessed through infrared spectroscopy seen in Figure 4. The FTIR spectra shown in Figure 5 depicts plausible Pb(II) binding sites in comparison to the biomass before and after Pb(II) treatment.

4 Conclusion

In this study, an efficient bioflocculant-producing strain, *Bacillus* sp. R1, was isolated; this strain fermented wheat bran hydrolysate into a highly efficient and absorbent bioflocculant. Our findings demonstrate, for the first time, bioflocculant production using wheat bran hydrolysate and its effective application in the flocculation of granular carbon particles and carbon fines, which are widely used in the treatment of organic pollutants. A yield of 3.71 g/L BF-R1 bioflocculant was achieved when 200 mL/L hydrolysate was used as the sole carbon and nitrogen source in 12 h; this would definitely reduce both raw material and time costs. Moreover, BF-R1 aggregated and precipitated the granular carbon particles and the carbon fines to a three-dimensional structure with a better settling capacity, thus accelerating NP absorption efficiency. As the characteristics and flocculation efficiency were examined at the lab-scale in this study, large-scale application tests are essential for industrial application of BF-R1.

Acknowledgement

Conflict of interest

There are no conflicts of interest to declare. This study did not involve any human participants.

References

Cornelissen, G., Gustafsson, O., Buchili, T.D., Jonker, M.T.O., Koelmans, A.A., and Van Noort, P.C.M., 2005. Extensive sorption of organic compounds to black carbon, coal, and kerogen in sediments and soils: Mechanisms and consequences for distribution, bioaccumulation, and biodegradation. Environmental Science and Technology, 39(18), 6881-6895.
https://doi.org/10.1021/es050191b

Yang, Y.F., Zhang, Y.B., Li, Z.Y., Zhao, Z.Q., Quan, X., and Zhao, Z.S., 2017. Adding granular activated carbon into anaerobic sludge digestion to promote methane production and sludge decomposition. Journal of Cleaner Production, 149, 1101-1108.
https://doi.org/10.1016/j.jclepro.2017.02.156

Lou, L.P., Yao, L.D., Cheng, G.H., Wang, L.X., He, Y.F., and Hu, B.L., 2015. Application of rice-straw biochar and microorganisms in nonylphenol remediation: Adsorption-biodegradation coupling relationship and mechanism. PLoS ONE, 10(9), e0137467.
https://doi.org/10.1371/journal.pone.0137467

Vijayalakshmi, S.P. and Raichur, A.M., 2003. The utility of *Bacillus subtilis* as a bioflocculant for fine coal. Colloids and

Surfaces B: Biointerfaces, 29(4), 265-275.
https://doi.org/10.1016/S0927-7765(03)00005-5

Wu, B., Wong, P.C.Y., and Fane, A.G., 2015. The potential roles of granular activated carbon in anaerobic fluidized membrane bioreactors: Effect on membrane fouling and membrane integrity. Desalination and Water Treatment, 53(6), 1450-1459.
https://doi.org/10.1080/19443994.2014.943057

Zhao, J.H., Chen, W., Zhao, Y.Q., Liu, C.Y., and Liu, R.B., 2015. UV/TiO$_2$ photocatalytic disinfection of carbon-bacteria complexes in activated carbon-filtered water: Laboratory and pilot-scale investigation. Environmental Letters, 50(12), 1274-1281.
https://doi.org/10.1080/10934529.2015.1055155

Kurane, R., Takeda, K., and Suzuki, T., 1986. Screening for and characteristics of microbial flocculants. Agricultural and Biological Chemistry, 50(9), 2301-2307.
https://doi.org/10.1080/00021369.1986.10867746

Salehizadeh, H. and Shojaosadati, S.A., 2001. Extracellular biopolymeric flocculants: Recent trends and biotechnological importance. Biotechnology Advances, 19(5), 371-385.
https://doi.org/10.1016/S0734-9750(01)00071-4

Guo, J.Y. and Ma, J., 2015. Bioflocculant from pre-treated sludge and its applications in sludge dewatering and swine wastewater pretreatment. Bioresource Technology, 196, 736-740.
https://doi.org/10.1016/j.biortech.2015.07.113

Abdel-Halim, E.S. and Al-Deyab, S.S., 2011. Removal of heavy metals from their aqueous solutions through adsorption onto natural polymers. Carbohydrate Polymers, 84(1), 454-458.
https://doi.org/10.1016/j.carbpol.2010.12.001

Yang, Q.H., Ming, H.M., Zhao, X.X., Zhang, J., Wei, Z., Zhao, C.Q., and Guan, X.Q., 2015. Screening of bioflocculant and preliminary application to treatment of tannery wastewater. Journal of Residuals Science and Technology, 12(3), 177-181.
http://doi.org/10.12783/issn.1544-8053/12/3/8

Guo, H.P., Hong, C.T., Zhang, C., Zheng, B.S., Jiang, D., and Qin, W.S., 2018. Bioflocculants' production from a cellulase-free xylanase-producing *Pseudomonas boreopolis* G22 by degrading biomass and its application in cost-effective harvest of microalgae. Bioresource Technology, 255, 171-179.
https://doi.org/10.1016/j.biortech.2018.01.082

Zhuang, X.L., Wang, Y.P., Li, Q.B., Yan, S., and He, N., 2012. The production of bioflocculants by *Bacillus licheniformis* using molasses and its application in the sugarcane industry. Biotechnology and Bioprocess Engineering, 17(5), 1041-1047.
https://doi.org/10.1007/s12257-012-0213-0

Lee, D.J. and Chang, Y.R., 2018. Bioflocculants from isolated stains: A research update. Journal of the Taiwan Institute of Chemical Engineers, 87, 211-215.
https://doi.org/10.1016/j.jtice.2018.03.037

Guo, J.Y., Yang, C.P., and Zeng, G.M., 2013. Treatment of swine wastewater using chemically modified zeolite and bioflocculant from activated sludge. Bioresource Technology, 143, 289-297.
https://doi.org/10.1016/j.biortech.2013.06.003

Wang, L., Ma, F., Lee, D.J., Wang A.J., and Ren, N.Q., 2013. Bioflocculants from hydrolysates of corn stover using isolated strain *Ochrobactium ciceri* W2. Bioresource Technol-ogy, 145, 259-263.
https://doi.org/10.1016/j.biortech.2012.11.020

Liu, W.J., Hao, Y., Jiang, J.H., Zhu, A.H., Zhu, J.R., and Dong, Z., 2016. Production of a bioflocculant from *Pseudomonas veronii* L918 using the hydrolyzate of peanut hull and its application in the treatment of ash-flushing wastewater generated from coal fired power plant. Bioresource Technology, 218, 318-325.
https://doi.org/10.1016/j.biortech.2016.06.108

Guo, J.Y., Yu, J., Xin, X., Zou, C.W., Cheng, Q.F., Yang, H.J., and Nengzi, L.C., 2015. Characterization and flocculation mechanism of a bioflocculant from hydrolyzate of rice stover. Bioresource Technology, 177, 393-397.
https://doi.org/10.1016/j.biortech.2014.11.066

Prückler, M., Sibenhandl-Ehn, S., Apprich, S., Höltinger, S., Haas, C., Schmid, E., and Kneifel, W., 2014. Wheat bran-based biorefinery 1: Composition of wheat bran and strategies of functionalization. LWT - Food Science and Technology, 56(2), 211-221.
https://doi.org/10.1016/j.lwt.2013.12.004

Bergmans, M.E.F., Beldman, G., Gruppen, H., and Voragen, A.G.J., 1996. Optimization of the selective extraction of (glucurono)arabinoxylans from wheat bran: use of barium and calcium hydroxide solution at elevated temperatures. Journal of Cereal Science, 23(3), 235-245.
https://doi.org/10.1006/jcrs.1996.0024

Maes, C. and Delcour, J.A., 2001. Alkaline hydrogen peroxide extraction of wheat bran non-starch polysaccharides. Journal of Cereal Science, 34(1), 29-35.
https://doi.org/10.1006/jcrs.2001.0377

Liu, Z.Y., Ying, Y., Li, F.L., Ma, C.Q., and Xu P., 2010. Butanol production by *Clostridium beijerinckii* ATCC 55025 from wheat bran. Journal of Industrial Microbiology and Biotechnology, 37(5), 495-501.
https://doi.org/10.1007/s10295-010-0695-8

Annamalai, N. and Sivakumar, N., 2016. Production of polyhydroxybutyrate from wheat bran hydrolysate using *Ralstonia eutropha* through microbial fermentation. Journal of Biotechnology, 237, 13-17.
https://doi.org/10.1016/j.jbiotec.2016.09.001

Bai, N.L., Wang, S., Abuduaini, R., Zhu, X.F., and Zhao, Y.H., 2016. Isolation and characterization of *Sphingomonas* sp. Y2 capable of high-efficiency degradation of nonylphenol polyethoxylates in wastewater. Environmental Science and Pollution Research, 23(12), 12019-12029.
https://doi.org/10.1007/s11356-016-6413-y

Zhao, C.Q., Zhao, X.X., Gu, H.B., Zhang, J., Zou, W., Liu, J., and Yang, Q.H., 2016. Qualitative analysis of components of bioflocculant prepared with *Bacillus* fusiformis for the treatment of tannery wastewater. Clean Technologies and Environmental Policy, 18(3), 973-978.
https://doi.org/10.1007/s10098-015-1085-8

Bai, N.L., Abuduaini, R., Wang, S., Zhang, M.N., Zhu, X.F., and Zhao, Y.H., 2017. Nonylphenol biodegradation characterizations and bacterial composition analysis of an effective consortium NP-M2. Environmental Pollution, 220, 95-104.
https://doi.org/10.1016/j.envpol.2016.09.027

Zhong, C.Y., Cao, G., Rong, K., Xia, Z.W., Peng, T., Chen, H.G., and Zhou, J.G., 2018. Characterization of a microbial polysaccharide-based bioflocculant and its anti-inflammatory

and pro-coagulant activity. Colloids and Surfaces B: Biointerfaces, 161, 636-644.
https://doi.org/10.1016/j.colsurfb.2017.11.042

Zhong. C.Y., Xu, A.H., Chen, L., Yang, X.H., Yang, B.K., Hong, W.T., Mao, K.W., Wang, B.Y., and Zhou, J.G., 2014. Production of a bioflocculant from chromotropic acid waste water and its application in steroid estrogen removal. Colloids and Surfaces B: Biointerfaces, 122, 729-737.
https://doi.org/10.1016/j.colsurfb.2014.08.006

Wu, J.Y. and Ye, H.F., 2007. Characterization and flocculating properties of an extracellular biopolymer produced from a *Bacillus subtilis* DYU1 isolate. Process Biochemistry, 42(7), 1114-1123.
https://doi.org/10.1016/j.procbio.2007.05.006

Poelmans, E. and Swinnen, J., 2011. A brief economic history of beer. In J.F.M. Swinnen (ed.), The Economics of Beer, 3-28, Oxford: Oxford University Press.
http://doi.org/10.1093/acprof:oso/9780199693801.003.0001

Deng, S.B., Yu, G., and Ting, Y.P., 2005. Production of a bioflocculant by *Aspergillus parasiticus* and its application in dye removal. Colloids and Surfaces B: Biointerfaces, 44(4), 179-186.
https://doi.org/10.1016/j.colsurfb.2005.06.011

Guo, J.Y. and Chen, C., 2017. Removal of arsenite by a microbial bioflocculant produced from swine wastewater. Chemosphere, 181, 759-766.
https://doi.org/10.1016/j.chemosphere.2017.04.119

Giri, S.S., Harshiny, M., Sen, S.S., Sukumaran, V., and Park, S.C., 2015. Production and characterization of a thermostable bioflocculant from *Bacillus subtilis* F9, isolated from wastewater sludge. Ecotoxicology and Environmental Safety, 121, 45-50.
https://doi.org/10.1016/j.ecoenv.2015.06.010

Field, S.J., Ryden, P., Wilson, D., James, S.A., Roberts, I.N., Richardson, D.J., Waldron, K.W., and Clarke, T.A., 2015. Identification of furfural resistant strains of *Saccharomyces cerevisiae* and *Saccharomyces paradoxus* from a collection of environmental and industrial isolates. Biotechnology for Biofuels, 8(1), 33.
https://doi.org/10.1186/s13068-015-0217-z

Liu, C., Wang, K., Jiang, J.H., Liu, W.J., and Wang, J.Y., 2015. A novel bioflocculant produced by a salt-tolerant, alkaliphilic and biofilm-forming strain *Bacillus agaradhaerens* C9 and its application in harvesting *Chlorella minutissima* UTEX2341. Biochemical Engineering Journal, 93, 166-172.
https://doi.org/10.1016/j.bej.2014.10.006

Yang, Q.H., Zhao, X.X., Zhang, J., Wang, Y., Zou, W., and Ming, H.M., 2015. Components of a bioflocculant for treating tannery wastewater. Journal of Residuals Science and Technology, 12(2), 99-103.
http://doi.org/10.12783/issn.1544-8053/12/2/9

Ugbenyen, A.M., Vine, N., Simonis, J.J., Basson, A.K., and Okoh, A.I., 2015. Characterization of a bioflocculant produced from the consortium of three marine bacteria of the genera *Cobetia* and *Bacillus* and its application for wastewater treatment. Journal of Water Sanitation and Hygiene for Development, 5(1), 81-88.
https://doi.org/10.2166/washdev.2014.181

El-Salam, A. E.A., Abd-EI-Haleem, D., Youssef, A.S., Zaki, S., Abu-Elreesh, G., and El-Assar, S.A., 2017. Isolation, characterization, optimization, immobilization and batch fermentation of bioflocculant produced by *Bacillus aryabhattai* strain

PSK1. Journal of Genetic Engineering and Biotechnology, 15(2), 335-344.
https://doi.org/10.1016/j.jgeb.2017.07.002

Shahadat, M., Teng, T.T., Rafatullah, M., Shaikh, Z.A., Sreekrishnan, T.R., and Ali, S.W., 2017. Bacterial bioflocculants: A review of recent advances and perspectives. Chemical Engineering Journal, 328, 1139-1152.
https://doi.org/10.1016/j.cej.2017.07.105

Jenny, A.S., Vidyadharani, G., Santhosh, S., and Dhandapani, R., 2018. Optimization and Characterisation of Thermo Stable Exopolysaccharide Produced from *Bacillus licheniformis* WSF-1 Strain. Journal of Polymers and the Environment, 26(9), 3824-3833.
https://doi.org/10.1007/s10924-018-1261-0

Hamdaoui, O. and Naffrechoux, E., 2007. Modeling of adsorption isotherms of phenol and chlorophenols onto granular activated carbon: Part I. Two-parameter models and equations allowing determination of thermodynamic parameters. Journal of Hazardous Materials, 147(1), 381-394.
https://doi.org/10.1016/j.jhazmat.2007.01.021

Camper, A.K., LeChevallier, M.W., Broadaway, S.C., and McFeters, G.A., 1986. Bacteria associated with granular activated carbon particles in drinking water. Applied and Environmental Microbiology, 52(3), 434-438.
http://aem.asm.org/content/52/3/434