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*Original Research*

# Resource Reuse of Waste-Activated Sludge as Raw Material of Microbial Flocculant Preparation: Methods and Comparison

**Zhiqiang Zhang[1, 2], Jiao Zhang[3], Siqing Xia[2]***

[1]Key Laboratory of Yangtze River Water Environment, Ministry of Education, College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China
[2]State Key Laboratory of Pollution Control and Resource Reuse, College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China
[3]School of Civil Engineering and Transportation, Shanghai Technical College of Urban Management, Shanghai 200432, China

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**Abstract**

Resource reuse of waste-activated sludge (WAS) from a municipal wastewater treatment plant (WWTP) was introduced as raw material of microbial flocculant (MBF) preparation. With WAS samples of three concentrations, MBF preparation by various methods – including cation exchange resin (CER), CER-ultrasonication, CER-ultrasonication-CER, ultrasonication, ultrasonication-CER, and ultrasonication-CER-ultrasonication methods – were comparatively investigated. According to the results of chemical compositions, concentrations, and flocculating activities of the prepared MBFs, the compound methods excelled the sole methods, and the CER-ultrasonication method was the best. Major compositions of the prepared MBFs contained polysaccharides, proteins, and nucleic acids. Improving sludge concentration was beneficial to extract MBF in high concentrations using the above methods. Appropriate MBF mass dosage was crucial its flocculating activity because less or more dosage would deteriorate the flocculation. The mechanism analysis indicated that CER treatment could provide a good pretreatment for ultrasonication, but became useless when it was set after ultrasonication.

**Keywords:** waste activated sludge, microbial flocculant, cation exchange resin, ultrasonication, compound method

## Introduction

As a concomitant of wastewater biological treatment, waste activated sludge (WAS) from a wastewater treatment plant (WWTP) has become a current problem and has been focused on for decades due to its huge production and potential environmental pollution [1, 2]. Minimization of sludge production coupled with recovery of valuable byproducts and bioenergy is becoming increasingly critical for sustainable sludge management [3].

Microbial flocculants (MBFs) are microorganism-produced organic macromolecule substances that can flocculate suspended solids, cells, colloidal solids, etc. [4]. With the advantages of high efficiency, innocuity, and biodegradability, MBFs recently have been given more attention [5]. However, the general method of MBF preparation is carried out by aerobic fermentation of a purified strain in a specific culture medium. The complex

*e-mail: siqingxia@tongji.edu.cn

process of screening, conservation, and culture of the purified strains usually causes a high cost of MBF preparation, which has become the bottleneck of the MBF application [6].

With regard to the reported strains producing MBFs, most of them were screened out from activated sludge [6]. The strains in activated sludge can produce natural organic macromolecule substances with flocculating activity during the biodegradation process of organic substances in wastewater, which play a key role in both sludge-floc formation and sludge sedimentation [6]. Tyagi et al. isolated 25 extracellular polymeric substances (EPS) producing bacterial strains from municipal wastewater sludge, and evaluated the bioflocculability of the obtained EPSs by measuring their flocculating activities to kaolin suspension [7, 8]. After treating waste sludge using hydrochloric acid (HCl) as a solubilizing reagent, Nomura et al. found that the supernatant could function in a similar manner to commercial flocculant in aggregating solid particles under gravity [9]. Liu et al. prepared EPS from the backwashed sludge of a laboratory biologically aerated filter (BAF) treating cassava wastewater by low frequency ultrasonic at 80 W for 10 min in an ice bath, and utilized it as a bioflocculant for dying wastewater treatment [10]. Yu et al. prepared the tightly bound EPS (TB-EPS) fraction from excess sludge by using ultrasound at 20 kHz and 480 W for 10 min, and found its flocculating activity to kaolin suspension [11]. Thus it can be seen that WAS might become a good raw material used to produce cheap MBFs. However, the reported methods preparing bioflocculants from sludge are rarely applied because they need largely consume either hydrochloric acid (HCl) or energy.

The structural component of activated sludge flocs can be categorized as microorganisms, organic matter (in addition to microbial cells), and inorganic cations and anions [12, 13]. Both cation exchange resin (CER) [12, 14-17] and ultrasonication [3, 18] are the most common and simple methods to break up the sludge flocs, which can cause subsequent release of extracellular organic macromolecule substances from sludge. CER can efficiently remove the cations from the sludge matrix, leading to breakup of the flocs and subsequent release of extracellular organic macromolecule substances [12, 14-17]. Ultrasonication can generate a repeating pattern of compressions and rarefactions by ultrasound wave propagating, which can arouse cavitation in the WAS [3, 18]. The sudden and violent collapse of huge numbers of micro-bubbles generates powerful hydromechanical shear forces in the bulk liquid surrounding the bubbles [18]. The extreme shear forces produced by the collapsing bubbles disrupted the sludge matrix and released extracellular organic macromolecule substances [7, 19]. As a matter of fact, most attention was paid to the dewaterability or the biodegradability of the disintegrated sludge. There are few reports about the two methods or their compound methods directly applied to MBF preparation from WAS.

In this paper, the resource reuse of WAS from municipal WWTP was introduced as raw material of MBF preparation. With the WAS samples of three concentrations (3.5, 6.4 and 9.3 g/L), MBF preparation by various methods, including CER, CER-ultrasonication, CER-ultrasonication-CER, ultrasonication, ultrasonication-CER, and ultrasonication-CER-ultrasonication methods, were comparatively investigated. The key parameters, like chemical compositions, concentrations, and flocculating activities of the prepared MBFs, were used to compare the preparation methods. The mechanisms of MBF preparation from WAS by these methods also were analyzed.

## Materials and Methods

### Waste-Activated Sludge

The WAS samples were collected from the secondary settling tank of a full-scale municipal WWTP in Shanghai, China. This plant treats about 60,000 $m^3$/d of wastewater using the anaerobic–anoxic-oxic process. The main parameters of the sludge are listed as follows: pH 6.8-7.5, TSS 7.0±1.5 g/L, and VSS/TSS 65±8%. The collected samples were transported to the laboratory within 30 min, and subsequently stored at 4ºC and analyzed within 2 days. Then fresh WAS would be sampled from the municipal WWTP. The low concentration WAS (about 3.5 g/L) was obtained by diluting the collected WAS sample by using deionized water. The high concentration WAS (about 9.3 g/L) was obtained by gravity thickening of the collected WAS sample.

### Reagents

Kaolin clay was purchased from Sinopharm Chemical Reagent Co., Ltd., China, and the average diameter of Kaolin clay particles was about 45 μm. Cation exchange resin (strong acidic styrene 001×7) was purchased from Shanghai Huizhu Resin Co., Ltd., China. Other reagents were of analytical grade.

### MBF Preparation

According to the previous study, the optimal treatment conditions of WAS by CER method for MBF preparation from WAS is as follows: resin dosage 60 g/g VSS, agitation speed 650 rpm, neutral pH, and treatment time 3 h [20]. Then the treated sludge samples were centrifuged at 12,000 rpm for 10 min at 4ºC. The bulk solutions were used as the crude liquid MBFs for the following flocculation experiments.

The optimal treatment conditions of WAS by ultrasonication method for MBF preparation from WAS is as follows: frequency 20 kHz, pulse style 4 s, power density 2.7 kW/L, neutral pH, and treatment time 2 min [21]. Then the treated sludge samples were centrifuged at 12,000 rpm for 10 min at 4ºC. The bulk solutions were used as the crude liquid MBFs for the following flocculation experiments.

For simplification, the CER treatment is denoted as *R*, the ultrasonication as *U*, and the centrifugation as *C*. With centrifugation (*C*) as a control, various methods – including the CER method (*R*-*C*), the ultrasonication method (*U*-*C*),

the CER-ultrasonication method (*R-U-C*), the CER-ultrasonication-CER method (*R-U-R-C*), the ultrasonication-CER method (*U-R-C*), and the ultrasonication-CER-ultrasonication method (*U-R-U-C*) – were carried out to extract MBF from WAS.

### Flocculation Experiments

Flocculating activity (*FA*) of prepared MBF was measured using a previous method in which Kaolin clay was chosen as the suspended solid [22, 23]. 4 g Kaolin clay, 3.0 ml of $CaCl_2$ (1%, w/v), and 2.0 ml of MBF, were added into 95 ml distilled water in 100-ml test tubes in turn. Then the mixture was vigorously stirred and allowed to stand for 5 min. The optical density (OD) of the clarifying solution was measured with a spectrophotometer (HACH, DR 2800) at 550 nm. A control experiment was prepared using the same method, but the MBF was replaced by distilled water. The flocculating activity was calculated according to Eq. (1). All experiments were performed in triplicate for the mean calculation.

$$FA = ((B\text{-}A)/B) \times 100\% \quad (1)$$

...where *FA* is flocculating activity, *A* the optical density of the sample experiment at 550 nm, and *B* the optical density of control experiment at 550 nm.

### Analytical Techniques

The sludge parameters, including total suspended solid (TSS) and volatile suspended solid (VSS), was analyzed following the standard methods [24]. The polysaccharide contents in the prepared MBFs were determined by the phenol-sulfuric acid method using glucose as the standard solution [25]. The protein contents in the prepared MBFs were measured by the Bradford method with bovine serum albumin (BSA) as the standard [26]. The nucleic acid contents in the prepared MBFs were determined by the diphenylamine colorimetric method using calf thymus deoxyribonucleic acid as the standard [27].

## Results and Discussion

### MBF Preparation from Low Concentration WAS

With the WAS sample of low concentration (about 3.5 g/L), various MBF preparation methods were comparatively investigated. Both the chemical compositions and the concentrations of the prepared MBFs from the low concentration WAS were analyzed. As shown in Fig. 1, no obvious effect on the MBF preparation was observed when the low concentration WAS was simply treated by centrifugation, meaning that MBF didn't lie in the supernatant of WAS [11]. Both the CER method and the ultrasonication method were effective in MBF preparation. The MBF preparation efficacy by any compound method was higher than that solely by the CER method or the ultrasonication method. The prepared MBFs mainly consisted of polysaccharides (about 30%), proteins (about 40%), and nucleic acids (about 30%). The highest MBF concentration (about 170 mg/L) was obtained when the CER-ultrasonication method was used to extract MBF from the low concentration WAS. The MBF yield was about 47 mg MBF/g TSS, and about 78 mg MBF/g VSS.

With the MBF volume dosage of 2% (v/v), the flocculating activities of the MBFs prepared from the low concentration WAS by various preparation methods were shown in Fig. 2. The flocculating activity of the MBF prepared by the ultrasonication method was markedly higher than that of the MBF prepared by the CER method. The flocculating activity of the MBF prepared by CER-ultrasonication method was over 70% under the MBF volume dosage of 2% (v/v) (0.84 mg MBF/g Kaolin), which is higher than that of the MBF prepared by any other method. The flocculating activities of the prepared MBFs by various methods were almost consistent with the MBF concentrations.

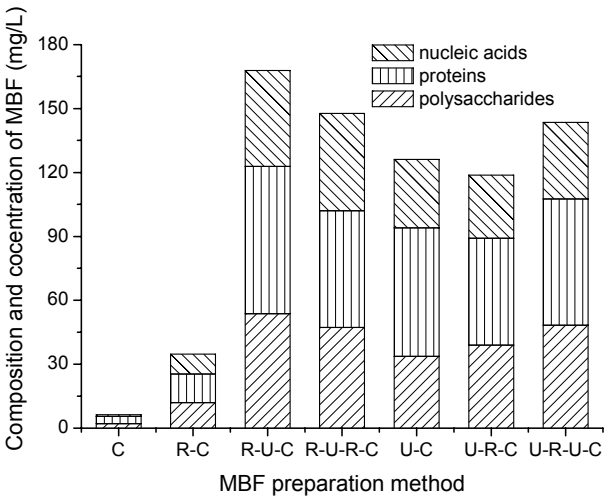


Fig. 1. Comparison of various methods in both compositions and concentrations of the prepared MBFs from the low-concentration WAS (3.5 g/L).

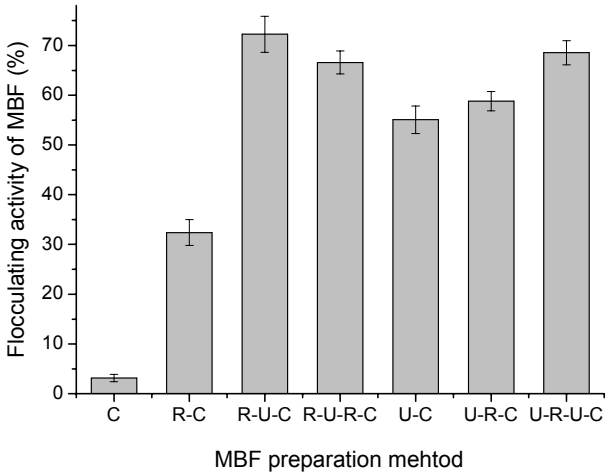


Fig. 2. Comparison of various methods in the flocculating activities of the prepared MBFs from the low-concentration WAS (3.5 g/L) at the MBF volume dosage of 2% (v/v).

## MBF Preparation from Middle Concentration WAS

With the WAS sample of middle concentration (about 6.4 g/L), various MBF preparation methods were comparatively investigated. As shown in Fig. 3, the prepared MBFs from the middle concentration WAS were similar with those from the low concentration WAS in chemical compositions, but showed higher concentrations. The highest MBF concentration (about 276 mg/L) was obtained when the CER-ultrasonication method was used to extract MBF from the high-concentration WAS. The MBF yield of middle concentration WAS was about 43 mg MBF/g TSS, and about 71 mg MBF/g VSS, a little lower than that of low concentration WAS.

With the MBF volume dosage of 2% (v/v), the flocculating activities of the MBFs prepared from the middle concentration WAS by various methods were shown in Fig. 4. Contrary with the results of the low concentration WAS, the flocculating activity of the MBF prepared by CER-ultrasonication method was only about 35% under the MBF volume dosage of 2% (v/v) (1.38 mg MBF/g Kaolin), lower than those of MBFs prepared by other methods. This might be caused by superfluous MBF mass dosage deteriorated by the flocculation effect [30].

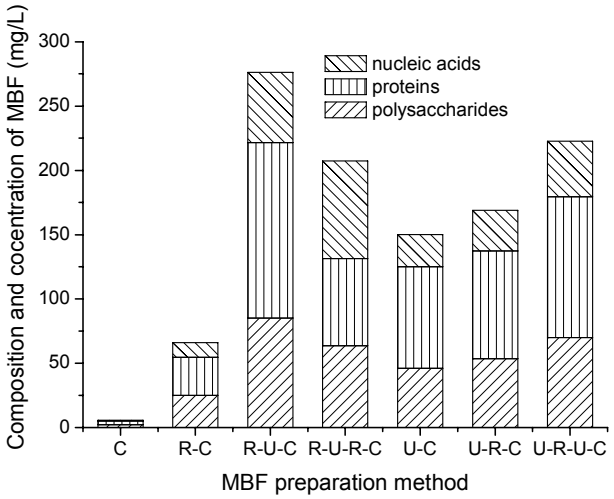


Fig. 3. Comparison of various methods in both compositions and concentrations of the prepared MBFs from the middle concentration WAS (6.4 g/L).

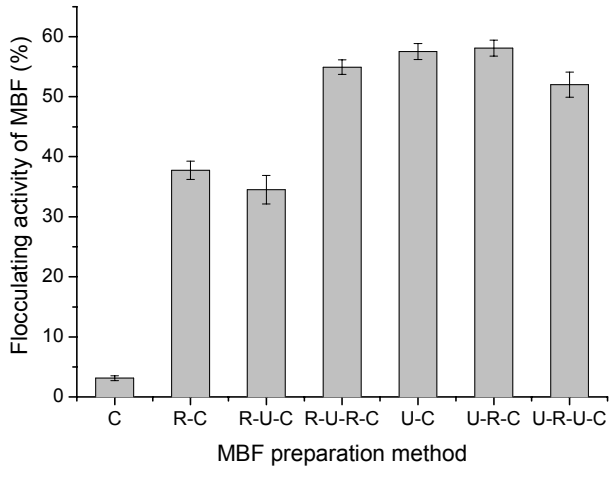


Fig. 4. Comparison of various methods in the flocculating activities of the prepared MBFs from the middle concentration WAS (6.4 g/L) at the MBF volume dosages of 2% (v/v).

## MBF Preparation from High Concentration WAS

To further examine the effect of WAS concentrations on MBF preparation methods, various MBF preparation methods with the WAS sample (about 9.3 g/L) were comparatively investigated. As shown in Fig. 5, the prepared MBFs from the high-concentration WAS also were similar to those from the low and the middle concentration WAS in composition, but showed highest concentrations.

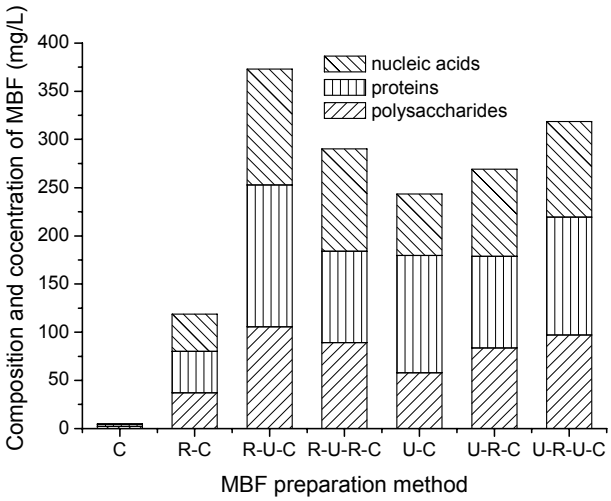


Fig. 5. Comparison of various methods in both compositions and concentrations of the prepared MBFs from the high concentration WAS (9.3 g/L).

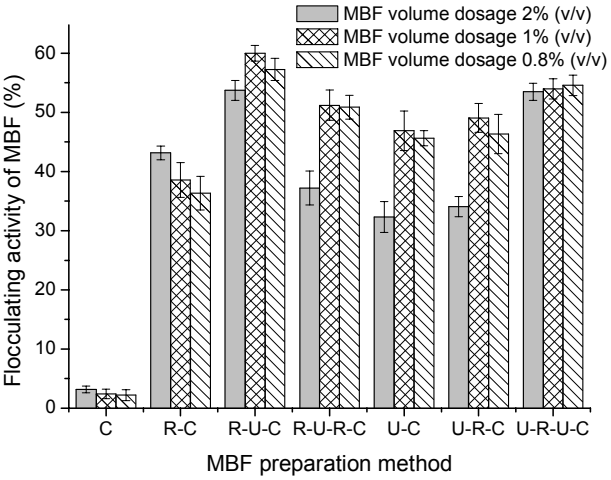


Fig. 6. Comparison of various methods in the flocculating activities of the prepared MBFs from the high concentration WAS (9.3 g/L) at the MBF volume dosages of 2%, 1% and 0.8% (v/v).

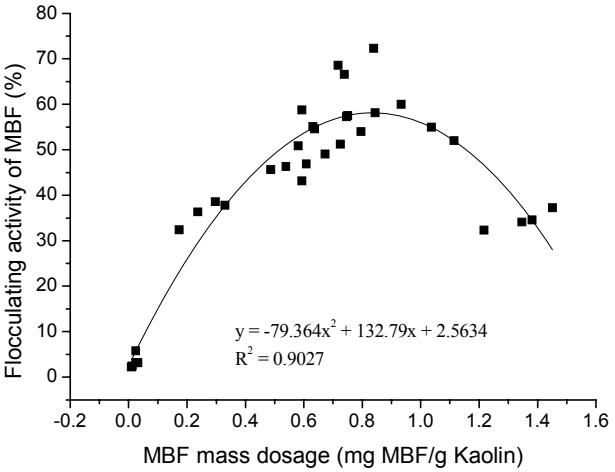


Fig. 7. Relationships between MBF mass dosage and its flocculating activity.

For each of the compound methods, the concentration of the MBF from the high concentration WAS is 2 to 3 times that of the MBF from the low concentration WAS. It indicates that all the prepared MBFs are of similar flocculation performance, and their flocculating activities might be mainly influenced by the MBF mass dosages. The highest MBF concentration (about 370 mg/L) also was obtained when the CER-ultrasonication method was used. Accordingly, improving sludge concentration was beneficial to extract high concentration MBF by the above methods. The MBF yield of high concentration WAS was about 40 mg MBF/g TSS, and 66 mg MBF/g VSS, lower than those of low concentration WAS and middle concentration WAS. Mass transferring efficiency in WAS was reduced little by little with rising sludge concentration, which might cause the decreasing MBF yield with rising WAS concentration.

Considering the effect of MBF mass dosage on the flocculation effect, with three MBF volume dosages (2%, 1%, and 0.8%, v/v), the flocculating activities of the MBFs prepared from the high concentration WAS by various preparation methods were shown in Fig. 6. The flocculating activities varied with the MBF volume dosage, and no obvious rules can be found. To clarify the phenomena, the mass dosage was calculated and presented in terms of mg MBF/g kaolin. Based on the results of Figs. 2, 4, and 6, the relationship between MBF mass dosage and flocculating activity was obtained and shown in Fig. 7. It can be found that appropriate MBF mass dosage is crucial to its flocculating activity because less or more dosage would deteriorate the flocculation, just like other flocculating agents [28, 29]. Less MBF mass dosage means not enough MBF molecules to adsorb the suspended Kaolin clay particles, and to bridge between them [30]. On the other hand, more MBF mass dosage would inhibit small flocs growing into big ones caused by the stronger repulsion force between them [30]. From Fig. 7, the optimal MBF mass dosage for its flocculating activity is about 0.84 mg MBF/g Kaolin.

## Mechanism Analysis of MBF Preparation by Compound Methods

From Figs. 1, 3, and 5, the MBF concentrations were markedly improved when various compound methods of the CER method and the ultrasonication method were used to extract MBF from WAS. For WAS samples of various concentrations, all the highest MBF concentrations were obtained by the CER-ultrasonication method, which exceeded the sum of the MBF concentrations obtained solely by the CER method and the ultrasonication method. This means that the CER method not only released the extracellular organic macromolecule substances, but also provided a good pretreatment for the subsequent ultrasonication method by breaking up the sludge matrix [14]. The reversal sequence treatment of this method is the ultrasonication-CER method. The MBF concentration obtained by the latter method is close to that obtained solely by the ultrasonication method, indicating that the subsequent CER treatment was nearly idle or even negative for the MBF preparation after ultrasonication disrupted the sludge matrix. This phenomenon also appeared when the CER-ultrasonication-CER method was used for MBF preparation from WAS. As for the ultrasonication-CER-ultrasonication method, it was equal to the addition of the ultrasonication treatment after the ultrasonication-CER method, the MBF concentration also increased because of the last ultrasonication after the CER treatment.

## Conclusions

Resource reuse of WAS from municipal WWTP was achieved by the CER method, the ultrasonication method, or their compound methods. Among these methods, the CER-ultrasonication method excelled at the MBF concentration. Containing polysaccharides, proteins, and nucleic acids, the major compositions of the MBFs prepared from WAS of various concentrations were similar. The MBF concentration increased with rising sludge concentration. The mechanism analysis indicated that CER treatment could provide a good pretreatment for ultrasonication, but became useless when it was set after ultrasonication.

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## References

1. YU G.H., HE P.J., SHAO L.M., HE P.P. Stratification Structure of Sludge Flocs with Implications to Dewaterability. Environ. Sci. Technol., **42**, 7944, **2008**.

2. VAXELAIRE J., CEZAC P. Moisture distribution in activated sludges: a review. Water Res., **38**, 2215, **2004**.
3. KHANAL S.K., GREWELL D., SUNG S., VAN LEEUWEN J. Ultrasound applications in wastewater sludge pretreatment: A review. Crit. Rev. Env. Sci. Technol., **37**, 277, **2007**.
4. ZHANG T., LIN Z., ZHU H.L. Microbial flocculant and its application in environmental protection. J. Environ. Sci., **11**, 1, **1999**.
5. ZHANG Z.Q., LIN B., XIA S.Q., WANG X.J., YANG A.M. Production and application of a novel bioflocculant by multiple-microorganism consortia using brewery wastewater as carbon source. J. Environ. Sci., **19**, 667, **2007**.
6. SALEHIZADEH H., SHOJAOSADATI S.A. Extracellular biopolymeric flocculants – Recent trends and biotechnological importance. Biotechnol. Adv., **19**, 371, **2001**.
7. SUBRAMANIAN S.B., YAN S., TYAGI R.D., SURAMPALLI R.Y. Extracellular polymeric substances (EPS) producing bacterial strains of municipal wastewater sludge: Isolation, molecular identification, EPS characterization and performance for sludge settling and dewatering. Water Res., **44**, 2253, **2010**.
8. SUBRAMANIAN S.B., YAN S., TYAGI R.D., SURAMPALLI R.Y., LOHANI B.N. Isolation and molecular identification of extracellular polymeric substances (EPS) producing bacterial strains for sludge settling and dewatering. J. Environ. Sci. Health. Part A Toxic/Hazard. Subst. Environ. Eng., **43**, 1495, **2008**.
9. NOMURA T., ARAKI S., NAGAO T., KONISHI Y. Resource recovery treatment of waste sludge using a solubilizing reagent. J. Mater. Cycles Waste Manage., **9**, 34, **2007**.
10. LIU W.J., YUAN H.L., YANG J.S., LI B.Z. Characterization of bioflocculants from biologically aerated filter backwashed sludge and its application in dying wastewater treatment. Bioresour. Technol., **100**, 2629, **2009**.
11. YU G.H., HE P.J., SHAO L.M. Characteristics of extracellular polymeric substances (EPS) fractions from excess sludges and their effects on bioflocculability. Bioresour. Technol., **100**, 3193, **2009**.
12. FR LUND B., PALMGREN R., KEIDING K., NIELSEN P.H. Extraction of extracellular polymers from activated sludge using a cation exchange resin. Water Res., **30**, 1749, **1996**.
13. JORAND F., ZARTARIAN F., THOMAS F., BLOCK J.C., BOTTERO J.Y., VILLEMIN G., URBAIN V., MANEM J. Chemical and Structural (2D) Linkage between Bacteria within Activated-Sludge Flocs. Water Res., **29**, 1639, **1995**.
14. D'ABZAC P., BORDAS F., VAN HULLEBUSCH E., LENS P.N.L., GUIBAUD G. Effects of extraction procedures on metal binding properties of extracellular polymeric substances (EPS) from anaerobic granular sludges. Colloids Surf., B, **80**, 161, **2010**.
15. RAS M., GIRBAL-NEUHAUSER E., PAUL E., SPERANDIO M., LEFEBVRE D. Protein extraction from activated sludge: An analytical approach. Water Res., **42**, 1867, **2008**.
16. MCSWAIN B.S., IRVINE R.L., HAUSNER M., WILDERER P.A. Composition and distribution of extracellular polymeric substances in aerobic flocs and granular sludge. Appl. Environ. Microbiol., **71**, 1051, **2005**.
17. LIU H., FANG H.H.P. Extraction of extracellular polymeric substances (EPS) of sludges. J. Biotechnol., **95**, 249, **2002**.
18. PILLI S., BHUNIA P., YAN S., LEBLANC R.J., TYAGI R.D., SURAMPALLI R.Y. Ultrasonic pretreatment of sludge: A review. Ultrason. Sonochem., **18**, 1, **2011**.
19. HE P.J., YU G.H., SHAO L.M., ZHU Y.S. Extracellular proteins, polysaccharides and enzymes impact on sludge aerobic digestion after ultrasonic pretreatment. Water Res., **42**, 1925, **2008**.
20. ZHANG Z.Q., ZHANG J. Waste activated sludge treated by the cation exchange resin method for microbial flocculant preparation. Adv. Mater. Res., **518-523**, 3585, **2012**.
21. ZHANG Y.J., ZHANG Z.Q., DAI X.Q., WANG C.H., XIA S.Q. Extraction of microbial flocculant from waste sludge by ultrasonic method. Chin. J. Environ. Eng., **6**, 1030, **2012**.
22. XIA S.Q., ZHANG Z.Q., WANG X.J., YANG A., CHEN L., ZHAO J.F., LEONARD D., JAFFREZIC-RENAULT N. Production and characterization of a bioflocculant by Proteus mirabilis TJ-1. Bioresour. Technol., **99**, 6520, **2008**.
23. KURANE R., TOEDA K., TAKEDA K., SUZUKI T. Culture conditions for production of microbial flocculant by *Rhodococcus erythropolis*. Agr. Biol. Chem., **50**, 2309, **1986**.
24. APHA, AWWA, WEF. Standard Methods for the Examination of Water and Wastewater, Americal Public Health Association/American Water Works Association/ Water Environment Federation, Washington DC, USA, **1998**.
25. CHAPLIN M.F., KENNEDY J.F. Carbohydrate Analysis (2nd edition), Oxford University Press, New York, USA, **1994**.
26. BRADFORD M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of proteindye binding. **72**, 248, **1976**.
27. SUN Y.D., CLINKENBEARD K.D., CLARKE C., CUDD L., HIGHLANDER S.K., DABO S.M. Pasteurella haemolytica leukotoxin induced apoptosis of bovine lymphocytes involves DNA fragmentation. Vet. Microbiol., **65**, 153, **1999**.
28. LI W.W., ZHOU W.Z., ZHANG Y.Z., WANG J., ZHU X.B. Flocculation behavior and mechanism of an exopolysaccharide from the deep-sea psychrophilic bacterium Pseudoalteromonas sp SM9913. Bioresour. Technol., **99**, 6893, **2008**.
29. YIM J.H., KIM S.J., AHN S.H., LEE H.K. Characterization of a novel bioflocculant, p-KG03, from a marine dinoflagellate, Gyrodinium impudicum KG03. Bioresour. Technol., **98**, 361, **2007**.
30. ZHANG Z.Q., XIA S.Q., ZHAO J.F., ZHANG J. Characterization and flocculation mechanism of high efficiency microbial flocculant TJ-F1 from Proteus mirabilis. Colloids Surf., B, **75**, 247, **2010**.