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Bioflocculation technology for total nitrogen removal from tannery wastewater

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ABSTRACT

This paper introduced the sources of total nitrogen pollutants in tannery wastewater, the status of the current total nitrogen treatment. Currently, there are two methods, clean production and biological treatment, to control the total nitrogen in tannery wastewater. And efficient biological treatment has become a research hotspot. Meanwhile, it was expounded for the research status of the application of a bioflocculation technique for removing total nitrogen pollutants from tannery wastewater. The component analysis indicated that the polysaccharide, protein, glycoprotein, glycosylamine, and lipids were found in the prepared bioflocculants. Furthermore, active component analysis results demonstrated that polysaccharide and protein were the active components for total nitrogen removal. Amount of studies on the action mechanisms of bioflocculants had established four theories for flocculation mechanisms, including adsorption and bridging theory, lectin hypothesis, cellulose fibril hypothesis, and chemical reaction hypothesis. The application of a bioflocculation technique in tannery wastewater treatment was proposed.

Keywords: Bioflocculation; Tannery wastewater; Total nitrogen

1. Introduction

The leather-making industry, as a type of light industry, can be the source of severe pollution, among which nitrogen was the main component. It is well-known that excess nitrogen causes water eutrophication. Therefore, the limitations of total nitrogen emissions from tannery wastewater had drawn increasingly wide attention. On December 27, 2013, Chinese officials released *Discharge Standard of Water Pollutants for Leather and Fur Making Industry* (GB30486 – 2013), in which total nitrogen was set as a new control index and the emission limits of total nitrogen were specified. Table 1 lists the direct emission limits of total nitrogen and ammonia nitrogen for existing and new leather treatment enterprises as well as unique protection regions. The treatment of total nitrogen in tannery wastewater can no longer be ignored, and in order to ensure nitrogen levels in tannery wastewater meet the new emission standard, it is urgent and necessary to develop an economical and highly efficient nitrogen removal technique.

Total nitrogen includes all nitrogen-containing compounds in wastewater, namely both organic nitrogen (such as protein, polypeptide, amino acid, and urea) and inorganic nitrogen (such as ammonia nitrogen, nitrite nitrogen, and nitrate nitrogen). Total nitrogen produced during the leather-making process mainly came from the following two sources: skin collagen, which became organic nitrogen after hydrolysis, and various ammonium salts [1,2]. In leather manufacturing, a minute quantity of nitrite nitrogen and nitrate nitrogen were produced, whose contents in tannery wastewater were generally lower than 3 mg/L and 50 μg/L, respectively [2–4]. Therefore, total nitrogen in tannery wastewater primarily includes organic nitrogen and ammonia nitrogen. Studies had shown that approximately 14 kg total nitrogen and 6 kg ammonia nitrogen were produced during the processing of 1 ton of raw materials [2].

Since the previously implemented *Integrated Wastewater Discharge Standard* (GB8978 – 1996) sets no emission limits on

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Table 1
Direct emission limits of total nitrogen and ammonia nitrogen in tannery wastewater

	Total nitrogen (mg/L)	Ammonia nitrogen (mg/L)
Special protection regions	20	15
New enterprises	50	25
Existing enterprises (Since January 1, 2016)	50	25
Existing enterprises (2014.7.1 – 2015.12.31)	70	35

total nitrogen, more importance was placed on the treatment of ammonia nitrogen; the treatment of total nitrogen in tannery wastewater was overlooked. Currently, total nitrogen in tannery wastewater is controlled using one of the following two methods: clean production and biological treatment [5–10]. The first method aims to control the pollution source, and scholars had aspired to develop clean deliming and tanning technologies in recent years to reduce the content of total nitrogen as much as possible [5,6]. Meanwhile, researchers explored biochemical methods for removing total nitrogen, which mainly include anaerobic/aerobic (A/O) technique and biomembrane reactor [7–10]. In order to lower the total nitrogen in tannery wastewater to meet the new national emission standard, a new high-efficiency wastewater treatment technique must be sought in addition to adopting clean production and reducing pollution caused by total nitrogen from the source.

2. Application of biological flocculants

Biological flocculants (hereinafter referred to as bioflocculants) possess advantages such as insensitivity to the external environment, high processing efficiency, wide application range, favorable biodegradability, and environmental protection. Furthermore, the industrial production of bioflocculants can be achieved using the method in bioengineering. Bioflocculants showed increasingly broader application prospects and became a new research interest in microbial technology [11,12].

Until now, few studies had been conducted on the application of biological flocculants in the disposal of tannery wastewater. Zhang et al. [13] found that adding flocculants produced from bacterial strain C-62 to the wastewater of a leather tanning industry reached a removal ratio of turbidity of 96%. Using the conventional bacteria isolation and purification method, Li et al. acquired six strains of bioflocculant-producing bacteria from wastewater, soils, and activated sludge. Of these, one was chosen for leather tannery wastewater treatment. Results showed that the removal ratios of chemical oxygen demand (COD), sulfur, turbidity, and chromaticity were 61%, 97.1%, 98.7%, and 99%, respectively [14]. Chai et al. screened out two strains of flocculant-producing bacteria from wastewater, soil, and activated sludge and then used them to purify leather-making wastewater. According to their research, the removal ratio of the suspended solids (SS) was as high as 96.3%, and when it was applied to wastewater from leather dye, the decolorization ratio was over 91% [15]. Qin et al. screened microorganisms from sludge and used them as flocculants for tannery wastewater disposal. In tannery wastewater with COD, SS, and Cr^{3+} concentrations of 3,200 mg/L, 3,100 mg/L, and 70 mg/L, and a pH value of 7.0, the removal ratios were 72.4%, 62%, and 63.5%, respectively, after the addition of the flocculants at a volume ratio of 0.2% [16]. Rajan et al. [17] isolated three strains of bioflocculant-producing bacteria from the pollution soils in a tannery and found both *Trichococcus flocculzformis* and *Pseudomonas fluorescens* showed satisfactory flocculation performances. Wang et al. screened out four bacterial strains and produced flocculants after mixing these bacteria. After using the produced flocculants for disposing tannery wastewater, it was found that the wastewater's chromaticity, turbidity, and COD decreased significantly [18]. Liu et al. [19] reviewed the performances and action mechanisms of various flocculants and presented their research status and application prospects in tannery wastewater disposal. Sam et al. [20] determined the composition of bioflocculants for tannery wastewater treatment and discovered they contained polysaccharides. Lin et al. [21] singled out two strains of molds with high flocculation activity from activated sludge and used them for tannery wastewater treatment; they observed obvious effects while processing the wastewater's chromaticity, ammonia nitrogen, COD, and Cr. Wei et al. [22] screened out and isolated a strain of high-efficiency flocculant-producing bacterium X-3 from activated sludge, and found that it demonstrated positive flocculation and sedimentation results in leather-making wastewater disposal. Kim et al. used a microorganism combination for removing COD, total nitrogen (TN), total phosphorus (TP), and Cr from leather tannery wastewater. Their results showed that, without any chemical pretreatments, the removal ratios of these pollutants reached 98.3%, 98.6%, 93.6%, and 88.5%, respectively [23]. Although studies regarding the removal of ammonia nitrogen in tannery wastewater had been conclusively reported, the flocculants used were microorganisms (molds), and the application of bioflocculants extracted from microorganisms in removing nitrogen from tannery wastewater had yet to be reported.

3. Component analysis of bioflocculants

This research was engaged in the treatment of tannery wastewater using bioflocculants. First, the bioflocculants were prepared from screened bacillus and then applied in tannery wastewater treatment. Results showed that using these bioflocculants, the total nitrogen, ammonia nitrogen, chromaticity, and COD were appreciably removed [24]. Through component analysis, polysaccharide, protein, glycoprotein, glycosylamine, and lipids were found in the prepared bioflocculants [25]. Furthermore, active component analysis results demonstrated that polysaccharide and protein were the active components for total nitrogen removal; in particular, polysaccharide was the most effective component that accounted for flocculation activity [26]. However, the structural composition of the polysaccharide and the action mode and mechanism of total nitrogen removal must be explored in depth. To date, no similar research has been conducted on bioflocculants for tannery wastewater treatment.

Many scholars had focused on the polysaccharide component of bioflocculants. Sam et al. [20] used preprocessed molasses to cultivate halobacteria to prepare a kind of bioflocculant; the component measurement results revealed the existence of polysaccharide in the bioflocculants. Li et al. [27] used bioflocculants produced from *Paenibacillus elgii* B69 to remove heavy metal ions from wastewater, and found that the polysaccharide in the flocculants was composed of glucose, uronic acid, mannose, and xylose. Zhang et al. identified the components of polysaccharide in a bioflocculant MBFGA1 that showed remarkable effects in disposing soil suspension, coal-washing wastewater, and landfill leachate. Through detection, it was discovered that the monosaccharide composition included xylose, mannose, glucose, and rhamnose [28]. Tang et al. [29] used *Paenibacillus mucilaginosus* GIM1 to prepare three bioflocculants for removing heavy metal ions from wastewater, and characterized their polysaccharide structure by means of infrared detection and electron scanning.

4. Mechanisms of bioflocculants

Extensive studies on the action mechanisms of bioflocculants conducted by international scholars had established four theories for flocculation mechanisms. The first theory is the adsorption and bridging theory, which assumes that the active groups in flocculant macromolecules adsorb the other colloidal particles via ionic bonds, hydrogen bonds, or Van der Waals' force, while two or more particles are bonded together through bridging, which leads to flocculation [30]. Fig. 1 illustrates the bioflocculants' adsorption and bridging process. The second theory is the lectin hypothesis, which is generally used for explaining saccharomycetes' flocculating mechanism. According to this theory, flocculation is induced by the specific bonding between special surface proteins on the flocculating yeast cell wall and mannose residues on the other yeast cell surface. The third theory is the cellulose fibril hypothesis, which proposes that flocculating agents produced by some microorganisms are not free from the bacterium culture solution, but are tightly attached to the cell surface to form capsule-shaped materials (such as yeast with flocculation ability and cellulase-producing bacteria). These capsule-shaped materials are similar to the fibrils outside the bacteria, and thus impose flocculation. This hypothesis provides a reasonable explanation for the flocculation mechanisms of cellulase-producing bacteria.

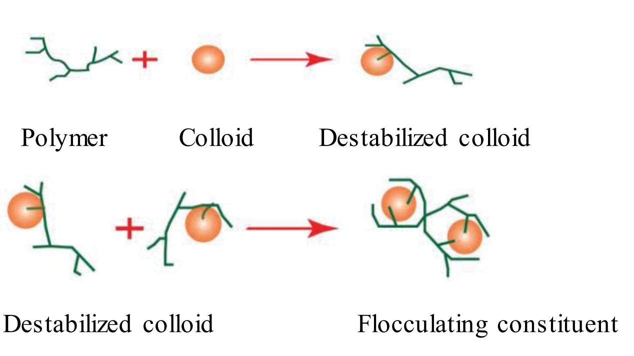

Fig. 1. Illustration of polymer's adsorption and bridging mechanism.

The final theory is a chemical reaction hypothesis in which active groups in flocculants' macromolecules chemically react with corresponding groups in the substance to be flocculated, and the agglomerated macromolecules are precipitated. The flocculation activity is greatly affected if the natural macromolecules are modified or active groups are removed or added, which suggests that the flocculation activity of these flocculants depends heavily on the active groups [31].

5. Future prospects

In order to gain an in-depth understanding of removal mechanisms of total nitrogen from tannery wastewater using bioflocculants and to further improve removal efficiency, it is necessary to analyze the polysaccharide component and the active ingredients in the bioflocculants, and to detect the action mode between the bioflocculants and the total-nitrogen pollutants in tannery wastewater. Future studies aim to elucidate the removal mechanisms of total nitrogen from tannery wastewater using the bioflocculation technique in order to provide sufficient theoretical bases for its application in tannery wastewater treatment.

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References

[1] S.L. Ding, X.L. Lei, L. Ling, Advances in treatment technique on ammonium removal of tannery wastewater, Leather Sci. Eng., 39 (2010) 166–167.
[2] K. Xiao, L.Y. Yu, X.S. Zhang, L. Yu, Rapid determination of nitrate in tannery effluent by reverse flow injection analysis, J. Soc. Leather Technol. Chem., 96 (2012) 215–219.
[3] H.P. Li, X.S. Zhang, L.Y. Yu, S. Chen, On-line determination of nitrite in tannery effluent by flow injection analysis, J. Soc. Leather Technol. Chem., 96 (2012) 195–199.
[4] X.W. Chai, Y.N. Wang, X.P. Liao, M.R. Cao, B. Shi, Determination of ammonia nitrogen in tannery wastewater and analysis of its origin (III), China Leather, 39 (2010) 21–24.
[5] J.H. Lu, Y.H. Zeng, X.P. Liao, M.R. Cao, B. Si, Comparison of deliming efficiency of boric acid, citric acid and commercial non-/low-ammonium deliming agents, China Leather, 40 (2011) 12–15.
[6] M. Yang, H.C. Yuan, Z.H. Shan, C. Hui, Y.C. Zhao, Tanning characteristics of Fe(II)-oxidation polysaccharide tanning agent, China Leather, 42 (2013) 19–22.
[7] H.R. Ma, K. Du, K.Z. Lian, H. Zhang, X.J. Ma, C.Y. Liu, Influence of HRT on nitrogen removal extent of tannery wastewater in A/O process, China Leather, 41 (2012) 56–59.
[8] X.Q. Chen, Y.X. Zhu, X.L. Chen, Q.H. Lu, Analysis on nitrogen removal technology in tannery wastewater treatment, West Leather, 34 (2012) 13–18.

[9] C.X. Chi, B. Shi, Y.Z. Pan, L.S. Ye, Study on advanced treatment of secondary effluent from tannery wastewater by porous carrier bioreactor, Leather Chem., 34 (2012) 13–18.
[10] J.G. Wang, Y.H. Liu, Study on the treatment of tannery wastewater with the high concentration of ammonia nitrogen by MBR, Appl. Mech. Mater., 71–78 (2011) 2186–2189.
[11] B. Lian, Y. Chen, J. Zhao, H.H. Teng, L.J. Zhu, S. Yuan, Microbial flocculation by *Bacillus mucilaginosus*: applications and mechanisms, Bioresour. Technol., 99 (2008) 4825–4831.
[12] J.H. Yim, S.J. Kim, S.H. Ahn, H.K. Lee, Characterization of a novel bioflocculant, p-KG03, from a marine dinoflagellate, *Gyrodinium impudicum* KG03, Bioresour. Technol., 98 (2007) 361–367.
[13] T. Zhang, H.L. Zhu, Z. Lin, Advances in research and application of biological flocculants, Chinese J. Appl. Environ. Biol., 2 (1996) 95–105.
[14] Z.L. Li, B.L. Zhang, J. Bei, Screening of flocculant-producing bacteria and the flocculating effects on wastewater, Chinese J. Appl. Environ. Biol., 3 (1997) 67–70.
[15] X.L. Chai, J. Chen, M. Wang, Z.S. Shen, Screening for flocculant-producing bacteria, Pollut. Control Technol., 13 (2000) 68–70.
[16] Y.M. Qin, Experimental study on the leather wastewater treatment by bio-flocculant, Ind. Water Treat., 26 (2006) 45–47.
[17] M.R. Rajan, C. Nithya, Isolation of bioflocculant producing microbes from tannery polluted soil, Ecol. Environ. Conserv. Paper, 12 (2006) 175–178.
[18] J.X. Wang, Y.C. Li, Selections of compounded microbial flocculant and its application in tannery wastewater treatment, China Leather, 38 (2009) 27–32.
[19] K.S. Liu, Y.S. Yang, S.Q. Shi, Y.J. He, Research progress and application of flocculants in tannery effluent treatment, Leather Chemicals, 28 (2011) 20–24.
[20] S. Sam, F. Kucukasik, O. Yenigun, B. Nicolaus, E.T. Oner, M.A. Yukselen, Flocculating performances of exopolysaccharides produced by a halophilic bacterial strain cultivated on agro-industrial waste, Bioresour. Technol., 102 (2011) 1788–1794.
[21] Y.H. Lin, Y.J. Yao, L.H. Teng, J. Zhang, Screening of high flocculating activity moulds and application in treatment of tannery wastewater, Amino Acids Biotic Resour., 34 2012 35–38.
[22] M.B. Wei, Q.G. Ren, X.J. Zheng, Screening of microbial flocculant-producing bacterium and study on its characteristics, Ind. Water Wastewater, 43 (2012) 60–63.
[23] I.S. Kim, K.I. Ekpeghere, S.Y. Ha, B.S. Kim, B. Song, J.T. Kim, H.G. Kim, S.C. Koh, Full-scale biological treatment of tannery wastewater using the novel microbial consortium BM-S-1, J. Environ. Sci. Health Part A Toxic/Hazard. Subst. Environ. Eng., 49 (2014) 355–364.
[24] Q.H. Yang, H.M. Ming, X.X. Zhao, J. Zhang, W. Zou, C.Q. Zhao, X.Q. Guan, Screening of bioflocculant and preliminary application to treatment of tannery wastewater, J. Residuals Sci. Technol., 12 (2015) 177–181.
[25] Q.H. Yang, X.X. Zhao, J. Zhang, Y. Wang, W. Zou, H.M. Ming, C.Q. Zhao, Components of a bioflocculant for treating tannery wastewater, J. Residuals Sci. Technol., 12 (2015) 99–103.
[26] C.Q. Zhao, X.X. Zhao, H.X. Gao, H.X. Gao, H.B. Gu, J. Zhang, W. Zou, Q.H. Yang, Determination of active ingredients in bioflocculants for treatment of tannery wastewater, J. Soc. Leather Technol. Chem., 100 (2016) 122–126.
[27] O. Li, C. Lu, A. Liu, L. Zhu, P.M. Wang, C.D. Qian, X.H. Jinag, X.C. Wu, Optimization and characterization of polysaccharide-based bioflocculant produced by *Paenibacillus elgii* B69 and its application in wastewater treatment, Bioresour. Technol., 134 (2013) 87–93.
[28] Y.Y. Zhang, Z.H. Yang, G.M. Zeng, L.K. Wang, J. Huang, S.M. Wei, J. Feng, Microbial flocculant MBFGA1 preliminary structure identification and research on flocculation mechanisms, China Environ. Sci., 33 (2013) 278–285.
[29] J.Y. Tang, S.J. Qi, Z.G. Li, Q. An, M. Xie, B. Yang, Y. Wang, Production, purification and application of polysaccharide-based bioflocculant by *Paenibacillus mucilaginosus*, Carbohydr. Polym., 113 (2014) 463–470.
[30] W.Y. Lu, T. Zhang, D.Y Zhang, C.H. Li, J.P. Wen, L.X. Du, A novel bioflocculant produced by *Enterobacter aerogenes* and its use in defecating the trona suspension, Biochem. Eng., 27 (2005) 1–7.
[31] X. Zhang, G.H. Zheng, J.L. Qiao, G.W. Gu, Initial findings in the mechanisms of bioflocculant, Jiangsu Environ. Sci. Technol., 20 (2007) 104–107, 110.