Biodegradation of Gallic Acid in Chinese Nutgall Processing Wastewater with a Sequencing Batch Reactor

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Abstract: *The degradation of gallic acid in the Chinese nutgall processing wastewater was carried out in a sequencing batch reactor (SBR). The biodegradability of gallic acid was monitored, and the effect of initial concentration, temperature, pH, and salinity on the degradation of gallic acid was studied. The result showed that the biodegradability of gallic acid was poor, but enough to meet the requirement of SBR process by further pre-treatment which would improve the biodegradability. A first-order Monod kinetic model was used to describe the degradation process of gallic acid. The removal efficiency decreased with the increase of the initial gallic acid concentration. The raise of temperature and pH were not conducive for the removal of gallic acid. When the salinity was 2 g·L^{-1} or less, the increase of the salinity content would improve the gallic acid removal efficiency, and the removal efficiency was higher than 75% when the salinity content was less than 10 g·L^{-1}.*

Keywords: *Gallic acid, Biodegradability, Salinity, Sequencing batch reactor.*

Introduction

Gallic acid (Fig. 1) is a naturally occurring polyphenol compounds which is widely used in biology, medicine, chemical, dyes, electronic, light industry and other fields [1]. It also has anti-inmammatory, anti-mutagenic, anti-oxidant, anti-free radical and other biological activities [6, 18]. The methods for producing gallic acid include acid hydrolysis process, alkali hydrolysis process, biological process and enzymatic process [23]. Generally, manufacturers use the alkali hydrolysis process for producing gallic acid, with Chinese nutgall as the raw material. The gallic acid contact in the wastewater generated by the alkali hydrolysis process was still up to 12-17 g·L^{-1}. However, effective treatment suitable for industrial applications has not been realized yet, serious environmental pollution problems still existed in the production process of gallic acid. The research and development of efficient and practical treatment technology for Chinese nutgall processing wastewater was of significant importance and urgency. After gallic acid was recovered from wastewater by solvent extraction [20], only less than 800 mg·L^{-1} gallic acid remained in the raffinate. Biological treatment could remove part of gallic acid in wastewater [9, 10], also there were studies indicating that the activity of microorganism would be inhibited by low concentration of gallic acid [8, 19, 16]. The composition of Chinese nutgall processing wastewater was

complicated, and its impact on the ecological environment was always overlooked.

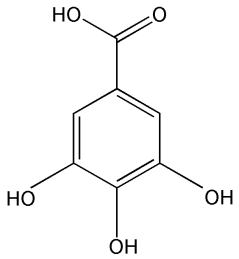

Fig. 1 Molecular structure of gallic acid

Gallic acid degraded when it was discharged into natural water bodies, dissolved oxygen would be decomposed and consumed, resulting in the death of aquatic organisms, which would deteriorate the water quality [15]. Gallic acid converted into halogenated hydrocarbons during the disinfection process of drinking water by chlorine, which was teratogenicity, carcinogenic and mutagenic to human body [7], making it a control objects for the safety of drinking water. Recently the environmental problems caused by gallic acid had attracted attention from scholars; the removal of gallic acid from wastewater was becoming a hot spot in the water pollution research.

During the gallic acid production by the alkaline hydrolysis process, approximately 100 $g \cdot L^{-1}$ NaCl was contained in the wastewater. Studies had shown that a certain concentration of NaCl would impact the effectiveness of biological treatment [5]. Because NaCl entered the wastewater eventually, salinity was one of the important factors affecting the treatment.

The aim of this study was to evaluate the biodegradation of gallic acid by activated sludge the effect of operation conditions on the biodegradation process. Batch experiments were carried out in a sequencing batch reactor (SBR), the effect of gallic acid concentrations; pH, temperature and salinity were evaluated. The results of this paper provided a theoretical basis for the biological treatment of Chinese nutgall processing wastewater.

Materials and methods

Reagents and materials

Standard gallic acid was provided by the China Pharmaceutical and Biological Products. Gallic acid and other reagents used were of analytical grade.

Analytical methods

High-performance liquid chromatography (HPLC) was used to analyze gallic acid [21], the sludge volume index (SVI), mixed liquid volatile suspended solids (MLVSS) and mixed liquid volatile suspended solids (MLVSS) of the sludge were measured by standard methods [17].

Preparation of the synthetic wastewater

The type and concentration of reagents in the synthetic wastewater were listed in Table 1. Gallic acid was added as the carbon source when the synthetic wastewater was already prepared.

Table 1. Types and concentrations of reagents in the synthetic wastewater

Reagent	Concentration, ($mg \cdot L^{-1}$)	Reagent	Concentration, ($mg \cdot L^{-1}$)
KCl	3 217	$NaNO_3$	2 804
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	2	NH_4Cl	193
K_2CrO_4	3	$MgCl_2$	239
$AlCl_3$	27	KH_2PO_4	654
H_3BO_3	7	K_2HPO_4	48
$MnCl_2 \cdot 4H_2O$	5	$FeCl_3 \cdot 6H_2O$	232
$ZnCl_2$	43	$CaCl_2 \cdot 2H_2O$	769

Experimental setup

The volume of the reactor was 5 L (Fig. 2), and the effective volume was 4 L. The flocculent sludge was acquired from an urban sewage treatment plant in Changsha, Hunan Province. The SVI of the active sludge was 47.9 $mL \cdot g^{-1}$, and MLVSS/MLSS was 0.85. Since the purpose of the experiment was to remove gallic acid from wastewater, in order to obtain accurate data, the wastewater was added into the reactor manually and instantaneous, and the reactor was run under restricted aeration.

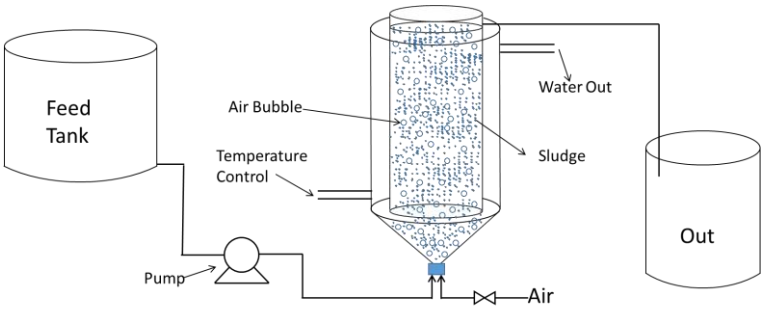

Fig. 2 Schematic diagram

The aeration time was 22 h, and the spate separation time was 2 h. The MLVSS was 5.0 ± 0.1 $g \cdot L^{-1}$. The air velocity was 4 $L \cdot min^{-1}$. The initial gallic acid levels was controlled at 50 ± 5 $mg \cdot L^{-1}$ except the kinetic study and the effect of the initial gallic acid concentration. The temperature was controlled at 30 °C except the temperature experiment, and the pH was kept at 7.0 ± 0.2 except the pH experiment.

Results and discussion

Kinetic of the biodegradable reaction of gallic acid

The microorganism concentration in the SBR reactor was changing all the time, but the change was little within one operating cycle, thus it was generally believed that the total amount of microorganisms was a constant [3], MLVSS was used to represent the microbial biomass indirectly. The aerobic reaction started instantly after the synthetic wastewater was added in and the aeration began immediately, which complied with the Monod kinetic equation namely during the aeration stage. The reactor was completely mixed and operated stably; theoretically, the relationship between the removal of the matrix and substrate concentration was of a first-order reaction, which could be expressed by the Monod equation:

$$\frac{dS}{dt} = -\frac{qSX}{K_s + S} \tag{1}$$

where S is the substrate concentration (mg·L^{-1}); K_s – half-saturation constant, the concentration of substrate when $V = V_{max}/2$ (mg·L^{-1}); q – maximum removal ratio of the matrix (d^{-1}); X – concentrations of sludge in the mixture of sludge and water (mg (MLVSS)·L^{-1}).

No more gallic acid was added after the aeration started; the change of the gallic acid concentration was equivalent to the degradation rate by microorganisms. The variation between gallic acid concentrations and time could be gained from the integration and consolidation of Eq. (1):

$$\frac{1}{tX}(S_0 - S) = \frac{K_s}{tX}\ln\frac{S_0}{S} - q \tag{2}$$

The value of K_s and q could be obtained by the linear regression analysis of Eq. (2) using the least square method with $\frac{1}{tX}(S_0 - S)$ as the vertical axis, and $\frac{1}{tX}\ln\frac{S_0}{S}$ as the abscissa axis.

The temperature was controlled at 30 °C. Other conditions: pH = 7.0 ± 0.1, the hydraulic retention time (HRT) was 24 h. Three sets of parallel experiments were conducted under the same conditions. The result was shown in Fig. 3.

The degradation of gallic acid by SBR was in line with the first-order Monod kinetic process ($Y = 17.65X - 0.24$, $R^2 = 0.9994$). The value of K_s was 17.65 mg·(g MLVSS·d)$^{-1}$, and q was 0.24 which was less than 0.3, indicating that gallic acid in the Chinese nutgall processing wastewater was difficult for the biological treatment. Pre-treatments such as hydrolysis acidification [13], ozone oxidation [12], or electrolysis [22] should be taken to improve the biodegradability before biological treatment.

Effect of the initial gallic acid concentration

The gallic acid removal efficiency decreased with the increase of substrate concentration (Fig. 4). The gallic acid levels in the influent is proportional to the effluent under the same reaction time, which was in line with the linear equation $Y = 0.13X + 5.13$ ($R^2 = 0.9853$).

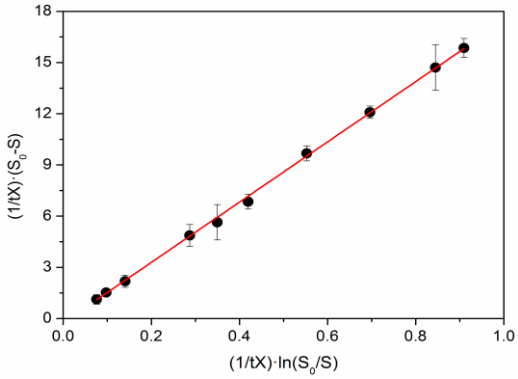

Fig. 3 The relationship of $K_s - q$

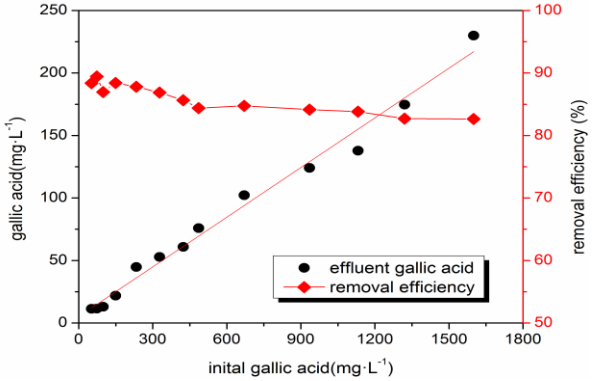

Fig. 4 The gallic acid removal efficiency under different initial gallic acid contents

Effect of temperature

The gallic acid removal efficiency decreased from 10 °C to 40 °C (see Fig. 5) when the reaction time was longer than 8 h, demonstrating that the activity of microorganism decreased with the increase of temperature, indicating the activity of the gallic acid-degrading-enzyme in microorganisms declined when the temperature raised. Therefore, the optimal temperature was 10 °C.

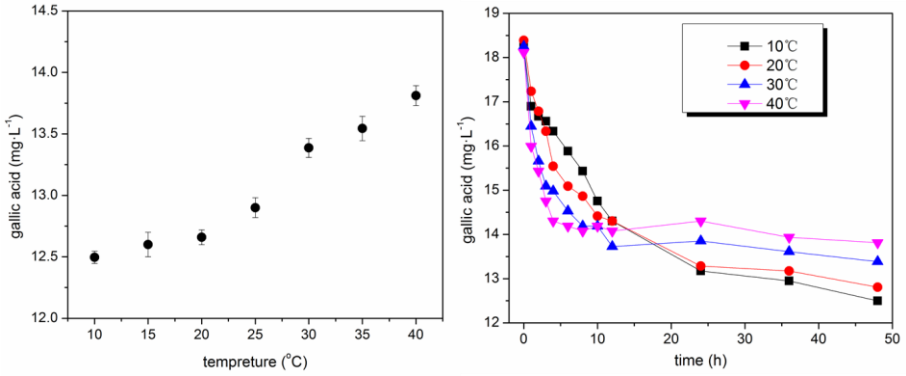

Fig. 5 The gallic acid removal efficiency under different temperature

The results showed that when the reaction time was less than 8 h, the gallic acid removal speed was accelerated with the raise of temperature, but after the equilibrium reached, the gallic acid concentrations left increased with the increase of temperature. The removal of gallic acid was primarily caused by the adsorption by activated sludge and biodegradation when the reaction time was shorter than 8 h. the removal speed was accelerated with temperature might because the adsorption capacity of aerobic sludge was positively correlated with temperature, also the Brownian motion of gallic acid exacerbated, so the binding speed of gallic acid onto the activated sludge was enhanced, then the absorption rate increased.

When the reaction time was shorter than 8 h, the degradability of active sludge was the highest when the temperature was 40 °C, and when the reaction time was longer than 8 h, the removal efficiency was the highest when the temperature was 10 °C.

Effect of pH

The pH was controlled in the range 5-9 during the experiment. The results were shown in Fig. 6.

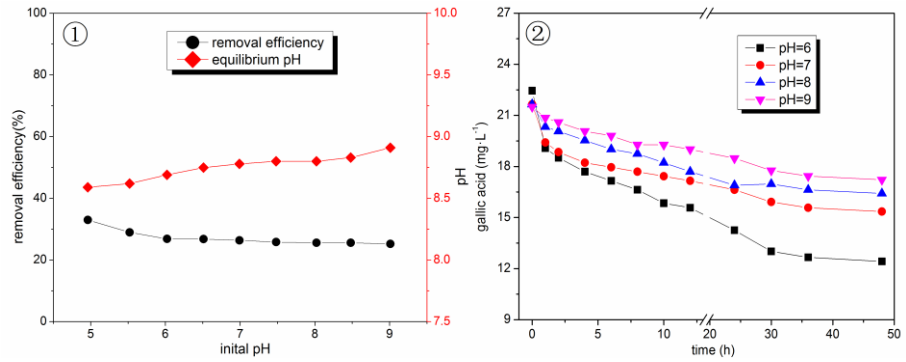

Fig. 6 ① the effect of pH on the gallic acid removal efficiency;
② the degradation curve of gallic acid under different pH

pH had significant effect on the metabolism of gallic acid. In the pH range 5-9, the ionic form of gallic acid were mainly AH_3^- and AH_3^-/AH_2^{2-} [11], the AH_2^{2-} proportion would increase

with the increase of pH, meanwhile the charge of the activated sludge would change, affecting the adsorption of gallic acid, thus the degradation of gallic acid was indirectly affected. The gallic acid concentration decreased significantly in the initial 0.5 h, which was not degraded but attached on the activated sludge. The gallic acid removal rate decreased with the increase of pH, demonstrating that the adsorption capacity of active sludge on AH_2^{2-} was lower than that of AH_3^-. Because the substrate concentration of biodegradation was restricted by the adsorption of activated sludge, and the substrate concentration was proportional to the degradation speed, the gallic acid degradation speed increased with the increase of pH.

The difference among final pH was small, compared with the initial pH, which was because the degradation of gallic acid would cause the elevation of pH. Thus when SBR was used for the treatment of wastewater containing gallic acid, the pH needs to adjust to an optimal range. Since the optimum pH range for biological treatment was 6-9, when applied for the treatment of actual Chinese nutgall wastewater, the pH could be adjusted to about 6.

Effect of salinity

Different concentrations of NaCl were added into the synthetic wastewater to generate different salinity. The result was shown in Fig. 7.

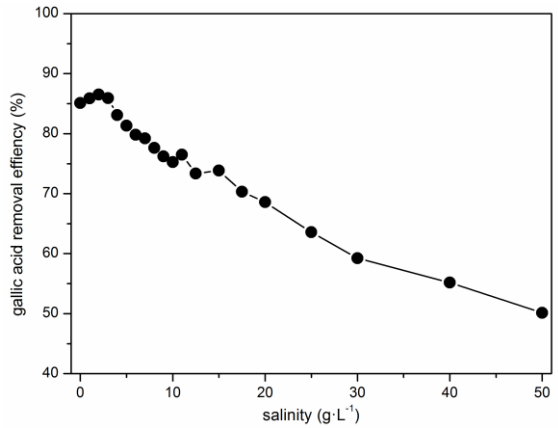

Fig. 7 Gallic acid removal efficiencies in SBR under different salinity

The gallic acid removal efficiency increased with the increase of salinity when the salinity was 2 $g \cdot L^{-1}$ or less, indicating that under appropriate salinity the activity of the degrading enzyme in the microorganisms could be promoted. The removal efficiency decreased with the increase of salinity when the salinity was higher than 2 $g \cdot L^{-1}$. Under the circumstances when the salinity was not more than 10 $g \cdot L^{-1}$, the degradation rate of gallic acid was higher than 75.27%.

The removal rate of gallic acid reduced in high-salinity wastewater, which was because the inhibit of the activity of aerobic microorganisms by salinity [2], under high salinity, even the structure of microorganisms could be destroyed [4, 14], their biological activity was inhibited and metabolism slowed down, thus the treatment efficiency decreased.

Conclusion

In summary, our results indicated that SBR was a feasible approach method for the treatment of Chinese nutgall processing wastewater, and could be constructed with limited resources in practically every location. The following conclusions can be drawn: gallic acid in the Chinese nutgall processing wastewater was difficult for the biological treatment, but could be proceed

if pre-treated by acidification, advanced oxidation or micro-electrolysis. The degradation of gallic acid was of a first-order Monod kinetic process. The gallic acid removal efficiency decreased with the increase of initial concentration. The rise of temperature and pH were not conducive for the removal of gallic acid. When the salinity was 2 $g \cdot L^{-1}$ or less, the increase of the salinity could lead to an improvement of the gallic acid removal efficiency, indicating an appropriate salinity could promote the activity of active sludge; the gallic acid removal efficiency was higher than 75.27% when the salinity was less than 10 $g \cdot L^{-1}$.

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References

1. Chang L. J., Z. Zhang, J. Huang, H. Xu, C. Zhong (2010). Review on Preparation and Application of Gallic Acid, Biomass Chemical Engineering, 44(4), 48-52.
2. Cui Y. W., J. R. Ding, Y. F. Chen, H. Su (2012). Assessment on Effect of Salt Toxicity on Activity of Activated Sludge in Wastewater Treatment Plant, CIESC Journal, 63(5), 1566-1573.
3. Galinha C. F., G. Carvalho, C. A. Portugal, G. Guglielmi, M. A. Reis, J. G. Crespo (2012). Multivariate Statistically-based Modelling of a Membrane Bioreactor for Wastewater Treatment Using 2D Fluorescence Monitoring Data, Water Research, 46(11), 3623-3636.
4. Kargi F., A. R. Dincer (1996). Effect of Salt Concentration on Biological Treatment of Saline Wastewater by Fed-batch Operation, Enzyme and Microbial Technology, 19(7), 529-537.
5. Jia Y. P., S. S. Wang, L. H. Zhang, X. M. Wang, J. B. Guo (2013). Effect of Different Salinity on Sludge Bulking by Using Sequencing Batch Reactor, Transactions of the Chinese Society of Agricultural Engineering, 29(19), 112-119.
6. Kuo C. L., K. C. Lai, Y. S. Ma, S. W. Weng, J. P. Lin, J. G. Chung (2014). Gallic Acid Inhibits Migration and Invasion of SCC-4 Human Oral Cancer Cells Through Actions of NF-κB, Ras and Matrix Metalloproteinase-2 and-9, Oncology Reports, 32(1), 355-361.
7. Li W. W., X. D. Li, K. M. Zeng (2009). Aerobic Biodegradation Kinetics of Tannic Acid in Activated Sludge System, Biochemical Engineering Journal, 43(2), 142-148.
8. Nguyen D. M .C., D. J. Seo, H. B. Lee, I. S. Kim, Kim K. Y., R. D. Park, W. J. Jung (2013). Antifungal Activity of Gallic Acid Purified from *Terminalia nigrovenulosa Bark* against *Fusarium solani*, Microbial Pathogenesis, 56, 8-15.
9. Nogales J., Á. Canales, J. Jiménez-Barbero, B. Serra, J. M. Pingarrón, J. L. García, E. Díaz (2011). Unravelling the Gallic Acid Degradation Pathway in Bacteria: The Gal Cluster from *Pseudomonas putida*, Molecular Microbiology, 79(2), 359-374.
10. Pepi M., S. Cappelli, N. Hachicho, G. Perra, M. Renzi, A. Tarabelli, R. Altieri, A. Esposito, S. E. Focardi, H. J. Heipieper (2013). *Klebsiella* sp. Strain C2A Isolated from Olive Oil Mill Waste is Able to Tolerate and Degrade Tannic Acid in Very High Concentrations, FEMS Microbiology Letters, 343(2), 105-112.
11. Powell H., M. Taylor (1982). Interactions of Iron (II) and Iron (III) with Gallic Acid and Its Homologues: A Potentiometric and Spectrophotometric Study, Australian Journal of Chemistry, 35(4), 739-756.
12. Qiao Y., A. Do, D. Yeh, M. J. Watt (2014). A Bench-scale Assessment of Ozone Pre-treatments for Landfill Leachates, Environmental Technology, 35(1-4), 145-153.
13. Rajagopal R., F. Beline (2011). Anaerobic Hydrolysis and Acidification of Organic Substrates: Determination of Anaerobic Hydrolytic Potential, Bioresource Technology, 102(10), 5653-5658.

14. Roeva O. (2012). Optimization of *E. coli* Cultivation Model Parameters Using Firefly Algorithm, International Journal Bioautomation, 16(1), 23-32.
15. Sakai S., M. Nakaya, Y. Sampei, D. Dettman, K. Takayasu (2013). Hydrogen Sulfide and Organic Carbon at the Sediment-water Interface in Coastal Brackish Lake Nakaumi, SW Japan, Environmental Earth Sciences, 68(7), 1999-2006.
16. Sarjit A., Y. Wang, G. A. Dykes (2015). Antimicrobial Activity of Gallic Acid against Thermophilic Campylobacter is Strain Specific and Associated with a Loss of Calcium Ions, Food Microbiology, 46, 227-233.
17. SEPA (2002). Water and Wastewater Monitoring and Analysis Methods, China Environmental Science Press, Beijing.
18. Spilioti E., M. Jaakkola, T. Tolonen, M. Lipponen, V. Virtanen, I. Chinou, E. Kassi, S. Karabournioti, P. Moutsatsou (2014). Phenolic Acid Composition, Antiatherogenic and Anticancer Potential of Honeys Derived from Various Regions in Greece, PloS ONE, 9(4), e94860.
19. Wang B., X. Ji (2015). Soft-sensing Modeling Based on MLS-SVM Inversion for L-lysine Fermentation Processes, International Journal Bioautomation, 19(2), 207-222.
20. Wu Y. D., K. G. Zhou, S. Y. Dong, W. Yu, H. Q. Zhang (2015). Recovery of Gallic Acid from Gallic Acid Processing Wastewater, Environmental Technology, 36(5), 661-666.
21. Weidenhamer J., M. Li, Allman J., R. Bergosh, M. Posner. (2013). Evidence does not Support a Role for Gallic Acid in Phragmites Australis Invasion Success, Journal of Chemical Ecology, 39(2), 323-332.
22. Xie R., Y. Xing, Y. A. Ghani, K. E. Ooi, S. W. Ng (2007). Full-scale Demonstration of an Ultrasonic Disintegration Technology in Enhancing Anaerobic Digestion of Mixed Primary and Thickened Secondary Sewage Sludge, Journal of Environmental Engineering and Science, 6(5), 533-541.
23. Zhang Y., J. Li, L. Liu, Z. Yu, Q. Ma, M. Li (2013). Research Process in Gallic Acid from Galla Chinensis, Science and Technology of Food Industry, 34(10), 386-390.

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