Nanoparticles TiO_2 promote the biofilm formation of *Escherichia coli*

Fang-Hui Chen[1], Yu-Lu Yang[1], Yu-Fan Zhan[1], Lu-Chan Gong[1, 2], Cheng-Hai Yan[1, *], Jun Wang[1, 2, *]
[1] School of biotechnology, Jiangsu University of Science and Technology, 212018 Zhenjiang, China;
[2] Sericultural Research Institute, Chinese Academy of Agricultural Sciences, 212018 Zhenjiang, China.
Key words: Nanoparticles, TiO_2, Biofilm, Extracellular Polymeric Substances, *Escherichia coli.*
Presenting author email: biojustych@163.com; wangjun@just.edu.cn

In the modern synthetic industry, problems such as the depletion of petrochemical resources, environmental pollution and greenhouse effect have always limited green development (Bilal et al., 2019). Enzyme is a kind of green and efficient catalyst, which is widely used in industrial chemical synthesis (Madavi et al., 2021). Compared with the catalytic reaction of extraction enzyme, the whole cell catalytic reaction keeps the state and specific position of the original living cell, and the enzyme is more stable and more adaptable (Imam et al., 2021). In addition, in recent years, biofilm have been used as a new type of whole cell catalyst for biotransformation. Zhuang et al. apply *Clostridium acetobutylicum* biofilm for acetone-butanol-ethanol fermentation， the results indicate that biofilm shows strong tolerance to acetic acid and butanol, and butanol catalytic yield increases by 61.08% (Zhuang et al., 2016). The microorganisms enclosed in the biofilm form stable colonies that can cope with various physiological pressures in adverse environments, while improving the efficiency of biocatalytics (Rosche et al., 2009). Therefore, it is expected to obtain low cost and high performance biocatalsts when it is applied to the efficient transformation of biomass base on the good catalytic performance of biofilm.

The three-dimensional structure of biofilm provides a natural barrier for bacteria, which has the characteristics of self-regeneration, sustainability and expansion (Jiang et al., 2018). However, biofilm catalysis faced with some problems that the ability and strength of the engineered strain to form biofilm is weak. The composition and properties of biofilm are dynamic, and its composition, structure and chemical properties are controlled by biochemical and physicochemical factors, which in turn are affected by external environmental conditions. Research shows that nanoparticles are toxic to bacteria in most cases, but low concentrations of nanoparticles promoted the formation of biofilms and enhanced bacterial stress resistance (Ou-Yang et al., 2020). Therefore, in order to properly utilize biofilm, it is necessary to understand how nanoparticles interact with biofilm and their behavior in biofilm.

In this study, *Escherichia coli* was exposed to TiO_2 nanoparticles of different concentrations (0-800 mg/L), and the effect of the nanoparticles on the growth of its biofilm was detected by crystal violet staining. Figures 1A and B show the growth state of biofilm before and after adding TiO_2 nanoparticles, the results showed that *E. coli* forms a compact biofilm after adding TiO_2 nanoparticles. Figure 1C showed the changing curve of the biofilm production of *Escherichia coli* with different TiO_2 concentration, showing a trend of first increasing and then decreasing. When the amount of TiO_2 nanoparticles was 400 mg/L, the biofilm content of *Escherichia coli* was 6.62 times higher than that of the control group. The toxicity of low concentration nanoparticles to growing bacterial cells was transient, and the presence of suspended cells may enhance the formation of biofilm (Capdevila, 2016). Therefore, the addition of low concentration TiO_2 nanoparticles is expected to enhance the growth of E. coli biofilm.

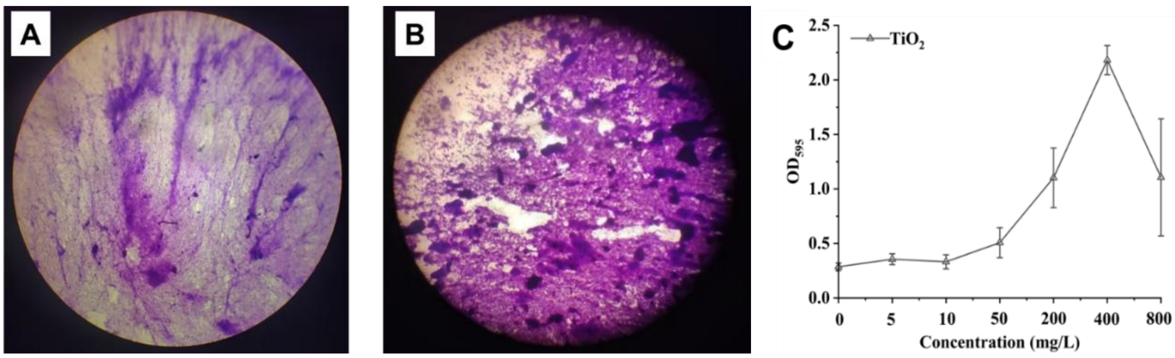

Figure. 1 Crystal violet staining of biofilm with untreated (A) and treated TiO_2 nanoparticles(B), and effects of TiO_2 nanoparticles concentration on *E. coli* biofilm formation(C)

As the main component of the biofilm, extracellular polymeric substances (EPS)can be subdivided into LB-EPS in the outer layer and TB-EPS in the inner layer, and the content of polysaccharide and protein accounts for 70-80% of the total EPS, which plays a major role in the formation of the biofilm. In order to evaluate the effect of TiO_2 nanoparticles on EPS secretion, the changes of protein and polysaccharide were analyzed in biofilm matrix after nanoparticle treatment. The results showed that the addition of TiO_2 nanoparticles increased the content of polysaccharide and protein in LB-EPS by 55.94% and 72.89%, while the content of TB-EPS polysaccharide and protein increased by 184.78% and 71.99%. Previous research has shown that compared with LB-EPS, TB-EPS played a greater role in the process of biofilm formation due to a more compact structure in the process of biofilm formation (Chen et al., 2022). Therefore, it is speculated that TiO_2 nanoparticles accelerate the formation of biofilm by promoting EPS secretion.

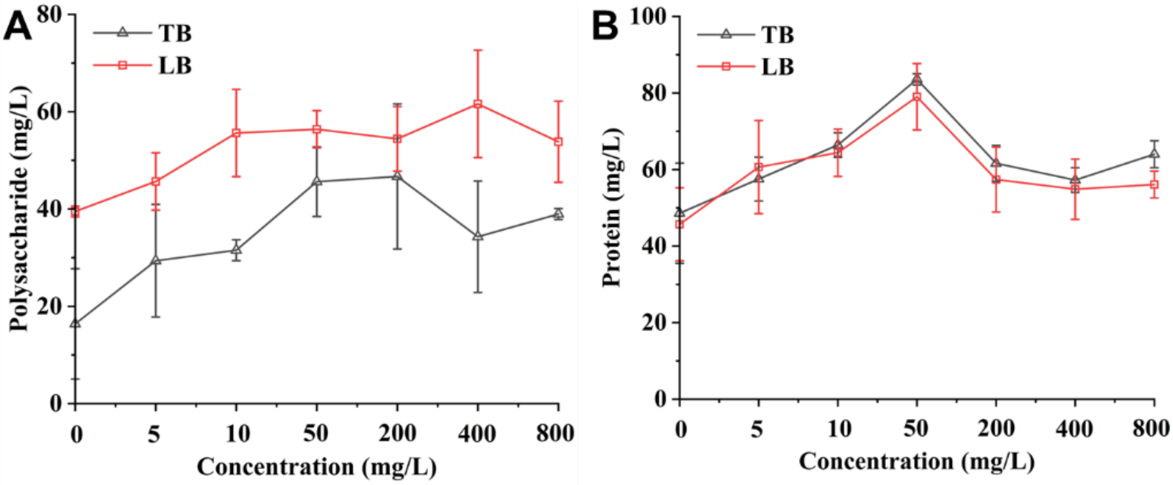

Figure. 2 Effects of TiO_2 nanoparticles concentration on EPS-polysaccharide (A) and EPS-protein (B)

Acknowledgements: This work was supported by the National Natural Science Foundation of China (22278196).

References:

[1] Bilal, M., Cui, J. D., Iqbal, H. M. N., 2019. 'Tailoring enzyme microenvironment: State-of-the-art strategy to fulfill the quest for efficient bio-catalysis'. *INTERNATIONAL JOURNAL OF BIOLOGICAL MACROMOLECULES.* 130: 186-196.

[2] Madavi, T. B., Chauhan, S., Keshri, A., Alavilli, H., Choi, K. Y., Pamidimarri, S. D. V. N., 2021. 'Whole-cell biocatalysis: Advancements toward the biosynthesis of fuels'. *BIOFUELS, BIOPRODUCTS AND BIOREFINING*, 16 (3): 859-876.

[3] Imam, H. T., Krasnan, V., Rebros, M., Marr, A. C., 2021. 'Applications of ionic liquids in whole-cell and isolated enzyme biocatalysis'. *BIOTECHNOLOGY ADVANCES*, 26 (16): 4791.

[4] Zhuang, W., Yang, J., Wu, J. L., Liu, D., Zhou, J. W., Chen. Y., Ying, H. J., 2016. 'Extracellular polymer substances and the heterogeneity of Clostridium acetobutylicum biofilms induced tolerance to acetic acid and butanol'. *RSC ADVANCES*, 6 (40): 33695-33704.

[5] Rosche, B., Li, X. Z., Hauer, B., Schmid, A., Buehler, K., 2009. 'Microbial biofilms: a concept for industrial catalysis'. *TRENDS IN BIOTECHNOLOGY*, 27 (11): 636-643.

[6] Jiang, L., Song, X. G., Li, Y. F., Xu, Q., Pu, J. H., Huang, H., Zhong, C., 2018. 'Programming integrative extracellular and intracellular biocatalysis for rapid, robust, and recyclable synthesis of trehalose'. *ACS CATALYSIS*, 8 (3): 1837-1842.

[7] Ou-Yang, K., Mortimer, M., Holden, P. A., Cai, P., Wu, Y. C., Gao, C. H., Huang, Q. Y., 2020. 'Towards a better understanding of Pseudomonas putida biofilm formation in the presence of ZnO nanoparticles (NPs): Role of NP concentration'. *ENVIRONMENT INTERNATIONAL*, 2020. 137: 105485.

[8] Capdevila, D. A., Wang, J. F., Giedroc, D. P., 'Bacterial strategies to maintain Zinc metallostasis at the host-pathogen interface'. *JOURNAL OF BIOLOGICAL CHEMISTRY*, 291: 20858–20868.

[9] Chen, H., Yan, C. H., Zhan, Y. F, Geng, L. T., Zhu, L. L., Gong, L. C., Wang, J., 2022. 'Boron derivatives accelerate biofilm formation of recombinant *Escherichia coli* via increasing quorum sensing system Autoinducer-2 activity'. *INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES*, 23: 8059.