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# A Biezymatic Cascade for the Complete Hydrolysis of Phthalic Acid Esters

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Phthalic acid esters (PAEs) are widely used as plastic additives to increase the flexibility and durability of plastics. Constantly leaching out from plastics, PAEs are ubiquitously found in the environment. As PAEs exhibit biological activities such as being endocrine disruptive, the quest for efficient degradation strategies continues. Here, we report a biezymatic degradation system for PAEs to phthalic acid (PA) using a cascade comprising two hydrolases, EstJ6 and P8219. The reaction

conditions were optimized with respect to concentrations of both enzymes, temperature, and initial pH. Finally, the substrate scope of the new cascade was investigated, revealing that particularly PAEs with relatively small alcohols were degraded to more than 90%. This present study provides a potential doable biocatalytic strategy for the complete hydrolysis of PAEs.

## Introduction

Phthalic acid esters (PAEs) are widely used in plastics, cosmetic products, agricultural and medical formulations. From these products, PAEs constantly leach out into the environment.<sup>[1,2]</sup> Therefore, PAEs can be detected in air,<sup>[3,4]</sup> soil,<sup>[5,6]</sup> drinking water,<sup>[7,8]</sup> and food products.<sup>[9,10]</sup> Various studies showed that PAEs exhibit toxic effects on the reproductive system, neuro-endocrine system, and immune system.<sup>[11–14]</sup> Therefore, intensive research efforts are dedicated to the efficient degradation of these persistent organic pollutants in the environment.

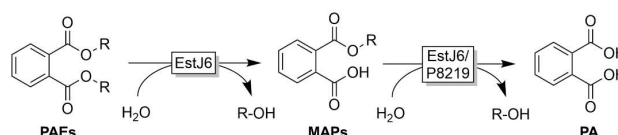
Biocatalytic hydrolysis to phthalic acid (PA) and simple alcohols is a promising degradation strategy.<sup>[15]</sup> More and more PAEs-degrading bacteria have been isolated and proposals for the responsible metabolic pathways including PAE hydrolysis and metabolic degradation of the hydrolysis products have been formulated.<sup>[16]</sup> The hydrolytic step is frequently reported to be non-complete accumulating monoalkyl phthalates

(MAPs),<sup>[15]</sup> which also exhibit reproductive toxicity.<sup>[17–19]</sup> Recently, several hydrolases capable of fully hydrolyzing PAEs have been reported,<sup>[20–24]</sup> but their efficiency seems unsatisfactory. Therefore, the need for efficient hydrolysis methods persists.

To the best of our knowledge, cascade reactions completely hydrolyzing PAEs into PA have been reported *in vivo* only.<sup>[25,26]</sup> In this study, a biezymatic cascade for efficient transformation of PAEs into PA is proposed. EstJ6<sup>[23]</sup> has previously been reported to efficiently hydrolyze PAEs to MAPs while P8219<sup>[27]</sup> is a promising catalyst for the second hydrolysis step. We therefore hypothesized that combining EstJ6 and P8219 may result in efficient full hydrolysis of PAEs (Scheme 1).

## Results and Discussion

In a first set of experiments we investigated the hydrolysis of dibutyl phthalate (DBP) using EstJ6 and P8219 in different molar ratios (Figure 1). In enzyme-free reactions, there was no product observed under the reaction condition here. Representative samples were analyzed using gas chromatography-mass spectrometry (GC-MS) to confirm the chemical nature of the hydrolysis products (Figure S1). DBP was converted to mono-butyl phthalate (MBP) in the presence of 2  $\mu$ M EstJ6 and there was no phthalic acid (PA) detected (Figure 1). P8219 could not catalyze the hydrolysis of DBP. As judged by the initial rates, EstJ6 appeared to be overall rate limiting the hydrolysis of DBP, and P8219 further accelerated the hydrolysis of DBP (Fig-



**Scheme 1.** Proposed biezymatic cascade for the complete hydrolysis of phthalic acid esters (PAEs) to phthalic acid (PA) via the monoalkyl phthalates (MAPs) catalyzed by the hydrolases EstJ6 and P8219.

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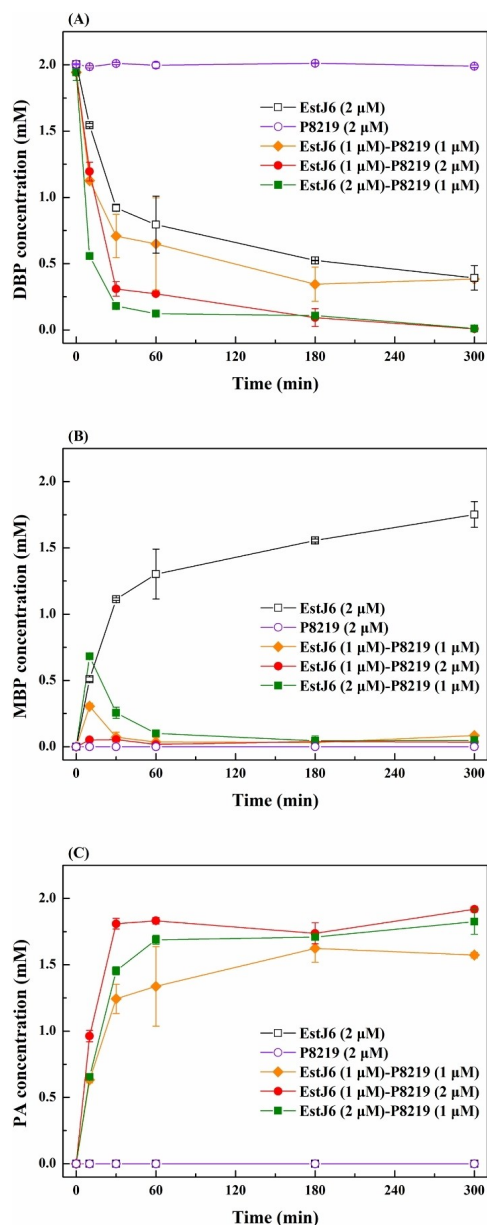
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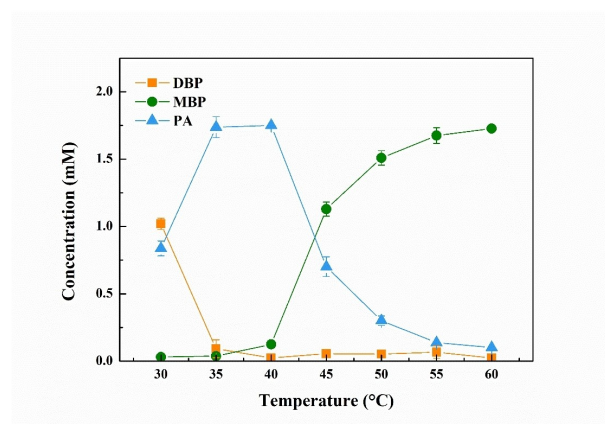


**Figure 1.** Hydrolysis of dibutyl phthalate (DBP) at different enzyme concentrations. Time courses of (A) DBP concentration, (B) MBP concentration and (C) PA concentration. Reaction conditions: total reaction volume: 1 mL (10 mM phosphate buffer, pH 7);  $[DBP]_0 = 2$  mM (40  $\mu$ L of a 50 mM stock solution in DMSO),  $T = 35^\circ\text{C}$ , 500 rpm. Negative controls that did not contain enzymes were carried out and no MBP or PA was detected.

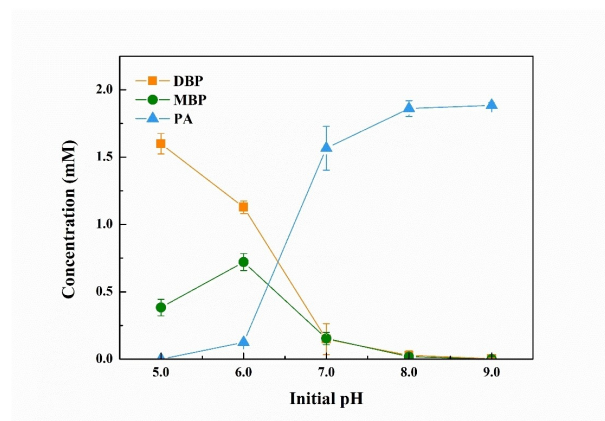
ure 1A). Compared with EstJ6, P8219 has a higher affinity towards MBP and has higher catalytic efficiency for MBP, which is concluded from their kinetic parameters (Table S1). The intermediate accumulation of the mono hydrolysis product (MBP) occurred within one hour of the beginning of the reaction and correlated with the ratio of EstJ6 and P8219 (Figure 1B). Compared with 1  $\mu$ M P8219, 2  $\mu$ M P8219 could quickly convert MBP into PA, avoiding the accumulation of MBP in the reaction system (Fig.1B). A combination of 1  $\mu$ M EstJ6 and 2  $\mu$ M P8219 was selected for further experiments.

Next, we systematically varied the reaction temperature for the bienzymatic hydrolysis of DBP limiting the reaction time to 3 h each (Figure 2). At the temperature lower than  $35^\circ\text{C}$  the hydrolysis was incomplete within the timeframe of the experiments (3 h) and ca. 50% of the initial DBP were recovered. Obviously, particularly EstJ6 did not work optimally at the temperature. Increasing the reaction temperature above  $40^\circ\text{C}$  resulted in increasing mono hydrolysis product indicating that P8219 exhibited poor thermal stability under these conditions. A similar temperature dependence was reported previously by Nishioka et al.<sup>[27]</sup> Due to the near-full conversion of DBP into PA (92.1%) at  $40^\circ\text{C}$ , we continued our investigations at this temperature.

When initial reaction pH values were higher than 7, the yield of PA was higher than 90% (Figure 3), indicating slightly



**Figure 2.** Selectivity of the DBP hydrolysis into MBP and PA at different temperatures. Reaction conditions: total reaction volume: 1 mL (10 mM phosphate buffer, pH 7);  $[DBP]_0 = 2$  mM (40  $\mu$ L of a 50 mM stock solution in DMSO),  $[EstJ6] = 1$   $\mu$ M,  $[P8219] = 2$   $\mu$ M,  $t = 3$  h, 500 rpm. Negative controls that did not contain enzymes were carried out and no MBP or PA was detected.



**Figure 3.** Effect of initial pH on the cascade reaction. Reaction conditions: total reaction volume: 1 mL (10 mM phosphate buffer);  $[DBP]_0 = 2$  mM (40  $\mu$ L of a 50 mM stock solution in DMSO),  $[EstJ6] = 1$   $\mu$ M,  $[P8219] = 2$   $\mu$ M,  $T = 40^\circ\text{C}$ ,  $t = 3$  h, 500 rpm. Negative controls that did not contain enzymes were carried out and no MBP or PA was detected.

alkaline reaction conditions favored the full hydrolysis of DBP into PA. The first hydrolysis step proceeded to some extent in slightly acidic media but the P8219-catalyzed second hydrolysis had a clear pH optimum above pH 7.<sup>[27]</sup> As shown in Figure S2, no significant spontaneous hydrolysis was observed in the absence of the biocatalysts (under otherwise identical conditions). We continued our investigations at an initial pH of 8.

Finally, we investigated the scope of PAEs hydrolyzed by the proposed bienzymatic reaction scheme. As EstJ6 had also been reported of exhibiting considerable catalytic activity on MAPs<sup>[23]</sup> we also performed the hydrolysis reaction with EstJ6 alone (Table 1). In addition, consistent with the initial report,<sup>[27]</sup> P8219 could not catalyze the hydrolysis of PAEs. In all cases except DMP the degree of hydrolysis achieved with the bienzymatic cascade surpassed the one on using EstJ6 alone significantly, indicating that bienzymatic cascade is an efficient way to boost the full hydrolysis of PAEs. The nature of the alcohol groups somewhat influenced the efficiency of the hydrolysis reaction. While small PAEs (up to pentyl) were converted smoothly, the degree of hydrolysis gradually decreased with the chain length of the alcohols. Also increasing the steric demand by utilizing branched alcohols decreased the hydrolysis rate considerably.

## Conclusion

The hydrolysis of PAEs does not seem to be as easy as it seems. For example, commercially available lipase B from *Candida antarctica* (CALB) could not catalyze their hydrolysis (Figure S3). The hydrolysis of PAEs into biodegradable PA and simple alcohols remains an active topic of environmental research. A range of hydrolases such as EstG,<sup>[20]</sup> CarEW,<sup>[21]</sup> and XtgR8<sup>[22]</sup> have been proposed. So far, however, complete hydrolysis has been difficult to achieve. The bienzymatic cascade proposed here may represent a practical approach to achieve the desired complete hydrolysis. Admittedly, the scope of PAEs hydrolyzed is yet limited to sterically little demanding starting materials and bulkier ones remain challenging. Further broadening the substrate scope of the two hydrolases proposed in this study will therefore be in focus of our future research interest. Also, adopting the optimal reaction conditions to practically relevant reaction conditions such as in waste water will be investigated. Finally, it is significant to find or design enzymes that can identify low substrate concentrations, because the concentration of PAEs is very low in actual contaminated samples. Overall, we are convinced having established a very promising degradation approach to eliminate dialkyl and monoalkyl phthalates from our environment.

## Experimental Section

### Chemical reagents and materials

Dimethyl phthalate (DMP), diethyl phthalate (DEP), dipropyl phthalate (DPRP), dibutyl phthalate (DBP), monobutyl phthalate (MBP), phthalic acid (PA), diisobutyl phthalate (DIBP), dipentyl phthalate (DPP), dihexyl phthalate (DNHP), diheptyl phthalate (DHP), dioctyl phthalate (DNOP), di-2-ethylhexyl phthalate (DEHP),

**Table 1.** Substrate scope of the enzymatic phthalate hydrolysis cascade using EstJ6/P8219 mix or either of them alone.

| Substrate | Structure | Conversion (%) <sup>*</sup> |                      |                      |
|-----------|-----------|-----------------------------|----------------------|----------------------|
|           |           | EstJ6-P8219 <sup>[a]</sup>  | EstJ6 <sup>[b]</sup> | P8219 <sup>[c]</sup> |
| DMP       |           | 98.8 ± 1.1                  | 98.3 ± 0.7           | N.D. <sup>[d]</sup>  |
| DEP       |           | 98.3 ± 1                    | 51.3 ± 3.8           | N.D.                 |
| DPRP      |           | 98.4 ± 0.3                  | 56.1 ± 4.7           | N.D.                 |
| DBP       |           | 99.6 ± 0.2                  | 49.7 ± 5             | N.D.                 |
| DIBP      |           | 94.2 ± 4.6                  | 29.6 ± 1.9           | N.D.                 |
| DPP       |           | 99.4 ± 8                    | 24.8 ± 5             | N.D.                 |
| DNHP      |           | 58.3 ± 6.7                  | 22.7 ± 4.1           | N.D.                 |
| DHP       |           | 54.2 ± 4.1                  | 12.7 ± 3.3           | N.D.                 |
| DNOP      |           | 13.1                        | 1.9 ± 0.1            | N.D.                 |
| DEHP      |           | 9.7 ± 0.7                   | 3.7 ± 0.8            | N.D.                 |

<sup>\*</sup> The conversion was calculated based on the the yield of complete hydrolyzed product PA. [a] Reaction conditions: total reaction volume: 1 mL (10 mM phosphate buffer, pH 8); [Substrate]<sub>0</sub> = 2 mM (40 μL of a 50 mM stock solution in DMSO), [EstJ6] = 1 μM, [P8219] = 2 μM, T = 40 °C, t = 12 h, 500 rpm. The detection information of 10 PAEs were shown in Table S2. [b] Reaction conditions: total reaction volume: 1 mL (10 mM phosphate buffer, pH 8); [Substrate]<sub>0</sub> = 2 mM (40 μL of a 50 mM stock solution in DMSO), [EstJ6] = 1 μM, [P8219] = 0 μM, T = 40 °C, t = 12 h, 500 rpm. [c] Reaction conditions: total reaction volume: 1 mL (10 mM phosphate buffer, pH 8); [Substrate]<sub>0</sub> = 2 mM (40 μL of a 50 mM stock solution in DMSO), [EstJ6] = 0 μM, [P8219] = 2 μM, T = 40 °C, t = 12 h, 500 rpm. [d] Not detected. Negative controls that did not contain enzymes were carried out and only substrates were detected.

pyridine, and N,O-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane were purchased from Sigma-Aldrich, Aladdin or Macklin in the highest purity available and used without further purification. Methanol and formic acid (purchased from Aladdin) were chromatographically pure. Water was purified with a UNIQUE-R20 purification system (UNIQUE, Xiamen, China).



## Preparation, isolation, and purification of EstJ6 and P8219

EstJ6 and P8219 were prepared according to the previous reports<sup>[23,27]</sup> with minor modification. The recombinant *Escherichia coli* was inoculated into lysogeny broth medium containing 50 mg/L kanamycin and kept at 37 °C and 220 rpm until cells grew to a density of 0.8–1.0 at OD600. Isopropyl- $\beta$ -D-thiogalactopyranoside (IPTG) was added to the medium to a final concentration of 0.2 mM. Then the cell culture was kept at 18 °C and 220 rpm for about 24 h. Cells were harvested by centrifugation at 5000 rpm and 4 °C for 30 min and then re-suspended using Buffer A (20 mM sodium phosphate buffer, 500 mM NaCl, 30 mM imidazole, pH 8.0) at a ratio of 1:10 w/v. The suspension was sonicated and centrifuged to obtain crude enzyme solution. The crude enzyme solution was then purified using affinity chromatography (HiPrep Q HP 16/10 column, GE healthcare, Upsala, Sweden) on an Äkta Pure system (Cytiva, Marlborough, USA). The 10 kDa cut-off ultrafiltration centrifuge tube (MilliporeSigma, Burlington, USA) was used to concentrate and desalt the enzyme solution. The obtained purified enzyme was stored at –30 °C. The molecular weight of EstJ6 and P8219 are 32.1 and 34.6 kDa, respectively. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) results of purified EstJ6 and P8219 are shown in Figure S4.

## PAEs degradation reaction

As one of the most used PAEs, DBP was as a model substrate to optimize the parameters. All reactions were carried out in a 4 mL glass vial submerged in a thermostatic oil bath for temperature control, and the reaction vial was closed. The reaction volume was 1 mL, containing 40  $\mu$ L of a 50 mM substrate stock solution in DMSO and 960  $\mu$ L of 10 mM phosphate buffer. Phosphate buffer contained enzymes as the experimental group, whereas phosphate buffer did not contain enzyme as the negative control. At specific time points, 100  $\mu$ L of 3 M HCl was used to terminate the reaction firstly. Then, 1 mL of ethyl acetate was used to extract products and residual substrates. Finally, high-performance liquid chromatography (HPLC) and GC-MS were used for the analysis of products and residual substrates.

## Detection of DBP, MBP, and PA

### HPLC Analysis

The qualitative and quantitative detection of DBP, MBP and PA was performed on a Waters e2695 system (Waters Corporation, Massachusetts, USA) equipped with a 2489 UV/Vis detector and a SunFire C18 column (4.6 mm  $\times$  250 mm, 5  $\mu$ m). The detection wavelength was 254 nm. Column temperature was set as 30 °C. The mobile phase was methanol/ultrapure water/formic acid (A; 90:10:1, v/v/v) and ultrapure water (B), and the flow rate was constant at 1.0 mL/min. The gradient elution procedure was as follows: 80% A (0 min), 100% A (5–10 min), 80% A (12–15 min). The injection volume of samples was 10  $\mu$ L. The retention times of DBP, MBP, and PA were 8.95, 5.05, and 3.26 min, respectively (Figure S5).

### GC-MS Analysis

MBP and PA contain active hydrogen thus they are thermally unstable at 200–300 °C. Therefore, prior to GC-MS measurements, the extract solution was chemically derivatized. The derivative reaction system contained 10  $\mu$ L extract solution, 100  $\mu$ L pyridine,

and 100  $\mu$ L derivatization reagents. The derivatization reagent is N,O-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane. After mixing, the derivative reaction system was placed at 65 °C for 40 min. The derivative solution was determined using GCMS-TQ8050 (Shimadzu, Kyoto, Japan) equipped with an AOC-20s auto sampler and an AOC-20i auto injector. A low-polarity SH Rxi-5Sil MS column (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu$ m) was used with helium (purity > 99.999%) as the carrier gas. The injection volume was 1  $\mu$ L with splitless injection mode, and the carrier gas ran at a constant flow rate of 1.0 mL/min. The oven temperature was programmed from 60 °C (holding for 1 min) to 220 °C (holding for 1 min) at 20 °C/min, to 250 °C (holding for 1 min) at 5 °C/min, and finally to 290 °C at 20 °C/min, keeping the final temperature for 7.5 min. Injector, GC interface and ion source temperatures were 260, 280 and 230 °C, respectively. Electron ionization was used at 70 eV. Acquisition was performed using scan mode to identify products and residual substrates.

## Supporting Information Summary

Supporting information summary includes GCMS results, summarized kinetic parameters, test methods and graphs of HPLC, and SDS-PAGE analysis.

## Author Contribution Statement

Liu Yang and Yebao Chen conceived, designed the study, and performed the experiments, Yunjian Ma analyzed the experimental data. Frank Hollmann assisted in data interpretation and manuscript formulation. Yonghua Wang designed and directed the whole project. All the authors contributed to scientific discussion. The article was written based on contributions from all authors.

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## Conflict of Interest

The authors declare no conflict of interest.

## Data Availability Statement

All data are available from the corresponding author upon reasonable request.

**Keywords:** phthalic acid esters · degradation · phthalic acid · hydrolase · cascade

- [1] U. Kotowska, J. Kapelewska, R. Sawczuk, *Environ. Pollut.* **2020**, *267*, 115643.
- [2] X. Wang, M. Song, M. Guo, C. Chi, F. Mo, X. Shen, *J. Environ. Sci.* **2015**, *37*, 67–74.
- [3] M. Song, C. Chi, M. Guo, X. Wang, L. Cheng, X. Shen, *J. Environ. Sci.* **2015**, *28*, 157–162.
- [4] X. Zhou, J. Lian, Y. Cheng, X. Wang, *Environ. Res.* **2021**, *194*, 110681.
- [5] D. Wang, Y. Xi, X.-Y. Shi, Y.-J. Zhong, C.-L. Guo, Y.-N. Han, F.-M. Li, *Environ. Pollut.* **2021**, *286*, 117546.
- [6] L. Wei, Z. Li, J. Sun, L. Zhu, *Sci. Total Environ.* **2020**, *726*, 137978.
- [7] W. Shi, X. Hu, F. Zhang, G. Hu, Y. Hao, X. Zhang, H. Liu, S. Wei, X. Wang, J. P. Giesy, H. Yu, *Environ. Sci. Technol.* **2012**, *46*, 1811–1818.
- [8] Q. Luo, Z. Liu, H. Yin, Z. Dang, P. Wu, N. Zhu, Z. Lin, Y. Liu, *Water Res.* **2018**, *147*, 362–372.
- [9] W. Xiang, Q. Gong, J. Xu, K. Li, F. Yu, T. Chen, S. Qin, C. Li, F. Wang, *J. Sci. Food Agric.* **2020**, *100*, 1124–1131.
- [10] J. A. Colacino, T. R. Harris, A. Schecter, *Environ. Health Perspect.* **2010**, *118*, 998–1003.
- [11] S. Benjamin, E. Masai, N. Kamimura, K. Takahashi, R. C. Anderson, P. A. Faisal, *J. Hazard. Mater.* **2017**, *340*, 360–383.
- [12] S. Kim, S. Eom, H.-J. Kim, J. J. Lee, G. Choi, S. Choi, S. Kim, S. Y. Kim, G. Cho, Y. D. Kim, E. Suh, S. K. Kim, S. Kim, G.-H. Kim, H.-B. Moon, J. Park, S. Kim, K. Choi, S.-H. Eun, *Sci. Total Environ.* **2018**, *624*, 377–384.
- [13] M. R. Holahan, C. A. Smith, B. E. Luu, K. B. Storey, *Toxicol. Sci.* **2018**, *165*, 512–530.
- [14] K. Chiu, S. T. Bashir, R. A. Nowak, W. Mei, J. A. Flaws, *Sci. Rep.* **2020**, *10*, 18788.
- [15] M. Bhattacharyya, S. Basu, R. Dhar, T. K. Dutta, *Environ. Microbiol. Rep.* **2022**, *14*, 333–346.
- [16] L. Ren, Z. Lin, H. Liu, H. Hu, *Appl. Microbiol. Biotechnol.* **2018**, *102*, 1085–1096.
- [17] N. Li, K. Liu, H. Yuan, J. Zhu, G. Yu, J. Xie, S. Fu, K. Guo, L. Ye, *Environ. Toxicol. Pharmacol.* **2015**, *39*, 643–650.
- [18] M. Ema, E. Miyawaki, *Reprod. Toxicol.* **2001**, *15*, 189–194.
- [19] R. Hauser, J. D. Meeker, N. P. Singh, M. J. Silva, L. Ryan, S. Duty, A. M. Calafat, *Hum. Reprod.* **2007**, *22*, 688–695.
- [20] W. Whangsuk, P. Sungkeeree, M. Nakasiri, S. Thiengmag, S. Mongkolsuk, S. Loprasert, *Int. Biodeterior. Biodegrad.* **2015**, *99*, 45–54.
- [21] J. Ding, C. Wang, Z. Xie, J. Li, Y. Yang, Y. Mu, X. Tang, B. Xu, J. Zhou, Z. Huang, *PLoS One* **2015**, *10*, 1–17.
- [22] J. Qiu, H. Yang, Z. Yan, Y. Shi, D. Zou, L. Ding, Y. Shao, L. Li, U. Khan, S. Sun, Z. Xin, *Int. J. Biol. Macromol.* **2020**, *164*, 1510–1518.
- [23] J. Qiu, Y. Zhang, Y. Shi, J. Jiang, S. Wu, L. Li, Y. Shao, Z. Xin, *Ecotoxicol. Environ. Saf.* **2020**, *190*, 110148.
- [24] J. Sarkar, A. Dutta, P. Pal Chowdhury, J. Chakraborty, T. K. Dutta, *Microb. Cell Fact.* **2020**, *19*, 1–12.
- [25] M. Lu, W. Jiang, Q. Gao, M. Zhang, Q. Hong, *Ecotoxicol. Environ. Saf.* **2020**, *195*, 110517.
- [26] H. Huang, X. Y. Zhang, T. L. Chen, Y. L. Zhao, D. S. Xu, Y. P. Bai, *J. Agric. Food Chem.* **2019**, *67*, 8548–8558.
- [27] T. Nishioka, M. Iwata, T. Imaoka, M. Mutoh, Y. Egashira, T. Nishiyama, T. Shin, T. Fujii, *Appl. Environ. Microbiol.* **2006**, *72*, 2394–2399.

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