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Highly sensitive microbial biosensor based on recombinant *Escherichia coli* overexpressing catechol 2,3-dioxygenase for reliable detection of catechol

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ABSTRACT

A highly sensitive whole cell based electrochemical biosensor was developed for catechol detection in this study. The *carE* gene of *Sphingobium yanoikuyae* XLDN2–5 encoding catechol 2,3-dioxygenase (C23O), a key enzyme in the biodegradation of aromatic compound, was cloned and over-expressed in *Escherichia coli* BL21 (*E. coli* BL21). Compared to *Sphingobium yanoikuyae* XLDN2–5, the recombinant *E. coli* BL21 over-expressed C23O exhibited higher catalytic activity towards catechol. Moreover, the whole cells provided a better environment for C23O to maintain its catalytic activity and stability compared with crude enzyme. The distinctive features of the recombinant *E. coli* BL21 over-expressed C23O made it an ideal bio-recognition element for the fabrication of a microbial biosensor. Additionally, nanoporous gold (NPG) with unique properties of structure and function was selected as a support to immobilized the recombinant *E. coli* BL21 over-expressed C23O. Based on the synergistic effect of C23O and NPG, the *E. coli* BL21-C23O/NPG/GCE bioelectrode showed a good linear response for catechol detection ranging from 1 μM to 500 μM with a high sensitivity of 332.24 μA mM^{-1} cm^{-2} and a low detection limit of 0.24 μM. Besides, the *E. coli* BL21-C23O/NPG/GCE bioelectrode exhibited strong anti-interference and good stability. For the detection of catechol in wastewater samples, the concentrations detected by the *E. coli* BL21-C23O/NPG/GCE bioelectrode were in good agreement with the standard concentrations that added in the wastewater samples, which make the *E. coli* BL21-C23O/NPG/GCE bioelectrode an ideal tool for reliable catechol detection.

1. Introduction

Environmental pollution caused by aromatic compounds such as catechol has become a major issue for human health. Catechol is a vital reagent for industry, and is inevitably applied in various fields, such as petroleum, chemicals, rubber, medicine, pesticide, and dyestuff (Wang et al., 2013; Yan et al., 2016). Additionally, catechol is also an important metabolite in aromatic pollutants biodegradation (Gai et al., 2007). Because catechol is water-soluble, it is easy to infiltrate and migrate in the environment, which make it widespread existence in soil and water resource and thus pose a threat to the environment and human health (Zhou et al., 2014; Kara et al., 2015). Owing to its biotoxicity, catechol can cause lympho-hematopoietic cancer, toxicity to central nervous system, and DNA replication inhibition (Topping et al., 2007; Zhou et al., 2014), and thus has been identified as one of human carcinogens by the International Agency for Research on Cancer (Palanisamy et al., 2016). Therefore, the sensitive detection of catechol is a great significance for the treatment of catechol pollution and environmental monitoring.

For catechol detection, enzyme based electrochemical biosensors have attracted wide attention given their unique advantages such as high selectivity, low cost, fast response, and continuous on-line detection (Attar et al., 2015). As reported, various enzymes were used as bio-recognition elements for catechol detection such as horseradish peroxidase (HRP) (Wu et al., 2016), tyrosinase (Qu et al., 2013), laccase (Zheng et al., 2018), and extradiol dioxygenase (Zhang et al., 2011). Among them, catechol 2,3-dioxygenase (C23O) belonging to extradiol dioxygenase family has attracted increasing attention in the fabrication of catechol biosensors. As a key enzyme in the microbial degradation of aromatic compounds, C23O could catalyze the incorporation of oxygen atoms from O_2 into catechol and the extradiol ring cleavage to form 2-hydroxymuconate semialdehyde (2-HMS) (Kita et al., 1999). As a bio-

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recognition element for catechol detection, C23O has two major advantages compared with tyrosinase, HRP, and laccase. Firstly, C23O shows high selectivity and sensitivity for catechol. By contrast, tyrosinase could catalyze both the conversion of phenol to catechol and the reaction of catechol to o-benzoquinone, which make it difficult to distinguish phenol and catechol in real samples (Portaccio et al., 2010). Besides, HRP and laccase showed poor selectivity because of their ability of catalyzing many phenols and aromatic amines (Liu et al., 2006; Kong et al., 2010). Secondly, additional mediators were non-requirement in the oxidation of catechol catalyzed by C23O. Unlike HRP, catechol could be directly catalyzed by C23O to reduce O_2 without the participation of additional mediators such as H_2O_2, which could improve the electron transfer efficiency from substrates to corresponding oxides (Zhang et al., 2018). In view of the unique properties of C23O, it could be a good choice as a recognition element for catechol detection. However, enzyme based biosensors also have some inherent drawbacks due to the properties of enzymes: 1) The purification process of enzyme was time-consuming and expensive; 2) As a recognition element, enzyme requires strict detecting conditions, extreme environmental factors such as pH, temperature, and toxic chemicals could affect the activity of enzyme and even causing the inactivation of enzyme; 3) Exposure to air, C23O is prone to self-oxidation of the ferrous ion nucleus as well as to dissociation of its tetrameric structure, which resulting in the extracellular C23O activity was much lower than intracellular C23O activity (Celesia et al., 2017; Felshia et al., 2017). Due to the inherent drawbacks of enzyme and the instability of C23O outside microbial cell, it is still a challenge to construct a C23O based biosensor for catechol detection.

Microbial biosensor using recombinant bacteria with over-expressed enzyme as bio-recognition element would be a new insight in the construction of biosensors for environment monitoring or clinical diagnosis. Because the whole cell with over-expressed enzyme could provide appropriate pH environment, cofactor, and energy for enzymatic reaction, which makes the over-expressed enzymes retain a high catalytic activity. Compared to enzyme based biosensors, the microbial biosensor constructed using recombinant bacteria with over-expressed enzyme has better stability and viability, which can easily adapt to the working environment. Furthermore, recombinant bacteria with over-expressed enzyme also have the advantages of easy preparation and cost-effectiveness, which make it a preferred candidate for the fabrication of an efficient biosensor. For example, Prathap et al. (2012) used genetically modified *E. coli* whole cell over-expressed lindane dehydrochlorinase as a bio-recognition element for lindane detection. Thus, the microbial biosensor based on gene recombination technique would be expected as an ideal choice for solving the limitations in the construction of enzyme based biosensor.

In this work, the *carE* gene of *Sphingobium yanoikuyae* XLDN2–5 encoding catechol 2,3-dioxygenase (C23O), a key enzyme in aromatic compound degradation, was cloned and over-expressed in *E. coli* BL21. In order to evaluate catalytic activity and stability, the recombinant *E. coli* BL21-C23O cell and crude enzyme (the supernatant of cell disruption) were compared. Nanoporous gold (NPG) with unique properties of structure and function was selected as an ideal support to immobilized the recombinant *E. coli* BL21-C23O cells. Then, a highly sensitive whole-cell-based electrochemical biosensor was developed for the detection of catechol. The performance of the whole-cell-based electrochemical biosensor was investigated in detail.

2. Experimental

NPG made by dealloying AgAu alloying (Wang et al., 2011) was employed as a support for microbial cells immobilization. A recombinant *E. coli* BL21 (DE3) strain overexpressed C23O was constructed and immobilized on NPG to fabricate an *E. coli* BL21-C23O/NPG biocomposite. Then an *E. coli* BL21-C23O/NPG/GCE bioelectrode was made by GCE modified with the *E. coli* BL21-C23O/NPG biocomposite. The details of the dealloying method of AgAu alloying, the cloning, overexpression, cell culture, activity assay of C23O, immobilization of recombinant *E. coli* BL21-C23O, construction of various bioelectrodes, and all electrochemical measurements were described in details in Supplementary material.

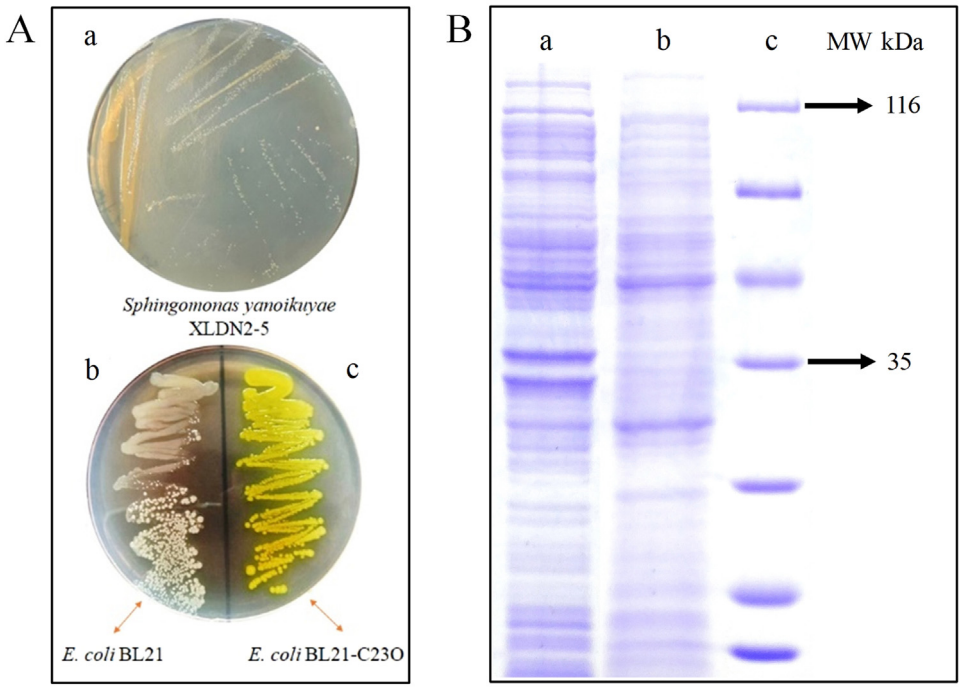
3. Results and discussion

3.1. Construction of recombinant E. coli BL21-C23O

The schematic illustration of a recombinant expression vector construction was shown in Fig. S1-a. To construct the recombinant *E. coli* BL21-C23O, the *carE* gene was amplified by PCR using *Sphingobium yanoikuyae* strain XLDN2–5 genomic DNA as a template. As shown in Fig. S1-b, the 933 bp PCR product was consistent with *carE* gene in size. Then, the PCR-amplified product was combined with a pEASY-Blunt vector to construct a recombinant pEASY-Blunt-*carE* cloning vector. The pEASY-Blunt-*carE* cloning vector was digested with endonucleases BamHI and NdeI, which produced a 933 bp *carE* gene and a 3.9 kb pEASY-Blunt vector (Fig. S1-c). The 933 bp *carE* gene, which generated by endonuclease digestion, was consistent with the PCR products in size (Fig. S1-b), which indicated that the *carE* gene was successfully inserted into the cloning vector. After sequencing to verify the *carE* gene's identity with the parent sequence, the *carE* gene digested by BamHI and NdeI was ligated into the expression vector pET-28a, which had been digested by the same two endonucleases. Fig. S1-d shows the restriction endonuclease digestion of the recombinant plasmid pET-28a-*carE* with BamHI and NdeI. The 933 bp *carE* fragment and 5.37 kb pET-28a linear plasmid also verified the correct insertion of *carE* gene. The above results demonstrated that the *carE* gene was successfully cloned into *E. coli* BL21 cells.

According to Kita et al. (1999), the enzyme C23O could catalyze a ring cleavage of catechol to form 2-HMS. This production of an intensely colored product from a colorless substrate has been used in a number of biotechnological assays. In this work, the specific color reaction was used to confirm the expression of C23O protein in different microbial cells. Compared to *Sphingomonas yanoikuyae* XLDN2–5 (Fig. 1A-a) and *E. coli* BL21 (Fig. 1A-b), the recombinant *E. coli* BL21-C23O colonies sprayed by catechol solution turned yellow in a short time (Fig. 1A-c), which proved the successful expression of C23O in *E. coli* BL21 cells. To further verify the *carE* gene expression in *E. coli* BL21 cells, SDS-PAGE was carried out as shown in Fig. 1B. The recombinant C23O protein matched the calculated molecular mass (~35 kDa, Fig. 1B, line c) of the C23O protein from wild strain *Sphingobium yanoikuyae* XLDN2–5. Compared to the wild strain *Sphingobium yanoikuyae* XLDN2–5 (Fig. 1B, lane b), the recombinant *E. coli* BL21-C23O cells could produce higher amounts of C23O protein (Fig. 1B, lane a). These results demonstrated that the C23O was successfully over-expressed in *E. coli* BL21 cells.

To further investigate the catalytic activity towards catechol and the expression level of C23O in *Sphingomonas yanoikuyae* XLDN2–5, *E. coli* BL21, and recombinant *E. coli* BL21-C23O, the spectrophotometric method was used to determine the accumulation of 2-HMS at 375 nm. As shown in Fig. 1C, no catalytic activity towards catechol was detected in cell suspension and crude enzyme of *E. coli* BL21, which indicated that *E. coli* BL21 did not contain related genes for C23O expression. For wild strain *Sphingomonas yanoikuyae* XLDN2–5, the activity of C23O was only found in crude enzyme with 85.9 U g^{-1}. The low catalytic efficiency towards catechol was due to the low-level expression of the C23O protein in *Sphingomonas yanoikuyae* XLDN2–5 (also confirmed in Fig. 1B, lane b). Besides, *Sphingomonas yanoikuyae* XLDN2–5 also require a very complex culture conditions such as mineral salt and trace metals solution (Gai et al., 2007). Thus, *Sphingomonas yanoikuyae* XLDN2–5 cells or its crude enzyme would not be a good choice for the construction of a catechol biosensor. In contrast, for the recombinant *E. coli* BL21-C23O, the activities of C23O were both found in cell



C

Strains	C23O specific enzyme activity		
	Bacterial suspension (U g^{-1})	Supernatant (U g^{-1})	Cell extract (U g^{-1})
Recombinant *E. coli* BL21-C23O	225.61	1.51	1096.64
Sphingomonas yanoikuyae XLDN2-5	undetected	undetected	85.95
E. coli BL21	undetected	undetected	undetected

Fig. 1. (A) The catalytic activity of C23O in different microbial cell were tested by spraying catechol solution. (B) The SDS-PAGE of C23O protein in recombinant *E. coli* BL21 cells (lane a) and *Sphingomonas yanoikuyae* XLDN2–5 (lane b); line c was protein molecular weight marker. (C) The catalytic activities of the recombinant *E. coli* BL21-C23O, *Sphingomonas yanoikuyae* XLDN2–5, and *E. coli* BL21 cells were determined by spectrophotometry method.

suspension with 225.6 U g^{-1} and crude enzyme with 1096.6 U g^{-1}, which were higher than that of crude enzyme extracted from wild strain. Additionally, *E. coli* BL21 strain is a low-cost, rapid breeding, and thoroughly studied bacterium. Moreover, the recombinant *E. coli* BL21 over-expressing C23O does not require complex culture conditions compared with the wild strain. Therefore, according to the level of enzyme activity, the recombinant *E. coli* BL21-C23O or its crude enzyme was suitable for the fabrication of a microbial or enzyme biosensor for catechol detection.

3.2. Enzymatic property of the recombinant C23O

Michaelis constant (K_m) is the substrate concentration at which the reaction rate is half of the maximum rate (V_{max}), which is an index of the substrate's affinity for the enzyme. To investigate the affinity between the recombinant *E. coli* BL21-C23O cell or its crude enzyme and the substrate of catechol, spectrophotometry was used to calculate the specific enzyme activity and K_m. As shown in Fig. 2A and B, the apparent K_m values of the recombinant *E. coli* BL21-C23O cell and its crude enzyme towards catechol were determined to be around 2.52 μM and 3.41 μM, respectively. The similar and low K_m value demonstrated that both the recombinant *E. coli* BL21-C23O cell and its crude enzyme have good affinity with catechol, which also indicated that they were suitable for the construction of a catechol biosensor.

The stability of a bio-recognition element is an important factor in a biosensor construction and its practical applications. As reported by Xi et al. (2018), free C23O is usually instability and cannot be easily reused, which makes the pure enzyme cannot be a good choice for a biosensor construction. To fully investigated the storage stability, the recombinant *E. coli* BL21-C23O cell and its crude enzyme were compared at 4 °C or room temperature. As shown in Fig. 2C, after 9 days storage at 4 °C, the catalytic activity of crude enzyme reduced to approximately 50% of its original enzymatic activity. After stored at room temperature for 9 days, the crude enzyme was completely inactivated because of the self-oxidation of the extracellular C23O when exposed to air (Celesia et al., 2017; Felshia et al., 2017). In contrast, after a 9-days storage, the catalytic activity of the recombinant *E. coli* BL21-C23O cells retained 90% at 4 °C and 40% at room temperature due to the engineered microbial cells combining the advantages of enzymes and recombinant bacteria (Fig. 2D). In brief, the engineered microbial cells could provide a stable internal environment such as appropriate pH and cofactor, which makes the over-expressed enzyme retain high catalytic activity and good stability. Thus, the recombinant *E. coli* BL21 over-expressed C23O cell was more suitable as the bio-recognition element

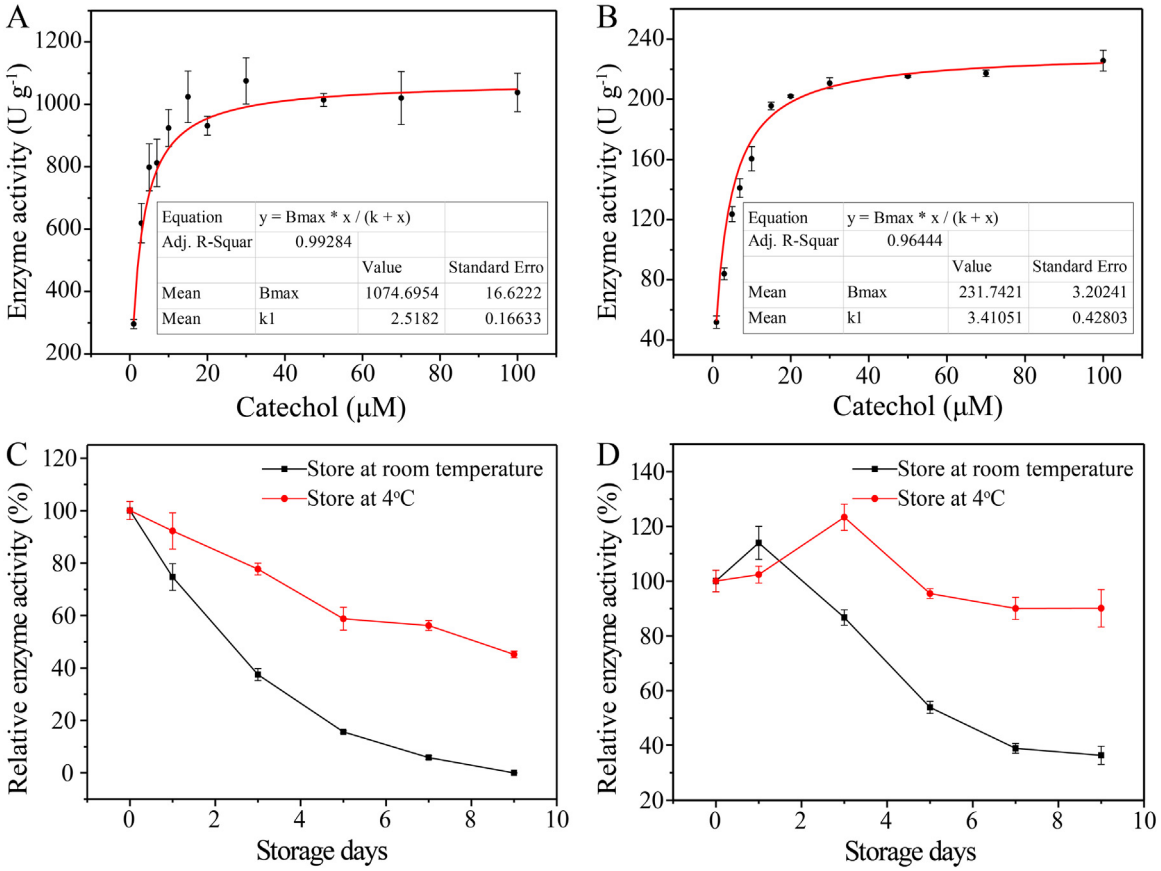


Fig. 2. The michaelis constants (K_m) towards catechol catalyzed by crude enzyme (A) and cell suspension (B). The storage stability of crude enzyme (C) and cell suspension (D) at different temperature.

for catechol determination.

3.3. Construction and characterization of the bioelectrodes

Based on the researches of the recombinant C23O enzyme properties, the *E. coli* BL21 cell and *E. coli* BL21-C23O cell were used to construct bioelectrodes respectively. Because the morphology of the *E. coli* BL21 cell was the same as the *E. coli* BL21-C23O cell, only the recombinant *E. coli* BL21-C23O cell loading on NPG was characterized by SEM. Fig. 3A showed that NPG has an open bicontinuous three-dimensional nanoporous structure with a pore size of ca. 35 nm, which could increase the adhesion of the recombinant *E. coli* BL21-C23O cells on NPG (Fig. 3B) and facilitate sufficient substrate and electron transfer. Besides, the abundant active gold atoms on the surface of NPG could covalently bind with amino group (–NH_2) and mercapto group (–SH) on the surface of *E. coli* BL21-C23O cells (Wang et al., 2011), which make the binding of the recombinant *E. coli* BL21-C23O cells on NPG more firm and stable. The SEM images provide a morphological evidence that the recombinant *E. coli* BL21-C23O cells were successfully immobilized onto the surface of NPG/GCE electrode.

To further confirm *E. coli* BL21 cells and *E. coli* BL21-C23O cells were successfully immobilized onto the surface of NPG/GCE electrode, the NPG/GCE electrode, *E. coli* BL21/NPG/GCE bioelectrode and *E. coli* BL21-C23O/NPG/GCE bioelectrode were compared in a PBS (50 mM, pH 7.0) using CV at a scan rate of 50 mV s^{-1}. As shown in Fig. 3C, it was clear that the redox peak current densities of the *E. coli* BL21/NPG/GCE bioelectrode and *E. coli* BL21-C23O/NPG/GCE bioelectrode were both lower than that of the NPG/GCE electrode. For the *E. coli* BL21/NPG/GCE bioelectrode and *E. coli* BL21-C23O/NPG/GCE bioelectrode, *E. coli* BL21 cells and *E. coli* BL21-C23O cells were insulated, which obstructed the interfacial electron transfer and thus decreased the current response of NPG. These results provided further evidences that the *E. coli* BL21 cells and *E. coli* BL21-C23O cells were successfully immobilized onto the surface of NPG/GCE electrode.

3.4. The bioelectrode reaction mechanism

In our previous work, it was found that NPG with bicontinuous three-dimensional nanoporous structure was an ideal support for the immobilization of bio-recognition elements such as enzyme or microbial cells (Wang et al., 2011; Wu et al., 2016; Liu et al., 2017). Besides, NPG could electrocatalytically oxidize catechol (Wu et al., 2016). Therefore, NPG was selected as a support for the immobilization of the recombinant *E. coli* BL21 over-expressed C23O in this study. Based on previous studies (Bui et al., 2015; Wu et al., 2016), the working principle of the NPG/GCE electrode during the electrochemical catalysis of catechol could be expressed as follows:

$$\text{Catechol} \xrightarrow{\text{NPG}} \text{o-Benzoquinone} + e^- + H^+ \quad (1)$$

$$\text{o-Benzoquinone} + e^- + H^+ \xrightarrow{\text{Electrode}} \text{Catechol} \quad (2)$$

In the oxidation reaction stage, catechol could be oxidized by NPG to o-benzoquinone and release H^+ (Eq. (1)). In this process, an electron was transferred from catechol to the surface of the NPG/GCE electrode. During the process of reduction reaction, the o-benzoquinone would accept an electron from electrode and then combine with H^+ to form catechol (Eq. (2)) as shown in Scheme 1. According to Kita et al. (1999) and Hupert-Kocurek et al. (2013), the active site of C23O is ferrous ion (Fe(II)), which could combine with catechol through double coordination bond. Then C23O with its active site Fe(II) was activated and could catalyze the bond cleavage of catechol between the carbon atoms at

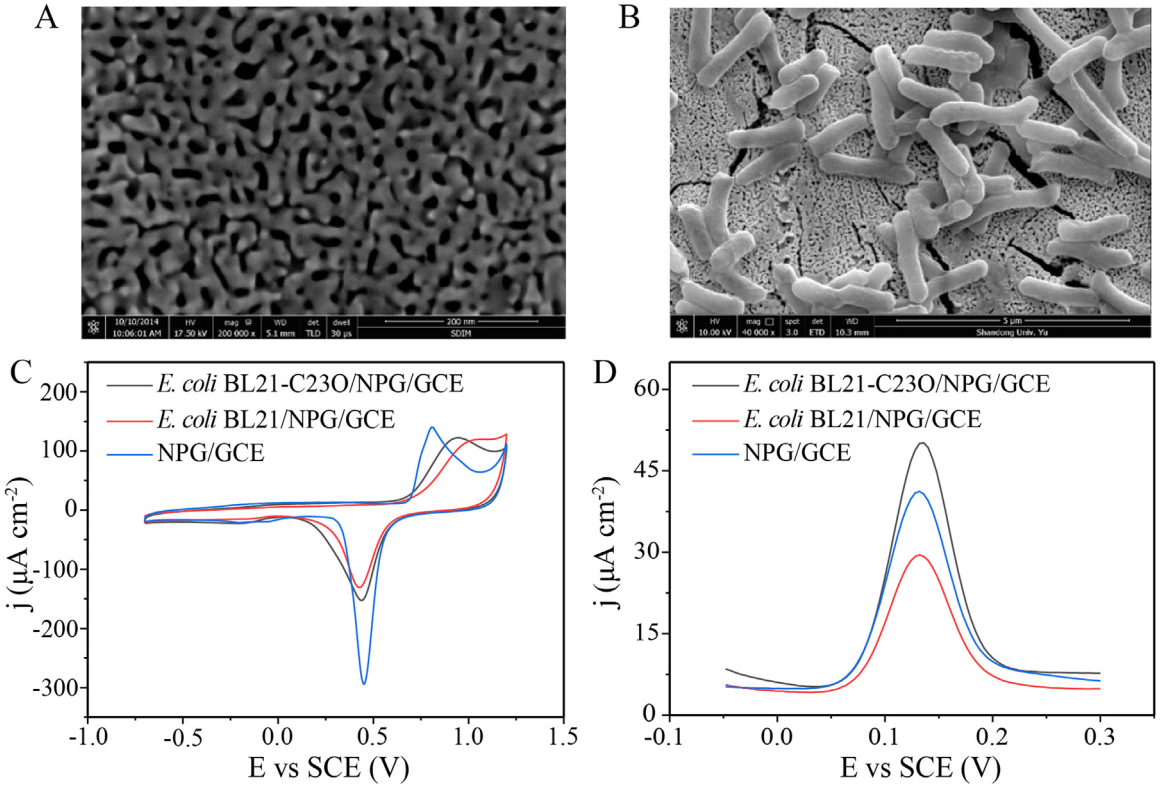
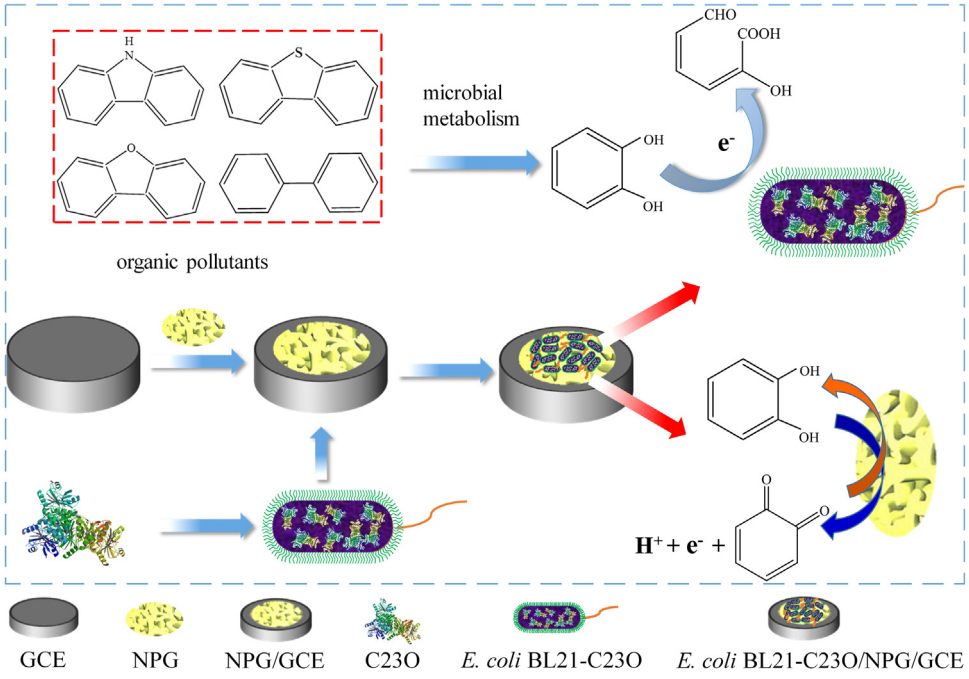


Fig. 3. SEM images of NPG before (A) and after (B) the recombinant *E. coli* BL21-C23O cells loading. (C) The CVs of the NPG/GCE electrode, *E. coli* BL21/NPG/GCE bioelectrode and *E. coli* BL21-C23O/NPG/GCE bioelectrode in a PBS (50 mM, pH 7.0). (D) The DPVs of the NPG/GCE electrode, *E. coli* BL21/NPG/GCE bioelectrode and *E. coli* BL21-C23O/NPG/GCE bioelectrode in a PBS (50 mM, pH 7.0) with 150 μM catechol.

positions of 2 and 3 to form 2-HMS with the insertion of two atoms of dioxygen (Eq. (3)). The bond cleavage of the catechol catalyzed by C23O was also clearly illustrated in Scheme 1. However, the C23O is an intracellular enzyme. To confirm that the enzyme which catalyze the catechol is intracellular enzyme rather than the enzyme that maybe secreted to the outside of the cell, the recombinant *E. coli* BL21-C23O cells were harvested by centrifugation at 4000 × *g* for 10 min, and washed twice with PBS (50 mM, pH 7.0) to remove the enzyme secreted to the outside of the cell. After spraying catechol solution, it was found that the coloration time of the washed recombinant *E. coli* BL21-C23O colonies was same as the non-washed recombinant *E. coli* BL21-C23O colonies (about 20 s). Further, the coloration time was shorter than the



Scheme 1. The schematic construction and reaction mechanism of the engineered *E. coli* BL21-C23O/NPG/GCE bioelectrode.

scanning time of DPV. Based on these results, although the C23O is an intracellular enzyme, the recombinant *E. coli* BL21-C23O still exhibit high catalysis activity towards catechol. The reason may be that catechol can be rapidly transported through the outer membrane, and this step is a requisite for its metabolism (Waigi et al., 2015). Therefore, the working principle of the recombinant *E. coli* BL21-C23O during the electrochemical catalysis of catechol could be expressed as follows:

$$\text{Catechol}+O_2 \xrightarrow{\text{C23O}} \text{2-HMS} \quad (3)$$

To test the effect of C23O and NPG on the electrochemical oxidation of catechol, an NPG/GCE electrode, an *E. coli* BL21/NPG/GCE bioelectrode, and an *E. coli* BL21-C23O/NPG/GCE bioelectrode were compared using DPV in a PBS (50 mM, pH 7.0) containing 150 μM catechol. As shown in Fig. 3D, an obvious oxidation peek current response for the NPG/GCE electrode was observed at +0.13 V, indicating NPG could catalyze the electrochemical oxidation of catechol. After *E. coli* BL21 cells immobilized on NPG, the oxidation peek current response of the *E. coli* BL21/NPG/GCE bioelectrode towards catechol was significantly decreased, which indicated that the *E. coli* BL21 cells loaded on NPG occupied parts of the active sites of NPG (as evidenced by the decreased redox peak current densities of NPG in Fig. 3C) and could not catalyze the catechol (confirmed the results of Fig. 1A-b and Fig. 1C once again). By contrast, for the *E. coli* BL21-C23O/NPG/GCE bioelectrode, though the immobilized *E. coli* BL21-C23O cells occupied parts of the active sites of NPG, the resulting bioelectrode still exhibited better sensing performance towards catechol than the NPG/GCE electrode due to the high catalytic activity of C23O towards catechol as shown in Scheme 1. Based on these results, the synergistic effect of C23O and NPG could efficiently generate and transfer more electrons on the surface of the bioelectrode, which in turn drastically enhance the sensing performance of the bioelectrode towards catechol. Thus, the sensitive determination of catechol would be achieved using the *E. coli* BL21-C23O/NPG/GCE bioelectrode.

To investigate the reaction kinetics of catechol, the *E. coli* BL21-C23O/NPG/GCE bioelectrode was explored by CV in a PBS (50 mM, pH 7.0) containing 150 μM catechol at different scan rates. As shown in Fig. 4A, a couple of redox peaks were emerged in the presence of 150 μM catechol and the peak current densities increased with the increase of scan rates. From the insert of Fig. 4A, the peak current densities were linearly correlated with the square root of scan rates, and the correlation coefficients of the cathodic and anodic peaks were 0.996 and 0.989, respectively. These findings demonstrated that the electron transfer process in the presence of catechol was a diffusion-controlled process, which was consistent with previous reports (Han et al., 2014; García-Hernández et al., 2016). Furthermore, according to Palomäki et al. (2015) and Liu et al. (2016), the electrochemical reaction of catechol on the surface of non-enzyme electrode was a reversible electrochemical process, which means that the ratio of the cathodic peak current density and the anodic one (Ipc/Ipa) would approximate to 1. In contrast, the cathodic peak current density of catechol was apparently higher than the anodic at the same scan rate due to the electrocatalytic oxidation of catechol by C23O in this study. These characteristics confirmed that the *E. coli* BL21-C23O/NPG/GCE bioelectrode was successfully fabricated, and the electrochemical oxidation of catechol on the *E. coli* BL21-C23O/NPG/GCE bioelectrode was attributed to the synergistic effect of C23O and NPG.

3.5. Catechol detection by the E. coli BL21-C23O/NPG/GCE bioelectrode

The pH value of buffer solution was an important factor to influence the electrochemical behavior of a microbial biosensor (Qi et al., 2014). To obtain the maximum current response of C23O and NPG towards catechol, the influence of pH on the electrochemical oxidation of catechol was investigated using DPV in the presence of 100 μM catechol with a pH range from 4.0 to 8.0. As shown in Fig. 4B, the current response of the *E. coli* BL21-C23O/NPG/GCE bioelectrode towards catechol increased with pH increasing, and the maximum current response was found at pH 7.0. Then, the oxidation peak current densities decreased when pH value was higher than pH 7.5. Hence, pH 7.0 was chosen as the optimal pH for the catalysis of C23O and NPG towards catechol. This result was different with the report that the optimal pH for the catalysis of C23O towards catechol is pH 8.4 (Xi et al., 2018), because the optimal pH value (pH 7.0) in this study was obtained based on the NPG and *E. coli* BL21-C23O cells rather than C23O alone. Additionally, the current response of the *E. coli* BL21-C23O/NPG/GCE bioelectrode towards catechol has a small change when pH ranges from 6.0 to 7.5. In this pH range, the current responses were less affected by the pH altering, because the *E. coli* BL21 cells over-expressed C23O was used as the bio-recognition element, which provide a stable internal environment that can reduce the effect of external environment changing on the enzyme activity.

To evaluate the sensing performance of the bioelectrode towards catechol under the optimal conditions, the *E. coli* BL21-C23O/NPG/GCE bioelectrode was explored by DPV in a PBS (50 mM, pH 7.0) containing a range of catechol concentrations. As shown in Fig. 4C, the peak current densities were increased gradually around +0.13 V with catechol concentration increasing. The inset profile in Fig. 4C showed that the plot of oxidation peak current densities versus catechol concentrations was constituted by two linear relationships with different slopes. When the concentrations ranged from 1 μM to 150 μM, the linear regression equation was $j(\mu A cm^{-2})=2.8564+0.33224\times C_{catechol}(\mu M)$ ($R^2 = 0.987$) with a high sensitivity of 332.24 $\mu A\, mM^{-1}\, cm^{-2}$. Besides, in the concentration range from 150 μM to 500 μM, the linear regression equation could be expressed as $j(\mu A cm^{-2})=36.9274+0.10768\times C_{catechol}(\mu M)$ ($R^2 = 0.991$) with the sensitivity of 107.68 $\mu A\, mM^{-1}\, cm^{-2}$. In addition to the wide linear range and high sensitivity, the *E. coli* BL21-C23O/NPG/GCE bioelectrode also had a low detection limit of 0.24 μM (S/N = 3). The relative standard deviation (RSD) for determining catechol was 0.97% (n = 5), which indicated that the catechol determination using the *E. coli* BL21-C23O/NPG/GCE bioelectrode was highly reproducible. By contrast, the sensing performances of other electrochemical biosensors for catechol were summarized in Table S1. Compared with other electrochemical biosensors, the resulting microbial biosensor based on the recombinant *E. coli* BL21 over-expressed C23O and NPG presented a larger linear range, higher sensitivity, and lower detection limit for catechol determination.

3.6. Anti-interference and stability of the E. coli BL21-C23O/NPG/GCE bioelectrode

The anti-interference is a critical factor for a microbial biosensor, which has a major impact in practical applications. To evaluate the anti-interference of the *E. coli* BL21-C23O/NPG/GCE bioelectrode, different concentrations of Mg^{2+}, Mn^{2+}, NO_2^-, Fe^{2+}, SO_4^{2-}, phenol, hydroquinone (HQ), carbazole (CA), dibenzofuran (DBF), and ethylenediaminetetraacetic acid (EDTA) were separately added into a PBS (50 mM, pH 7.0) containing 20 μM catechol. Fig. 4D showed that the addition of these interferents mentioned above caused negligible changes of current signal, and the change was less than 4.88%. These results indicated that the *E. coli* BL21-C23O/NPG/GCE bioelectrode presented a strong anti-interference capability due to the high specificity and efficient catalysis of C23O towards catechol.

Stability is a basic requirement for a biosensor. The *E. coli* BL21-C23O/NPG/GCE bioelectrode was preserved in a culture solution at 4 °C and measured 150 μM catechol weekly to test its stability. After 4 weeks of storage, the *E. coli* BL21-C23O/NPG/GCE bioelectrode retained 93.8% of its original current response. Compared to the recombinant *E. coli* BL21-C23O cells retained 90% of its original catalytic activity after a 9 days storage at 4 °C as shown in Fig. 2D, the *E. coli* BL21-C23O/NPG/GCE bioelectrode also maintained high catalytic activity after a long storage.

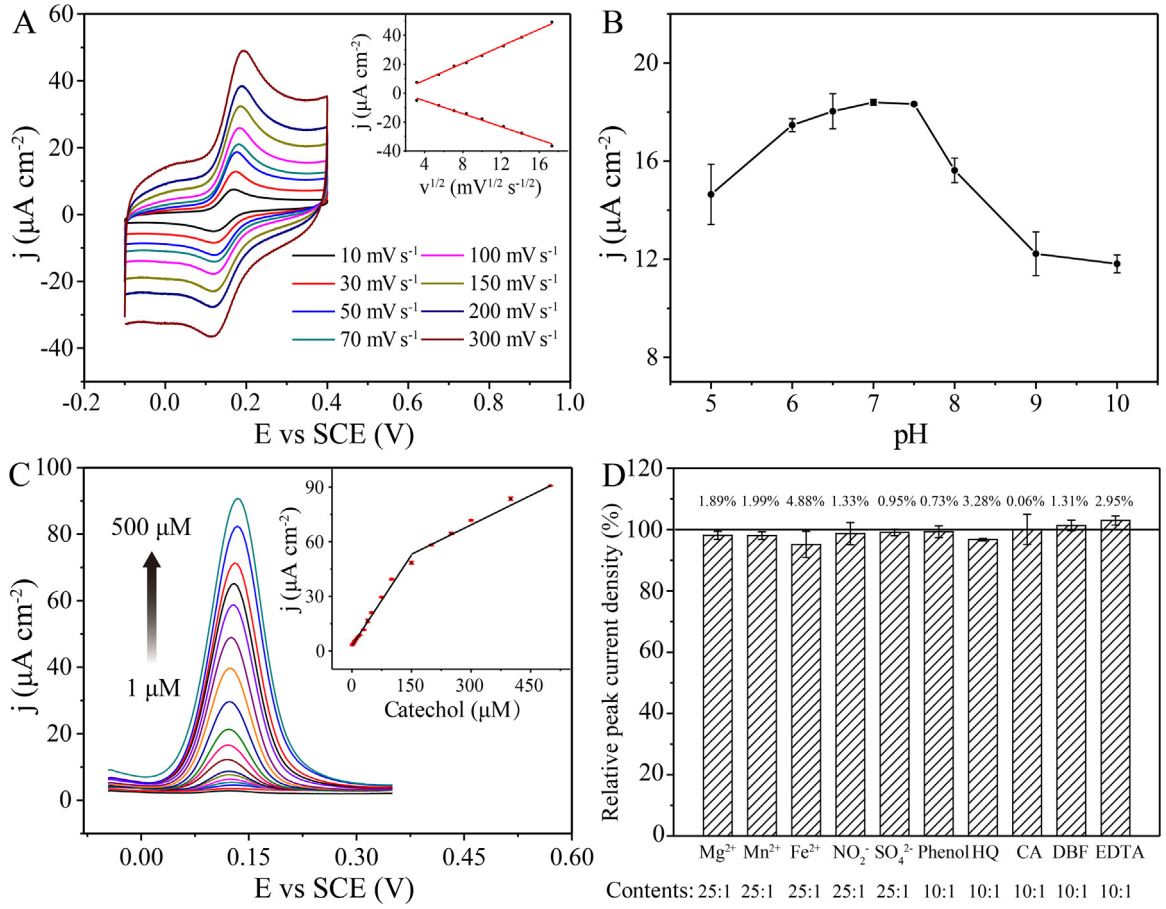


Fig. 4. (A) The CVs of the *E. coli* BL21-C23O/NPG/GCE bioelectrode at different scan rates in a PBS (50 mM, pH 7.0) with 150 μM catechol. (B) The effect of pH on catechol detection using the *E. coli* BL21-C23O/NPG/GCE bioelectrode. The inset profiles show the peak current density as a function of the square root of scan rate. (C) The electrochemical response of the *E. coli* BL21-C23O/NPG/GCE bioelectrode as a function of catechol concentration. The inset profiles show the calibration curve between the peak current density and catechol concentration. (D) The anti-interference of the *E. coli* BL21-C23O/NPG/GCE bioelectrode (HQ: hydroquinone; CA: carbazole; DBF: dibenzofuran; EDTA: ethylenediaminetetraacetic acid). Content is the molar ratios of interferents to catechol.

3.7. The detection of catechol in seawater and wastewater

The practical performance of the *E. coli* BL21-C23O/NPG/GCE bioelectrode for catechol detection was analyzed using standard addition method. The synthetic water samples containing 5 mL seawater (Cape of Good Hope, South Africa) or 5 mL wastewater (Jining wastewater treatment plant, China) and 10 mL PBS (50 mM, pH 7.0) were measured using the *E. coli* BL21-C23O/NPG/GCE bioelectrode. Prior to electrochemical analysis, certain amount of catechol was added into the synthetic water samples. The concentrations of catechol detected by the *E. coli* BL21-C23O/NPG/GCE bioelectrode were compared with the standard concentrations of catechol that added into synthetic water samples. For seawater samples, the recoveries of catechol were in the range of 99.98–104.67% and the deviation rate was less than 5% (Table 1). For wastewater samples, the recoveries were between 95.67% and 103.98% (Table 1). Compared to seawater samples, the larger changes in recoveries were due to the complicated wastewater composition. Nevertheless, the deviation rate of wastewater was also less than 5%. These results suggested that the *E. coli* BL21-C23O/NPG/GCE bioelectrode was practical and reliable for catechol detection in actual water samples.

Table 1
Determination of catechol in seawater and wastewater samples.

Samples		Added (μM)	By proposed biosensor (μM)	Recovery (%)	Deviation rate (%)
Seawater	1#	15	15.70 ± 0.05	104.67	+4.67
	2#	55	54.99 ± 0.08	99.98	–0.02
	3#	125	126.19 ± 0.25	100.95	+0.95
Wastewater	1#	15	14.35 ± 0.01	95.67	–4.33
	2#	75	74.50 ± 1.95	99.33	–0.67
	3#	215	223.56 ± 0.22	103.98	+3.98

4. Conclusion

In summary, a highly sensitive microbial biosensor was constructed using the recombinant *E. coli* BL21-C23O cell as bio-recognition element for catechol detection. Based on the synergistic effect of C23O and NPG, the resulting microbial biosensor exhibited good performance for catechol detection with high sensitivity, strong anti-interference and good stability. However, the proposed microbial biosensor can only detect catechol due to the overexpression of C23O in *E. coli* BL21. In fact, many kinds of pollutants exist in the environment at the same time. Therefore, a microbial biosensor that could simultaneously detect several pollutants is urgently needed for environmental monitoring in the future work.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2018.10.050.

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