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Development of an *Escherichia coli*-based electrochemical biosensor for mycotoxin toxicity detection

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ABSTRACT

Mycotoxin contamination in food and feed is a global concern because mycotoxin contamination can cause both acute and chronic health effects in humans and animals. In the present work, an *Escherichia coli*-based biosensor is described for the toxicity assessment of aflatoxin B_1 (AFB_1) and zearalenone (ZEN). In this electrochemical biosensor, *E. coli* is used as the signal recognition element, *p*-benzoquinone is used as the mediator, and a two-step reaction procedure has been developed to separate the mediator from the mycotoxins. The current value of the as-prepared microbial biosensor exhibits a linear decrease with concentrations of AFB_1 and ZEN in the range of 0.01–0.3 and 0.05–0.5 μg/mL, with detection limits reaching 1 and 6 ng/mL, respectively. The IC_{25} values obtained by the present method are 0.25 and 0.40 μg/mL for AFB_1 and ZEN, which shows that the cytotoxicity of AFB_1 to *E. coli* is more severe than the cytotoxicity of ZEN to *E. coli*. The combined toxic effect of these two mycotoxins has also been explored, and synergistic biotoxicity has been observed. Moreover, the biosensor is successfully applied to the toxicity evaluation of mycotoxins in real samples, including peanut and corn oils. This work could provide new insight into mycotoxin and microorganism interactions and could establish a new approach for future mycotoxin detection.

1. Introduction

Mycotoxins are toxic secondary metabolites produced by organisms of the fungus kingdom such as *Aspergillus*, *Penicillium* and *Fusarium* species [1,2]. As an exogenous toxicant in food and livestock feed, mycotoxins normally do not show acute toxicity in trace amounts but do have strong cumulative, teratogenic, carcinogenic and mutagenic effects [3,4]. To date, the problem of mycotoxin contamination is a global concern; more than 400 mycotoxins have been found in the food ingested daily and in animal feeds [5]. Commonly harmful mycotoxins that can cause human and animal illness include aflatoxins (AFs), ochratoxin A (OTA), deoxynivalenol (DON), zearalenone (ZEN), T-2 toxin (T-2) and fumonisins (FBs) [6], of which aflatoxin B_1 (AFB_1) and zearalenone (ZEN) are two toxins broadly causing concern. AFB_1 is a derivative of dihydrofuran oxaphthalene, which has been classified as a human carcinogen by the International Agency for Research on Cancer (IARC) because of its strong toxic and carcinogenic effects compared to other aflatoxins [7]. ZEN is a non-steroidal, oestrogenic mycotoxin that is most widely distributed in our natural environment [8]. These mycotoxins are capable of many toxic effects, including hepatotoxicity, cytotoxicity, immunotoxicity and tumourigenicity, which seriously threaten human health and cause huge economic losses in the animal husbandry industry [9]. Many countries have released strict national standards to limit the mycotoxin content for food safety. Therefore, accurate and sensitive mycotoxin toxicity evaluation methods are urgently needed for daily food monitoring and environmental detection.

To date, the traditional analytical methods, such as high-performance liquid chromatography (HPLC) [8] and ultra-high-pressure liquid chromatography coupled to triple quadrupole mass spectrometry technique (UHPLC-MS/MS) [10], are still the first choice for qualitative and quantitative mycotoxin detection in the laboratory owing to their accuracy and ultrasensitive ability. However, many biological techniques, such as immunochip technology [11], the enzyme-linked immunosorbent assay (ELISA) [12], the photoelectrochemical immunoassay [13], the portable

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glucometer-based detection method [14] and the molecular imprinting technique (MIP) [15] are also applied to mycotoxin detection. These methods can achieve qualitative or semiquantitative detection of mycotoxins. These biological methods and electrochemical biosensors are also employed in mycotoxin measurement due to their advantages, such as high sensitivity, low cost, simplicity and sample pretreatment processes [16]. Mammalian cells [7,17], DNA aptamers [18] and antibodies [19] are used as the recognition elements in these electrochemical biosensors to achieve the specific and rapid detection of mycotoxins [20–22]. However, the abovementioned recognition elements (e.g., enzymes, antibodies, cells) are expensive, complicated to integrate and hard to store, which limits their practical applications [23].

In addition, simultaneous detection of multiple mycotoxins or their toxicity assessment is of great importance because of the coexistence of mycotoxins in the natural environment [24–27]. Thus, exploring the combined toxicity effects of various mycotoxins is more instructive for reducing the harm of mycotoxins to humans or animals. Bioassay methods based on enzymes, antibodies, or DNA cannot discriminate the coexistence of mycotoxins because these recognition elements lack detection specificity for different individual mycotoxins [28–30]. Limited efforts have been made so far to develop methods and instruments for the total toxicity detection of these mycotoxins [31]. Electrochemical biosensors appear to be a very promising alternative technique [7,9]. Bacteria as independent living organisms can serve as a simple and cost-effective substitute for the recognition element. Bacteria possess a unique physiological structure and have the merits of easy cultivation and utilization [32–35]. In addition, employing bacteria as the biological recognition elements can also help to investigate the influence of mycotoxins on the native organisms, which can directly observe the toxic effects of mycotoxins on living organisms and provide some indications of the biotoxicity of mycotoxins in humans.

Herein, we describe a novel biosensor for the toxicity assessment of AFB_1 and ZEN that combines the advantages of both the electrochemical method and the unique characteristics of bacteria (*E. coli)* as the bio-recognition elements. The toxicity of mycotoxin AFB_1 and ZEN in bacteria are evaluated based on the inhibition of *E. coli* metabolic activity, and the combined toxic effects of the above two mycotoxins on *E. coli* are also explored. The recovery experiments of real oil samples suggest that the as-prepared biosensor is applicable in the real sample mycotoxin detection. Furthermore, the morphology changes of *E. coli* after co-culture with mycotoxins are investigated to unravel the toxicity mechanism of AFB_1 and ZEN in bacteria.

2. Experimental section

2.1. Reagents and instruments

Electrochemical mediators: *p*-benzoquinone was purchased from Adamas-beta (Shanghai, China), potassium ferricyanide was acquired from Acros Organics (Beijing, China), and thionine acetate was obtained from Alfa Aesa (Tianjin, China). Phosphate buffer solution (0.1 M, PBS) with pH = 7.0 was purchased from Beijing Leagene Biotech. Co., Ltd. Mycotoxin Aflatoxin B_1 was purchased from J&K Chemical Ltd, Beijing, China, and zearalenone was obtained from Aladdin Industrial Corporation, Shanghai, China. Mycotoxins were dissolved in methanol (chromatographic grade) and further diluted with PBS. Other chemical reagents were purchased from Beijing Lanyi Chemical Products Co., Ltd., China. Peanut and corn oil samples were purchased from a local supermarket. All solutions are prepared with Milli-Q water system unless specific description.

The optical density (OD_{600}) of the *E. coli* suspension was measured with a UV–Vis spectrophotometer (SECOMAM UVIKONXL) at 600 nm. All electrochemical characterizations were performed on the electrochemical workstation (Princeton 263A, Princeton Applied Research, USA). The morphologies of the *E. coli* cells were characterized by scanning electron microscopy (Hitachi SU8000 and S-4800, Hitachi Co., Ltd., Japan).

2.2. Preparation of glassy carbon electrode and E. coli

The glassy carbon electrode (GCE) was first polished with alumina powder (1 μm and 0.05 μm) and then ultrasonically cleaned consecutively with nitric acid solution (v:v = 1:1), ethanol and deionized water for 10 min each. Polished electrodes were dried at room temperature before use.

E. coli (ATCC 25922) was supplied by China General Microbiological Culture Collection Centre (CGMCC). Lysogeny broth (LB) (10 mg/mL peptone, 5 mg/mL beef extract, 5 mg/mL NaCl) at pH = 7.3–7.5 was used for *E. coli* cultivation. *E. coli* was replanted in 100 mL LB medium and cultured aerobically at 37 °C for 16 h. The bacteria were harvested by centrifugation at 6,000 rpm for 5 min, then washed twice with NaCl solution (0.85%, w/v) and finally dispersed in the NaCl solution. The concentration of *E. coli* was measured by UV–Vis spectrophotometry at a wavelength of 600 nm (OD_{600}).

2.3. Optimization of experimental conditions

A two-step reaction method [36] was used to detect mycotoxins; the experimental procedure is shown in Scheme 1. In brief, *E. coli* cells are first co-cultured with mycotoxins for a certain time period, centrifuged at 6,000 rpm for 6 min and washed twice with a 0.85% (w/v) NaCl solution to fully remove the mycotoxin residue. Subsequently, 10 mL mediator solution is added to react with *E. coli* for a certain time period, and then the mixture is centrifuged at 6,000 rpm for 8 min. The steady-state current of obtained supernatant is measured by chronoamperometry as an indication of bacterial viability. Thus, in this experiment, four experimental conditions need to be optimized: the mediator, the *E. coli* concentration, the mycotoxin and *E. coli* co-culture time and the *E. coli* and mediator co-culture time. The specific optimization process is described in detail in the supporting information.

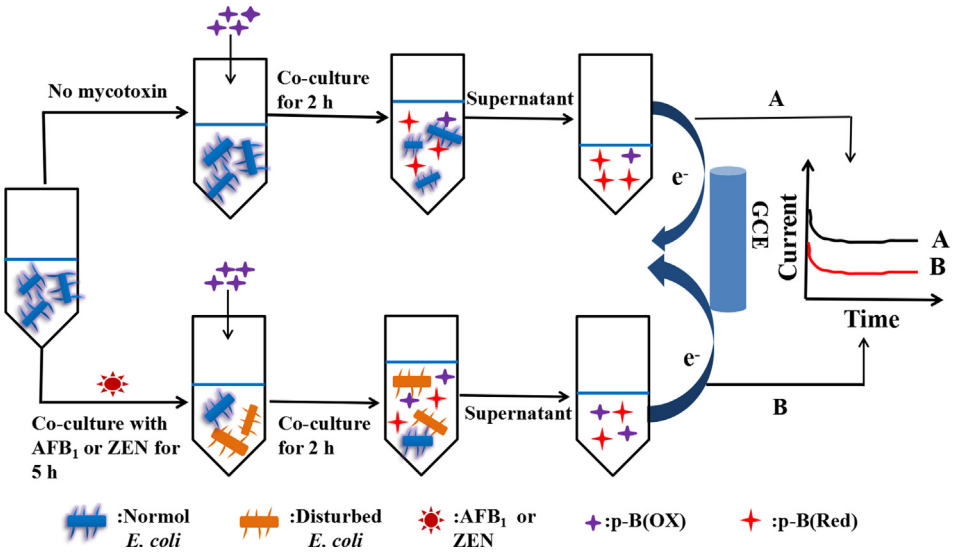
2.4. Electrochemical biotoxicity analysis of AFB_1, ZEN and their combinations

The above mentioned *E. coli*-based electrochemical biosensor is used to detect biotoxicity of mycotoxins AFB_1 and ZEN under optimal conditions (the detailed procedure is depicted in the supporting information). The inhibition rate of the mycotoxin at a certain concentration is calculated according to Eq. (1):

$$\text{Inhibition}\ (\%) = (i_c - i_e)/i_c \times 100\% \quad (1)$$

where i_e is the steady current in the experimental groups (added mycotoxins), and i_c is the steady current in the control groups (added PBS).

In the combined toxicity effect experiment, four concentration mixtures of AFB_1 + ZEN were detected to estimate the toxic interactions of AFB_1 and ZEN. *E. coli* cells that are not exposed to mycotoxins serve as the control group. The estimated inhibition value is calculated according to Eq. (2), and the experimental inhibition rate is detected when both mycotoxins act simultaneously on



Scheme 1. The principle of mycotoxin toxicity assessment by electrochemical biosensor based on *E. coli.*

E. coli. The significance of difference is calculated using the *t*-test, and $P < 0.05$ is considered statistically significant.

$$\text{Estimated inhibition rate (\%)} = \text{inhibition rate}_{AFB1}\ (\%) + inhibitionrate_{ZEN}(\%) \quad (2)$$

2.5. Detection of AFB_1 and ZEN in real samples

To evaluate the detection capability of the fabricated biosensor in real samples, different concentrations of AFB_1 and ZEN in oil were measured using the electrochemical biosensor that had been developed. Known concentrations of AFB_1 and ZEN standard solutions were added to the pre-treated oil sample as the experimental group, and the pre-treated oil sample added to the PBS was used as the control group. The pre-treatment process for the peanut and corn oil list is as follows: weigh 5 g of sample in a 50 mL centrifuge tube, and then add 20 mL 70% methanol solution. Sonicate for 20 min to mix evenly, and finally, centrifuge at 6,000 rpm for 10 min to collect the supernatant. The supernatants are placed in a −4 °C refrigerator for further use.

3. Results and discussion

3.1. Detection principle and feasibility test

As described above, a two-step reaction method [36] is applied to detect AFB_1 and ZEN. As shown in Scheme 1, the principle of the method is to isolate the mycotoxins with mediators to avoid interference. *E. coli* is a prokaryote with no mitochondria; as such, aerobic respiration occurs mainly in the cytoplasmic matrix and cell membrane. *p*-Benzoquinone, as the mediator, can compete with dissolved oxygen in the water to extract electrons from the respiratory chain and reduce to hydroquinone. Hydroquinone is re-oxidized on the electrode and generates current, which reflects the bacterial activity. Thus, the activity of *E. coli* is inhibited, and respiration is weakened after co-cultivation with mycotoxins for a certain time period. The reaction between the *p*-benzoquinone and disturbed *E. coli* leads to a decrease in the amount of hydroquinone, which ultimately reduces the oxidation current.

The feasibility of the biosensor based on *E. coli* is confirmed by the toxicity test performed with AFB_1 and ZEN, as shown in the *i*-t curves of Fig. 1. The steady current of the experimental group receiving 7 μg/mL AFB_1 or ZEN is significantly lower than the steady current of the control group, indicating that the above biosensor can be applied to mycotoxin toxicity detection.

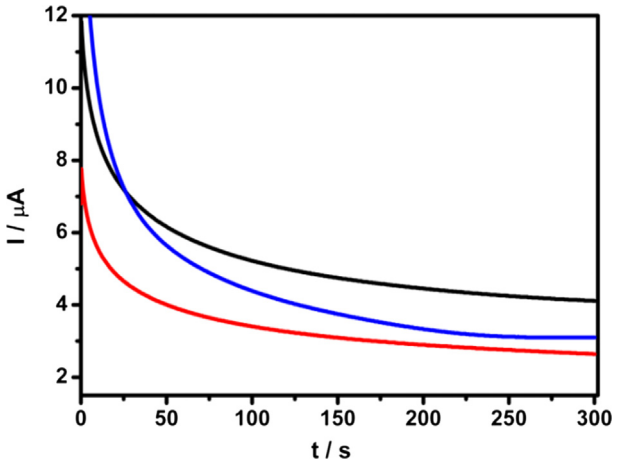


Fig. 1. Chronoamperometry curves of normal bacteria (black line) and bacteria after co-culture with AFB_1 (red line) or ZEN (blue line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. Optimization of experimental conditions

Four experimental conditions were optimized before subsequent experiments: mediator, *E. coli* concentration, mycotoxin and *E. coli* co-culture time and the *E. coli* and mediator co-culture time. First, the steady-state currents of four mediators, potassium ferricyanide, *p*-benzoquinone, thionine and neutral red, were compared after reaction with *E. coli* for 1 h (Fig. 2(a)). The oxidation currents of neutral red and thionine are negligible due to low solubility and short incubation time, and this oxidation current cannot transport electrons from *E. coli* to the electrode effectively. However, both the potassium ferricyanide and *p*-benzoquinone supernatants show obvious oxidation currents. The lipophilicity and good solubility of *p*-benzoquinone exert these advantages to access the *E. coli* respiratory chain and capture electrons, so the current intensity of the *p*-benzoquinone supernatant is larger than

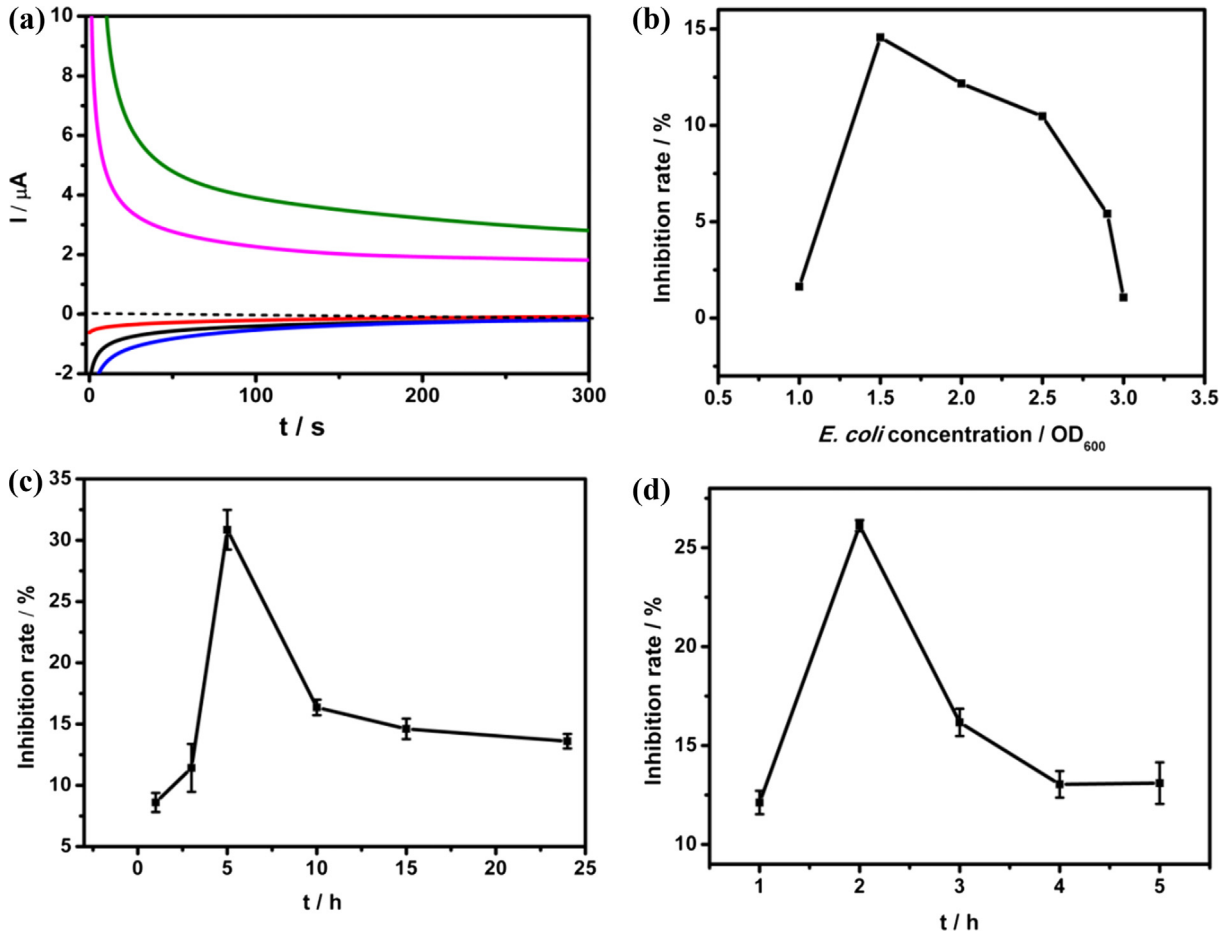


Fig. 2. Optimization of experimental conditions. (a) *i*-t curves of different mediators after reaction with *E. coli* for 1 h. Black line: control; blue line: thionine; red line: neutral red; pink line: $K_3Fe(CN)_6$; green line: *p*-benzoquinone. (b) Inhibition rate curve at different *E. coli* concentrations; (c) inhibition rate curve for different mycotoxin and *E. coli* co-culture times; (d) inhibition rate curve for different *p*-benzoquinone and *E. coli* reaction times. The mycotoxin added in these optimization experiments is 7 μg/mL AFB_1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the current intensity of potassium ferricyanide. Therefore, *p*-benzoquinone was selected as the mediator of this *E. coli*-based biosensor in subsequent experiments.

The sensitivity of mycotoxin detection may be affected by the *E. coli* concentration, so the *E. coli* concentration was optimized according to the OD_{600} value. The inhibition rates were measured under different OD values by adding the same concentration of AFB_1 and culturing for 3 h. Fig. 2(b) shows that the inhibition rate reaches the highest point at OD = 1.5, but the current intensity is dose-dependent on the bacterial concentration, as shown in Fig. S1. Therefore, considering both inhibition rate and current intensity factors, OD = 2.0 was chosen as the optimal concentration of *E. coli*. The co-culture time of the fungal toxin with *E. coli* was also optimized at 37 °C, and the inhibition rate-time curve in Fig. 2(c) suggests that 5 h is the best co-culture time for mycotoxin detection. Similarly, the mediator and bacterial co-culture time was also optimized according to the inhibition rate at 7 μg/mL AFB_1, and 2 h was selected as the optimal time, as shown in Fig. 2(d).

In summary, *p*-benzoquinone was utilized as the mediator, and the following optimal conditions were selected for the subsequent experiments: OD = 2.0, mycotoxin and *E. coli* co-culture time of 5 h, and *p*-benzoquinone and *E. coli* reaction time of 2 h.

3.3. Biotoxicity evaluation of AFB_1, ZEN and their combinations

Under the optimal conditions, the toxicity determination of AFB_1 and ZEN is performed using chronoamperometry. The current value decreases as AFB_1 (or ZEN) concentration increases, and the developed electrochemical toxicity evaluation system exhibits a dose-dependent response (Figs. 3(a), 4(a)). The linear range of 0.01–0.3 μg/mL (R^2 = 0.984) is observed for AFB_1 (Fig. 3(b)), and the limit of detection (LOD) is 1 ng/mL based on 3 S/N. Similarly, the linear range of ZEN is 0.05–0.5 μg/mL (R^2 = 0.999) with an LOD of 6 ng/mL (Fig. 4(b)). These results indicate that both AFB_1 and ZEN have a toxic effect on *E. coli*. IC_{25} is defined the mycotoxin concentration at 25% inhibition rate; the obtained IC_{25} values are 0.25 and 0.40 μg/mL for AFB_1 and ZEN, respectively. The data show that the toxic effect of AFB_1 on *E. coli* is more severe than that of ZEN. The detection sensitivity of the present biosensor is compared with other previously reported biosensors, as shown in Table 1. The present *E. coli*-based electrochemical biosensor exhibits lower LOD values for both AFB_1 and ZEN. However, the linear ranges are relatively narrow compared to other methods, possibly because *E. coli* is sensitive to mycotoxins in a low concentration range, and the tolerance of *E. coli* to mycotoxins at high concentrations is relatively higher than the tolerance of human cells.

For real samples that are contaminated by mycotoxins, there is always more than one kind of mycotoxin. Thus, the total biological toxicity of the AFB_1 + ZEN mixture was evaluated. There are three kinds of combined toxicity effects of toxicants, namely, additive, synergistic and antagonistic effects. These effects are determined based on whether the measured inhibition rate value is equal, significantly above or below the estimated inhibition rate value [9]. In this experiment, four combinations (AFB_1 + ZEN of 0.01 + 0.05 μg/mL, 0.

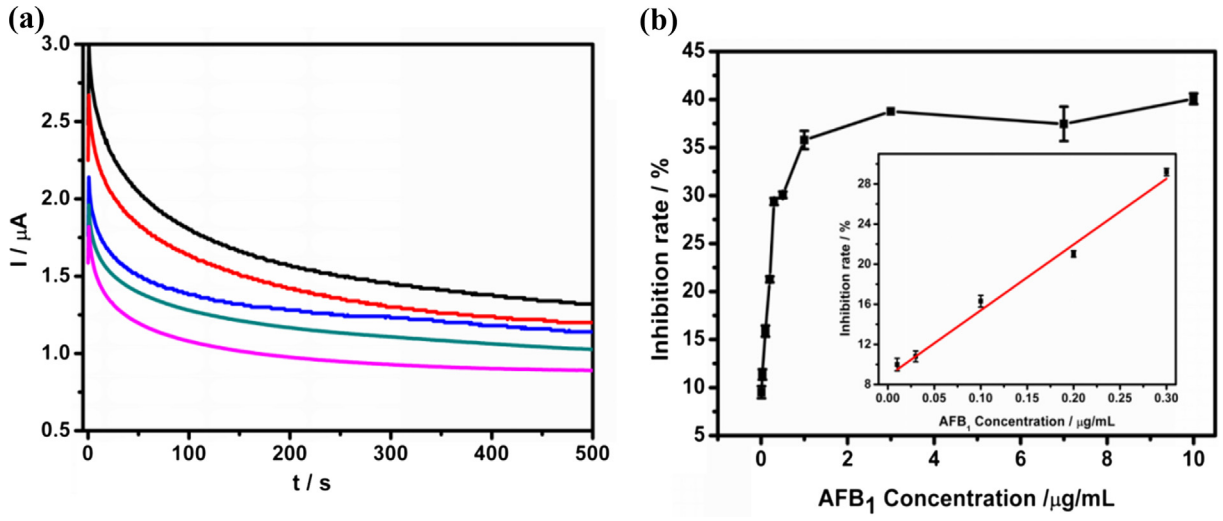


Fig. 3. (a) Chronoamperometry curves of different concentrations of AFB_1. Black line: control; red line: 0.01 μg/mL; blue line: 0.1 μg/mL; green line: 0.2 μg/mL; pink line: 0.3 μg/mL (b) Inhibition rate curve of different concentrations of AFB_1 to *E. coli*. The inset presents the calibration curve for AFB_1, y = 65.63 × + 8.84, R^2 = 0.984. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

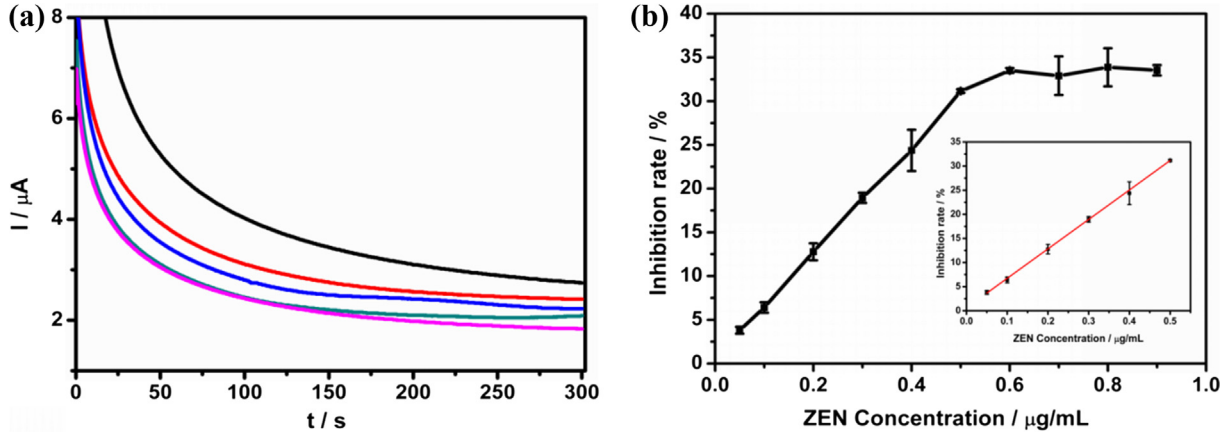


Fig. 4. (a) Chronoamperometry curves of different concentrations of ZEN. Black line: control; red line: 0.2 μg/mL; blue line: 0.3 μg/mL; green line: 0.4 μg/mL; pink line: 0.5 μg/mL (b) Inhibition rate curve of different concentrations of ZEN to *E. coli*. The inset presents the calibration curve for ZEN, y = 61.05 × + 0.62, R^2 = 0.999. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Comparison of different mycotoxin detection methods.

Mycotoxins	Methods	Detection limit	Linear range	Ref.
AFB_1	*E. coli*, *i*-t	1 ng/mL	0.01–0.3 μg/mL	This study
	HepG2 cells, EIS	–	0.1–3.5 μg/mL	[7]
	AchE, amperometry	2 ng/mL	0.01–0.06 μg/mL	[37]
	LMOF, PL intensity	46 ng/mL	–	[38]
ZEN	*E. coli*, *i*-t	6 ng/mL	0.05–0.5 μg/mL	This study
	HepG2 cells, EIS	–	0.1–50 μg/mL	[7]
	BEL-7402 cells, EIS	50 ng/mL	0.1–50 μg/mL	[9]
	Antibodies, iSPR	24 ng/g	–	[39]

EIS: electrochemical impedance spectroscopy; AchE: acetylcholinesterase; LMOF: luminescent metal-organic framework; PL: photoluminescent; iSPR: imaging surface plasmon resonance.

01 + 0.1 μg/mL, 0.03 + 0.05 μg/mL and 0.03 + 0.1 μg/mL) were used to estimate the cytotoxic interactions of AFB_1 and ZEN. As observed in Fig. 5, after exposure to AFB_1 + ZEN mixtures for 5 h, all mixing samples exhibited a significant increase in the level of inhibition rate compared to the simple addition of AFB_1 or ZEN separately. Thus, a synergistic toxicity effect is observed between AFB_1 and ZEN when using *E. coli* as the recognition element. In general, this result is consistent with the reported combined effects of AFB_1 and ZEN [27,40]. However, some previous studies reported an antagonistic effect of AFB_1 and ZEN that may be attributed to the differential sensitivity and experimental conditions [7,41]. Therefore, further studies are still needed to reveal the underlying mechanisms.

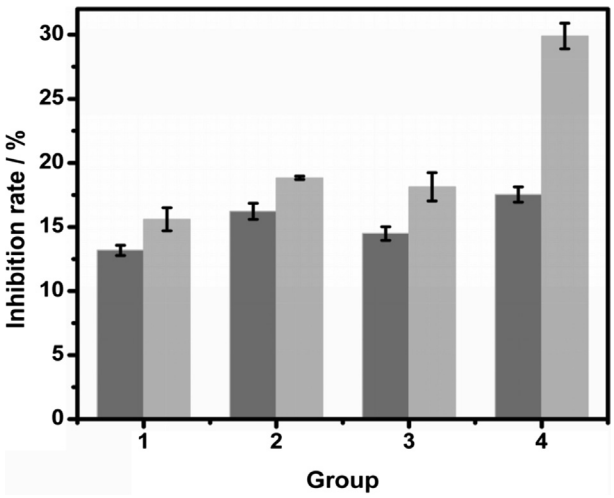


Fig. 5. Histogram of the estimated and detected combined toxicity of AFB_1 + ZEN; group 1 to 4 represent mixtures of 0.01 + 0.05 μg/mL, 0.01 + 0.1 μg/mL, 0.03 + 0.05 μg/mL and 0.03 + 0.1 μg/mL AFB_1 + ZEN, respectively. Grey: estimated values, Light grey: measured values.

3.4. Toxicity mechanism of mycotoxins to E. coli

Since methanol is often used to extract the mycotoxin from many contaminated agricultural samples, methanol was chosen as the solvent to dissolve AFB_1 and ZEN. Thus, before the study of the mycotoxin toxicity mechanism with *E. coli,* the morphology of the bacterial cells after the treatment with methanol was first characterized (Fig. S2). The result shows that the structure of *E. coli* treated with methanol for 3 h still maintains a rod shape similar to that of normal *E. coli*. Moreover, the results obtained have demonstrated (Fig. S3) that there is no significant difference in the electrochemical response of samples treated with methanol or PBS. The above results indicate that a small amount of methanol has no significant effect on the morphology and activity of *E. coli*.

To explore the underlying mechanism of AFB_1 and ZEN on *E. coli*, the cell structure change was captured by SEM. As shown in Fig. 6 (a), normal *E. coli* cells exhibit a rod-like structure and an uneven surface. After co-cultivation with AFB_1 or ZEN, the surface morphology of *E. coli* changes significantly. An obvious perforation and cell collapse (Fig. 6(b) and (c)) can be observed, which means the AFB_1 and ZEN may destroy the cell wall of *E. coli* and inhibit *E. coli* cell respiratory activity.

Penicillin has been reported to have a similar wall-breaking effect on *E. coli* [42], and AFB_1, ZEN and penicillin are all metabolites of fungi. The effects of penicillin on the morphology of *E. coli* are due to the competitive reaction of the penicillin molecule and the enzyme substrate with the enzyme active centres [43]. The reactive groups in penicillin bind to the penicillin-binding protein on the cell membrane, which causes the reticulocyte wall to fail to form properly [44] and leads to the changes in cell morphology [42]. Moreover, the outer membrane of the *E. coli* surface is composed of both integral and lipid-linked membrane proteins [45,46], and the simulation of electronic cloud distribution (Fig. 7) and Bader charge calculation (Tables S1, S2) shows that the active groups of AFB_1 and ZEN are hydroxyl or ester groups. The activity of these groups is proportional to the difference between the electron density and the number of charges. The specific simulation process is listed in the supporting information. Thus, we hypothesize that the mechanism of AFB_1 and ZEN affecting the morphology of *E. coli* is similar to penicillin. The reactive group on the AFB_1 and ZEN binds to proteins on the membrane by bonding or other interactions, which may destroy the integrity of the reticulocyte wall of *E. coli*. In addition, due to the limited studies on the mechanism of the toxicity of mycotoxins to microorganisms, some other factors such as mycotoxin-induced oxidative stress or the efficiency of the bio-transformative pathways of different kinds of mycotoxins or some specific receptors may also influence the toxicity response of mycotoxins. The detailed mechanism needs further investigation.

3.5. Detection accuracy for standard samples

The accuracy of AFB_1 and ZEN calibration curves were verified by blank recovery experiments. The AFB_1 and ZEN standard solutions with known concentrations were prepared before the electrochemical tests, and the measured concentration is calculated by the calibration curve. The test results of these blank recovery experiments are shown in Tables S3 and S4. The recovery rates of AFB_1 standard samples vary from 94.4% to 101.3%, and the relative standard variations (RSDs) are in the range of 1.89–6.15%. For mycotoxin ZEN, the recovery rates are from 102.4% to 106.0% with the RSD in the range of 4.24–11.07%. These results confirmed the accuracy of the linear fitting curves of AFB_1 and ZEN and laid a foundation for the further detection of these two mycotoxins in real samples.

3.6. Practical application in oil samples

The feasibility of biosensor described above for toxicity detection of AFB_1 and ZEN in real oil samples was verified by recovery assay. Mycotoxin at a specific concentration was added to the pre-treated oil samples, and the actual obtained concentration was calculated by a calibration curve based on the current value. As listed in Table 2, the recovery rates for AFB_1 in peanut oil were from 90% to 112%, and the RSD values were in the range of 0.34–8.99%. The recovery rates in corn oil varied from 98% to 108% with the RSD between 3.06 and 5.99%. The biosensor also showed good sensitivity for ZEN (Table 3), as the recovery rates in peanut oil ranged from 92% to 94.3% and the RSD values ranged between 3.44

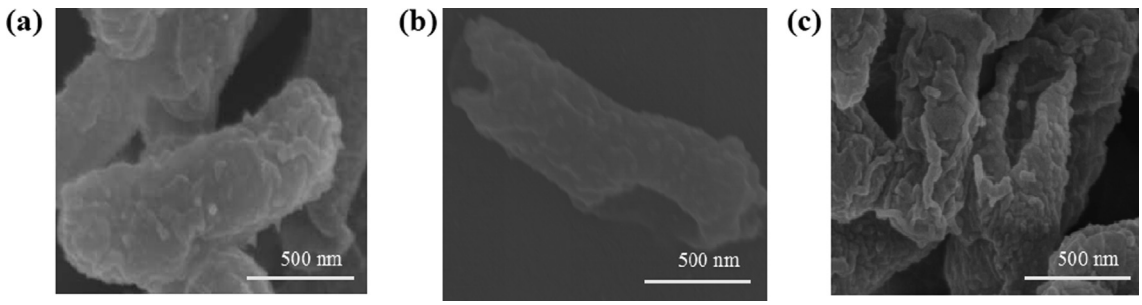


Fig. 6. Images of *E. coli* cellular morphology. (a) Normal *E. coli* cells; (b) *E. coli* treated with AFB_1 for 5 h; (c) *E. coli* treated with ZEN for 5 h.

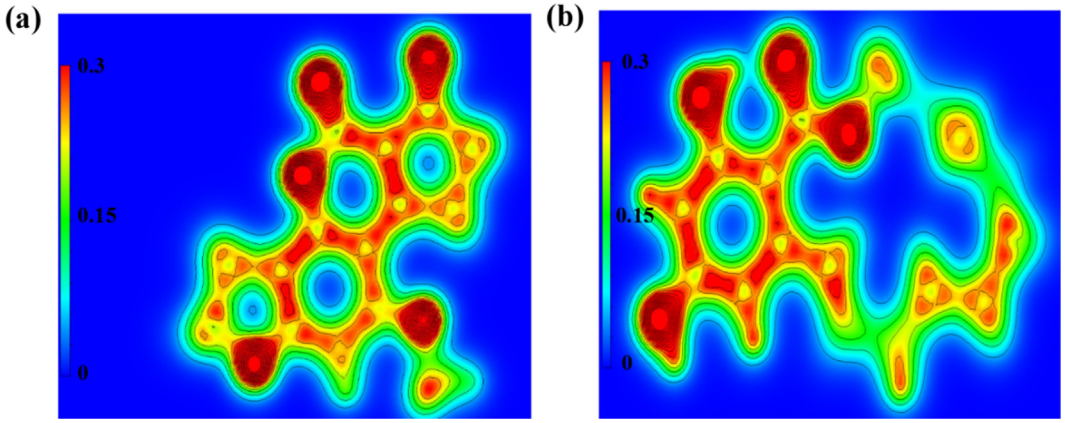


Fig. 7. Simulation images of electronic distribution of AFB_1 and ZEN. (a) electronic cloud distribution of AFB_1 (b) electronic cloud distribution of ZEN.

Table 2
Recovery of AFB_1 from peanut oil and corn oil.

Sample	Added AFB_1 (μg/mL)	Found (μg/mL)	Recovery (%)	RSD (%, n = 3)
peanut oil	0.01	0.009	90	2.12
	0.05	0.056	112	8.99
	0.25	0.241	96.4	0.34
corn oil	0.02	0.020	100	3.12
	0.05	0.054	108	5.99
	0.25	0.245	98	3.06

Table 3
Recovery of ZEN from peanut oil and corn oil.

Sample	Added ZEN (μg/mL)	Found (μg/mL)	Recovery (%)	RSD (%, n = 3)
peanut oil	0.05	0.047	94	11.93
	0.2	0.184	92	3.96
	0.3	0.283	94.3	3.44
corn oil	0.05	0.048	96	11.63
	0.3	0.286	95.3	9.84
	0.5	0.521	104.2	4.95

and 11.93%. For corn oil, the recovery rates varied from 95.3% to 104.2%, and RSD values were in the range of 4.95–11.63%. The above results exhibited good accuracy for the *E. coli*-based biosensor in real oil sample mycotoxin detection, indicating a great potential for this method to be applied in food and feed detection in daily life.

4. Conclusions

In this study, a sensitive and convenient electrochemical biosensor based on *E. coli* was developed for the toxicity detection of mycotoxins AFB_1 and ZEN. This is the first time that *E. coli* bacteria were used as the recognition element for the detection of mycotoxins. A two-step reaction strategy was applied to the biosensor design to eliminate the mutual interference of mediator, analyte and buffer solution. The above biosensor exhibited good sensitivity in standard sample detection and excellent accuracy in real oil sample analysis. The toxicity inhibition effect of AFB_1 and ZEN on *E. coli* can better reflect the harm of mycotoxins to individual organisms. Moreover, the synergistic toxic effect of the AFB_1 and ZEN mixtures also provides a warning that the co-existence of more than one mycotoxin may cause more severe damage. This work may provide a new way for multiple mycotoxin toxicity evaluation in food and feed by using bacteria as the biological recognition element.

Declaration of Competing Interest

The authors declare they have no conflicts of interest to disclose, have full control of all the primary data and consent to the journal reviewing their data if requested.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioelechem.2019.107453.

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