Supporting information

Profiling of co-metabolic degradation for tetracycline by the bio-cathode in microbial fuel cells

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Materials and Methods

Degradation experiment of TEC by MFC bio-cathode

TEC was applied to quantify the degradation activity of MFC bio-cathode. In this paper, initial concentration of TEC, type of carbon sources, concentration of carbon sources, and aeration strength were the investigated factors that influenced TEC degradation of MFC bio-cathode. The initial concentration array was set as 2, 5, 10, 20 and 30 mg/L. The selected carbon sources were sodium acetate, glucose, sodium bicarbonate and sodium acetate/sodium bicarbonate complex. The concentration array of carbon source (sodium acetate) was set as 0, 0.2, 0.5, 0.7 and 1.0 g/L. The aeration strength array was set as 0, 6, 12, 18 and 24 L/h.

The process of each degradation experiment at different conditions was basically the same. Take the experiment of initial concentration as an example. Firstly, add TECs at demanded concentration into the cathode chambers of five operational MFC reactors. Next, replenish the catholyte to prevent the reactor from drying up. Then, at given time intervals (4 h), 1 mL aliquot was sampled and filtered through 0.22 μm membranes for HPLC analysis, containing a BEH C18 column and a UV–vis detector. Acetonitrile and 0.01 mol/L oxalic acid (31:69, v/v) were used as the mobile phase to elute TEC from the HPLC columns at a flow rate of 1 mL/min. The temperature of oven was set at 30 °C. The detecting wavelength was 355 nm and the sample volume injected was 20 μL. **All the samples were obtained by at least three times of experiments to reduce errors.**

Determination of TEC intermediates

Degradation products were determined with Thermo Fisher Scientific Vanqiush liquid chromatography tandem Q Exactive Plus mass spectrometry (LC-MS/MS) equipped with an electrospray ionization (ESI). HPLC column used was BEH-C18. The HESI source parameters were set as follows: negative ion mode (ESI-), 320°C capillary temperature, 1 L/min gas flow rate, 41°C sheath gas heater, 6 L/min sheath gas flow, 3000 V spray voltage. The mobile phase was composed of methanol (solvent A) and ultrapure water (solvent C). The test flow rate was 0.3 mL/min and the taken volume was 10 µL. The product ion scan range was set as 50-500 m/z.

Analysis of TEC degradation process

To in-depth study the mechanism of TEC degradation on MFC bio-cathode, it was essential to distinguish the contributions of adsorption[1], hydrolysis[2], bioelectrochemical degradation[3] and biodegradation to TEC degradation on bio-cathode. Firstly, the MFC containing cathode liquid only was run to explore the hydrolysis of TEC in the cathode chamber. Next, the control group containing inactivated microbes was used to explore the adsorption of carbon felt and microbes for TEC. Then, the open circuited MFC was used to explore the effect of microbial activities on TEC degradation. Finally, the MFC bio-cathode under normal conditions was run as a reference. All the experiments were carried out under the conditions that initial concentration of TEC was 10 mg/L; concentration of sodium acetate was 0.5 g/L; aeration rate was 12 L/h.

Analysis and measurement methods

Toxicity analysis

The initial concentration of TEC was set as 10 mg/L and the samples were collected at the degradation time of 0 h, 12 h, 24 h and 36 h. These samples were coated on LB agar medium respectively. Then, the *Escherichia coli* (*E. coli*) in the period of logarithmic growth were diluted to 7-10 times and were coated on LB agar medium. After 12-hour culture in a biochemical incubator, colony count was carried out and the values between 30 and 300 were valid.

In this experiment, normal saline without TEC was set as blank group. The relative inhibition rate over *E. coli* treated in blank group was used to characterize the toxicity of the samples.

Data analysis methods

The calculations of degradation rate and kinetics for TEC are shown in Equations (1) and (2):

$$Degradation\ (\%) = \left(1 - \frac{C_t}{C_0}\right) \times 100\% \quad (1)$$

$$\ln\left(\frac{C_t}{C_0}\right) = -\ k_{obs} \times t \quad (2)$$

where, C_0 is the initial concentration of TEC in the whole reaction system; C_t is the concentration of TEC after the reaction time t; k_{obs} is the reaction rate constant.

The calculation of the growth inhibition rate of *E. coli* is shown in Equation (3):

$$Growth\ inhibition\ rate\ (\%) = \frac{(N_{blank} - N_{sample})}{N_{blank}} \times 100\% \quad (3)$$

All the experiments were repeated for three times and the average value was taken as the result. The experimental data and the error analysis were calculated using the software Microsoft Excel and Origin 2018, and then drew diagrams.

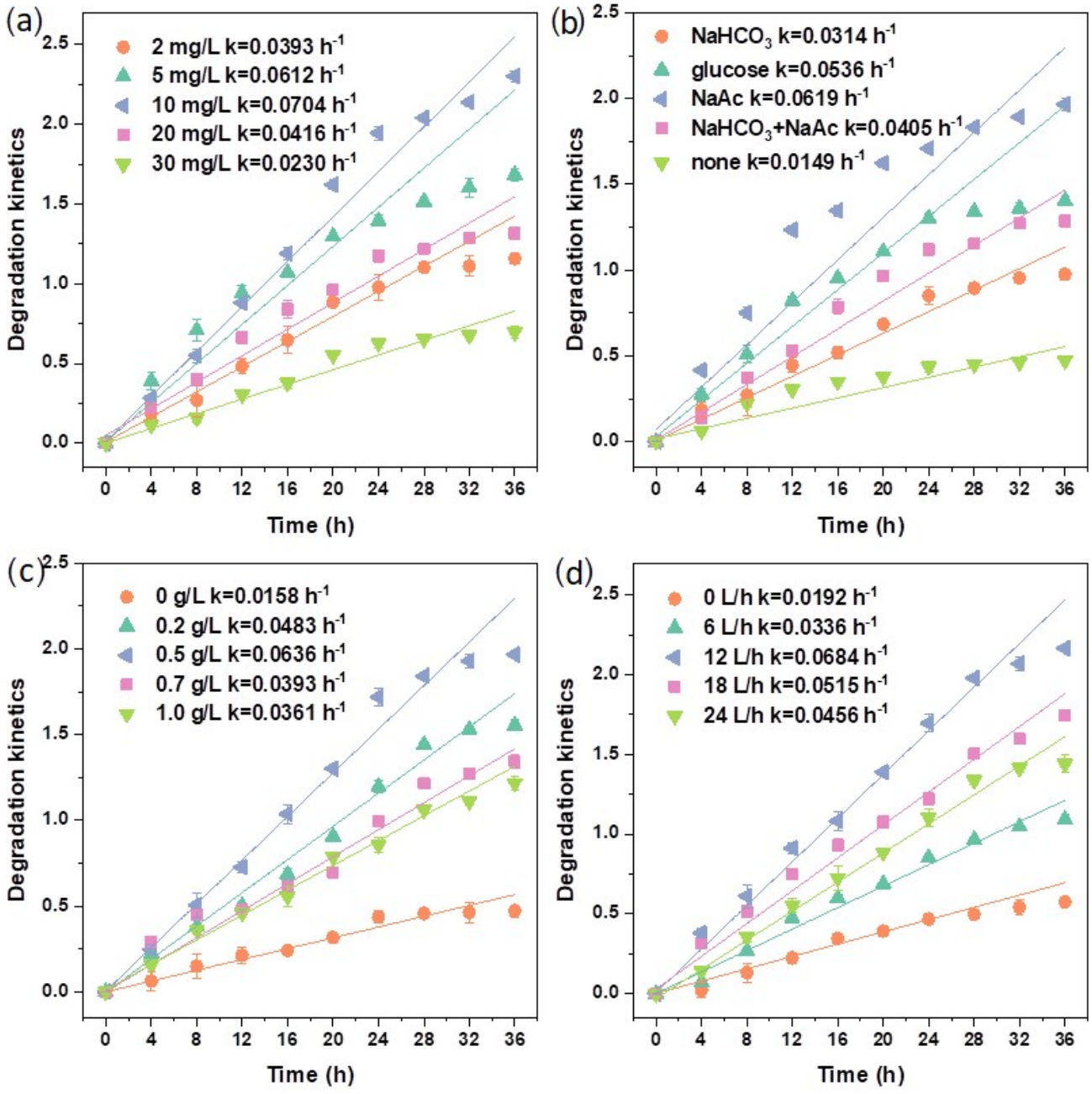


Fig. S1. Kinetics fitting curve: (a) initial concentration, (b) type of carbon sources, (c) concentration of carbon sources, (d) aeration intensity.

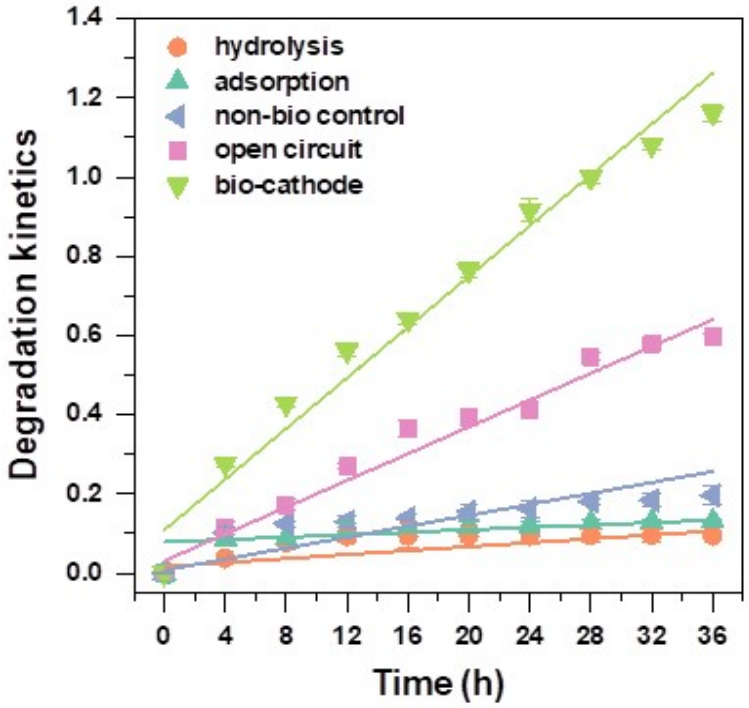


Fig. S2. Kinetics fitting curve of degradation processes in different units.

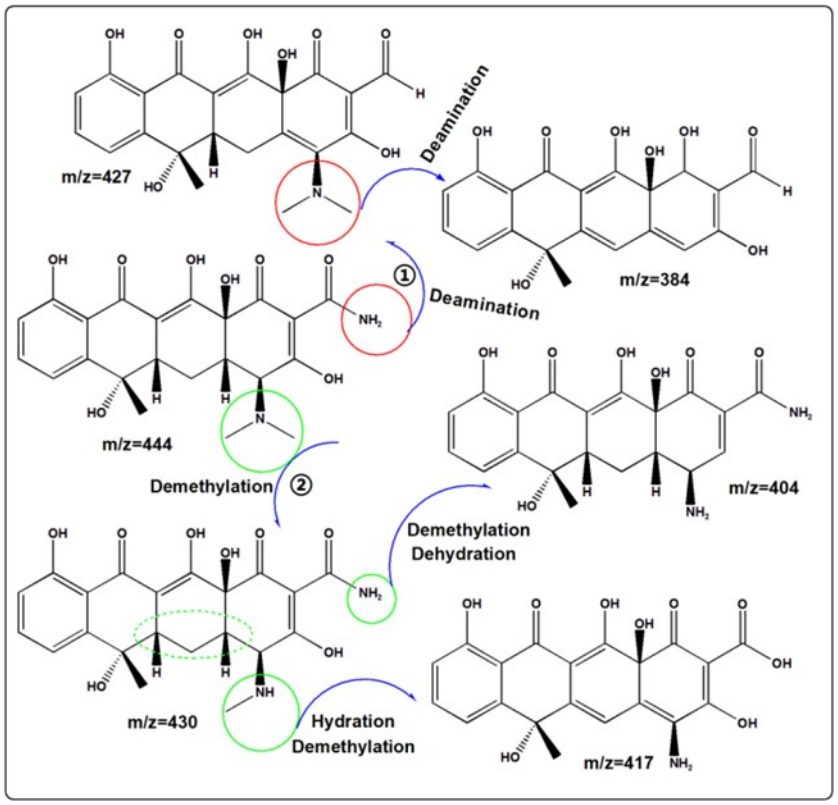


Fig. S3. Degradation pathways of TEC on MFC bio-cathode.

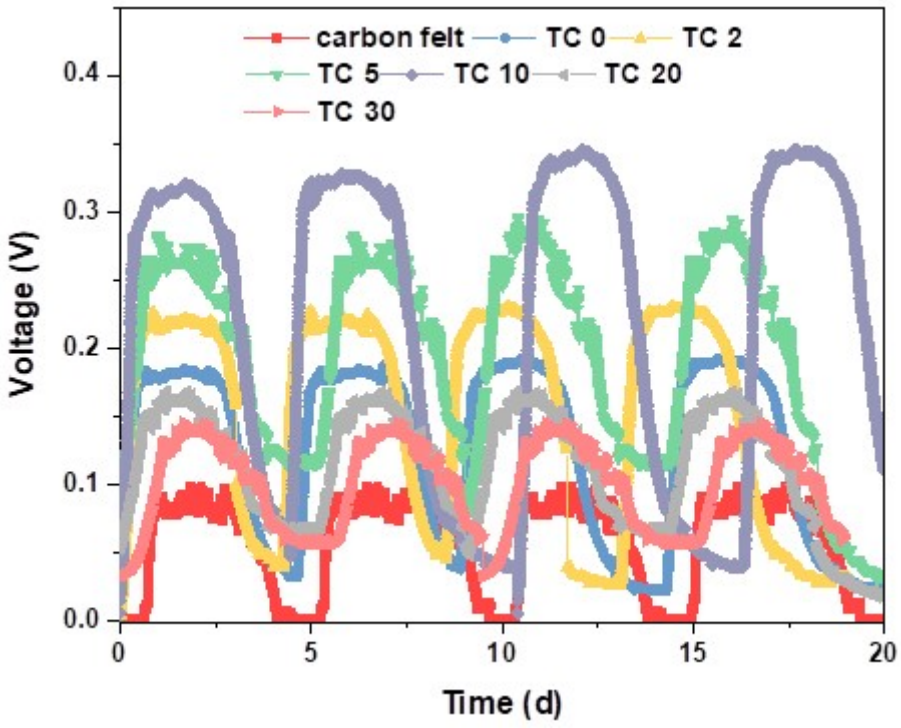
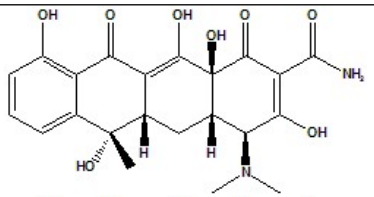
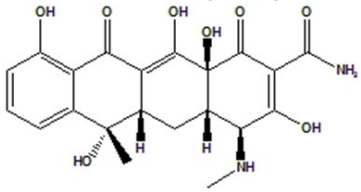
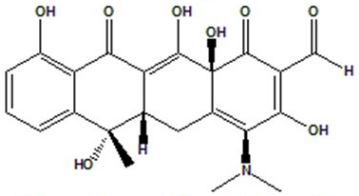
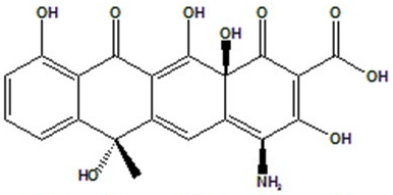
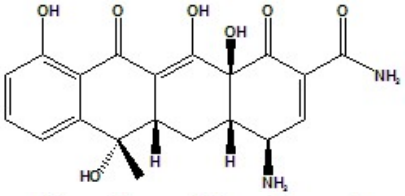
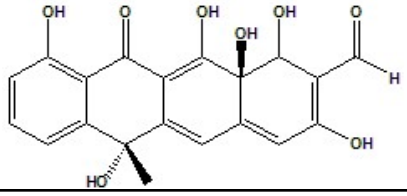


Fig. S4. Electricity generation curves of bio-cathodes at different TEC concentrations.

Table S1. List of main intermediates of TEC degradation.

Name	Chemical structure	Retention time (min)	Molecular weight (MW)
TEC (A444)		1.43	444
B430		1.76	430
C427		1.87	427
D417		2.46	417
E404		2.76	404
F384		4.09	384

Reference

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