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Kinetic study on the cometabolic degradation of 17β-estradiol and 17α-ethinylestradiol by an *Acinetobacter* sp. strain isolated from activated sludge

Wei Cai, Yi Li*, Lihua Niu*, Qing Wang, Yue Wu

Key Laboratory of Integrated Regulation and Resource Development on Shallow Lakes, Ministry of Education, College of Environment, Hohai University, Xikang Road #1, Nanjing 210098, P.R. China, Tel. +86 015151828685; Fax: +86 25 83786251; email: helloweicai@gmail.com (W. Cai), Tel. +86 013776417768; Fax: +86 25 83786251; email: envly@hhu.edu.cn (Y. Li), Tel. +86 018251920036; Fax: +86 25 83786251; email: Nlhnq55@163.com (L. Niu), Tel. +86 015805178499; Fax: +86 25 83786251; email: 349038658@qq.com (Q. Wang), Tel. +86 018751958597; Fax: +86 25 83786251; email: 251090723@qq.com (Y. Wue)

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ABSTRACT

A bacterial strain has been isolated from activated sludge and was demonstrated to be capable of cometabolic biodegradation of 17β-estradiol (E2) and 17α-ethinylestradiol (EE2). Strain LY1, identified as *Acinetobacter*, can use E2 as the sole carbon source, while was unable to grow on EE2. However, with E2 (1–60 mg/L) as the growth substrate, the strain was capable of cometabolizing EE2 (1–20 mg/L) under the concentration ratio of EE2/E2 varying from 0.25 to 5.00. It was observed that the EE2 removal efficiency increased with the increasing excess of E2, whereas the E2 removal efficiency decreased with the increasing amounts of EE2. The kinetic model of cometabolic biodegradation of E2 and EE2 was proposed on the basis of our previous studies for the first time, considering the substrate interaction occurring, and satisfactorily predicted the cometabolic degradation progress with correlation coefficient (R^2) larger than 0.90 in batches.

Keywords: Cometabolism; Biodegradation; 17β-estradiol; 17α-ethinylestradiol; *Acinetobacter*; Model

1. Introduction

Endocrine disrupting compounds (EDCs), particularly natural and synthetic estrogens such as 17β-estradiol (E2) and 17α-ethinylestradiol (EE2), are the major issues of increasing public concern since the exposure of these compounds may adversely affect wildlife survival [1]. These estrogens of high estrogenic activity are continuously released into the environment mainly through wastewater treatment plant (WWTP) effluents, agricultural sewage sludge, and surface non-point run-off of manure [2]. Due to incomplete removal during the waste treatment process, estrogen residues are being detected in surface water, groundwater, sediment, and aquatic biota across the world [3]. It has been reported that E2 and EE2 of low concentration ranging from 0.1 to 1 ng/L can produce reproductive disorders in aquatic organisms [4]. As for their high bioactivity, ubiquitous nature and toxicity, it is of great importance to investigate and study pathways to degrade these substances in the environment.

Among different degradation pathways of estrogens, biodegradation, especially cometabolism was suggested to be an important removal mechanism

*Corresponding authors.

which is cost-effective and eco-friendly during the wastewater treatment processes [5–7]. A number of studies have reported that different types of bacteria were able to degrade estrogen. The first evidence of estrogen mineralization was reported by Layton et al. [8]. Several bacteria capable of degrading both E2 and EE2 at μg/L to mg/L were then isolated from activated sludge, compost, and soil, belonging to *Novosphingobium*, *Rhodococcus zopfii*, *Rhodococcus equi*, *Pseudomonas aeruginosa*, *Phyllobacterium myrsinacearum*, *Acinetobacter*, *Ralstonia picktettii*, *Sphingobacterium*, and *Nitrosomonas europaea* [4,9–12]. To the best of our knowledge, only one previous study by Pauwels et al. [4] has investigated the cometabolic biodegradation of E2 and EE2 concentrations by six strains (among the above-mentioned bacteria) isolated from compost. The first-order kinetic equations were established to describe the degradation progress. However, further research on the cometabolic degradation dynamics has not been conducted.

Cometabolism acts as a superior biological mechanism to degrade many compounds of environmental and toxicological significance. It is catalyzed by existing microbial enzymes and yields no carbon or energy benefits to the transforming cells. Leadbetter and Forster [13] firstly addressed cometabolic mechanism for oxidation of ethane by methane-utilizing bacteria, after which cometabolic degradation has been investigated to tap the potential of the application to environmental systems for biologically resistant compound treatment [14–16]. Cometabolic degradation kinetics can be of major practical importance for better understanding of degradation reaction and bioremediation application. Besides, the cometabolic degradation model developed based on the degradation kinetics can also be a vital point for planning and monitoring site bioremediation [17]. Several models have been proposed on the cometabolism of synthetic and toxic organic chemicals [18,19]. However, there was no specific model that comprehensively depicted the cometabolic biodegradation of E2 and EE2.

Therefore, the main objective of the present study was to investigate the cometabolic kinetics of E2 and EE2 by a strain of bacteria isolated from activated sludge. The strain was identified and the cometabolic degradation of E2 and EE2 under different concentrations was studied. In addition, a kinetic model was developed based on the interaction between E2 and EE2 to describe the cometabolic degradation progress, which may contribute to providing a theoretical basis for the practical bioremediation application of the cometabolism of E2 and EE2.

2. Material and methods

2.1. Enrichment, isolation, and cultivation of the bacterial strain

The estrogen-degrading strain was obtained using enrichment culture of activated sludge samples collected from four WWTPs in Nanjing, China. The mineral salts (MS) medium contained (per liter) 7 g $Na_2HPO_4{\cdot}12H_2O$, 1 g KH_2PO_4, 10 mg $CaCl_2{\cdot}2H_2O$, 2 mg $FeCl_3$ and 20 mg $MgSO_4{\cdot}7H_2O$. These sludge samples were used as seeds of the enrichment culture and then mixed with MS medium. The culture was grown at 28˚C with a pH of approximately 7.0. A 250-mL Erlenmeyer flask containing 100 mL of the MS medium with 2 mg/L E2 and 2 mg/L EE2 as carbon and energy source was inoculated with 20 mL of activated sludge and placed on shaker at 28˚C for two week. Liquid culture above was then picked and spread out on MS medium plates with E2 and EE2 as the carbon source at 28˚C for another two weeks. Single colonies that developed on the plates were picked and inoculated into MS medium plates. The process was repeated until a pure culture was obtained. After enrichment culture, 1-mL sample of liquid culture was diluted tenfold with ultrapure water and plated on MS medium plates containing 10 mg/L EE2 and 10 mg/L E2, which was then subsequently cultured at 28˚C for 24 h by shaking (aerobically) to yield the strain. Medium with no substrate was used as a control in parallel cultivation.

2.2. E2-degrading capacity of the isolated strain

Liquid cultures of strain LY1 (an initial optical density OD_{600} of 0.01) were exposed to E2 as the only carbon and energy source. Then, 5 mL of a liquid culture was inoculated in 200-mL MS medium in a 250-mL Erlenmeyer flask. Table 1 summarizes the biodegradation batch experiments of Group A, B, and C. The experimental tests of Group A were conducted with 6 flasks as described above containing E2 of initial concentration 1, 2, 5, 10, 20, and 60 mg/L, respectively. All the tests were repeated in duplicate. These flasks were wrapped in aluminum foil and incubated at 28˚C on a rotary shaker (100 rpm). The E2 concentration was monitored over time (0, 9, 18, 27, 36, 45, 54, 63, and 72 h) using HPLC with UV and fluorescence detection. The cell concentration was also determined periodically.

Table 1
Summary of tests of biodegradation

Tests	Approximate concentration ratio of EE2/E2	Initial EE2 concentration (mg/L)	Initial E2 concentration (mg/L)
A1	0/1	0	1.08
A2	0/2	0	2.11
A3	0/5	0	4.77
A4	0/10	0	9.83
A5	0/20	0	20.43
A6	0/60	0	61.28
B1	1/0	1.12	0
B2	2/0	1.94	0
B3	6/0	6.12	0
B4	10/0	10.23	0
B5	15/0	15.37	0
B6	20/0	19.89	0
C1	5/1	5.05	1.03
C2	5/5	5.05	5.11
C3	5/15	5.05	15.08
C4	5/25	5.05	25.01
C5	20/5	20.07	5.05
C6	20/25	20.07	24.89
C7	20/45	20.07	45.10
C8	20/60	20.07	59.30

2.3. EE2-degrading capacity by the isolated strain

Analogous to the procedure as above, liquid culture of the isolated strain was exposed to EE2. The tests of EE2 metabolic degradation by strain LY1 (OD_{600}=0.01) were also conducted in 6 250-mL Erlenmeyer flasks. The samples of experimental Group B containing 1, 2, 6,10, 15, and 20 mg/L EE2, respectively, were incubated in 200-mL MS medium in the flasks at 28˚C at 100 rpm on the shaker for 72 h. Liquid samples were collected over time and analyzed for estrogen and cell concentrations as described. All degradation tests were performed in duplicate.

2.4. Identification of the isolated strain by 16S rDNA sequences

Phenotypic and biochemical characterization of strain LY1 was done by standard techniques according to diagnostic table of Cowan and Steel [20] and Bergey's Manual of Determinative Bacteriology [21]. DNA of the isolated strain was extracted with a FastDNA spin kit for soil (Bio 101; Qbiogene, Inc., Carlsbad, CA) as described in the manufacturer's instructions. BOX-PCR fingerprints were obtained with the BOXA1R primer as described by to Seurinck et al. [22], from which one isolate strain was determined to obtain the unique BOX-PCR patterns. The 16S rRNA gene fragments were amplified from the extracted total DNA with primers P63f and P1378r [23]. ITT Biotech-Bioservice (Bielefeld, Germany) could conduct the DNA sequencing of the PCR fragments. DNA sequences and homology searches were analyzed with standard DNA sequencing programs and the BLAST server of the National Center for Biotechnology Information (NCBI) [22].

2.5. Cometabolic degradation of E2 and EE2

Substrate cometabolic utilization kinetic tests were setup in a series of 250-mL Erlenmeyer flasks. The experimental tests of Group C were conducted with a series of strain LY1 cultures (OD_{600} = 0.01) under different initial concentrations of substrates (Fig. 1). A 5 mL of liquid strain LY1 culture in minimal medium from the previous experiment was inoculated in 200-mL MS medium in every flask. The initial concentrations of E2 ranged from 1 to 60 mg/L and for EE2, the concentrations ranged from 1 to 20 mg/L. After the addition of cell suspension, the flasks were wrapped with aluminum foil and shaken with a rotary shaker (100 rpm) in dark at 28˚C. It was monitored over time sacrificed for estrogen analyses for 72 h. All the experiments were performed in duplicate.

2.6. Analytical methods

A sample of 3 mL was taken from each test at different time intervals for analysis. The concentration of

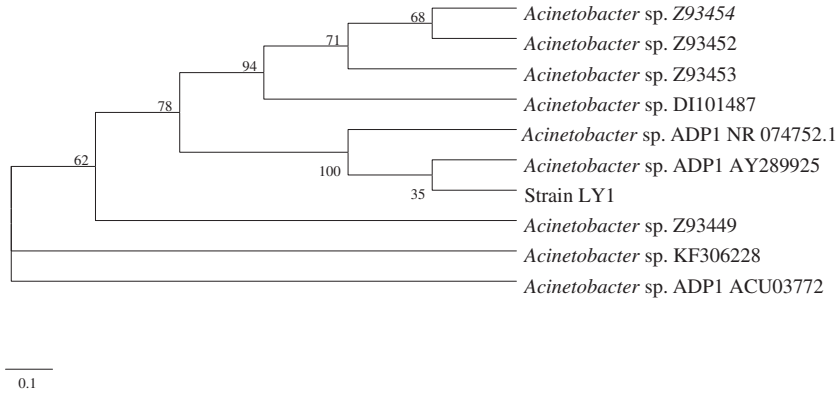

Fig. 1. Phylogenetic tree based on 16S rRNA sequence analysis. The tree constructed using neighbor-joining method with bootstrapping 1,000.

cell growth was determined from optical density (OD) measurements at 600 nm using a Xinmao ultraviolet spectrophotometer UV-7504. The following formula was used to obtain the dry cell density: DCW (mg/L) = 314.5 × OD_{600}. The concentration of E2 and EE2 was measured using a Dionex HPLC (Sunnyvale, CA, USA) with a RF2000 fluorescence detector. The detection limit for the two estrogens was 10 μg/L and column temperature was set at 28˚C. Two kinds of solvent were prepared. One was acetonitrile–acetate (100:0.1 v/v, both Sigma, Bornem, Belgium) served as solvent A, and the other was water–acetate (100:0.1 v/v) served as solvent B. The columns were eluted as follows: the solvent composition was 45% A and 55% B at the beginning, while it has been decreased to 35% B in 10 min. A linear increase was made to 57% B up to 15 min, followed by an isocratic elution kept up to 20 min. For all samples, fluorescence was measured at excitation/emission wavelengths of 230 nm/290 nm.

3. Results and discussion

3.1. Phylogenesis determination of strain LY1

An estrogen-degrading bacterium, designated as strain LY1, was successfully isolated from activated sludge. The morphological and physiochemical characteristics of the strain are shown in Table 2. The phylogenetic tree constructed for strain LY1 and related strains by the neighbor-joining method with bootstrapping 1,000 is shown in Fig. 1. The 16S rRNA sequence obtained from strain LY1 (about 1,400 bases) was identical to that of a registered strain *Acinetobacter* (sp. ADP1 no. AY289925) with 99.9% similarity. Thus, the strain was identified as *Acinetobacter* sp. *Acinetobacter* species have been previously described as the degrading bacterium capable of totally cometabolizing EE2 in the presence of E1, E2, and E3 [4]. Furthermore, they were also well known for their capacity in the cometabolism of 3- and 4-chlorophenol [24], phenol and chlorophenol [25], and 3, 4-dichlorobenzoate and 4-chlorobenzoate [26].

3.2. Biodegradation of E2 as the sole substrate

Fig. 2 shows the degradation process of E2 under different initial concentrations from 1 to 60 mg/L. It was found that E2 was nearly biodegraded within 72 h by strain LY1 for all the tests, which indicated high E2-alone degradation activity of the strain. In the study of Pauwels et al. [4], a faster degradation rate of E2 at an initial concentration of 5 mg/L was achieved. None of E2 could be detected within 48 h in the culture media of six strains isolated, including two *Acinetobacter* genera. Fig. 3(A) shows the time-dependent curves of cell growth and E2 degradation when E2 was used as the sole carbon sources at an initial concentration of 5 mg/L. Cell grew simultaneously with the transformation of E2 occurring and was identified as an exponential biodegradation phase, followed by a decline of cell concentration as E2 was depleted. These results indicated that E2 can be utilized as the sole carbon source by strain LY1.

3.3. Biodegradation of EE2 as the sole substrate

In tests with EE2 alone at concentration ranging from 1 to 20 mg/L, no EE2 removal was observed even after 100 h. While in the abiotic control, it showed no major changes in EE2 concentration. These results may be due to that EE2 was not the growth substrate for strain LY1 and thus contributed little to the cell growth. Briefly, it indicated that strain LY1

Table 2
Taxonomic description of *Acinetobacter* sp. LY1

Tests	Results	Tests	Results
Colony morphology		Carbohydrate utilization tests	
Configuration	Circular	Lactose	+
Margin	Entire	Xylose	+
Elevation	Raised	Maltose	+
Surface	Rough	Fructose	+
Density	Opaque	Dextrose	+
Pigments	Orange	Galactose	+
Gram' reaction	Negative	Raffinose	+
Shape	Rods	Trehalose	+
Size	Moderate	Melibiose	+
Arrangement	Single	Sucrose	+
Motility	+	L-arabinose	+
Fluorescence	–	Mannose	+
Growth at temperature (℃)		Inulin	–
20	+	Sodium gluconate	+
30	+	Glycerol	–
37	+	Salicin	+
Growth at pH		Glucosamine	+
6.0	+	Dulcitol	–
7.0	+	Sorbitol	+
8.0	+	Mannitol	+
9.0	+	Adonitol	+
10.0	–	α-Methyl-D-glucoside	+
Biochemical tests		Ribose	+
Hydrolysis of		Rhamnose	+
Tween 20	–	Melezitose	+
Tween 80	+	Cellobiose	+
Lipid	+	α-Methyl-D-mannoside	+
Starch	–	Xylitol	+
Casein	–	ONPG	–
MacConkey agar	–	Esculin	–
Oxidase	–	D-arabinose	+
Catalase	+	Citrate	+
Oxidation/fermentation (O/F)	–	Malonate	+
Gelatin liquefaction	–	Sorbose	+

cannot grow on EE2 as the sole carbon source. Consistently, no removal of EE2 was observed with an initial EE2 concentration of 1 mg/L for at least 500 h by the culture media of the genera of *Phyllobacterium*, *Ralstonia*, *Pseudomonas* and *Acinetobacter* [4].

3.4. Cometabolic degradation of E2 and EE2

3.4.1. Effect of E2 concentration on EE2 biodegradability

In Fig. 3(B), it is shown that the existence of E2 was likely beneficial to EE2 degradation as an EE2 removal up to 93.7% was observed in 72 h. Fig. 4 shows the degradation performance when E2 and EE2 coexisted under different initial concentrations. In Fig. 4(B), with EE2 at an initial concentration of 5 mg/L, it was observed that EE2 removal efficiency increased from 8.9 to 93.8% as the initial concentration of E2 increased from 1 to 25 mg/L. Similar results were also observed at an initial EE2 concentration of 20 mg/L. Pauwels et al. [4] concluded that the EE2/E2 concentration ratio was significant for the occurrence of EE2 biodegradation, and a higher EE2/E2 ratio tended to result in a lower overall degradation efficiency of EE2. As shown in Fig. 4(B), under the same EE2/E2 concentration ratio of 1/3, EE2 removal efficiency showed an improvement from 70.6 to 83.2% with the increasing initial E2 concentration from 15 to 60 mg/L, which suggested that high concentration of

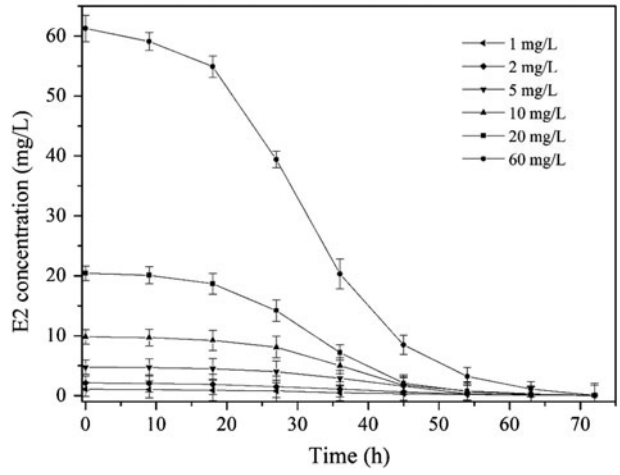

Fig. 2. Degradation profile of E2 as the sole substrate under different initial concentrations.

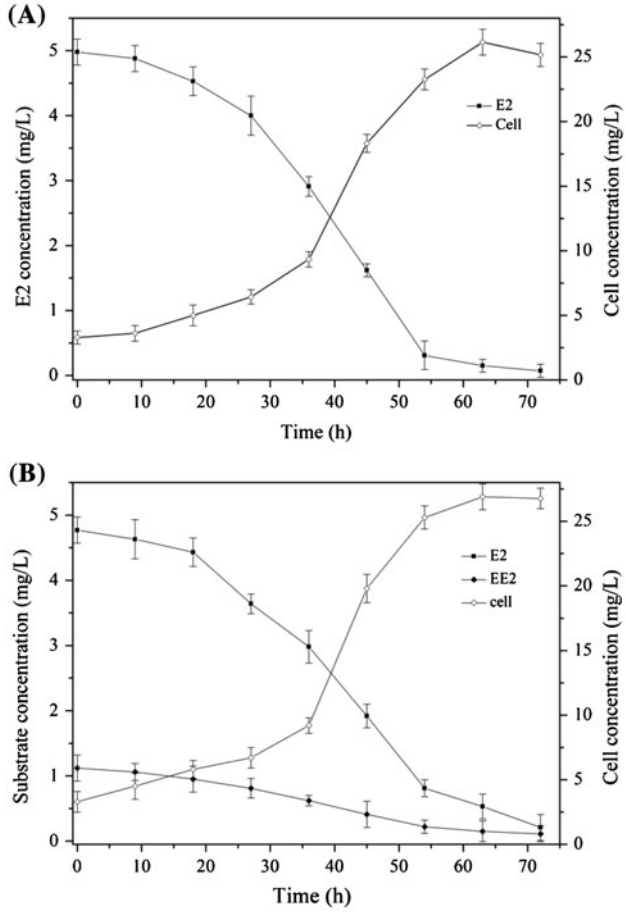

Fig. 3. Time-dependent curves of cell growth and different substrates as carbon sources: (A) E2 (the initial concentration of 5 mg/L); (B) E2 (the initial concentration of 5 mg/L) and EE2 (the initial concentration of 1 mg/L).

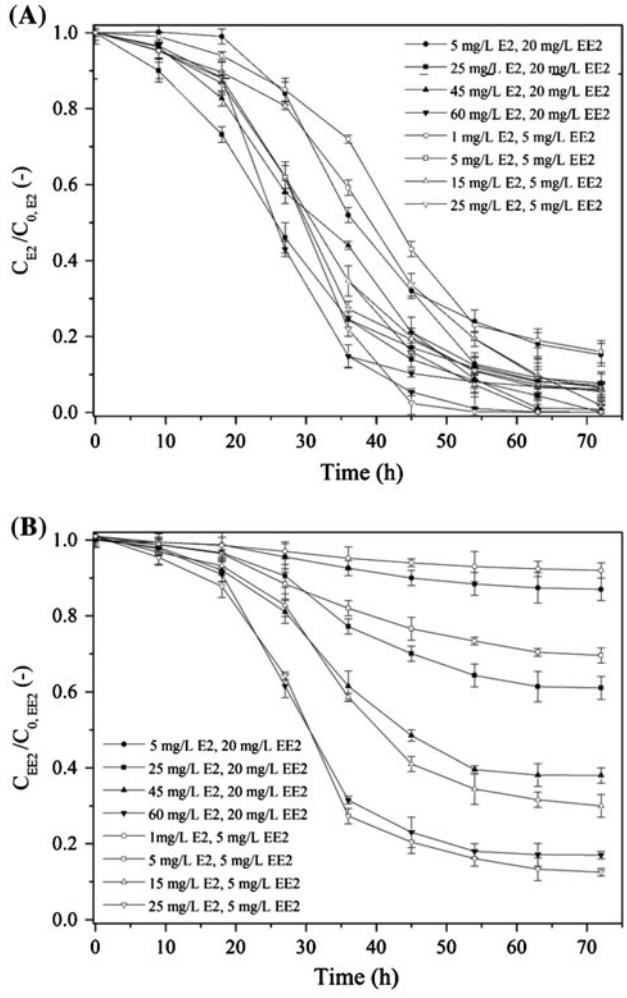

Fig. 4. Cometabolic degradation of E2 and EE2 by strain LY1 under different initial concentrations of E2 and EE2.

E2 might promote the EE2 biodegradation. Therefore, both the concentration ratio and the initial E2 concentration may have an influence on the EE2 degradation.

3.4.2. Effect of EE2 concentration on E2 biodegradability

Comparisons of the degradation profile over time for tests of single substrate (E2) reaction and cometabolic degradation of E2 and EE2 are shown in Fig. 3. It was observed that at an initial concentration of 4.77 mg/L for E2 conducted as the sole energy source, 99.0% of E2 was exhausted within 72 h (Fig. 3(A)). However, with an initial concentration of 1 mg/L for EE2, the degradation efficiency of E2 decreased to 94.9% (Fig. 3(B)). According to Fig. 4(A), under the initial E2 concentration of either 5 or

20 mg/L, the E2 removal efficiency showed a decline when the EE2 concentration was increased from 5 to 20 mg/L. It was suggested that EE2 might act as a competitive substrate or an inhibitor of the bacteria activity, thus had the potential to inhibit the biodegradation of E2.

3.5. Model simulation

3.5.1. Degrading kinetics of E2 by strain LY1

A single-specie, single-substrate reaction can be described by the basic mass-balance equations as follows:

$$-\frac{dS_1}{Xdt} = q_s \tag{1}$$

$$-\frac{dX}{Xdt} = q_x \tag{2}$$

To describe utilization kinetics for E2, the Monod kinetics equations introduced with a biomass growth rate were as follows, controlled by synthesis and maintenance yields [27]:

$$q_s = \frac{q_m S_1}{K_s + S_1} \tag{3}$$

$$q_x = \frac{Y_s(-q_s)}{X} - b \tag{4}$$

Combining Eqs. (1)–(4) yields:

$$-\frac{dS_1}{Xdt} = \frac{q_m S_1}{K_s + S_1} \tag{5}$$

$$-\frac{dX}{Xdt} = \frac{Y_s(-\frac{dS_1}{dt})}{X} - b \tag{6}$$

Eq. (6), when the value of b is much smaller that can be neglected compared to $[Y_S(-dS_1/dt)]/X$, is integrated to yield the following equation:

$$-\frac{dX}{dt} = Y_S\left(-\frac{dS_1}{dt}\right) \tag{7}$$

$$X = X_0 + Y_S(S_{10} - S_1) = Y_S S_1 + C \tag{8}$$

As indicated in Eq. (7), a linear relationship exists between biomass and E2 concentrations. The Y_s can be described as follows by rearranging Eq. (8):

$$Y_s = \frac{X - X_0}{S_{10} - S_1} = \frac{\Delta X}{\Delta S} \tag{9}$$

In the kinetic tests, we could plot the experimental data as the q_s value against the S_1 value. By dividing the initial E2 utilization rates over biomass added, the values of q_s were obtained. And the initial substrate utilization rates were also determined by dividing the mass difference of E2 over the incubation period. Over the whole incubation period, no biomass changes were observed. The values of q_m and K_S were determined through curve fitting using Sigmaplot 8.0 (SPSS Inc.) [28].

As shown in Fig. 5, both K_S and q_m could be estimated by nonlinear regression. The results showed that K_S and q_m were 3.163 mg/L and 0.452 h^{-1}, respectively, under 95% confident interval (R^2 was observed to be 0.97). Then the following model was acquired:

$$q_s = \frac{0.452 S_1}{3.163 + S_1} \tag{10}$$

3.5.2. Cometabolism model of EE2 degradation by strain LY1

Table 3 summarizes the detailed cometabolic degradation data. The observation leads to two conclusions. First, the values of q_0 were always higher than q_n, indicating that the presence of EE2 obviously inhibited the process of E2 biodegradation, thus Eq. (10) was not able to satisfy the experimental data

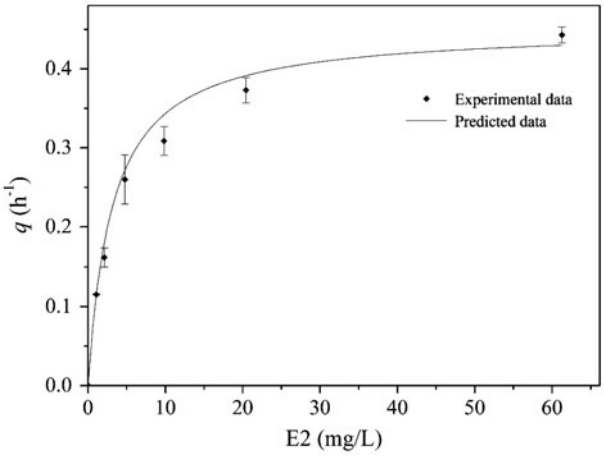

Fig. 5. Specific utilization rate of E2 as the sole substrate by strain LY1.

under cometabolic conditions. Second, it was observed that a higher value of S_2/S_1 resulted in a higher f value, in which degree the specific E2 utilization rate was also related to the concentration ratio of EE2/E2 besides the initial concentration of E2. Actually, it has been reported that the EE2/E2 concentration ratio was of importance for the cometabolic degradation of E2 and EE2 [4]. The relatively higher ratio may lead to a lower degradation efficiency of EE2. In addition, the concentration ratio of substrates was also reported to play a considerable role in the cometabolic degradation of phenol and chlorophenol by *Acinetobacter* species. Hao et al. [25] found that the mass ratio of phenol (growth substrate) and chlorophenol (non-growth substrate) was a deciding factor for the success of the chlorophenol biodegradation in the cometabolic process. The chlorophenol degradation was incomplete at lower mass ratios and a phenol/chlorophenol concentration ratio of 4 was proved to be necessary to guarantee the complete breakdown of chlorophenol.

Therefore, Eq. (10) was thus not suitable to describe the E2 biodegradation in dual-substrate system. According to our previous studies [29], it was probably concluded that the presence of EE2 affected the Monod coefficients q_m and K_S for E2 via interaction terms of the form $\left[1 + Z_1^{n_1}/K_1\right]$, where the function $Z_i = S_2$ or S_2/S_1 (Z_1 is for q_m and Z_2 is for Ks), transform Eq. (3) to:

$$q_s = -\frac{dS_1}{X dt} = \frac{\frac{q_m}{\left[1+\frac{Z_1^{n_1}}{K_1}\right]} S_1}{K_s\left[1 + \frac{Z_2^{n_2}}{K_2}\right] + S_1} \tag{11}$$

It has been reported that the non-growth substrate was transformed through the electrons, which were generated by the growth substrate oxidation or by biomass oxidation [19]. The rate of non-growth substrate (EE2) transformation ($-dS_2/dt$) could be:

$$\frac{dS_2}{dt} = \alpha\frac{dS_1}{dt} - \beta b X \tag{12}$$

When the decay is negligible, Eq. (11) can be simplified as:

$$\frac{dS_2}{dt} = \alpha\frac{dS_1}{dt} \tag{13}$$

Then Eq. (13) can be integrated analytically to give:

$$S_2 = S_{20} - \alpha(S_{10} - S_1) \tag{14}$$

Combining Eqs. (2), (11), and (14), then the kinetic of E2 in dual-substrate system is as follows:

$$-\frac{dS_1}{dt} = \frac{\frac{q_m}{\left[1+\frac{[S_{20}-\alpha(S_{10}-S_1)]^{n_1}}{K_1}\right]}[X_0 + Ys(S_{10} - S_1)]S_1}{K_S\left[1 + \frac{[S_{20}-\alpha(S_{10}-S_1)/S_1]^{n_2}}{K_2}\right] + S_1} \tag{15}$$

The kinetic of EE2 transformation in dual-substrate system is as follows by combining Eqs. (13) and (15):

$$-\frac{dS_2}{dt} = \alpha\frac{\frac{q_m}{\left[1+\frac{[S_{20}-\alpha(S_{10}-S_1)]^{n_1}}{K_1}\right]}[X_0 + Y_s(S_{10} - S_1)]S_1}{K_S\left[1 + \frac{[S_{20}-\alpha(S_{10}-S_1)/S_1]^{n_2}}{K_2}\right] + S_1} \tag{16}$$

The values for q_m and K_S were obtained from the E2 alone biodegradation tests as $q_m = 0.452\ h^{-1}$, $K_S = 3.163$ mg/L. Values of the model parameters K_1, n_1, K_2, and n_2 are shown in Table 4 estimated using differ-

Table 3
Pattern of binary substrates degradation

Tests	S_1 (mg/L)	S_2 (mg/L)	S_2/S_1 (mg/mg)	X (mg/L)	ΔX (mg/L)	q_0 (h^{-1})	q_n (h^{-1})	$f = \frac{\lvert q_0 - q_n \rvert}{q_0}$
C1	1.03	5.05	4.90	5.08	1.94	0.1112	0.0187	0.83
C2	5.11	5.05	1.03	12.29	9.15	0.2789	0.1345	0.52
C3	15.08	5.05	0.25	27.07	23.93	0.3761	0.2846	0.24
C4	25.01	5.05	0.20	38.99	35.85	0.4013	0.3892	0.03
C5	4.05	20.07	4.04	9.48	6.34	0.2789	0.0539	0.81
C6	24.89	20.07	0.81	37.76	33.62	0.4013	0.2355	0.41
C7	45.10	20.07	0.44	55.43	52.29	0.4226	0.3134	0.26
C8	59.30	20.07	0.34	68.58	65.44	0.4293	0.3387	0.21

Table 4
Summery of model parameter values for mixtures of E2 and EE2

Tests	α^a (mg/mg)	$Y_s{}^a$ (mg/mg)	$K_1{}^a$ (mg/L)	$n_1{}^a$	$K_2{}^a$ (mg/L)	$n_2{}^a$	R^2
C1	0.41	1.88	18.73	3.64	39.42	2.61	0.91
C2	0.32	1.84	14.89	3.51	35.82	2.52	0.93
C3	0.23	1.44	12.88	3.27	32.81	2.30	0.90
C4	0.20	1.43	10.14	3.01	30.23	2.13	0.92
C5	0.51	1.28	8.85	2.35	28.49	1.87	0.93
C6	0.32	1.34	7.76	1.28	26.48	1.63	0.94
C7	0.28	1.16	4.05	1.03	23.86	1.43	0.92
C8	0.29	1.11	1.94	0.82	20.83	1.25	0.91

[a]Parameters were calculated in Matlab using the curve fitting tool software cftool (version R2011a, MathWorks, Inc., Natick, MA).

ent initial conditions for the biodegradation phase with the help of Matlab software (version R2011a, MathWorks, Inc., Natick, MA). Least squares estimate for α were also provided through the slope of a linear regression between S_1 and S_2. Fig. 6 shows the experimental results and the linear regression for Test C4, while the other tests of Group C hold the similar behavior. It was clearly seen that the E2 and EE2 concentrations were linearly related, which demonstrated that the EE2 transformation rate was dependent on the E2 biodegradation rate.

The experimental data and model simulations for E2 and EE2 degradation are shown in Fig. 7. As it shows, the model satisfactorily predicted the cometabolic degradation of E2 and EE2 with correlation coefficient (R^2) larger than 0.90. The kinetic equations including growth and non-growth substrate inhibition were suitable to describe the biodegradation behavior of E2 and EE2 in dual-substrate system at mg/L level. The experimental concentration range of E2 and EE2 in our study was also used in previous studies [29–32] for the degradation of estrogens by bacteria. Actually,

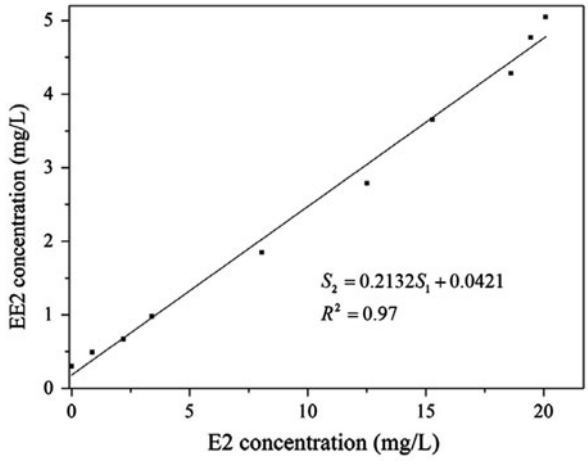

Fig. 6. Linear regression used to estimate the parameter for α (the initial concentrations of E2 and EE2 are 25 mg/L and 5 mg/L, respectively).

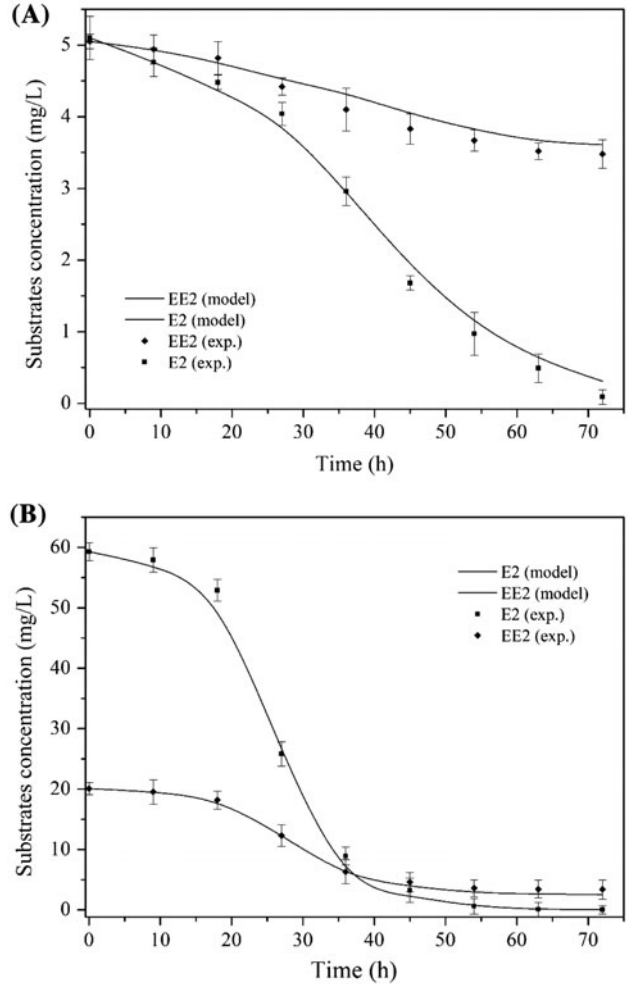

Fig. 7. Experimental dates and model simulations for E2 and EE2 degradation with different initial concentrations of E2 and EE2: (A) 5 mg/L E2 and 5 mg/L EE2; (B) 60 mg/L E2 and 20 mg/L EE2.

steroid estrogens (E1, E2, E3, and EE2) have always been found in aquatic environment (e.g. surface water, groundwater, drinking water, and wastewater treatment plant effluents) at ng/L level [33]. However, in our study, we aimed at investigating the characteristics of cometabolic degradation process of E2 and EE2, including the interaction of substrates on their degradation efficiency. Whether the initial concentrations of these substrates were at ng/L or mg/L level, the relationship between E2 and EE2 in the cometabolism process might not significantly change.

In this study, a better understanding was acquired on the substrate interactions in the mixture based on these findings and model predictions. Furthermore, it is also practically significant in process control and design of a cometabolism system. To our knowledge, the model developed on the basis of models described by our previous studies [34] was the first kinetic model that was sufficiently comprehensive to simulate the cometabolic biodegradation of E2 and EE2.

4. Conclusions

An estrogen-degrading bacteria, strain LY1 was isolated from activated sludge to cometabolize E2 and EE2 with a sufficient ratio of EE2/E2 varying from 0.25 to 5.00. Batch experiments demonstrated that the EE2 degradation efficiency increased with the increasing excess of E2, while the E2 degradation efficiency showed a decline with the increasing amounts of EE2. The E2 and EE2 transformation data were described using a kinetic model of cometabolism, which successfully identified and quantified the cometabolic process by strain LY1. Further studies are needed to optimize the biodegradation process and models, as well as to evaluate the mechanism of cometabolic degradation and toxicity of metabolites generated.

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Notations

α	—	amount ratio of EE2 transformed to E2 consumed
β	—	amount ratio of EE2 transformed to biomass oxidized
b	—	decay coefficient h^{-1}
f	—	variable rate of the specific E2 utilization rates
K_1	—	inhibition constant related to the maximum specific E2 utilization rate
K_2	—	inhibition constant related to the half-maximum rate E2 concentration
K_S	—	half-velocity constant
n_1, n_2	—	positive exponents
q_s	—	E2utilization rate, h^{-1}
q_x	—	biomass growth rate, h^{-1}
q_0	—	specific E2 utilization rates in the degradation of E2 alone, h^{-1}
q_n	—	specific E2 utilization rates in the cometabolic degradation of E2 and EE2, h^{-1}
R^2	—	correlation coefficient
S_1	—	concentration of E2, mg/L
S_2	—	concentration of EE2, mg/L
S_{10}	—	initial concentration of E2, mg/L
S_{20}	—	initial concentration of EE2, mg/L
t	—	experiment time, h
X_0	—	initial biomass concentration, mg/L
X	—	concentration of biomass when E2 degradation stopped, mg/L
ΔX	—	concentration variation of biomass when E2 degradation stopped, mg/L
Y_S	—	yield coefficient of biomass per unit of E2 consumed, mg cell/mg substrate

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