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Ultrasonic-assisted Biodegradation of Endocrine Disrupting Compounds in Soil by *Pseudomonas putida*: the Importance of Rhamnolipid for Intermediate Product Degradation

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Abstract The present study aimed to completely remove estrogens, including oestrone(E1), oestradiol(E2), oestriol(E3), 17α-ethinylestradiol(EE2) and bisphenol-A(BPA), from soil using *Pseudomonas putida*(*P. putida*). A central composite design was developed to determine the optimal conditions of three variables(ultrasonication time, quantity of *P. putida*, and concentration of added rhamnolipid) for the removal of the estrogens, and the biodegradation rates of the estrogens were investigated under the optimum conditions. Moreover, a quantitative structure-biodegradation relationship(QSBR) was used to analyze the effect of the estrogenic physicochemical properties on the enhancement of the biological degradation. The optimal conditions were an ultrasonication time of 3 min, a *P. putida* quantity of 8 mL, and a rhamnolipid concentration of 100 mg/L. These conditions resulted in removal of 100%, 94.86%, 94.90%, 96.56% and 94.56% of E1, E2, EE2, BPA and E3, respectively after 7 d. The degradations were more rapid and complete than those reported in previous studies, indicating the suitability of the adaptation of *P. putida* to estrogen degradation under conditions of ultrasonic-assistance and adding rhamnolipid; improvement was particularly apparent from the complete degradation of E3. Based on a Pearson correlation analysis, the estrogen molecule polar surface area(PSA) and surface tension were significantly related to the biodegradation effect. An analysis of the QSBR model with the estrogen biodegradation rates as a dependent variable and the PSA and surface tension as independent variables indicated that larger PSA caused decreased estrogen biodegradation, while the biodegradation progress was dominated by the surface tension of the estrogens. The interaction of PSA and surface tension had an antagonistic effect on the biodegradation of estrogens. Therefore, rhamnolipid/ultrasonication can significantly improve the biodegradation rates of oestrogens in soil, while simultaneously adjusting other environmental conditions would influence and control the biodegradation processes of estrogens.

Keywords Estrogen; Biodegradation; Rhamnolipid; Ultrasonic-assistance; Quantitative structure-biodegradation relationship(QSBR)

1 Introduction

Endocrine disrupting compounds(EDCs) in the environment interfere with biosynthesis, metabolism, reproduction and hormonal secretion by wildlife by blocking natural hormones[1]. Because of their strong hormonal effects, EDCs have aroused worldwide attention. Oestrone(E1), oestradiol(E2), and oestriol(E3) are classified as steroid hormones, and are predominantly found in secretions from female livestock. E1, E2, and E3 can affect the vitellogenin of male fish and reverse the gender of aquatic animals even at a μg/L level[2—4]. Both 17α-ethinylestradiol(EE2) and bisphenol A(BPA) are synthetic estrogens. EE2 is often used in oral contraceptive pills, and its toxicity is about 10—50 times higher than those of E1 and E2[5,6]. Owing to water shortages, much EDC-contaminated wastewater is reclaimed for irrigating cropland, and this transfers EDCs into the soil where they accumulate. The above EDCs are hydrophobic; their fate and bioavailability in soil mainly depend on the interactions between soil and water[7]. EDCs can be removed from soil-water systems by physical adsorption, biodegradation, and chemical oxidation. However, most previous research into EDC biodegradation concerned aquatic environments and wastewater systems, and studies about their biodegradation in soils are scarce[8—10].

At present, activated sludge process are widely used for wastewater treatment. The majority of EDCs can be removed by activated sludge(efficiency 70%—90%), but traces of them remain in wastewater and cause potential risk[11,12]. In

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activated sludge, 4-*n*-NP effectively adsorbed onto suspended solids, while BPA did to a lesser extent; the activated sludge process had a high removal rate(up to 90%) for trace-level BPA[13]. In previous studies focusing on the physical adsorption, biodegradation, and biotransformation of EDCs in soil, the removal rate of EDCs could reach 90% after 15—20 d[14]. The previous studies were mainly focused on the EDCs' degradation in the sewage treatment, but few involved with the biodegradation of EDCs by microorganism in soil. In a study of microbial degradation, di-*n*-butyl phthalate ester was removed by indigenous microorganisms in mangrove sediment; the effects of temperature, oxygen availability, and pH on the rate constant were explored, but the biodegradation effectiveness was not determined[15]. Robinson[16] employed native microbial communities for biodegrading E2, EE2 and BPA in seawater and sediment, but reported low degradation rates of the EDCs in the sediment. Above all, previous studies indicate the need for a long time for complete biodegradation of EDCs, and the biodegradation was limited by the efficiency of the native microorganisms.

However, in one study, blast furnace dust was used to promote the simultaneous biodegradation of chlorinated organic and endocrine disrupting compounds; the blast furnace dust could absorb some pollutants, and the removal efficiency therefore increased[17]. In the present study, we seek approaches that improve microbial biodegradation of EDCs.

The previous studies indeed brought an attempt at the degradation of EDCs in soil[18,19], sediment[20] or water environment[21,22]. However, there were some problems of EDCs biodegradation: EDCs could not be biodegraded completely by activity sludge, and only a small part of EDCs' removal rate was up to 90%; the biodegradation of native microorganism required a relative long time; and removal rate of EDCs when microorganism was only existed was low. Liu[23] attempted to use *Pseudomonas putida*(*P. putida*) for the biodegradation of EDCs, but the degradation effects were not obvious. Zhang[24] assessed the effects of calcium alginateand ultrasonication on the biodegradation of estrogens in soil, finding that the biodegradation levels of E1, EE2 and BPA were up to 90%, while the removal rates of E2 and E3 were <70%. Chen *et al.*[25] assessed the effect of sodium alginate-immobilized bacteria and ultrasonic assistance on the biodegradation of estrogens in soil. They found that the biodegradation rates of E1, E2, EE2 and BPA were up to 90%, but the removal rate of E3 was only 51.87%.

Ultrasound energy can increase the interaction between phases in a system by cavitation caused by the collapse of bubbles, whereas the ultrasonic jet disrupts the boundary phase and causes emulsification[26,27]. When applied in aqueous solutions or suspensions, ultrasound increases mixing, shearing, and mass transfer rate of the system, reducing process time when compared with other conventional mixing techniques. In biotechnological processes, ultrasound has been applied for some enzymatic reactions, wastewater treatment and biofuels production with very good results[28]. Compared with previous studies, the biological degradation technology has the advantages of cost saving, environment friendly, no secondary pollution, permanent degradation and high degradation efficiency. The aim of this study was to completely remove the EDCs(E1, E2, EE2, E3 and BPA) using *P. putida*, and, in particular, to improve the biodegradation of E3. In the biodegradation system, rhamnolipid was introduced and combined with ultrasonication to enhance the biodegradation efficiency. A central composite design was developed to optimize the conditions for EDCs biodegradation, and the removal rates of EDCs were investigated under the optimum conditions. Moreover, Pearson correlation analysis was used for screening the major parameters of the quantitative structure-biodegradation relationship(QSBR) model. The effect of the estrogenic physicochemical properties on the enhancement of the biological degradation mechanism was analysized by utilizing QSBR model.

2 Experimental

2.1 Chemicals and Instruments

E1(≥97% purity), E2(≥97%), EE2(≥97%), E3(≥99%) and BPA(≥98.3%) were purchased from Sigma-Aldrich (Steinheim, Germany). Methanol and acetonitrile(HPLC grade) were obtained from Burdick & Jackson(Honeywell, Morristown, NJ, USA). NaOH(A. R. grade) and HCl(A. R. grade) were purchased from Beijing Chemical Engineering Factory(Beijing, China). $CaCl_2$ was purchased from Xilong Chemical Plant of Shantou, Guangdong. NaN_3(A. R. grade) was obtained from Tianjin Fuchen Chemical Reagents Factory(Tianjin, China). Soil sediments[25] were collected from the Songhua River in Jilin Province, China.

A 1200-HPLC(Agilent Company, Santa Clara, CA, USA), equipped with two model pumps(G1312A), an in-line degasser(G1322A), a column oven(G1316A), and a fluorescence detector(G1321A), was used for HPLC analysis. The injection loop volume was 20.0 μL, and a Zorbox SB-C18 column(250 mm×4.6 mm; 5 μm) was used for the separations. A 2-16K centrifuge(Sigma, Munich, Germany), an FA-1004 analytical balance(Shanghai Hengping Science Instrument Company, Shanghai, China), and Milli-Q ultrapure water(Millipore, Billerica, MA, USA) were also used.

2.2 Enrichment Culture of *P. putida*

Luria-Bertani(LB) liquid medium was prepared before biological experiments, containing 10.0 g/L of tryptone, 5.0 g/L of yeast extract, and 10.0 g/L of NaCl, pH=7.0. The minimal salts medium(MSM) for degradation was made up as follows: 4.35 g/L of K_2HPO_4 $3H_2O$, 1.70 g/L of KH_2PO_4, 0.20 g/L of $MgSO_4$, 2.10 g/L of NH_4Cl, 0.05 g/L of $MnSO_4$, 0.01 g/L of $FeSO_4$ $7H_2O$, and 0.03 g/L of $CaCl_2$ $7H_2O$. The LB medium, minimal salts medium solution and all the apparatus were autoclaved at 121 °C for 15 min.

A pure strain of *P. putida*[25] was activated in LB liquid medium at 37 °C for 24 h, and the bacterium was enriched three times. Then, the LB liquid medium containing *P. putida* was incubated at 37 °C for 24 h, with shaking at 60 r/min.

2.3 Enhanced Biodegradation of EDCs with Rhamnolipid

MSM(30 mL), 1.27, 4, 8, 12 or 14.73 mL of *P. putida*(OD_{600nm}≈0.8), and different quantities of rhamnolipid were added into a conical flask, respectively. The mixture was shaken up and put into the ultrasonic apparatus at 30 W for 1.32, 2, 3, 4 or 4.68 min, respectively. The samples were placed in a constant temperature incubator, and shaken for 7 d. Duplicate experiments were conducted to account for experimental error and to investigate the reproducibility of the results. A blank experiment was performed as a control. Experiments for optimizing all parameters were carried out at an initial EDCs concentration of about 5 mg/L.

2.4 HPLC Analysis

The EDCs were separated and quantified using a gradient elution procedure. The mobile phase was composed of water(A) and methanol(B). The UV wavelength for compound detection was 280 nm. The elution procedure settings for all the EDCs were as follows: 0—5 min, 70% B; 5—10 min, 70%—85% B; and 10—18 min, 85% B. The flow rate of the mobile phase was 1.0 mL/min. The column temperature was maintained at 30 °C. The excitation wavelength of the fluorescence detector was fixed at 230 nm, and the emission wavelength was set to 315 nm.

3 Results and Discussion

3.1 Optimization of Enhanced Biodegradation of EDCs Using Rhamnolipid

This study employed a central composite design to achieve the enhanced biodegradation of multiple EDCs(E1, E2, EE2, E3 and BPA) and investigated the effect of the following factors: ultrasonication time(X_1, min), quantity of *P. putida*(X_2, mL), and concentration of rhamnolipid(X_3, mg/L) on the biodegradation rates of EDCs in soil. Each of the effect factors was studied at five levels. Based on preliminary experiments, the factor values of each level are shown in Table 1.

Table 1 Factors and levels of central composite design based on rhamnolipid

Factor	Ultrasonic time/min	Quantity of *P. putida*/mL	Concentration of rhamnolipid/(mg L^{-1})
–1.682	1.32	1.27	15.91
–1	2	4	50
0	3	8	100
+1	4	12	150
+1.682	4.68	14.73	184.09

In the central composite design, X_1, X_2, and X_3 were set as influence factors, and the biodegradation rates of EDCs were set as responses(Y, %); the arrangement of the experiment is shown in Table 2.

Table 2 Central composite design of the enhanced degradation of EDCs based on the rhamnolipid

No.	Ultrasonic time/min	Quantity of *P. putida*/mL	Concentration of rhamnolipid/(mg L^{-1})
1	2	4	50
2	2	4	150
3	2	12	50
4	2	12	150
5	4	4	50
6	4	4	150
7	4	12	50
8	4	12	150
9	1.32	8	100
10	4.68	8	100
11	3	1.27	100
12	3	14.73	100
13	3	8	15.91
14	3	8	184.09
15	3	8	100
16	3	8	100
17	3	8	100
18	3	8	100
19	3	8	100
20	3	8	100

The experimental results showed that the biodegradation of E1 and E2 reached about 100% after 4 d; therefore, E1 and E2 were considered biodegraded after 3 d for optimization. EE2, E3, and BPA were considered biodegraded after 7 d. Table 3 shows the biodegradation rates of the EDCs on the basis of the central composite design.

Table 3 Biodegradation rates of EDCs enhanced by rhamnolipid

No.	Biodegradation rate of EDCs(%)				
	E3	BPA	EE2	E2	E1
1	99.62	84.19	97.44	99.41	100
2	90.54	83.09	96.86	99.41	100
3	99.53	94.73	99.95	99.27	100
4	47.78	87.07	99.95	99.27	100
5	99.62	94.30	98.94	99.41	100
6	47.60	91.27	99.96	99.41	100
7	99.53	95.07	99.95	99.27	100
8	50.81	95.76	99.95	99.27	100
9	99.57	88.21	97.53	99.34	100
10	82.29	91.09	97.84	99.34	100
11	96.32	69.69	89.41	99.45	100
12	75.58	98.42	99.94	99.22	100
13	53.96	88.82	97.14	99.34	100
14	99.57	87.41	93.41	99.34	100
15	99.57	85.07	96.42	99.34	100
16	99.57	70.75	88.50	99.34	100
17	99.57	88.69	97.05	99.34	100
18	68.65	80.42	94.37	99.34	100
19	99.57	94.47	98.11	99.34	100
20	99.57	99.57	95.33	99.34	100

The multiple regression models of EDC biodegradation were established at significance level of 0.05. Statistical analysis systems(SAS) software[25] was used to construct a response surface figure of the biodegradation rates of the

EDCs. Considering the ultrasonication time(X_1), the biodegradation rates of EE2 and BPA initially showed a decrease and then increased with ultrasonication time. This demonstrated that the ultrasonication time was important for the EDC biodegradation rate, and that the rate increased as the permeability of cells was enhanced. However, if the ultrasonication time was too long or short, this compromised the biodegradation by *P. putida*. As shown in Fig.1, as the quantity of *P. putida*(X_2) increased, the biodegradation rate of BPA and EE2 gradually increased, but the biodegradation rates of E2 and E3 showed a decreased trend. A large quantity of *P. putida* obviously promoted the biodegradation of synthetic EDCs, while natural EDCs required a smaller amount of *P. putida*. When the concentration of rhamnolipid(X_3) increased, the biodegradation rate of E3 first increased and then decreased; BPA and EE2 showed the opposite trend.

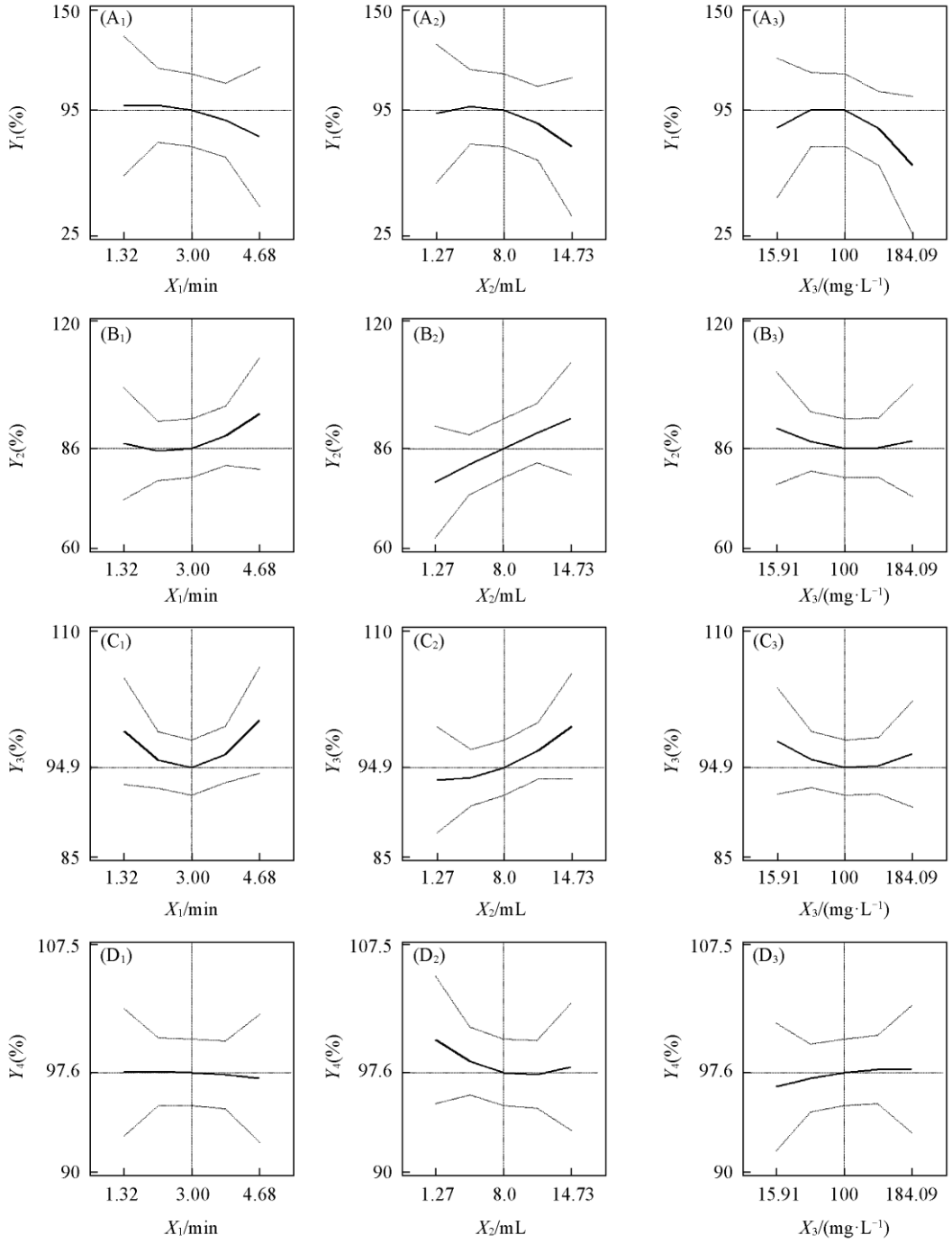

Fig.1 Effect tendency of factors(X_1, A_1—D_1; X_2, A_2—D_2; X_3, A_3—D_3) for EDCs biodegradation

Y_1(A_1—A_3), Y_2(B_1—B_3), Y_3(C_1—C_3) and Y_4(D_1—D_3) were the degradation rate of E3, BPA, EE2 and E2, respectively.

From the above observations, the optimum conditions for the EDCs(E1, E2, EE2, E3 and BPA) biodegradation were deduced to be: 3 min of ultrasonication, 8 mL of *P. putida*, and 100 mg/L rhamnolipid. Under the optimized conditions, the biodegradation rate of E1 reached 100% after 2 d, and the biodegradation rates of E3, BPA, EE2 and E2 were 94.56%, 94.56%, 94.90% and 94.86%, respectively after 7 d.

To assess the simultaneous biodegradation of E1, E2, EE2, E3 and BPA, the biodegradation rates of the above EDCs were explored under the optimum conditions(Table 4). The

biodegradation rates of E1, E2, EE2 and BPA reached>80% after 1 d, while the biodegradation rate of E3 was <40% after 3 d, then increased to 68.83% after 4 d, and 94.42% after 7 d.

Table 4 Law of biodegradation under the optimum conditions based on the rhamnolipid

Time/d	Biodegradation rate of EDCs(%)			
	E3	BPA	EE2	E2
1	18.11	80.55	93.48	98.9
2	23.77	87.47	97.48	97.76
3	32.36	90.35	99.95	99.34
4	68.83	81.9	93.84	100
5	76.79	91.59	98.64	100
6	90.47	86.97	97.67	100
7	94.42	86.50	94.96	100

3.2 Mechanism of Enhancing Biodegradation Under Optimum Conditions

Previous study demonstrated that E2 and EE2 were biodegraded into E1 and then transformed into E3 on aerobic biodegradation in soil[14]. In this study the level of E3 remained relatively high over the first 3 d, which indicated a large amount of E3 was generated by degradation of E2 and EE2; the generated E3 was then completely biodegraded by *P. putida* from days 4 to 7. EE2 and E1 are secondary metabolites, and E3 is the ultimate metabolic product. The oestrogen activity of these compounds ranks as follows: E2>EE2>E1>E3[29]. This observation agrees with the literature, which suggests that E3 is the intermediate degradation product of other oestrogen compounds[30]. The specific processes of estrogen degradation are shown in Table 5[31].

Rhamnolipid is a type of biological surfactant, which has the solubilization and dispersibility of a traditional surfactant, and also good emulsifiability[32]. In the biodegradation progress, rhamnolipid was beneficial for the growth of *P. putida*, hence the biodegradation of EDCs could be improved by the presence of rhamnolipid. In contrast to traditional biodegradation methods, the biodegradation levels of E1, E2, EE2 and BPA could reach >80% after 1 d[33]. To understand the mechanism of enhancement of the degradation, we investigated the surface tension of the rhamnolipid-containing solutions at different rhamnolipid concentrations(Fig.2). We can infer that the surface tension of rhamnolipid solutions stayed the same when the concentration was >100 mg/L and 100 mg/L was the critical micelle concentration(cmc) of the rhamnolipid. Previous study showed that EDC passage between microorganisms and water could be improved above the cmc[34]. The hydrophilic side of rhamnolipid bonds with weakly hydrophobic regions on the surface of the microorganism, and affinity between the microorganism surface and the EDCs is therefore promoted, which favors the biodegradation of the EDCs[35].

Table 5 Transformation of the selected EDCs[31]

Compound	Precursor	Intermediate product	Ultimate product
BPA	Polycarbonate, epoxy resin	—	4-Isopropenyl or BPA trimer, tetramer
E1	Conjugated oestrogens, E2, EE2	E3	2-Hydroxy E1, 16α-hydroxy E1 etc.
E2	Oestradiolvalerate, oestradiolcypionate	E1, E3	2-Hydroxy E2, testosterone analogues etc.
EE2	Mestranol	E1	C2-6 Molecule binary carboxylic acid
E3	Nylestriol, nilestriol etc.	—	4-Methoxy E3 etc.

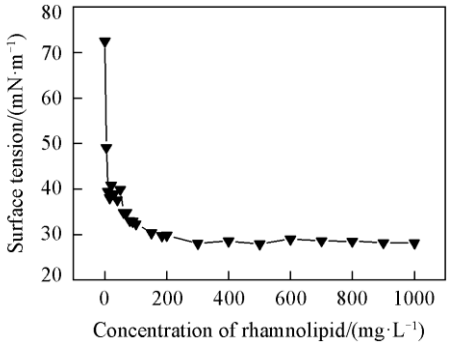

Fig.2 Surface tension of solvent at different rhammolipid concentrations

3.3 Parameter Selection and Analysis of the Biodegradation Mechanism of EDCs Based on the Physicochemical Properties of EDCs

3.3.1 Effect of Estrogenic Properties on Biodegradation

To clarify the mechanism of EDC biodegradation from the perspective of physicochemical parameters, a QSBR model was built. There are many physicochemical parameters(PPs) of EDCs, so a Pearson correlation analysis between the biodegradation rate and 28 PPs was performed to select relevant parameters(Table 6). A coefficient of $0.01<P<0.05$ was regarded as very significant, and a coefficient of $P<0.01$ was regarded as more significant. It can be inferred from Table 6 that the coefficients that showed a significant correlation with the biodegradation rate were "polar surface area"(PSA) and "surface tension" of the EDCs(at the level $0.01<P<0.05$), while other parameters did not show significance. Therefore, the PSA and surface tension of EDCs were chosen as the variables for QSBR study.

Table 6 Pearson correlation of the correlation between estrogenic properties and biodegradation rates of estrogens

Property	Heat of formation	Gibbs energy/ (kJ mol^{-1})	Ideal gas thermal capacity	Formal charge	Connolly accessible area	Connolly molecular area	Connolly solvent excluded volume/ (cm^3 mol^{-1})	Exact mass/ (g mol^{-1})
Pearson correlation	–0.167	0.208	0.555	—	0.431	0.508	0.600	0.610
Significant[a]	0.788	0.738	0.331	—	0.468	0.383	0.285	0.274
N[b]	5	5	5	5	5	5	5	5

To be continued on the next page.

Property	Mass	Mol weight	Ovality	Principal moment	Mol refractivity	Partition coefficient	Balaban index	Cluster count
Pearson correlation	0.610	0.610	–0.720	0.427	0.603	–0.089	0.235	0.600
Significant[a]	0.274	0.274	0.170	0.473	0.281	0.887	0.703	0.284
N^b	5	5	5	5	5	5	5	5

Property	Molecular topological index	Num rotatable bonds	Polar surface area	Radius	Shape attribute	Shape coefficient	Sum of degrees	Sum of valence degrees
Pearson correlation	0.521	–0.145	–0.888[a]	–0.022	0.600	0.022	0.706	0.361
Significant[a]	0.368	0.816	0.044	0.972	0.285	0.972	0.183	0.551
N^b	5	5	5	5	5	5	5	5

Property	Topological diameter	Total connectivity	Total valence connectivity	Wiener index	lgK_{oc}	lgK_{ow}	Polarizability	Surface tension
Pearson correlation	–0.022	-0.853	-0.645	0.485	0.806	0.598	0.784	–0.901[a]
Significant[a]	0.972	0.066	0.240	0.408	0.100	0.287	0.116	0.037
N^b	5	5	5	5	5	5	5	5

a. The coefficient statistically significant was at $0.01<P<0.05$(n=10); *b*. *N*: sample size.

3.3.2 Analysis of Estrogenic Biodegradation Mechanism Based on QSBR Model

A QSBR model was built to study the correlation between the estrogenic properties and the removal of estrogenic contaminants. We hypothesized that PPs had both primary effects and binary interaction effects on the biodegradation of the EDCs; the QSBR could be expressed as follows:

$$Y=-76.8563+7.46139x_2-0.034x_1\times x_2-2.31x_1\times x_1-0.079x_2\times x_2 \quad (1)$$

(R^2=0.9047, n=10, F=7.1209, P=0.0693)

The correlation coefficient of the QSBR model was 0.9897, and the model was robust. In this model, x_1 is the PSA of the EDC molecule, which included the surface areas of oxygen atoms, nitrogen atoms, and hydrogen atoms[36]; x_2 is the surface tension of the EDC molecule, which is one of the most powerful techniques that provide information about surfaces and intermolecular interaction[37]. $x_1\times x_1$ represents the second order interaction effect of PSA on the biodegradation rate in the system; $x_2\times x_2$ represents the second order interaction effect of surface tension on the biodegradation rate in the system; and $x_1\times x_2$ represents the interaction effect of PSA with surface tension on the biodegradation rate in the system.

In medicine and chemistry, the PSA represents the total surface area of polar molecules, including oxygen, nitrogen, and the hydrogen bonds. The PSA negatively correlated with the penetrability of cells, which was detrimental to growth and reproduction of microorganisms. A larger PSA indicates more hydrogen bonds and hydrophilic groups, which negatively impacts microbial degradation efficiency[38,39]. In Pearson correlation analysis, the PSA correlated negatively with the biodegradation rate. However, in the QSBR model, PSA had little primary effect on the biodegradation rate of the EDCs. Surface tension exists at the boundary interface between a liquid and a gas. The molecules in liquid maintain an equilibrium distance from each other; long distance causes attraction, while short distance causes repulsion. Therefore, liquid molecules cannot diffuse in an unlimited way like gaseous molecules, and they move around their equilibrium position. In this study, the surface tension(x_2) positively correlated with the biodegradation rate(Y), and its own second order interaction effect($x_2\times x_2$) was negative, decreasing the biodegradation rate. The binary interaction of PSA and surface tension showed an antagonistic effect on the biodegradation of the EDCs.

3.4 Comparison Between the Effects of Rhamnolipid on EDC Degradation and Those of Sodium Alginate and Calcium Alginate

As may be seen from Table 7, Zhang[24] studied the effects of calcium alginateand ultrasonication on the biodegradation of estrogens in soil, finding that the biodegradation levels of E1, EE2 and BPA were 100%, 96.90% and 96%, respectively, while the removal rates of E2 and E3 were <70%. Chen *et al*.[25] assessed the effect of sodium alginate-immobilized bacteria and ultrasonic assistance on the biodegradation of estrogens and found removal rates of 100%, 100%, 93%, 96.47% and 51.87% for E1, E2, EE2, BPA and E3, respectively. In this paper, the removal rates of E1, E2, EE2, BPA and E3 were 100%, 94.86%, 94.90%, 96.56% and 94.56%, respectively. These levels of degradation were achieved more rapidly than those reported by Chen *et al*.[25] and Zhang[24], indicating the value of using *P. putida* to degrade estrogens under conditions of ultrasonic-assistance and in the presence of rhamnolipid. In particular, there was also a marked improvement in the level of degradation of E3.

Table 7 Removal rates of rhamnolipid on EDCs' degradation with those of sodium alginate and calcium alginate under optimal conditions

Additive	Degradation rate of EDCs(%)					Dose of key additive under optimal condition/mol
	E1	E2	E3	EE2	BPA	
Rhamnolipid	100	94.86	94.56	94.90	96.56	4.61×10^{-6}
Calcium alginate[24]	100	67	52.65	96.90	96	2.57×10^{-3}
Sodium alginate[25]	100	100	51.87	93	96.47	4×10^{-4}

Compared with use of sodium alginate and calcium alginate, the dose of rhamnolipid under optimal conditions is the lowest, indicting the effect of rhamnolipid on EDC degradation is the best. The rhamnolipid can enhance the degradation, dissolution, diffusion and absorption of E3, increase the cell hydrophobic surface, and change the cell surface charge

properties. Hydrogen bonds would form between rhamnolipid and *P. putida*, which would enhance the affinity between the microorganism and E3 in solution and further promote the degradation of E3. Sodium alginate frees fixed microorganisms, such as *P. putida*, in a limited amount of space, which significantly improves the microbial concentration per unit volume in the reaction system[40]. However, after a period of time, with the loss of Ca^{2+}, the gel structure of sodium alginate is destroyed, following the stability of the sphere, strength and other properties decline. Due to E3 is an intermediate product of the degradation of E1, E2, EE2 and BPA, E3 will be produced when E1, E2, EE2 and BPA are degraded. Therefore, it will take longer time if E3 is completely degraded. However, by this stage, the stability and strength of sodium alginate spheres has declined. This explains why E3 was not completely degraded in reaction systems that contain sodium alginate. Moreover, rhamnolipid can be used by microorganisms as a carbon and energy source, which promoted the amount of *P. putida* and enhanced the degradation of E3, whereas sodium alginate is not used by *P. putida* as a carbon and energy source[41]. The function of calcium alginate is similar to that of sodium alginate, which has also been mixed with microorganisms. However, the effects of calcium alginate on EDC degradation were not as great as those of sodium alginate.

The QSBR model was built to study the correlation between the properties of the EDCs and the removal of the EDCs. Chen *et al.*[25] showed that the PSA negatively correlated and hydrophobicity(represented by $\lg K_{ow}$) positively correlated with EDC removal, and these effects were synergistic. Zhang[24] reported that the PSA negatively correlated with EDC removal. In this paper, the PSA negatively correlated and the surface tension positively correlated with the EDC degradation, and these effects were antagonistic. Therefore, PSA negatively correlated with EDC removal when any of sodium alginate, calcium alginate and rhamnolipid was added to enhance the degradation of EDCs. The greater the PSA, the lower the mobility of the molecule. Surfactant can increase the mobility of molecules[42], reduce the PSA and enhance the degradation of EDCs. Sodium alginate and calcium alginate increase the amount of bacteria per unit area, but rhamnolipid has the ability to decrease the PSA. This is a reason why the effect of rhamnolipid on the biodegradation of E3 is greater than those of sodium alginate or calcium alginate.

4 Conclusions

In the present study, rhamnolipid and ultrasonication were used to enhance the biodegradation efficiency of EDCs. The biodegradation conditions for multiple EDCs in soil were optimized by central composite design. The mechanism of enhanced biodegradation and differences in the biodegradation of different EDCs were also analyzed. The conclusions can be summarized as follows: in contrast to traditional biodegradation techniques, the biodegradation of E1, E2, EE2, E3 and BPA could reach >90% after 7 d in the system with rhamnolipid; of particular note was the complete degradation of E3. A suitable ultrasonication time was a key factor for high biodegradation efficiency of EDCs in soil. A long period of ultrasonication might lead to rupture of the bacterial cells, decreasing the biodegradation. PPs of EDCs were an important reason for the differences in EDC biodegradation. A larger PSA caused a decrease in the EDC biodegradation rate; the biodegradation progress was dominated by the surface tension of the EDCs. Binary interaction of PSA and surface tension showed an antagonistic effect in the biodegradation of EDCs.

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