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Original Research

Sodium Alginate/Ultrasonic-Assisted Biodegradation of Oestrogens in Soil

Ying Chen[1, 2], Chen Zhang[3], Yaling Zeng[1, 2], Yu Li[1, 2]*

[1]Resource and Environment Institute of North China Electric Power University, Beijing 102206, China
[2]The State Key Laboratory of Regional Optimization of Energy Systems,
North China Electric Power University, Beijing 102206, China
[3]State Environmental Protection Key Laboratory for Lake Pollution Control,
Chinese Research Academy of Environmental Sciences, Beijing 100012, China

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Abstract

The aim of this study was to assess the effect of sodium alginate-immobilized bacteria and ultrasonic assistance on the biodegradation of oestrogens in soil. The studied oestrogens were oestrone (E1), oestradiol (E2), oestriol (E3), 17α-ethinylestradiol (EE2), and bisphenol-A (BPA). A central composite design was developed to determine the optimal conditions of the three variables (ultrasound time, sodium alginate concentration, and amount of sodium alginate beads) for the removal of oestrogens. Moreover, the experiment utilized a quantitative structure-biodegradation relationship (QSBR) to analyze the effect of the estrogenic physicochemical properties on the enhancement of the biological degradation mechanism. The results indicated that the optimal conditions are an ultrasound time of three min, a sodium alginate concentration of 3%, and 4 g of sodium alginate beads. These conditions resulted in removal rates of 100%, 100%, 93%, 96.47, and 51.87% for E1, E2, EE2, BPA, and E3, respectively, after seven days. These rates were 1.7, 1.4, 1.3, 1.2, and 2.1 times the microbial degradation rate of the suspended state, respectively. Based on a Pearson correlation analysis, the oestrogen molecule polar surface area (PSA) and hydrophobicity (represented by logKow) were significantly related to the effect of biodegradation. An analysis of the OSBR model (with the oestrogen biodegradation rates as a dependent variable and PSA and logKow as independent variables) indicated the following: PSA negatively correlated and logKow positively correlated with oestrogen removal, and these effects were synergistic. Therefore, sodium alginate/ultrasound assistance can significantly improve the biodegradation rates of oestrogens in soil, while simultaneously adjusting other environmental conditions would influence and control the biodegradation processes of oestrogens.

Keywords: oestrogen, sodium alginate, ultrasonic-assistant, biodegradation, QSBR

Introduction

Endocrine-disrupting chemicals (EDCs) are naturally occurring or man-made compounds present in the environment that can bind to oestrogen receptors [1] and interfere with normal endocrine effects in animals and humans at ng·L^{-1} levels [2]. Oestrone (E1), oestradiol (E2), and oestriol (E3) are naturally occurring oestrogens that can cause serious reproductive and developmental disorders and interfere with endocrine regulation in the whole body, the nervous system, and immune system, and lead to cancer [3].

*e-mail: liyuxx8@hotmail.com

17α-ethinylestradiol (EE2) and bisphenol A (BPA) are man-made oestrogens. EE2 is the main ingredient in aquaculture feed, oral contraceptives, and hormone supplements that cause feminization [4] in several species of fish [5], molluscs [6], amphibians [7], birds [8], and mammals [9] at concentrations as low as 0.1 $ng \cdot L^{-1}$ [2]. BPA can lead to malignant tumours, and these effects are compounded in combination with cadmium and ultraviolet light [10, 11].

As a frequently detected environmental pollutant, oestrogens are not only found in water but also in soil. To date, most oestrogen degradation studies have mainly focused on the estrogenic transformation and control in water [12, 13]. EDCs can be removed by the action of wastewater treatment plants (WWTPs) [14, 15], including the nitrification of activated sludge [4] and removal by aerobic and anaerobic treatment processes in a membrane bioreactor [16]. Oestrogens can undergo several fates after entering soil, including microbial immobilisation and mineralisation, abiotic transformation, sorption to the solid phase, uptake by plants, leaching, and runoff [17]. Several researchers have studied estrogenic sorption courses in soil [18, 19]. However, sorption is a pollutant transfer process, and only some oestrogens pass through this process; biodegradation allows for the complete removal of the contaminant. Hence, Liu Jianlin [20] attempted to use *Pseudomonas putida* for the biodegradation of oestrogens, but the degradation effects were not obvious. Therefore, this study aimed to control and remove EDCs in soil.

Sodium Alginate (SA) is famous for its cost effectiveness, relatively low cytotoxicity, exceptional immobilisation and mass transfer performance, and high enrichment in microbial cells. Simultaneously sodium alginate is widely used as a gelling agent due to its ability to form gels under mild conditions with divalent cations such as calcium, and is useful as a carrier of microorganisms [21, 22]. Therefore, it has become one of the most widely used embedding media. Moreover, ultrasound has emerged as an alternative source of homogenizing energy with potential applications in enzymatic reaction control. Ultrasound energy acts by increasing 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. 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 biofuel production, with very good results [23]. The aim of this study was to assess the effects of sodium alginate-immobilised bacteria and ultrasound assistance on the biodegradation of oestrogens in soil, to determine the optimal conditions for this process and to provide a theoretical foundation and basis for oestrogen removal.

Table 1. The factors and levels of central composite design for estrogenic biodegradation in soil.

Factor	-1.682	-1	0	+1	+1.682
Ultrasound time (min)	1.32	2	3	4	4.68
Sodium alginate concentration (%, w/v)	1.32	2	3	4	4.68
Amount of sodium alginate beads (g)	2.32	3	4	5	5.68

Materials and Methods

Chemicals and Instruments

E1 (≥97%), E2 (≥97%), EE2 (≥97%), E3 (≥99%), and BPA (≥98.3%) were purchased from Sigma-Aldrich (Steinheim, Germany). Sodium alginate was of analytical grade and obtained from J&K Chemical (Beijing, China). The biodegradation metabolites were analysed with high-performance liquid chromatography (HPLC, Agilent, 1200) coupled with a numerical control ultrasonic cleaner (KQ-100) from Kun Shan Ultrasonic Instruments Co. Ltd., China.

Methods

Preparation of Sodium Alginate Beads

The *Pseudomonas putida* cells were grown in enrichment medium for 24 h and mixed with a sodium alginate solution. To immobilise *Pseudomonas putida*, a mixture of sodium alginate and seed culture was dropped into a 2% (w/v) $CaCl_2$ solution using syringes. Subsequently, the sodium alginate beads were stored after immobilisation in a refrigerator at 4ºC for 12h, washing with normal saline and draining on sterile filter paper.

Experimental Design and Optimization of Estrogenic Biodegradation

A certain amount of oestrogens was added to a mineral medium and mixed with different amounts of sodium alginate beads according to central composite design schedule. The samples were then oscillated (avoid light) in the incubator after ultrasonic assistance.

To determine the optimal conditions of the three variables (ultrasound time, sodium alginate concentration, and amount of sodium alginate beads) for the removal of oestrogens, a central composite design was developed and a response surface method was used (the above three variables served as influencing factors, and the biodegradation rates of oestrogens served as response values). The coding of each influence factor, high and low level of specific values is shown in Table 1.

According to the central composite design factors level table, a schedule for enhancing biodegradation rates of oestrogens was obtained and is demonstrated in Table 2.

Table 2. The central composite design for estrogenic biodegradation in soil.

No.	Ultrasound time (min)	Sodium alginate concentration (%, w/v)	Amount of sodium alginate beads (g)
1	2	2	3
2	2	2	5
3	2	4	3
4	2	4	5
5	4	2	3
6	4	2	5
7	4	4	3
8	4	4	5
9	1.32	3	4
10	4.68	3	4
11	3	1.32	4
12	3	4.68	4
13	3	3	2.32
14	3	3	5.68
15	3	3	4
16	3	3	4
17	3	3	4
18	3	3	4
19	3	3	4
20	3	3	4

Results and Discussion

Optimisation of Oestrogenic Biodegradation Based on Response Surface Method

Statistical Analysis System (SAS) software was used to construct a response surface figure of the enhancing biodegradation rates of oestrogens (Fig. 1).

As shown in Fig. 1, the biodegradation rates of E1 and E3 negatively correlated with the ultrasonic time and were minimised after three min, at which point they increased again; the rates of EE2 and BPA increased with the treatment time, and the biodegradation rate of E2 slightly decreased. E1 is one of the intermediate products of the degradation of E2 and EE2; E3 not only is one of the intermediate degradation products of E1, E2, and EE2, but also is the ultimate metabolic product. Appropriate intensity ultrasound accelerated growth and improved the ability of microorganisms to absorb and degrade oestrogens. With the addition of appropriate intensity ultrasound, the degradation rates of other oestrogen compounds increased, producing E1 and E3, that is increasing the amounts of E1 and E3 at the same time, so their degradation rates decreased.

The degradation rate of E1 positively correlated with the sodium alginate concentration, while that of E2 was relatively constant; the degradation rates of EE2 and E3 at first tended to negatively correlate with the sodium alginate concentration and were minimised at 3%; the degradation rate of BPA first increased and then decreased and was maximised at a sodium alginate concentration of 3%. Although sodium alginate frees fixed microorganisms in a limited amount of space, which significantly improves the microbial concentration per unit volume in the reaction system with the optimal dosage of sodium alginate; above this dosage, sodium alginate's balling with microorganisms will be relatively difficult, meaning the ability of immobilized microorganisms also decreased, so the degradation rate of BPA first increased and then decreased.

The degradation rate of E3 was also decreased with the addition of sodium alginate. E3 not only is one of the intermediate degradation products of other oestrogen compounds, but also is the ultimate metabolic product. With the addition of sodium alginate, the degradation rates of other oestrogen compounds increased with more E3 produced, increasing the amounts of E3 in the system so that the degradation rate of E3 decreased with the addition of sodium alginate.

The biodegradation rate of E1 slightly decreased as the amount of immobilised *Pseudomonas putida* cells increased, and was minimised at a cell load of 5.68 g; the degradation rate of E2 at first tended to increase as the cell load decreased and was maximised at a load of 4 g; the degradation rates of EE2 and E3 decreased and were minimised at a cell load of 4 g; the degradation rate of BPA first increased and then exhibited a change in trend, reaching a maximum value at 3%.

Comparing all experimental conditions, the biodegradation ratios of the five oestrogens were simultaneously maximised at an ultrasound treatment time of 3 min, sodium alginate concentration of 3%, and sodium alginate beads load of 4 g. At optimum conditions, the biodegradation ratios of E1 and E2 were 100% after 3d; and those of EE2, E3, and BPA were 93%, 51.87%, and 96.47% after 7d, respectively.

The Rule of Enhancing Biodegradation under Optimum Conditions

Fig. 2 shows the biodegradation behaviour of E1, E2, EE2, and E3, and BPA for 7d at optimum conditions.

As shown in Fig. 2, the biodegradation ratio of E1 reached 100% after 1d, the ratio suddenly decreased due to partial E2 oxidation into E1 [13] on 3d, and then increased to 100% again; over time, the BPA, EE2, and E3 ratios continued to increase after 7d and reached 96.47%, 93%, and 51.87%, respectively; the EE2 ratio was 100% after 2d at optimum conditions. Therefore, E1, E2, EE2, and BPA reached biodegradation ratios above 90% after 7d, while that of E3 was lower than 60% at optimum conditions.

At optimum conditions, the removal rates of E1, E2, EE2, BPA, and E3 reached 100%, 100%, 93%, 96.47, and 51.87% after 7d, respectively, and were 1.7, 1.4, 1.3, 1.2,

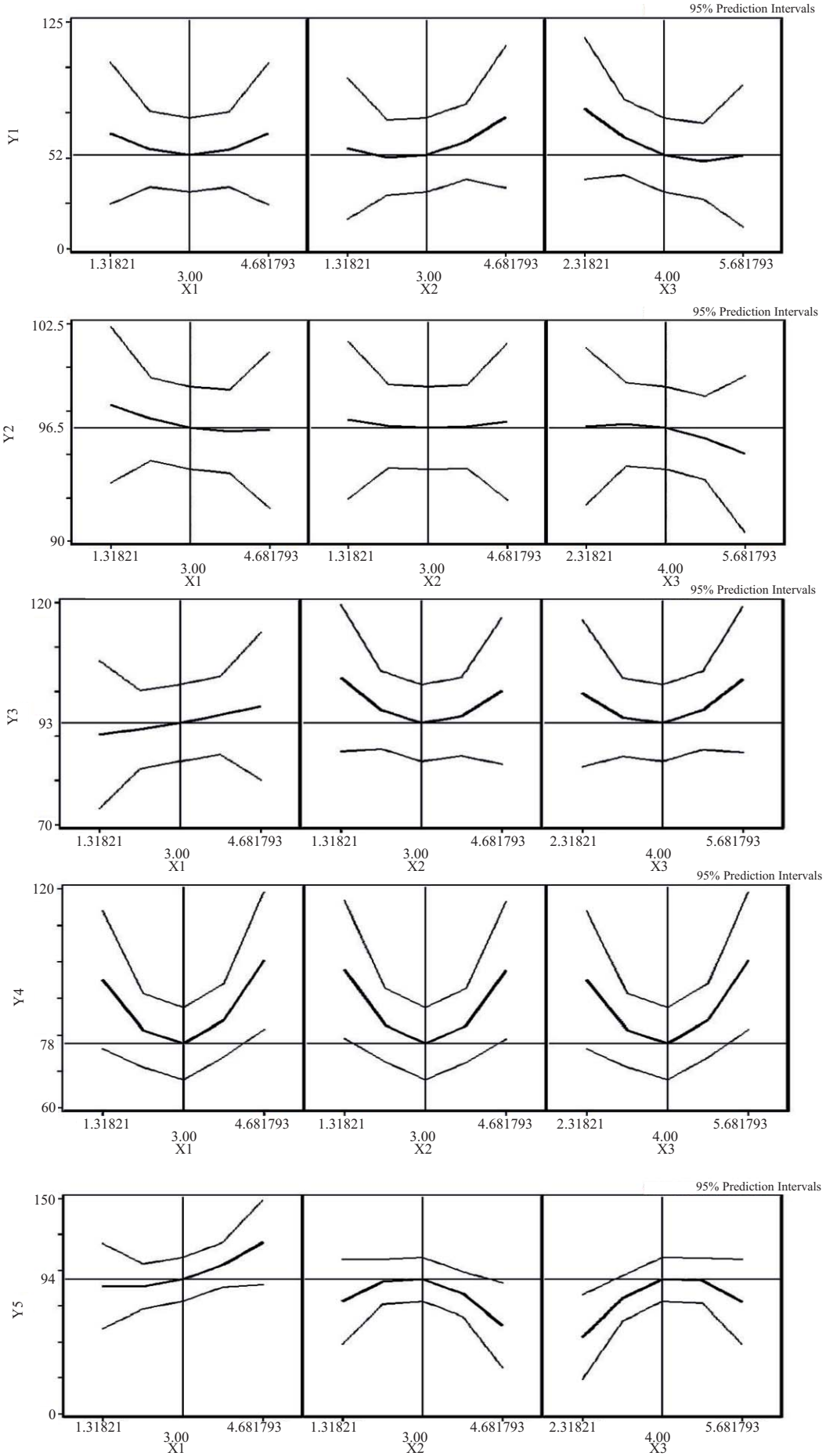

Fig. 1. The effect of factors on the biodegradation of EDCs (Y1-Y5 are the degradation rates of E1, E2, EE2, E3, and BPA, respectively).

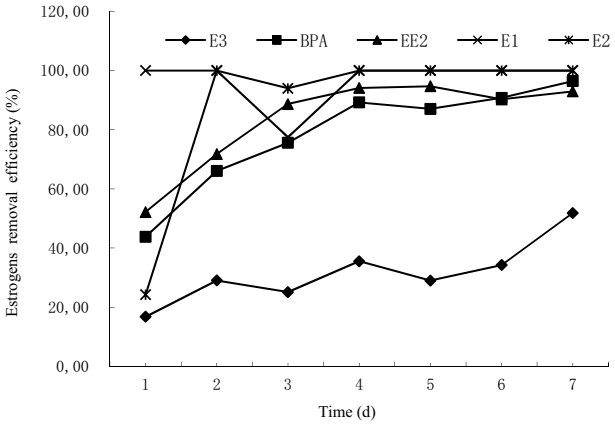

Fig. 2. The oestrogens biodegradation behaviour at optimum conditions.

and 2.1 times that of the suspended state [20]. By improving microbial stability to enhance the degradation of oestrogens, sodium alginate frees fixed microorganisms, such as *Pseudomonas putida*, in a limited amount of space, which significantly improves the microbial concentration per unit volume in the reaction system [24]. Furthermore, the application of immobilized microorganisms not only helps maintain high bacterial cell concentrations, but also increases the biological activity of the microorganisms, makes control separation easy, and protects cells against toxic substances and changes in environment factors [25, 26]. Microorganisms are attached to the carrier in the form of colonies, and the microbial fixation of sodium alginate improves their stabilities and benefits their growth. In addition, immobilization that allows achievement of an active and stable enzyme, with good specificity by the substrate, generally eliminates most of the disadvantages of the use of enzymes [27].

The immobilisation of intact enzymes on solid supports is important to help strengthen and prolong the biodegradation ability of microorganisms [28]. It was reported that low-intensity ultrasound improved microbial metabolism and stimulated physiological activities [29]. In addition, appropriate intensity ultrasound can increase the permeability of cell membrane, amplify the cells inside and outside the transportation of material, and improve the efficiency of microbial cell metabolism to promote enzyme activity, accelerate growth, and improve the ability of microorganisms to absorb and degrade organic matter [23, 30].

The maximum degradation rate of E3 reached only 51.87% but showed the largest variation, mainly because E3 is one of the intermediate products of the degradation of other oestrogen compounds; E2 is a primary metabolite that can produce E3 by hydration [31]; 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 [32]. This observation agrees with the literature, which suggests that E3 is the intermediate degradation product of other oestrogen compounds [19]. The specific processes of oestrogens are shown in Table 3.

Analysis of the Biodegradation Mechanism of Oestrogens

The Selection of Estrogenic Properties on Biodegradation Ratios

As shown in Table 4, the Matlab software was used to conduct a preliminary analysis and select the physicochemical properties of oestrogens, which demonstrated an apparent correlation between the estrogenic properties and the removal of estrogenic contaminants.

As shown in Table 4, the hydrophobicity (logKow) of oestrogen ($P = 0.05 \leq 0.05$) was significantly related to the biodegradation ratios, and the polar surface area (PSA) ($P = 0.002 < 0.01$) significantly correlated with this hydrophobicity. Therefore, the occurrence and types of PSA and logKow appear to be important factors that govern the removal efficiency of oestrogens.

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. In this model, the properties served as independent variables and the biodegradation served as the dependent variable.

The QSBR model can be expressed as follows:

$$Y=-95.48*X_1+1242.34*X_2+4.61X_1*X_2 +0.84X_1*X_1 -196.23X_2*X_2 \quad (1)$$

$$(R^2=0.9897, n=10, F=71.8542, P=0.0026)$$

...where X_1 is PSA, X_2 is logKow, and Y represents the biodegradation ratios. The PSA represents the total surface

Table 3. Transformation of select EDCs.

Compound	Precursor	Intermediate product	Ultimate product
BPA	polycarbonate, epoxy resin	-	4-isopropenyl or BPA trimer, tetramer
E1	conjugated oestrogens, E2 [31], EE2 [33]	E3 [31]	2-hydroxy E1, 16α-hydroxy E1 etc.
E2	oestradiol valerate, oestradiol cypionate	E1, E3 [31]	2-hydroxy E2, testosterone analogues etc. [31, 34]
EE2	mestranol	E1 [31]	C2-6 molecule binary carboxylic acid [31]
E3	nylestriol, nilestriol etc.	-	4-methoxy E3 etc.

Table 4. Pearson correlation analysis of the correlation between estrogenic properties.

Properties	Heat of Formation	Gibbs Energy	Ideal gas thermal capacity	Formal Charge	Connolly Accessible Area	Connolly Molecular Area	Connolly Solvent Excluded Volume	Exact Mass
Pearson correlation	0.662	0.574	-0.286	-	-0.257	-0.247	-0.209	-0.303
Significant	0.224	0.312	0.640	-	0.676	0.688	0.736	0.620
N	5	5	5	5	5	5	5	5
Properties	Mass	Mol Weight	Ovality	Principal Moment	Mol Refractivity	Partition Coefficient	Balaban Index	Cluster Count
Pearson correlation	-0.303	-0.30	-0.31	-0.23	-0.14	0.69	-0.28	-.244
Significant	0.621	0.62	0.62	0.70	0.82	0.20	0.65	.693
N	5	5	5	5	5	5	5	5
Properties	Molecular Topological Index	Num Rotatable Bonds	**Polar Surface Area**	Radius	Shape Attribute	Shape Coefficient	Sum of Degrees	Sum of Valence Degrees
Pearson correlation	-0.174	0.197	**-0.987***	0.158	-0.244	-0.158	-0.208	-0.411
Significant	0.779	0.751	**0.002**	0.800	0.692	0.800	0.737	0.492
N	5	5	**5**	5	5	5	5	5
Properties	Topological Diameter	Total Connectivity	Total Valence Connectivity	Wiener Index	logKoc	**logKow**	Polarisability	Surface tension
Pearson correlation	0.158	0.134	0.318	-0.258	0.661	**0.878**	-0.293	-0.866
Significant	0.800	0.830	0.602	0.675	0.225	**0.050**	0.632	0.058
N	5	5	5	5	5	**5**	5	5

Bold: the result of a preliminary analysis about the physicochemical properties of oestrogens, which demonstrated an apparent correlation between the estrogenic properties and the removal of estrogenic contaminants.
* The coefficient statistically significant (P) located between 0.01and 0.05.

area of polar molecules, including oxygen, nitrogen, and the hydrogen bonds; logKow is described as a physical property, i.e., that oestrogen molecules repel each other in water. X_1*X_1 represents its own second-order interaction effect on the biodegradation rate in the system; X_2*X_2 represents its own second order interaction effect on the biodegradation rate in the system; X_1*X_2 represents their interaction effects on the biodegradation rate in the system. The R^2 of the model was calculated to be 0.9897, which indicates that the model is robust.

As shown in Eq. (1), the PSA negatively correlated and the logKow positively correlated with oestrogen removal, and these effects were synergistic. Compared with the main effect of PSA (X_1), the PSA (X_1) was negatively correlated with the biodegradation rate (Y), and its own second order interaction effect (X_1*X_1) was a positive value, recognized as synergistic effect to the biodegradation rate (Y), although this effect is not significant. In the same way, logKow (X_2) was positively correlated with oestrogen removal, and its own second order interaction effect (X_2*X_2) was not only significant, but also recognized as an antagonism to the biodegradation rate (Y). Because the coefficient X_1*X_2 is a second-order interaction effect of both X_1 and X_2, being a positive value, the joint effect of PSA and logKow on the biodegradation rate (Y) was recognized as a synergistic effect.

The existing research showed that the value of logKow directly corresponds to the hydrophobicity of oestrogen compounds. The surface hydrophobicity of microbial cells is an important property that directly affects the efficiency of various bioprocesses, such as bioremediation, waste treatment, and green biotechnologies, using whole microbial cells [35]. Compounds with higher logKow values can improve the efficiency of microbial degradation, which would enhance its adhesion to microbial cells, modulated by the type of growth substrate, growth phase, and the presence of biosurfactants/chemical surfactants, and promote the growth and reproduction of microorganisms and production of microflora [36-38]. The PSA negatively correlated with the penetrability of cells, which was detrimental to the growth and reproduction of microorganisms. A larger PSA indicates more hydrogen bonds and hydrophilic groups, which negatively impacts microbial degradation efficiency [39, 40].

Conclusions

Sodium alginate/ultrasound-assistance could significantly improve the biodegradation rates of oestrogens in soil,

while oestrogenic PSA and logKow were significantly related to the biodegradation rates. Moreover, the PSA negatively and the logKow positively correlated with oestrogen removal, and these effects were synergistic. This study has provided a theoretical foundation showing that adjusting the environmental conditions would appropriately influence and control the biodegradation processes of oestrogens.

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