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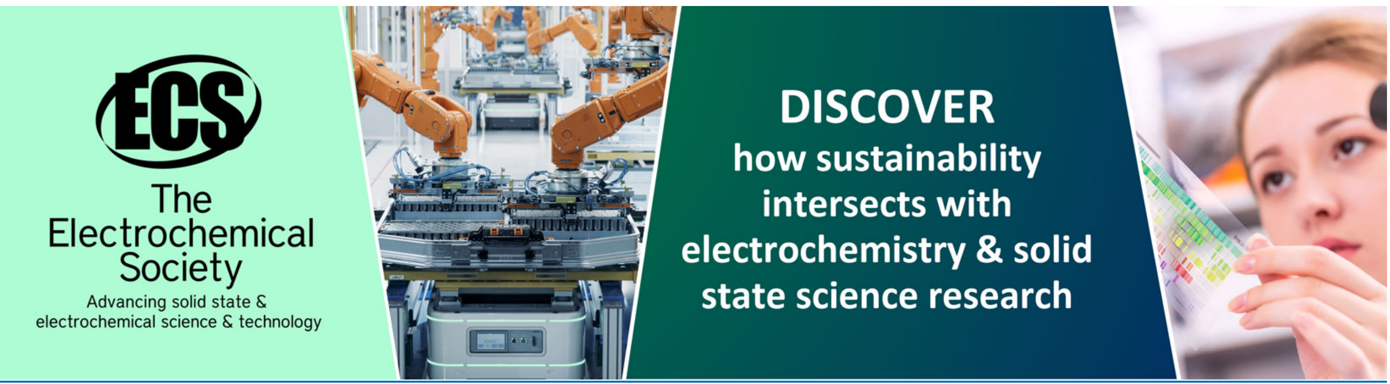

Oxytetracycline removal from water by novel microbial embedding gel beads

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Abstract. As a common antibiotic in aquatic environment, excessive oxytetracycline (OTC) is urgent to be removed due to its great biological toxicity. Compared with the traditional activated sludge, microbial embedding can enhance the treating efficiency. In this study, novel microbial embedding gel beads were produced with the additional agent of cyclodextrin (CD). Results show that CD could increase the mass transfer of OTC into gel beads, possibly because of its strong affinity for organic matters. In terms of OTC biodegradation, gel beads with CD were comparable to gel beads without CD, while the former's sucrose removal efficiency was higher than the latter. The biodegradation of OTC only occurred in the presence of sucrose. The respiration test also confirmed these findings. Overall, the produced novel gel beads modified with CD could improve the removal performance of OTC.

1. Introduction

Oxytetracycline (OTC), as one kind of typical tetracyclines, is widely used in clinical treatment and livestock industry, and as growth promoter due to its broad spectrum of activity and low cost [1]. Residues of antibiotics have been detected in surface water, sediment, soil and other environments. The presence of antibiotics in the environment not only brings chemical pollution, but also could provide selective conditions for the transfer and spread of antibiotic resistance [2].

Embedding technology is to prepare the gel carrier by cross-linking bacteria with embedding agents. As one kind of immobilization technology, the embedding technique has received increasing attention in the removal of organic contaminants [3]. There are various embedding agents, such as polyvinyl alcohol (PVA), sodium alginate (SA), etc..

Cyclodextrin (CD) is a kind of oligosaccharide, and the inner cavity is relatively hydrophobic, while all hydrophilic groups stay outside. CD can form clathrate compound with other substances embedded in the inner cavity like pollutants. The application of β-CD in environmental protection is

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common, due to low cost and appropriate inner diameter. For example, β-CD polymer was used to remove dissolved estrogens over a wide range of concentrations in the presence of various contaminants such as wastewaters [4].

In this paper, β-CD is introduced to PVA and SA embedding agents to prepare a novel microorganisms embedding material. The feasibility of this novel embedding material on OTC removal from water is evaluated.

2. Materials and Methods

2.1. Microbial embedding technique

PVA (hydrolyzed 97+%), SA and β-CD were used to prepare gel beads. These chemicals were purchased from Guangfu Fine Chemical Research Institute, Tianjin, China. Firstly, 7% (w/v) PVA and 1% (w/v) SA were completely melted and mixed by heating with water bath at 90°C, and 2% (w/v) β-CD was then added. The gel solution was cooled to 35°C and concentrated sludge was dispersed in gel solution. Furthermore, the gel solution was slowly added into solidifying solution 50% (w/v) $NaNO_3$ and 2% (w/v) $CaCl_2$ to make spherical beads that were continuously immersed in solidifying solution at 4°C for 12 h. Finally, gel beads were washed with distilled water.

2.2. Experiment of oxytetracycline biodegradation

200 g gel beads with embedded microbial were put into a 6 L cylinder bioreactor. Synthetic wastewater continuously flew to bioreactor to feed microbial: containing 200 mg/L sucrose, 2 mg/L OTC, 100 mg/L NH_4Cl, 27 mg/L KH_2PO_4, 500 mg/L $NaHCO_3$, 180 mg/L $CaCl_2{\cdot}2H_2O$, 300 mg/L $MgSO_4{\cdot}7H_2O$. And 1 mL trace element (625 mg/L EDTA, 190 mg/L $NiCl_2{\cdot}6H_2O$, 430 mg/L $ZnSO_4{\cdot}7H_2O$, 220 mg/L $NaMoO_4{\cdot}2H_2O$, 240 mg/L $CoCl_2{\cdot}6H_2O$, 990 mg/L $MnCl_2{\cdot}4H_2O$, 250 mg/L $CuSO_4{\cdot}5H_2O$) was added into per liter feeding solution. After one month of start-up, the water flow rate was successively set as 0.39 L/h, 0.66 L/h and 0.91 L/h for each two weeks. Then the ratio between sucrose and OTC was successively set as 100/2, 50/2 and 0/2. The influent and effluent were collected each day to measure OTC and chemical oxygen demanded (COD).

OTC was purchased from Sigma and the measured by HPLC system with UV detector (Shimadzu). An shim-pack VP-ODS (250 mm × 4.6 mm, 5 μm) was used as the analytical column at a flow rate of 0.8 ml/min. 0.01 mol/L oxalic acid solution as mobile phase A and acetonitrile with methanol (2:1) as mobile phase B were set and the detection wavelength was 355 nm.

2.3. Adsorption test

The dried gel beads without embedded microbial were added into 200 mL 100 mg/L OTC solution. Water was sampled in interval to analyze OTC concentration. The dispersion coefficient (De) was calculated by Eq. (1). Then, OTC concentration at the end of test was detected to calculate the specific adsorbed mass (Q) by Eq. (2).

$$-\ln\left(\frac{C_t - C_f}{C_0 - C_f}\right) = \frac{\pi^2 De}{R^2} t - \ln\frac{6}{\pi^2} \tag{1}$$

Where C_0 is the initial OTC concentration (mg/L), C_f is the final OTC concentration (mg/L), C_t is the detected OTC concentration (mg/L), De is the dispersion coefficient (cm^2/s), R is the diameter of gel bead (cm).

$$Q = \frac{(C_0 - C_f)V}{M} \tag{2}$$

Where V is liquid volume (mL), M is the mass of gel beads (g).

2.4. Respiration test

Gel beads with embedded microbial were firstly immersed in distilled water to calculate the endogenous respiration rate by a dissolved oxygen (DO) meter (Oxi3210, WTW, Germany) with stable temperature of 20°C. When the stable endogenous respiration rate was obtained, OTC was added to calculate the exogenous respiration rate in a unique carbon source of OTC. Finally, the synthetic wastewater in section 2.2 was added to calculate the exogenous respiration rate in carbon source of both OTC and sucrose. In terms of biomass of microorganisms embedded in gel beads, specific endogenous and specific exogenous respiration rates were measured to evaluate the activity of embedded microorganisms by Eq. (3).

$$\mathrm{SOUR} = \frac{C_i - C_e}{\Delta t}\frac{1}{\mathrm{VSS}} \tag{3}$$

Where SOUR is specific respiration rate (mg/(gVSS·h)), C_i is the initial DO concentration (mg/L), C_e is the final DO concentration (mg/L), Δt is the time between C_i and C_e (hour), VSS is the biomass concentration of microorganisms (g/L).

3. Results and Discussions

3.1. Adsorption of oxytetracycline

Fig. 1 shows the changing plots of cumulative mass transfer capacity of OTC into gel beads. The slop of fitting curve for gel beads with CD was larger than that of gel beads without CD. The dispersion coefficient and adsorbed OTC mass of gel beads with CD arrived at 6.1×10^{-7} cm^2/s and 8.1 mg/g gel bead, respectively. While in the case of gel beads without CD, these two values only stayed at 4.9×10^{-7} cm^2/s and 7.6 mg/g gel bead, respectively. The results indicate that CD could increase the mass transfer of OTC into gel beads, possibly due to its strong affinity for organic matters. It has been reported that CD could form the host-guest complexes with several organic matters. For example, the introduction of benzoic acid into the internal hydrophobic β-CD cavity was implied to cause the formation of 1:1 axial inclusion complexes of the host-guest type [5]. Thus CD is supposed to form the same inclusion complexes with OTC like other organic matters.

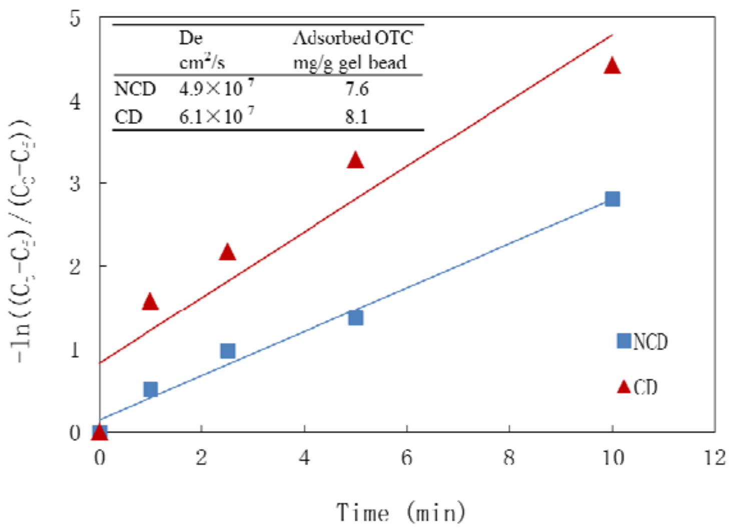

Figure 1. Cumulative mass transfer capacity of OTC into gel beads (NCD and CD represent gel beads without and with CD)

3.2. Biodegradation of oxytetracycline
Three flow rates were tested to observe the effects of water flow rates on OTC removal performance, as shown in Table 1. With the decrease of the flow rate, the effluent OTC concentration gradually decreased. The removal efficiency of OTC by gel beads without CD increased from 11% to 33%, and the OTC removal efficiency by gel beads with CD increased from 18% to 37%. The main reason is that the contact time between microorganisms and pollutants in gel beads grows with the increase of hydraulic retention time. In addition, the OTC removal efficiency by gel beads with CD is higher than gel beads without CD, which may be resulted by the adsorption of CD and the protection for microorganisms by CD.

In terms of COD biodegradation, the removal efficiency by gel beads without CD was relatively high, proving that there are still many heterotrophic microbial surviving even in the presence of OTC. Similarly, the COD removal efficiency by gel beads with CD was also high, at around 90%. However, with the increase of flow rate, the COD removal efficiency by gel beads without CD decreased from 83% to 63%. From the above results, it can be seen that metabolic mode of OTC biodegradation belongs to co-metabolism.

Table 1. Summary of OTC removal performance

Water flow rate (L/h)	Ratio of OTC/ sucrose	Influent OTC (mg/L)	Effluent OTC (mg/L)		OTC removal efficiency %		COD removal efficiency %	
			NCD	CD	NCD	CD	NCD	CD
0.91	200/2	2.15	1.91±0.08	1.77±0.17	11	18	63	87
0.66	200/2	2.08	1.73±0.10	1.68±0.10	17	19	78	90
0.39	200/2	2.25	1.48±0.24	1.43±0.19	33	37	83	89
0.39	100/2	2.05	1.17±0.10	1.18±0.10	43	42	67	77
0.39	50/2	2.05	1.76±0.09	1.78±0.01	14	13	40	53
0.39	0	2.05	1.99±0.02	1.97±0.02	3	4	-	-

In order to investigate the removal of OTC when lacking of easily biodegradable carbon source, the effects of OTC/sucrose ratio on OTC removal performance is evaluated as well (Table 1). With the decrease of the OTC/sucrose ratio, the effluent OTC concentration increased, and the OTC removal efficiency significantly reduced from about 40% to 3%-4%. It is further proved that co-metabolism contributed mainly to the degradation of OTC. In this study, OTC was not found to be degraded in the absence of sucrose. However, the heterotrophic microorganisms in gel beads can normally degrade sucrose in the presence of antibiotics, while the removal efficiency of COD decreased to some extent. Moreover, it is found that the OTC removal efficiencies by gel beads with and without CD were comparable. It is supposed that the adsorption effect of CD was not obvious possibly due to low OTC concentrations.

3.3. Microbial activity
Table 2 summarizes the respiration rates of microorganisms in suspended sludge, and gel beads with and without CD. It can be found that the maximum exogenous respiration rate and specific maximum exogenous respiration rate reached the maximum values after adding sucrose. The endogenous respiration rate was consistent with the exogenous respiration rate by the sole carbon source of OTC, which was consistent with the results of OTC degradation. Regarding with the specific maximum exogenous respiration rate by the carbon source of OTC and sucrose, the value for gel beads with CD was 1.22 $mgO_2/(gVSS \cdot h)$, the value for gel beads without CD was 0.61 $mgO_2/(gVSS \cdot h)$, and the lowest value belonged to the suspended sludge of 0.07 $mgO_2/(gVSS \cdot h)$. Results showed that the addition of CD could increase the activity of microorganism obviously. The reason may be that CD can alleviate the damage to microorganisms from embedding agents and embedding process.

Table 2. Comparison of biological respiration rates of suspended sludge and gel beads with and without CD

	[1]$R_{endo.}$	[1]$R_{max.exo.S1}$	[1]$R_{max.exo.S2}$	[2]Con.	[3]$SR_{endo.}$	$SR_{max.exo.S1}$	$SR_{max.exo. S2}$
	$mgO_2/(L\cdot h)$	$mgO_2/(L\cdot h)$	$mgO_2/(L\cdot h)$	g/L	$mgO_2/(gVSS\cdot h)$	$mgO_2/(gVSS\cdot h)$	$mgO_2/(gVSS\cdot h)$
[4]SS	0.04	0.07	0.21	3.17	0.01	0.02	0.07
[5]NCD	0.07	0.08	0.22	0.36	0.19	0.22	0.61
[6]CD	0.04	0.08	0.44	0.36	0.11	0.22	1.22

[1] R means respiration rate, endo. means endogenous, max.exo.S1 means maximum exogenous by OTC, max.exo.S2 means maximum exogenous by OTC and sucrose
[2] Con. means biomass concentration
[2] SR means specific respiration rate
[3] SS means suspended sludge, NCD means gel beads without CD, CD means gel beads with CD

Fig. 2 shows the change of dissolved oxygen consumption rate with time. 0-24 min is the process of endogenous respiration, 24-292 min is the process of exogenous respiration rate by the sole carbon source of OTC, and then from 292 min is the process of exogenous respiration rate after the addition of both OTC and sucrose.

It can be seen that the exogenous respiration rate and the endogenous respiration rate were equivalent after OTC addition, indicating that there is almost no degradation of OTC or microorganisms that can utilize OTC at that time. But once the sucrose was added, the respiration rate rose rapidly, rising from 0.07 $mgO_2/(L\cdot h)$ to 0.44 $mgO_2/(L\cdot h)$ and remained 180 min, then slowly dropped back to 0.09 $mgO_2/(L\cdot h)$.

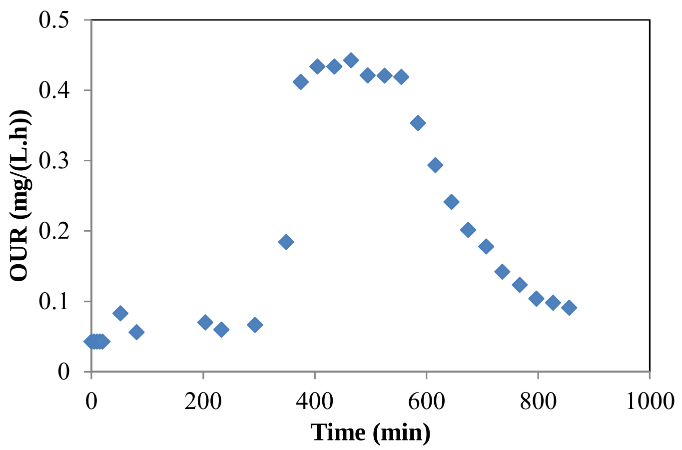

Figure 2. Variation of oxygen uptake rate with time

4. Conclusion

Gel beads had good adsorption effects on OTC, especially under the addition of CD. The difference of OTC removal efficiency by gel beads with and without CD was not significant, while their COD removal efficiency and microbial activity were distinct with better performance for the gel beads with CD. According to the results, the CD addition can improve the traditional gel beads' OTC removal behavior.

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