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ORIGINAL ARTICLE

Aerobic biodegradability of hydroxypropyl-β-cyclodextrins in soil

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Abstract Hydroxylpropyl-β-cyclodextrins (HP-β-CDs), hydroxyalkyl derivatives of β-CD, used in a broad range of applications in food, pharmaceutical, agriculture and bioremediation of soil because of their specific chemical properties. The possibility of varying the biodegradation rate of HP-β-CDs by changing the DS and substitution pattern makes HP-β-CDs suitable for various applications. Therefore, their biodegradation fate has been of great concern. In this study, the biodegradation of various HP-β -CDs, which have different degrees and patterns of substitution in different soil ecosystems, was investigated. The degree and pattern of substitution of HP-β-CDs were determined by the reductive-cleavage method and methylation analysis. Two common soils and a contaminated soil were used in the biodegradation test. All CDs were found to be more or less biodegradable. Increasing the degree of substitution (DS) had negative effect on the biodegradation rate of HP-β-CDs. The substitution pattern affected the biodegradation, too. The biodegradation rates of CDs in the contaminated soil were higher than that obtained in the uncontaminated soils. The contamination removing ability of CDs was highly affected by their own biodegradation fate in soil.

Keywords Hydroxylpropyl-β-cyclodextrins · Substitution · Methylation analysis · Biodegradation · Soil · Contamination

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Abbreviations

β-CD	β-cyclodextrin
HP-β-CD	Hydroxylpropyl-β-cyclodextrin
FDA	U. S. Food and Drug Administration
DS	Degree of substitution
CDase	Cyclodextrin-degrading enzyme
CGTase	Cyclodextrin glycosyl transferase
WHC	Water holding capacity
TS	Total dry solids
GC-MS	Gas chromatography-mass spectrometry
SEM	Solvent extractable material content
STD	Standard deviation

Introduction

Cyclodextrins (CDs) are macrocylic oligosugars obtained by enzymatic degradation of starch [1]. They are composed of a number of (1,4)-linked α-D-glucose units of which CDs with six, seven and eight glucose units are well known as α-, β- and γ-CD, respectively [2]. CDs are molecules with a shape of truncated cone, having hydrophobic cavity. Many hydroxyl groups are situated at the two edges of the ring, which makes the CDs both lipophilic and soluble in water. As a result, CDs are able to form inclusion complexes with a wide variety of hydrophobic compounds, and thus change the physical-chemical properties of the guest molecules [3, 4].

Hydroxylpropyl-β-cyclodextrin (HP-β-CD) a hydroxyalkyl derivative, is an alternative to α-, β- and γ-CD, with improved water solubility property [5] and may be slightly more toxicologically benign [6]. As the

first approved CD derivative by FDA, HP-β-CD has wide applications in food, pharmaceuticals and agriculture etc [7, 8]. HP-β-CDs are prepared by reacting β-CD with propylene oxide in alkaline aqueous solutions. High alkali concentration favors alkylation at O(6) and low alkali concentration at O(2) [9]. Still the products are always substituted randomly concerning the distribution among the different glucose units. And ratio of reactants, reaction time and temperature affect the average degree of substitution (DS). It follows that the composition of HP-β-CDs shows high variability, which is reflected in the chemical and physical properties, too [10].

The CD-degrading enzymes (CDase) are responsible for the cleavage of the CD ring. CD glycosyl transferase (CGTase) enzymes are able to catalyse the cyclisation of maltooligosacharides to yield CDs. It has been reported that most of the CGTase-producing bacteria (e.g., *B. coagulans*, *B. macerans* and *B. subtilis*) show CDase activity as well [11, 12]. The formation, uptake and intracellular degradation of CDs is a beneficial starch-degradation pathway for bacteria harboring both CD-forming and CD-degrading enzymes [13].

Oros et al. found several commonly occurring strains of plant-associated bacteria (*Agrobacterium*, *Bradyrhizobium*, *Xanthomonas* and *Corynebacterium*) as well as a soil fungi (*Trichoderma*) metabolising CDs as sole carbon source. The CD derivatives were more resistant than native CDs. For β-CD, the order of biodegradability was: unsubstituted > carboxymethyl > hydroxypropyl > trimethyl > polymer > dimethyl [14]. In another report, a controlled composting biodegradation test at 58°C was carried out by Verstichel et al. [15]. Three parent CDs (α-, β- and γ-CD) were completely and readily biodegradable, the CD derivatives reduced biodegradability significantly. Fully acetylated CDs were found to be non-biodegradable, and a HP-β-CD (DS = 4) was only partly biodegraded at a percentage of 20% during 45 days of composting. The biodegradation of several CDs in soils has been investigated by Fenyvesi et al. [16]. All the CDs were found to be more or less biodegradable, HP-β-CD (DS = 4) was completely biodegraded after 280 days in their test.

Recently new research has been performed on the incorporation of preservatives encapsulated in CDs and their derivatives into biodegradable packaging [17]. This resulted in polymers with antibacterial properties. But, until now only limited research has been performed on the biodegradation of CDs. In addition, more and more investigations are focusing on the environmentally safe bioremediation technologies using CDs and their derivatives as bioavailability enhancing additives to enhance the biodegradation of certain soil contaminants [18, 19]. HP-β-CDs are the mostly used agents to improve the solubility of the pollutants in soil washing technologies. When concentrated, (5–20%) HP-β-CD solutions are used for the removal of pollutants from aquifers [20]. It cannot be avoided that some of the HP-β-CD remains in soil. The increasing application of HP-β-CDs in soil remediation requires a deeper understanding of their biodegradability in soil.

There hasn't been any research done on the fate and behavior of various HP-β-CDs within soil, although it is widely known that the DS and substitution pattern should affect the properties of HP-β-CDs.

In this study, the biodegradation of several HP-β-CDs with varying DS and substitution pattern was studied in different soils using a standard biodegradation test. Comparing the trends in the rate of biodegradation for different HP-β-CDs in various soil ecosystems, some new conclusions are drawn.

Experimental

Materials

HP-β-CD samples were prepared in the laboratory by reacting β-CD with propylene oxide under two different base concentrations, that is, group A (samples 1–3) under 18%; group B (samples 4–6) under 5%, except one (sample 7) purchased from Wako Pure Chemical Industries, Ltd. (Chuoku, Osaka, Japan). All other materials were of analytical grade and used without further purification. The water used was deionized.

Soils

Three soil samples (S1, S2 and S3) were used in the biodegradability test. Table 1 represents the characteristics of the soils. Soil S1 was a mixture of one sandy soil and three woodland soils all from Wuxi in China. Soil S2 was a woodland soil derived from Guangzhou in China. While S3 used in the laboratory scale bioremediation experiment was a sandy soil derived from a garage (Wuxi, Jiangsu, China). It was contaminated with motor and diesel oil. All three soil samples were sieved through a 2 mm sieve to remove stones, plant debris and thoroughly mixed. The imperative pH of the soils used in the standard biodegradation test should be between 7.0 and 9.0; and water between 40 and 60% of its water holding capacity (WHC). Those characteristics of three soils used in the test fulfilled the requirement. The C/N ratio

Table 1 Physico-chemical characterics of soils

Characteristics	S1	S2	S3
Total solids (TS, %)	88.5	82.2	90.1
Ash content (% on TS)	67.8	61.7	68.4
pH	7.3	7.6	7.8
Water holding capacity (WHC, %)	26.0	48.3	24.1
Total N (g/kg, TS)	0.84	1.45	0.95
C/N ratio	13.4	15.3	36.7
Aerobic heterotrophic cells (CFU/g, TS)	6.5×10^6	1.7×10^7	2.8×10^7
Extractable material content (SEM, mg/kg, TS)	n.m.	n.m.	24000

of S3 was too high to perform the test, additional N was applied in the form of NH_4NO_3 fertilizer to optimize it.

Soil analysis

The total dry solids (TS) content was obtained by taking a known amount of test materials and drying them at 105°C to constant weight. The ash content on dry weight was calculated by incinerating a known amount of test soil samples at 550°C. The pH of the soil samples diluted with water at a ratio of 5:1 was measured with a pH-electrode [21]. The soil samples were immersed in water for 12 h and then the excess water were removed by centrifugation (2440 rpm, 40 min), the total water holding capacity (WHC) was determined by drying a known amount of the centrifugated soil samples at 105°C.

The total N was determined by Kjeltec apparatus [22]; C/N ratio was determined by elementary analysis. The concentration of the aerobic heterotrophic cells was determined by plating on agar gel and colony counting. The soil solvent extractable material content (SEM) was determined by extracting the soil samples with ether and then weighing the extract residue after drying.

Determination of substitution pattern and DS of HP-β-CDs

The pretreatment of samples were carried out according to Ciucanu and Kerek's reductive-cleavage method and methylation analysis [23]. HP-β-CD samples were permethylated, hydrolyzed and acetylated. The obtained acetylated D-glucitol ethers were analyzed by GC-MS. GC-MS were performed on a Finnigan Trace MS system with helium as the carrier gas. An OV1701 capillary column (30 m × 0.25 mm × 0.25 μm) was used.

Biodegradation experiments

The biodegradation test was performed according to ISO 17556 (2003) [24]. The biodegradation of β-CD and 7 types of HP-β-CDs was evaluated in a controlled test. Two control reactors and two reference reactors were included in each test series. The control reactors contained only soil without the added test material. Microcrystalline cellulose was used as the positive reference. The biodegradation of CDs was evaluated in duplicates, using 400 g soil (S1 and S2) and 1% of test or reference items in each reactor. All the CDs were evaluated in S1 soil, while only β-CD and group A HP-β-CDs were determined in S2 soil.

All the reactors (1000 ml) were incubated at 25 ± 1°C in the dark. Sufficient oxygen was provided by the ventilating air while CO_2 was removed by 1 M KOH trap. During the aerobic biodegradation, the solid carbon of the test materials was converted into CO_2. The produced CO_2 was led to traps by silicone rubber pipes and captured by 1 M KOH in the traps. The amount of the captured CO_2 was measured by titrating the $KHCO_3$ (pH 8.0) to KCl and CO_2 (pH 3.9) using 1 M HCl with a 702 SM Titrino automatic titrator. The percent biodegradation was calculated as the percentage of elemental carbon in the test substrate that was mineralized to form CO_2.

The biodegradation of the CDs in contaminated soil (S3) was carried out in a similar manner as described above, except that the amount of soil used in this test series is 200 g in each reactor. β-CD and HP-β-CD sample 2 were studied. Here, the captured CO_2 was the sum of CO_2 derived from CDs and waste oil contained in the contaminated soil. The amount of biodegraded waste oil was calculated by the difference between the values of SEM determined by two times. The equivalent of CO_2 to waste oil was measured by the proportion of the amount of captured CO_2 and biodegraded waste oil in the control reactors. Hence the amount of CO_2 derived from waste oil was calculated. The amount of CO_2 derived from CDs was obtained by subtracting the amount of CO_2 derived from waste oils, from the captured CO_2. The percent biodegradation of CDs and waste oil was calculated as the percentage of elemental carbon in the test substrates that were mineralized to form CO_2.

Results

Substitution pattern and DS of HP-β-CDs

The mass spectra of the acetylated D-glucitol ethers were analyzed to obtain the configuration information of HP-β-CDs. The fragmentation of the acetylated D-glucitol ethers on electron impact follows established principles. The results of the analyses are summarized in Table 2. The distribution of HP-substituents was generally similar to the study reported earlier [9], i.e., the substitution pattern depends on the alkali concentration used in the preparation. Weak alkaline conditions favored alkylation at more acidic C(2) hydroxyls, while strong alkaline conditions favored alkylation at the more accessible C(6) hydroxyls. The DS and ratio of alkylation at different points were calculated. In group A, the DS value of O(6) (DS (6)) was the highest, but at O(2) and O(3) positions also had many substituents. The ratio of DS (2 + 3) to DS (6) is close to 1. The distribution of substituents on primary (DS (6)) and secondary hydroxyl groups (DS (2 + 3)) was even, because the three free hydroxyl groups were all active under strong alkali conditions. The O(6) position got a little more opportunity to be substituted due to steric hindrance. Different from this group, the substituents concentrated at O(2) in group B. The DS value of secondary hydroxyl groups was about three times of primary face.

Biodegradability test in uncontaminated soils

Table 3 shows the percentage of the biodegradation fractions of the test items studied in the standard biodegradability test. The biodegradation percentages of β-CD and HP-β-CD samples as a function of time are presented in Figs. 1, 2, 3. A standard biodegradability test can be considered valid if the biodegradation percentages of the reference items are more than 60% at the end of the test. The reference cellulose reached high biodegradation percentages in all experiments. After 133 days of incubation, more than 90% of the cellulose was converted into CO_2. At the end of the test, biodegradation rates of 102.7% and 100.9% in S1 and S2 respectively were obtained for cellulose. The results fulfilled the requirement for a valid test.

The parent β-CD samples were degraded fast and completely (Figs. 1 and2). Biodegradation percentages greater than 90% were obtained within 100 days, and 99.2%, 103.9% in S1 and S2 respectively at the end of the test. In S1 soil, all the CDs were found to be more or less biodegradable. For the S2 soil, only β-CD and sample 1, 2, and 3 were studied. The initial rate of biodegradation decreased in the order of β-CD > cellulose > HP-β-CD 1 > HP-β-CD 2 > HP-β-CD 3. The purchased HP-β-CD (sample 7) had a similar biodegradation curve to the HP-β-CD prepared. It was obvious, at the beginning of the test that the biodegradation of all items started immediately at different rates. During the progression of the test, the biodegradation rates of all test items decreased gradually, and became very slow in the last weeks of the test. It might be assumed that the biodegradation of CDs were paused in this test. Also, there might be exhausted if sufficient time for microorganisms which could biodegrade CDs in the soil is provided [16].

In several instances, biodegradation percentages greater than 100% were reached at the end of the test (Table 3). From the STD values shown in Table 3, it can be drawn that the results might be due to an experimental error. It has been reported that CDs might mobilize organic compounds present in the soil, making them more bioavailable and therefore becoming biodegradable by microorganisms to produce CO_2 [25, 15]. This resulted in a net CO_2 production that was not exclusively produced by the test items. The cavity of CD is extended by HP substitution, which can either enhance the complex forming ability or sterically hinder the organic compounds into the cavity. The CO_2 production from HP-β-CDs was observed to be much slower than from β-CD. On considering that the evolution of biodegradation of β-CD was similar to that of

Table 2 Distribution of substituents and the respective relative DS value of hydroxyl groups in HP-β-CDs

Samples	Group A			Group B			7
	1	2	3	4	5	6	
DS(3)	0.18	0.57	0.98	0.88	1.18	1.90	1.43
DS(2)	1.24	2.51	3.24	1.59	2.97	3.61	2.85
DS(2 + 3)	1.43	3.08	4.22	2.47	4.15	5.51	4.28
DS(6)	1.78	2.78	4.48	0.66	0.91	1.67	1.25
DS(2 + 3)/ DS(6)	0.80	1.11	0.94	3.73	4.55	3.30	3.43
Molecular DS	3.21	5.77	8.68	3.14	5.06	7.19	5.53
Molecular Weight	1321	1470	1638	1317	1428	1551	1456

Table 3 Carbon content, net CO_2 production, and biodegradation of the test items

Test series	Theoretical carbon content (%)	Net CO_2 production (mg/g test item)	Biodegradation (%)	
			Average	STD
1. test in S1 (133 days)				
Cellulose	44.4	1672 ± 62	102.7	3.8
β-CD	44.4	1615 ± 77	99.2	4.7
HP-β-CD1	46.9	1273 ± 158	74.0	9.2
HP-β-CD2	48.4	973 ± 126	54.8	7.1
HP-β-CD3	49.8	471 ± 53	25.8	2.9
HP-β-CD4	46.8	1138 ± 130	66.3	7.6
HP-β-CD5	48.1	864 ± 90	49.0	5.1
HP-β-CD6	49.2	559 ± 67	31.0	3.7
HP-β-CD7	48.3	811 ± 120	45.8	6.8
2. test in S2 (133 days)				
Cellulose	44.4	1642 ± 138	100.9	8.5
β-CD	44.4	1692 ± 113	103.9	6.9
HP-β-CD1	46.9	1317 ± 124	76.6	7.2
HP-β-CD2	48.4	1051 ± 170	59.2	9.6
HP-β-CD3	49.8	508 ± 86	27.8	4.7
3. test in S3 (70 days)				
β-CD	44.4	1216 ± 103	74.7	6.3
HP-β-CD2	48.4	726 ± 105	40.9	5.9

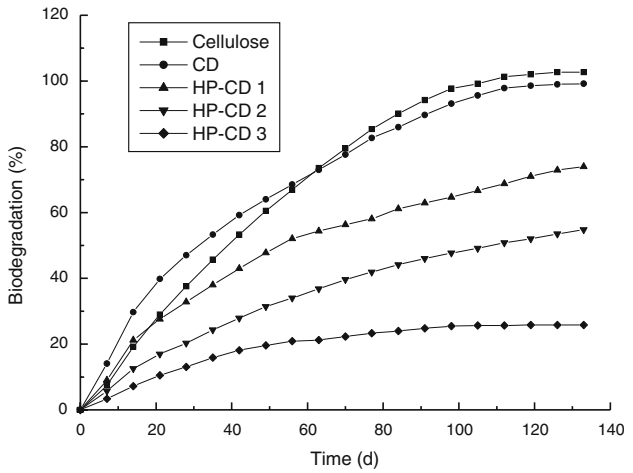

Fig. 1 Evolution of the biodegradation of cellulose, β-CD and group A HP-β-CDs in soil S1

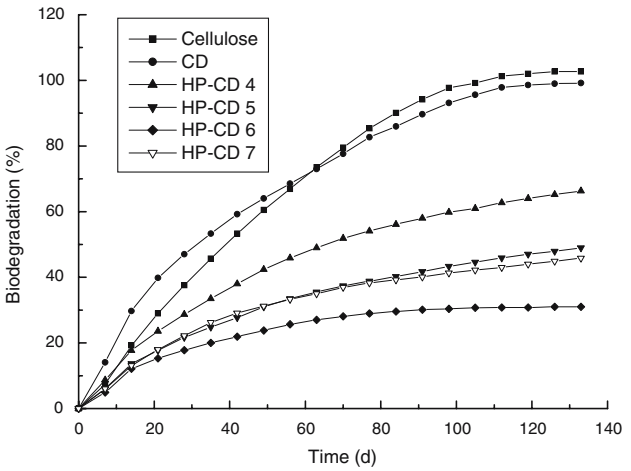

Fig. 2 Evolution of the biodegradation of cellulose, β-CD, group B HP-β-CDs and a commercial HP-β-CDs sample 7 in soil S1

cellulose, it may be assumed that no obvious mobilizing effects exited in this test. The CO_2 seemed to be derived primarily from the biodegradation of CDs. Fig. 3

Figure 4 shows the DS courses for percent biodegradation of CDs at the end of the test. It seems that the DS value had negative effect on the degree of biodegradation. The increasing amount of HP substituents at CD ring sterically hindered the approach of CD-degrading enzymes. In the same soil (S1), the biodegradation percentage of group B samples was lower than that of group A samples at the same DS values. The HP substituents of group B samples which excessively concentrated at O(2) and O(3) positions might cause them more difficult to be catalysed by the CD-degrading enzymes produced by microorganisms in the soil, hence the biodegradation rates decreased. The percent biodegradation of sample 7 was similar to that of group B samples as they resemble in configurations. The effect of soil on the degree of biodegradation was less than that of DS in this test. However, it could also be observed that, on the same CD, the biodegradation rate in loamy S2 soil was higher than in sandy S1 soil. Several factors might bring about this result, for instance, the number of microorganisms biodegrading CDs, soil conditions and the mobilizing effects.

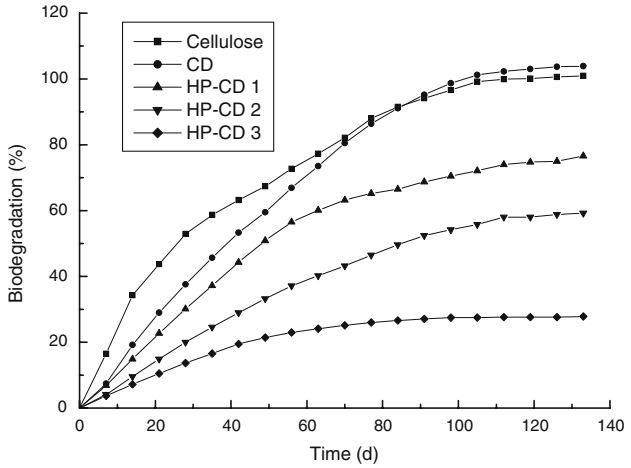

Fig. 3 Evolution of the biodegradation of cellulose, β-CD and group A HP-β-CDs in soil S2

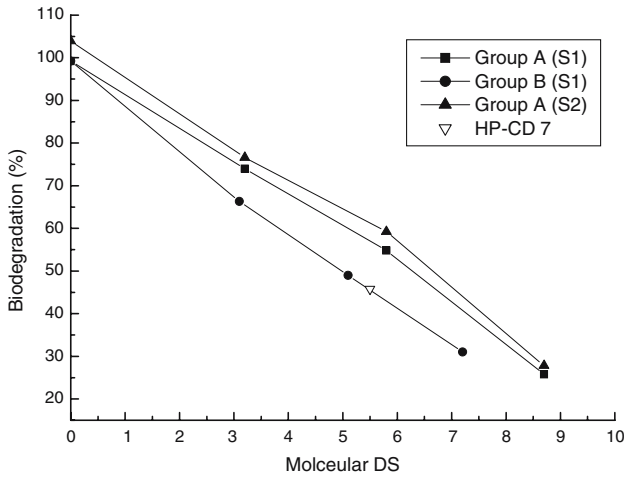

Fig. 4 The effect of molecular DS on biodegradation of CDs

Biodegradation experiments in contaminated soil

The aim of the CDs added to the contaminated soil was to enhance biodegradation of the poorly biodegradable motor and diesel oil. The CDs were found to be biodegraded in all experiments. The β-CD was degraded very fast. Only about 50% of the initial β-CD remained after 30 days, 74.7% was degraded at the end of the test. The biodegradation rate of HP-β-CD sample was 40.9% after 70 days. Both results were higher than those obtained in the uncontaminated soils (Table 3).

The SEM contents in control and CD-treated soils are given in Fig. 5. The oil removal rate was higher in the CD-treated soils than in the control. In the first 40 days, the waste oil in β-CD-treated soil biodegraded faster than HP-β-CD-treated soil. The cell concentration in β-CD-treated soil might be higher due to the

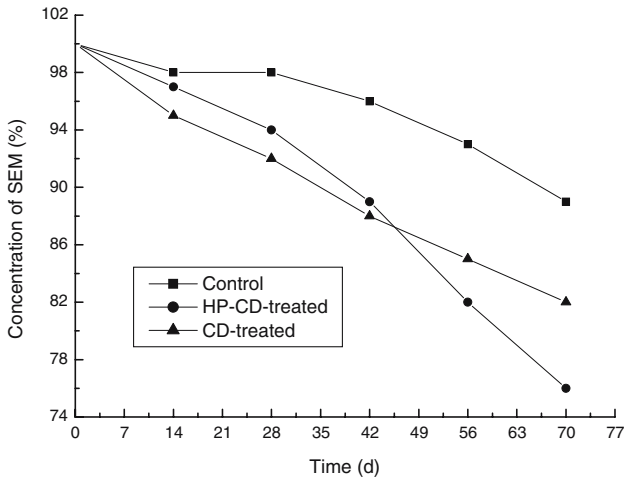

Fig. 5 The decrease in extractable material content of control, β-CD-treated and HP-β-CD-treated contaminated soil (S3)

easily biodegradable β-CD which functions as a nutrient for them, while a higher cell concentration led to faster oil removal rate. The efficacy of β-CD in accelerating the biodegradation of the waste oil decreased due to its decreasing concentration in the soil. During the last 4 weeks of the test, the decrease of contaminations in β-CD-treated soil was approximately parallel to that of control soil because there were few β-CD residues in the soil. This result could partly prove the beneficial effect of β-CD on the biodegradation of soil contaminated with hydrocarbons. A similar conclusion could be drawn to HP-β-CD. In addition, the removal rate of waste oil treated with HP-β-CD was higher than that treated with β-CD at the end of the test due to the higher solubilizing effect (higher bioavailability-enhancing effect) and the lower biodegradation rate of HP-β-CD.

Discussion

HP-β-CDs were partly biodegradable in soil in this test. They might be exhausted if sufficient time for microorganisms in the soil which could biodegrade CDs is provided. HP substituents affected the biodegradability strongly. The biodegradation percentage of HP-β-CDs had negative relationship with the DS values. The increasing amount of HP substituents on CD ring made them to be more difficult to catalyse by the CD-degrading enzymes produced by microorganisms in the soil. Even the substitution pattern affected the bioavailability of HP-β-CDs. It seems that the HP-β-CDs with HP substituents concentrated at O(2) and O(3) positions were more difficult to be biodegraded

due to steric hindrance of CD-degrading enzymes approaching the substrates. The soil characteristics might also slightly affect the biodegradation of HP-β-CDs.

The biodegradation rate of CDs in the S3 soil was higher than that obtained in uncontaminated soils. The experiments for the contaminated soil indicated that both β-CD and HP-β-CDs enhanced the bioavailability of contaminants in soil. The efficacy of CDs in accelerating the biodegradation of contaminations was highly affected by their own biodegradation fate in soils.

The possibility of varying the biodegradation rate of HP-β-CDs by changing the DS and substitution pattern makes HP-β-CDs suitable for applications in which biodegradation is desired at different rates. The low biodegradation rate HP-β-CDs might be of particular interest for use in soil bioremediation, aqueous solubilizer or waste water treatment. The high biodegradation rate and low DS value HP-β-CDs might be applied in food, pharmaceutical or biodegradable packing.

References

1. Del Valle, E.M.M.: Cyclodextrins and their uses: a review. Process Biochem. **39**, 1033–1046 (2004)
2. Szejtli, J.: Introduction and general overview of cyclodextrin chemistry. Chem. Rev. **98**, 1743–1753 (1998)
3. Schneiderman, E., Stalcup, A.M.: Cyclodextrins: a versatile tool in separation science. J. Chromatogr. B. **745**, 83–102 (2000)
4. Dodziuk, H.: Rigidity versus flexibility. A review of experimental and theoretical study pertaining to the cyclodextrin nonrigidity. J. Mol. Struct. **614**, 33–45 (2002)
5. Uekama, K., Hirayama, F., Irie, T.: Cyclodextrin drug carrier system. Chem. Rev. **98**, 2045–2076 (1998)
6. Sarah, G., Robert, C.S.: 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD): a toxicology review. Food Chem. Toxicol. **43**, 1451–1459 (2005)
7. Singh, M., Sharma, R., Banerjee, U.C.: Biotechnological applications of cyclodextrins. Biotechnol. Adv. **20**, 341–359 (2002)
8. Szente, L., Szejtli, J.: Highly soluble cyclodextrin derivatives: chemistry, properties, and trends in development. Adv. Drug Deliv Rev. **36**, 17–28 (1999)
9. Pitha, J., Rao, C.T.: Distribution of substituents in 2-hydroxypropyl ethers of cyclomaltoheptaose. Carbohydr. Res. **200**, 429–435 (1990)
10. Rao, C.T., Fales, H.M., Pitha, J.: Pharmaceutical usefulness of hydroxypropylcyclodextrins: ''e pluribus unum'' is an essential feature. Pharm. Res. **7**, 612–615 (1990)
11. De Pinto, J.A., Campbell, L.L.: Purification and properties of the cyclodextrinase of *Bacillus macerans*. Biochemistry **7**, 121–125 (1968)
12. Bender, H.: Cyclodextrin glucanotransferase from *Klebsiella pneumoniae* Part 3. Carbohydr. Res. **65**, 85–97 (1978)
13. Usanov, N.G., Loginov, O.N., Melent, A.I.: Synthesis of cyclodextrin glucanotransferases by microorganisms utilizing cyclodextrins as the only source of carbon. Dokl. Akad. Nauk. SSSR. **310**, 1489–1492 (1990)
14. Oros, G., Cserháti, T., Fenyvesi, E., Szejtli, J.: Microbial decomposition of some cyclodextrin derivates by bacteria associated with plants. Int. Biodeter. Biodegr. **26**, 34–42 (1990)
15. Verstichel, S., De Wilde, B., Fenyvesi, E., Szejtli, J.: Investigation of the aerobic biodegradability of several types of cyclodextrins in a laboratory controlled composting test. J. Polym. Environ. **12**, 47–55 (2004)
16. Fenyvesi, E., Gruiz, K., Verstichel, S., De Wilde, B., Leitgib, L., Csabai, K., Szaniszlo, N.: Biodegradation of cyclodextrins in soil. Chemosphere. **60**, 1001–1008 (2005)
17. Jung, H.H.: Antimicrobial Food Packaging. Food Technol. **54**(3), 56–65 (2000)
18. Steffan, S., Tantucci, P., Bardi, L., Marzona, M.: Effects of Cyclodextrins on Dodecane Biodegradation. J. Incl. Phenom. **44**, 407–411 (2002)
19. Fava, F., Di Gioia, D., Marchetti, L.: Cyclodextrin effects on the ex-situ bioremediation of a chronically polychlorinated biphenyl contaminated soil. Biotechnol. Bioeng. **58**(4), 345–355 (1998)
20. Blanford, W.J., Barackman, M.L., Boving, T.B., Klingel, E.J., Johnson, G.R., Brusseau, M.L.: Cyclodextrin-enhanced vertical flushing of a trichloroethene contaminated aquifer. Ground Water Monit. Rem. **21**(1), 58–66 (2001)
21. ISO 10390, Soil quality—Determination of pH (1994)
22. ISO 5563, Water quality—Determination of Kjeldahl nitrogen—Method after mineralization with selenium (1984)
23. Ciucanu, I., Kerek, F.: A simple and rapid method for permethylation of carbohydrates. Carbohydr. Res. **131**, 209–217 (1984)
24. ISO 17556, Determination of the ultimate aerobic biodegradability in soil by measuring the oxygen demand in a respirometer or the amount of carbon dioxide evolved (2003)
25. Boving, T.B., Wang, X., Brusseau, M.L.: Cyclodextrinenhanced solubilization and removal of residual-phase chlorinated solvents from porous media. Environ. Sci. Technol. **33**, 764–770 (1999)