STUDY ON THE DEGRADATION OF DIOXINS BY THE STEVIA EXTRACT

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Introduction

Dioxins are the most toxic organic compounds and found in various matrices such as soil, water, atmosphere and food. Because of their high toxicity, the reduction of dioxin levels in the environment is very important and urgent goal.

By this time, many types of microorganisms showing degradation ability of dioxins are well known[1, 2]. But there has been no report on the natural products that have activity as for the detoxification of the environmental samples containing dioxins.

In recent years, there has been considerable interest in stevia extract which have ability of degradation of dioxins[3, 4]. Stevia rebaudiana is a shrub, native to Paraguay and is grown in the Far East, South America and many other areas of the world[5]. Stevioside is a major component of stevia leaves and has been used as natural source sweetener.

This work places on the application of the extract to the degradation of toxic congeners of PCDDs/PCDFs. The applicability of this mild technique to the detoxification of soil and ash samples is demonstrated.

Experimentals

(1) Preparation of extracts

China-grown dried stevia leaves were used for obtaining the extracts. 500 g of dried stevia leaves were placed in a 4 L of flask and extraction was done with 2.5 L of water at boiling temperature. The extract was filtered and diluted with water until water ratio was maintained at the range of 80 – 83 %(w/w).

(2) Heterogeneous reaction

100 ml of the stevia extract was added to 5.49 g of soil or 1 g of ash in a 250 ml of Erlenmeyer flask. Both flasks were stirred for 3 days at room temperature.

(3) Homogeneous reaction

For liquid-liquid reaction with stevia extract, about 1 g of ash sample was extracted with toluene in a soxhlet apparatus for 24 hrs. The toluene fraction was completely evaporated by rotary evaporator and then dissolved with 1ml of methanol in a 250 ml of Erlenmeyer flask. Homogeneous reaction was performed by adding 100 ml of the stevia extract to the flask, and then was stirred for 3 days at room temperature.

(4) Clean-up and analysis

After all the reaction, the solutions were filtered with filter paper. Resulting liquid fractions

were transported into separatory funnels respectively. Then a mixture of fifteen ^{13}C-labeled internal standards was added to the funnels and extracted with methylene chloride. The funnels were shaken vigorously for 5 min with a shaker. The phases were separated, and water of the organic phase was removed by adding anhydrous Na_2SO_4. Simultaneously, as clean-up spike, $^{37}Cl_4$-2,3,7,8-TCDD was added respectively to the filter papers including treated soil or ash. Then the solid fractions were extracted with toluene in a soxhlet apparatus for 24 hrs. The solvents from the extraction steps were combined and evaporated. The remainder was cleaned by the EPA method 1613. The untreated soil and ash samples were also extracted and cleaned as above mentioned procedure.

All the samples were analyzed by high-resolution GC-mass spectrometry(SIM mode, resolution:10,000) with a HP6890 GC system(Hewlett- Packard, USA) coupled to an Autospec-ultima(Micromass, Manchester, UK) and a fused silica SP-2331 capillary column(60m x 0.25mm i.d.). The GC temperature program was 120 °C(held for 3 min), increased at 20 °C/min to 220 °C(held for 5 min), increased at 4 °C /min to 260 °C(held for 25 min). Injector temp. was 260 °C and injection was made on splitless mode(1 min). Helium at a pressure of 25 psi was used as carrier gas.

Results

(1) Degradation efficiency of dioxins in fly ash sample

The concentrations of toxic 17 isomers after the heterogeneous or homogeneous reactions between stevia extract and solid ash or ash extract are shown in Table 1. In the two reactions the degradation efficiency is 46.4 % and 68.8 % respectively. As shown in Table 1, the concentrations of all the isomers are uniformly decreased. The difference of degradation efficiency on fly ash between two reactions can be explained as high solubility of dioxins in the homogeneous phase.

Table 1. Degradation of dioxins in fly ash treated by stevia extract

Unit : ng-TEQ/g

	Dioxins Conc. In fly ash sample	Heterogeneous reaction	Homogeneous reaction
Congener	Before	After	After
2,3,7,8-TCDF	0.040	0.022	0.013
1,2,3,7,8-PeCDF	0.098	0.052	0.031
2,3,4,7,8-PeCDF	1.241	0.706	0.386
1,2,3,4,7,8-HxCDF	0.329	0.186	0.100
1,2,3,6,7,8-HxCDF	0.377	0.201	0.112
2,3,4,6,7,8-HxCDF	0.619	0.323	0.189
1,2,3,7,8,9-HxCDF	0.045	0.020	0.031
1,2,3,4,6,7,8-HpCDF	0.224	0.128	0.066
1,2,3,4,7,8,9-HpCDF	0.036	0.018	0.011
OCDF	0.012	0.006	0.005
2,3,7,8-TCDD	0.094	0.047	0.032
1,2,3,7,8-PeCDD	0.271	0.142	0.082
1,2,3,4,7,8-HxCDD	0.086	0.045	0.028

1,2,3,6,7,8-HxCDD	0.176	0.081	0.059
1,2,3,7,8,9-HxCDD	0.124	0.061	0.040
1,2,3,4,6,7,8-HpCDD	0.160	0.075	0.046
OCDD	0.041	0.020	0.012
Total Conc.	3.973	2.132	1.241
Degradation Efficiency(%)	-	46.4	68.8

(2) Degradation efficiency of dioxins in soil sample

The concentrations of toxic 17 isomers after the duplicate reactions between stevia extract and soil sample are shown in Table 2. As shown in Table 2, the average degradation efficiency in soil sample is 80.8%. The difference of the efficiency between soil and fly ash samples is ascribed that dioxins level of soil sample is much lower than that of fly ash. It seems that the degradation capability of stevia extract is varied with the dioxin levels and kinds of sample matrices.

Table 2. Degradation of dioxins in soil treated by stevia extract

Unit : pg-TEQ/g

	Dioxins Conc. in soil sample	1st reaction	2nd reaction
Congener	Before	After	After
2,3,7,8-TCDF	0.59	0.04	0.17
1,2,3,7,8-PeCDF	0.24	0.03	0.03
2,3,4,7,8-PeCDF	3.53	0.58	0.58
1,2,3,4,7,8-HxCDF	0.65	0.17	0.06
1,2,3,6,7,8-HxCDF	0.58	0.19	0.11
2,3,4,6,7,8-HxCDF	0.70	0.39	0.12
1,2,3,7,8,9-HxCDF	0.14	0.13	0.01
1,2,3,4,6,7,8-HpCDF	0.27	0.11	ND
1,2,3,4,7,8,9-HpCDF	0.02	0.02	0.01
OCDF	0.02	0.02	0.01
2,3,7,8-TCDD	0.61	0.17	0.09
1,2,3,7,8-PeCDD	1.29	0.16	0.12
1,2,3,4,7,8-HxCDD	0.15	0.06	ND
1,2,3,6,7,8-HxCDD	0.30	ND	0.06
1,2,3,7,8,9-HxCDD	0.24	0.11	0.04
1,2,3,4,6,7,8-HpCDD	0.20	0.06	0.03
OCDD	0.11	0.02	0.02
Total Conc.	9.65	2.26	1.46
Degradation Efficiency(%)	-	76.6	84.9

* ND : Not Detected

In conclusion, the stevia extract as a dioxins degradation agent shows a good performance.

Although the mechanism of degradation is unknown, it can be used for the restoration of soil and the detoxification of ashes produced from incinerators. Now, the work is in progress to elucidate the mechanism of degradation.

References

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