Biodegradation of dioxins by activated sludge

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Introduction

Dioxins are one of the most harmful man-made chemicals. Especially in Japan, many dioxin-contaminated sites have been found around incineration facilities, with the degree of contamination reaching over 8,000 pg/g. Contaminated soil has been gathered and stocked in special institutes until the toxicity has decreased to below the environmental guard line or treated by remediation methods.

While the number of analyses of soil contamination site has been increasing, the stock capacity has been limited to a small volume of highly concentrated dioxin-contaminated soil.

In general, dioxins are present in the soil at low concentrations and are spread broadly over the land surface. These low concentrations in the soil, however, translate into high concentrations in the human body as the dioxins pass through the food chain.

Therefore, effective dioxin treatment methods are necessary. Physio-chemical treatment methods using large amounts of energy and requiring the construction of special facilities have been tested, but as of yet there is no quick, effective method to be applied to the remediation of dioxin-contaminated soil. Therefore, as of yet, physio-chemical methods are expensive and time-intensive to be used for soils with only low to moderate levels of contamination.

Biological methods, for example, the use of microorganisms for dioxin degradation, can be ideal for remediation because they don't require special facilities and they can treat large amounts of contaminated soil on site; these methods, however, are comparatively slow to be applied on a large scale.

Currently, physio-chemical methods have been well developed by many researchers around the world as applications, while microbial degradation has not.

Some studies have been carried out to further develop our understanding of the fate of dioxins in the environment under anaerobic conditions, especially under methane-producing conditions (1). Under anaerobic conditions, it has been found that most toxic dioxins, specifically polychlorinated dibenzo-p-dioxins, are dechlorinated by microorganisms, but that this activity is very slow, taking a month or more. In addition, the related degradation rate is not sufficient to decrease the toxicity to less than the environmental guard line. Other researchers have isolated some aerobic bacteria and fungi from dioxin-contaminated soil (2-3). Aerobic bacteria, Pseudomonas and Sphingomonas, have been found to degrade some kinds of dioxins more quickly than to anaerobic. These strains can degrade non- to three-chlorinated dioxins. Fungi can degrade non- to octa-chlorinated dioxins, but the degradation takes a week or more. Last year in this congress we reported two novel dioxin-degrading bacterial strains and the application of these strains in a bioreactor along with activated sludge using degradation methods. In these results, bacteria could degrade non- to tetra-chlorinated dioxins in 4 days (4). In addition, activated sludge under denitrification conditions could degrade tetra- to octa-chlorinated dioxins in 1 month (5). From these experiments we confirmed the possibility of degrading dioxins through the use of microorganisms and speculated about the possibility of using these methods in large-scale bioreactors.

In this report we discuss the use of activated sludge in the degradation of dioxins.

Materials and Methods

Chemicals

PCDD/PCDF STANDARD MIXTURES EDF-4931 (Cambridge Isotope Laboratories Inc.) was used for the quantification of dioxins. The other chemicals used in this experiment were all chemical grade.

Laboratory-scale reactor system

The laboratory-scale reactor system, which has 700 ml of working volume and contains the mobilizing strains in the reactor by means of a ceramic membrane, was used for the reactor experiment. A schematic representation of the reactor system is shown in Fig. 1.

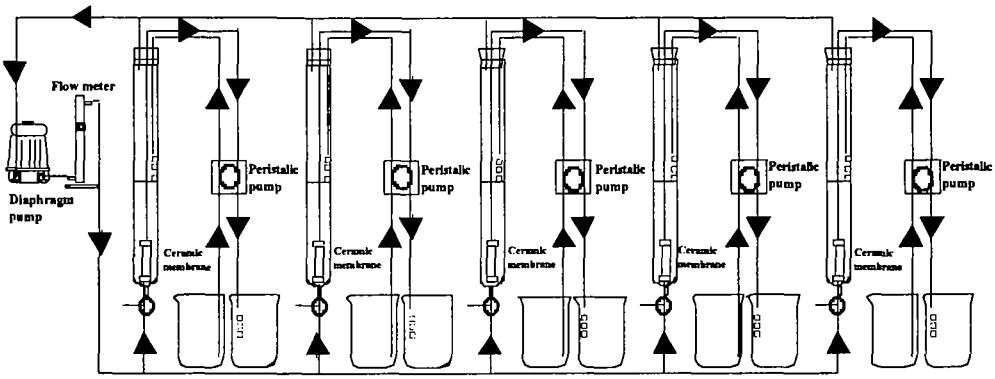

Figure 1. Schematic diagram of a laboratory-scale reactor system used for the degradation of dioxins.

Cultivation condition

The medium for this experiment contained $NaNO_3$ 2,430 mg, CH_3OH 2,200 mg, K_2HPO_4 21.7 mg, KH_2PO_4 8.5 mg, $Na_2HPO_4 \cdot 12H_2O$ 44.6 mg, NH_4Cl 1.7 mg, $MgSO_4 \cdot 7H_2O$ 22.5 mg, $CaCl_2$ 27.5 mg, and $FeCl_3 \cdot 6H_2O$ 0.25 mg per liter; the pH was adjusted to 7.0. A total of 700 ml of activated sludge (suspended solids, SS; 5,000 mg/*l*) was added to the reactor and sealed from outer air. Feed of the medium was fixed at 350 ml per day, and a 350-ml quantity of effluent was gathered per day. The amount of suspended solids, DO, ORP, pH, and NO_3 were checked every 3 days.

Preparation and analysis procedure

After cold-dried sludge was extracted with a soxhlet extractor, the effluent was extracted with 50 ml of toluene, then dried by anhydrous sodium sulfate, after which the toluene solution was washed by hydroxyl sulfite until the yellow pigment in hydroxyl sulfite was eliminated. The cleaned toluene solution was rinsed twice with distilled water and then dried by anhydrous sodium sulfate. The solvent was changed to hexane for the silica gel (3 g) column chromatography. The applied hexane solution was eluted with 150 ml of hexane. The hexane was then substituted with toluene, and its volume decreased to 100 µl under nitrogen gas flow. As a result of these procedures, approximately 100% of the spiked dioxins were recovered from the culture medium.

One microliter out of 100 µl was measured using a GC-MS apparatus (ThermoQuest GCQ plus equipped with TRACE GC 2000) on GC-MS/MS mode (6).

Results and Discussion

Effects of the dioxin concentration gradient

Last year we confirmed the presence of dioxin degradation activity in activated sludge under denitrification conditions in a 15-*l* reactor volume (5). Over 96% (15 ng/*l* of total dioxins, Suspended solid 700 mg/*l*) of absorbed dioxins in the activated sludge was degraded in 1 month. In the present study, the percentage of dioxins degraded by the activated sludge was estimated by a scale-downed glass column reactor (15 *l* to 700 ml). To change the added amount of dioxins (total amount) from 0 (not addition of dioxins) to 190 ng, the reactors were maintained under denitrification conditions for 1 week.

After cultivation, almost all of the dioxins were degraded, with only the inoculation column not being sufficiently degraded. This result indicated that the dioxin contents in the activated sludge was too low to be analyzed by the GC-MS/MS apparatus. In this experiment, the potential for dioxins to be degraded by activated sludge could not be estimated.

Effect of time course on the degradation of dioxins

Next, the dioxins added to the reactor were fixed at a concentration of 600 ng/700 ml and incubated for 0 to 5 weeks (sampling was done every week). From this study, approximately 99% of the dioxins (total amounts) were degraded in 1 week. From Total Toxicity Quantity (TEQ), dioxins were decreased by about one order in 1 week. This decrease was maintained for 3 weeks and stopped by analytical limits.

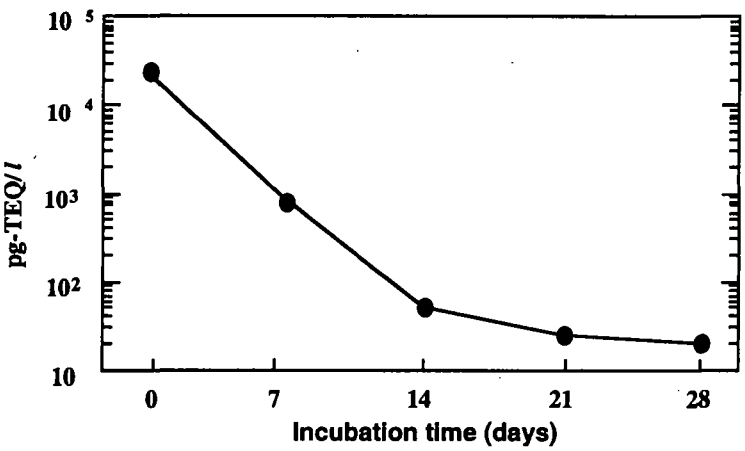

Figure 2. Time course of dioxin degradation under denitrification conditions

Usually dioxins were adsorbed on the sludge as soon as possible, and its density in water was decreased to very low levels. During the water treatment, the dioxins in the water were easily degraded as they were absorbed by the sludge, and the effluent was quite clean. The environmental standard for dioxins in water is 4 pg/*l*. As the capacity of the activated sludge to degrade the dioxins was at a rate of 600 ng /*l* per week, approximately 150,000 *l* of dioxin-containing water was treated by this activated sludge during a 1-week period.

Continuous cultivation

To examine the continuous treatment of dioxins, with 600 ng of dioxins were added to the reactor per week over a cultivation period of 1 to 4 weeks. From these results, it was determined that approximately one order of dioxins was eliminated from the reactor, and this level of activity was maintained for 4 weeks. Regarding the degradation of Tetrachloroethene (TCE), the activity of the strain was decreased due to the TCE toxicity (7); the degradation of

dioxins by the activated sludge activity, however, did not decrease but instead increase over the 4-week period. The activity of the activated sludge was not only high but also remained stable over time, indicating that this method could possibly be applied to large-scale operations.

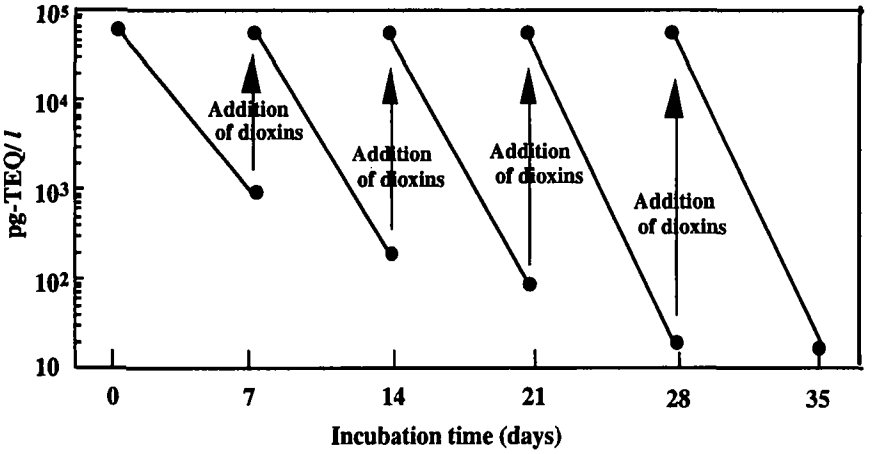

Figure 3. Time course of dioxin degradation with continuous cultivation

Effect of air on the degradation of dioxins

We also estimated the degradation of dioxins under non-denitrification conditions. Similar to the description above, 600 ng of dioxins was added to the reactors, which contained 700 ml of activated sludge (pre-cultivated under denitrification conditions for 1 week with the DO values kept below 0.1). To open the seal of the apparatus, DO was increased to 3.6, and the cultivation was begun with the addition of dioxins (600 ng) to the reactors. In addition, aerobic conditions were maintained for 4 weeks. Nitrate levels did not decrease over the 4-week period. Sampling was done for every week.

For 4 weeks, the added dioxins were slowly deg raded, and approximately 90% of the dioxins were degraded during this period. From these results, it appears that the degradation of dioxins is affected by the presence of oxygen.

Acknowledgements

Our study was supported by the Ministry of Health and Welfare of Japan. We would like to thank Dr. Sadayori Hoshina of Jikeikai University of Medicine and Dr. Ikuo Souda of Kanagawa Prefecture Environmental Research Center for their helpful discussion. Thanks are also due to Mr. Toshio Kawanishi of KUBOTA Co. Ltd., and Dr. Hirosi Goda of TOWA KAGAKU Co. Ltd.

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