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Development of bioreactor system for treatment of dioxin-contaminated soil using *Pseudallescheria boydii*

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Abstract

We developed a conceptual feasible design of bioreactor system for treatment of dioxin-contaminated soils that uses the dioxin-degrading fungus *Pseudallescheria boydii* (*P. boydii*) we had isolated. The dioxin-degradation conditions in bioreactor treatment was established by clarifying the inhibiting factors for the growth of *P. boydii* using both real contaminated and laboratory prepared soils mixed with fly ash. In addition, ethanol extraction process as post-treatment methods for the remaining dioxins, and the sterilization conditions of *P. boydii*, i.e., a weakly pathogenic fungus, in the residue was investigated. The better growth conditions of *P. boydii* were found to be chloride ion concentration of less than 10 g/L and a pH of less than 9. Under these conditions, 7310 pg-TEQ/g of the soil was treated to 2860 pg-TEQ/g by the bioreactor process, and the dioxin concentration was further decreased to 580 pg-TEQ/g by ethanol extraction, resulting to total removal ratio of 92%. Furthermore, development of an effective sterilization method for living *P. boydii* in the residue increased the applicability of our bioreactor system for practical use in dioxin-contaminated sites.

Keywords: Polychlorinated dibenzo-*p*-dioxins and dibenzofurans; Biodegradation; Inhibitors; Sterilizing condition; Bioreactor system

1. Introduction

Large amounts of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) were emitted from municipal solid waste (MSW) incinerators in Japan. In 1997, the government decided to abolish small incinerators because these were mainly responsible for the emission of dioxins (PCDD/Fs). Consequently, the emission of dioxins has decreased dramatically, from 7680 to 8135 g-TEQ/year in 1997 to 376–404 g-TEQ/year in 2003 [1].

However, there are many cases where soil around a MSW incinerator is contaminated by dioxins. For example, serious soil and surface water contamination was caused by scattered mist containing high concentrations of dioxins from a wet gas scrubber at the MSW incinerator in Nose, Osaka. The concentration of dioxins in the soil was about 8000 pg-TEQ/g. Other sites were contaminated by piling or burying fly ash containing dioxins around a MSW incinerator. In addition, an old landfill site that has been receiving non-treated fly ash is also believed to be one of the causes of contamination. In these two cases, the contaminated soil is mixed with fly ash. Immediate and cost-effective countermeasures are therefore required at these dioxin-contaminated sites. This study focused on the development of a biological treatment process, which is relatively inexpensive as compared to the existing physicochemical methods such as incineration or melting.

We isolated a fungus that is able to degrade PCDD/Fs from the activated sludge in a leachate treatment facility at a MSW landfill site [2] and identified it as *Pseudallescheria boydii* (*P. boydii*) by 18S rDNA analysis and morphological analysis [3]. *P. boydii* has the ability to degrade about 80% of pure dioxins (tetra to octachlorodibenzo-*p*-dioxins and furans) during the 3 days of incubation [2]. Heptachlorodibenzo-*p*-dioxin (HpCDD) was detected as one of the degradation by-products of octachlorodibenzo-*p*-dioxin (OCDD) by *P. boydii* [2]. This means that a dechlorination process has occurred. Non-chlorinated dibenzo-*p*-dioxin/furan (DD and DF) were oxidized and transformed to hydroxydibenzo-*p*-dioxin/furan,

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respectively, by *P. boydii* [4]. *P. boydii* has the abilities of both dechlorination and oxidation. In addition, *P. boydii* removed about 80% of tetra to octachlorodibenzo-*p*-dioxins and furans in the soil sampled from Nose after the soil was adjusted to slurry with water content of 70% and agitated in an Erlenmeyer flask [5]. *P. boydii* is a novel fungus, which has the unique ability to degrade dioxins.

Other researchers have studied the biodegradation of dioxins. For example, Valli et al. [6] tried to degrade 2,7-dichlorodibenzo-*p*-dioxin using the lignin-degrading basidiomycete, *Phanerochaete chrysosporium*. Takada et al. [7] showed that PCDD/Fs were degraded by the white rot fungus *Phanerochaete sordida* YK-624. Other studies revealed that fungi degraded DD, DF, 2,7-dichlorodibenzo-*p*-dioxin or 2,8-dichlorodibenzo-*p*-dioxin [8–11]. Nakamiya et al. [12] found that the cyclic ether degrading fungus, *Cordyceps sinensis*, degraded not only DD but also 2,3,7-trichlorodibenzo-*p*-dioxin and OCDD. However, these experiments were conducted to clarify the degradation mechanism of dioxins by fungi. Application of such dioxin-degrading fungi to the development of a real treatment process has never been attempted. Therefore, this study aimed to develop a bioreactor system using *P. boydii*, by clarifying the following three issues:

(1) Better growth conditions for *P. boydii* should be determined by identifying the inhibitors for growth of *P. boydii* in contaminated soils. Based on these conditions, degradation rate of dioxins should be determined by degradation tests in the bioreactor.
(2) A post-treatment process such as solvent extraction should be developed, supposing that the concentration of dioxins in the soil treated in the bioreactor does not meet the Japanese environmental quality standard level (1000 pg-TEQ/g in soil).
(3) To guarantee the safety associated with the treated soil, the conditions for sterilizing *P. boydii* should be determined to prevent emission of living *P. boydii* or its spores from the treatment system. Note that *P. boydii* is a weakly pathogenic fungus, but categorized as the lowest priority according to Japanese guidelines.

With these considerations, the objectives of this study are (1) to investigate incubation conditions for the growth of *P. boydii* in bioreactors with three kinds of contaminated soil, (2) to conduct degradation tests in the bioreactor and solvent extraction tests as post-treatment process, (3) to determine conditions suitable for sterilization of soil containing *P. boydii*, and (4) consequently to develop a conceptual feasible design of bioreactor system for dioxin-contaminated soils.

2. Materials and methods

2.1. Dioxins-contaminated soil

Three kinds of contaminated soil were used in this study. Soil A was sampled from the actual contaminated site in Nose as described above and dried at 100 °C for more than 2 h and then passed through a 2 mm mesh sieve. The concentration of dioxins was 7310 pg-TEQ/g in Soil A. Soils B-1 and B-2 were prepared in our laboratory by mixing uncontaminated soil with two types of fly ash. The uncontaminated soil was surface soil from within the Hokkaido University grounds. It was also dried and sieved as described above. The fly ash samples were taken from electric precipitators of different mechanical stoker-type MSW incinerators. The concentrations of dioxins in the samples of soil mixed with fly ash were 173 pg-TEQ/g in Soil B-1 and 2210 pg-TEQ/g in Soil B-2.

2.2. Conditions for incubation of P. boydii

P. boydii was incubated normally in 100 mL of medium containing glucose (1.0 g), $(NH_4)_2SO_4$ (0.2 g), NaCl (0.2 g), K_2HPO_4 (0.1 g), $MgSO_4{\cdot}7H_2O$ (0.1 g), and $CaCO_3$ (0.2 g). It also contained 0.1 mL of a trace element solution ($FeSO_4{\cdot}7H_2O$ (0.01 g), $MnCl_2{\cdot}4H_2O$ (0.01 g) and $ZnSO_4{\cdot}7H_2O$ (0.01 g) per 10 mL of distilled water). We call this the "normal medium". *P. boydii* was pre-cultivated in Erlenmeyer flasks for 48 h at 30 °C before all the experiments were conducted.

2.3. Test for inhibition of growth of P. boydii

From our experiments, it was assumed that *P. boydii* might degrade dioxins during its logarithmic growth phase. It is important to find out the identity of possible inhibitors of the growth of *P. boydii* for field application. In general, fly ash contains a lot of salts, which might inhibit the growth of *P. boydii* [13]. In addition, the pH of eluate of fly ash is high. We investigated the effect of salts concentration and pH on the growth of *P. boydii*.

In this study, *P. boydii* was incubated in a medium with (1) high chloride ion concentration using NaCl, $CaCl_2$ and/or KCl, (2) high pH using NaOH, and (3) in a medium where an eluate of fly ash was used instead of distilled water. The growth of *P. boydii* was then monitored periodically. However, monitoring the growth of *P. boydii* is very difficult because *P. boydii* is a filamentous fungus and therefore uncountable. Therefore, we assumed that a decrease in glucose concentration corresponded to the growth of *P. boydii*. The glucose concentration was measured as total organic carbon (TOC) because the concentration of other organic matter is very low or negligible.

2.4. Bioreactor test

A 5-L stainless steel cylindrical reactor was used, as shown in Fig. 1. A mixture of the soil and the medium (slurry with water content of 70%) was agitated with an angled turbine at 200 rpm. The position of the turbine and the number of rotations was determined by preliminary experiments. The reactor was maintained at 30 °C using a water jacket. About 10 mL of slurry was periodically obtained as a sample from the sampling location shown in the Fig. 1.

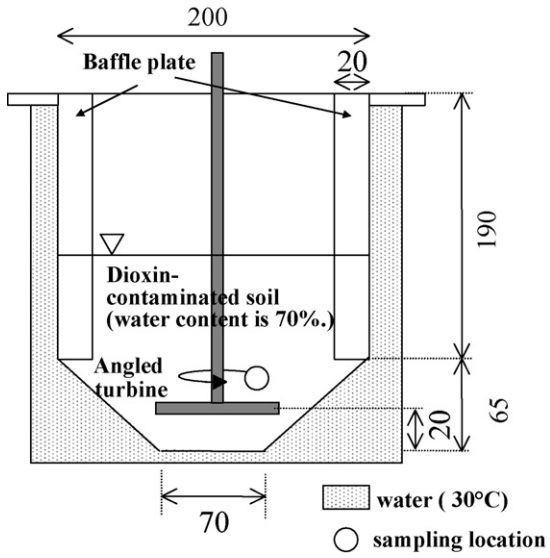

Fig. 1. Bioreactor apparatus.

2.5. Solvent extraction test

To treat the remaining dioxins after the bioreactor test, solvent extraction tests were conducted as post-treatment. The slurry obtained in the bioreactor test was freeze dried. Mixture of 3.6 g of the freeze-dried soil with 36 mL of ethanol (80% (v/v, water)) or 36 mL of isopropanol (60–100% (v/v, water)) or 36 mL of citric acid solution (20% (v/v, water)) were agitated in a 50 mL sealed Teflon vial for 10 min. Each solvent was heated to near its boiling point before the experiments. The extraction ratio was then calculated from the concentrations of dioxins in the solid and liquid phases.

2.6. Sterilizing test

We tried to sterilize *P. boydii* in the treated soil by heat and/or ethanol. Heating and using ethanol are well known as the simplest and the most general sterilizing methods. Since the sterilizing effect of ethanol might depend on the presence of medium or soil, we tested the following three cases: (a) only *P. boydii*, (b) *P. boydii* in the medium, and (c) *P. boydii* in the soils. In case (a), 48 h incubated *P. boydii* was separated from the mixture of cell and medium, and sterilized. In the other cases (b) and (c), 48 h incubated *P. boydii* in the medium or soil was sterilized directly. For 2 h, the temperature conditions were 25, 29, 50, 54, 60 or 74 °C and the net concentrations of ethanol in the mixture were 0, 70, 80, or 87.5% (v/v, water).

To confirm whether *P. boydii* had been completely sterilized, the samples after sterilization using the above methods were incubated for 7 days at 30 °C in the new medium. If no growth of *P. boydii* was observed, a part of the medium was incubated further for another 5 days using the Petri plate method. If no growth of *P. boydii* was observed, we decided that *P. boydii* had been sterilized completely.

2.7. Analytical methods

2.7.1. Dioxins

Samples (slurry) from the bioreactor and solvent extraction tests, were treated with acid (HCl and/or H_2SO_4) to extract dioxins from the cells and soil as much as possible, and the solid and liquid phases were separated with a suction funnel. The solid phase was then freeze dried. Dioxins in the dried solid phase were extracted with toluene using a Soxhlet extractor, and those in the liquid phase were extracted three times with toluene. The extracted dioxins in the toluene phase were dissolved into hexane, and were applied to a multilayer silica gel column. This was filled in sequence from the bottom to the top with 0.5 g of silica gel, 3.0 g of 2% potassium hydroxide-impregnated silica gel, 0.5 g of silica gel, 4.5 g of 44% sulfuric acid-impregnated silica gel, 6.0 g of 22% sulfuric acid-impregnated silica gel, 0.5 g of silica gel, 3.0 g of 10% silver nitrate-impregnated silica gel, 0.5 g of silica gel and 3.0 g of sodium sulfate. The dioxins in the column were eluted with 150 mL of hexane, and dissolved in 0.1 mL of toluene. Analysis of dioxins was carried out with an HRGCMS apparatus [14] or GC/MS/MS (ThermoQuest GCQ plus ion trap mass spectrometer and TRACE GC 2000 gas chromatograph) in accordance with the method of Kemmochi and Tsutsumi [15]. The degradation of dioxins was estimated from the decrease in the toxic equivalent quantity (TEQ) based on the peak area of each congener.

2.7.2. Other characteristics

The TOC concentration in the medium was determined by TOC meter (TOC-V CSH/CSN, Shimadzu Corporation). Chloride ion in the medium was analyzed by ion chromatography (DX-120, Dionex).

2.8. Chemicals

For calibration and clean-up, a PCDDs/DFs standard mixture, EDF-4931 (CIL, Inc.), and the isotopically labeled chlorodioxin standard, ED-900 (Wellington Lab.), were used, respectively. The other chemicals were laboratory grade.

3. Results and discussion

3.1. Monitoring growth of P. boydii

The relationship between the weight of *P. boydii* after drying and the TOC concentration is shown in Fig. 2. *P. boydii* mass increased with the decrease in the TOC concentration because *P. boydii* uses glucose as its carbon source. Therefore, we can monitor the growth of uncountable *P. boydii* in the medium by monitoring the decrease in TOC concentration.

3.2. Effect of salts concentration and pH on the growth of P. boydii

Fig. 3 shows the effect of chloride ion concentration and pH on the growth of *P. boydii*. In those cases, where the decreasing rate in TOC concentration is slower than that of the normal

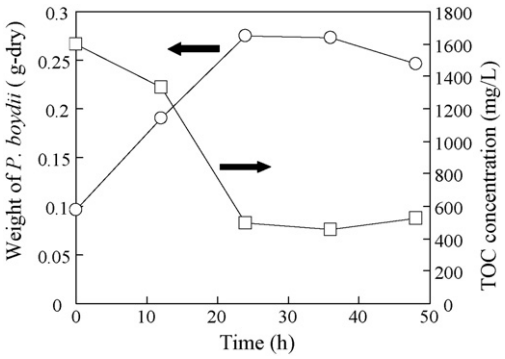

Fig. 2. Relationship between weight of *P. boydii* and TOC concentration.

condition using the "normal medium", the growth of *P. boydii* has been inhibited. It is noted that in preliminary experiments, we added NaCl, KCl, and $CaCl_2$ to the normal medium, respectively, in order to show difference according to the cation involved. However, since there was no difference in the growth of *P. boydii* with any of the cations tested, the inhibition can be explained only by chloride ion. Based on the results in Fig. 3, it was found that chloride ion concentration of less than 10 g/L and a pH of less than 9 in the medium did not inhibit the growth. In addition, the growth rate of *P. boydii* was very low in the medium where an eluate of fly ash was used instead of distilled water. Since the chloride ion concentration and the pH in the medium were 10.8 g/L and 10, respectively, it was concluded that pH affected the growth of *P. boydii* in this case.

Therefore, if the chloride ion concentration of fly ash is high, a pretreatment process is needed to reduce such concentration before the bioreactor process. In addition, pH control is needed during operation of the bioreactor.

3.3. Bioreactor test

Fig. 4 shows the result of bioreactor tests. The degradation ratio was calculated by dividing the concentration of dioxins by the initial concentration in the slurry, based on TEQ. In each experiment using the Soils A, B-1, and B-2, dioxin levels decreased with time. Simultaneously, the TOC concentration also decreased until 48 h after the initiation of test, indicating that *P. boydii* consumed glucose and degraded dioxins. The degradation ratios up to 48 h were 60% (Soil A), 65% (Soil B-1), and 39% (Soil B-2), respectively. With regard to concentration, 7310 pg-TEQ/g in Soil A decreased to 2860 pg-TEQ/g, 173 pg-TEQ/g in Soil B-1 to 66 pg-TEQ/g, and 2210 pg-TEQ/g in Soil B-2 to 1340 pg-TEQ/g.

In order to confirm that further degradation could occur, glucose and other constituents of the medium were added after 48 h of sampling. However, further decrease in TOC concentration was not observed after addition of glucose, as shown in Fig. 4. This indicates that *P. boydii* did not continue to grow and the reason why dioxins were not degraded. Although it was postulated that some inhibitors were produced during degradation of dioxins, the reason for the failure of growth of *P. boydii* could not be defined.

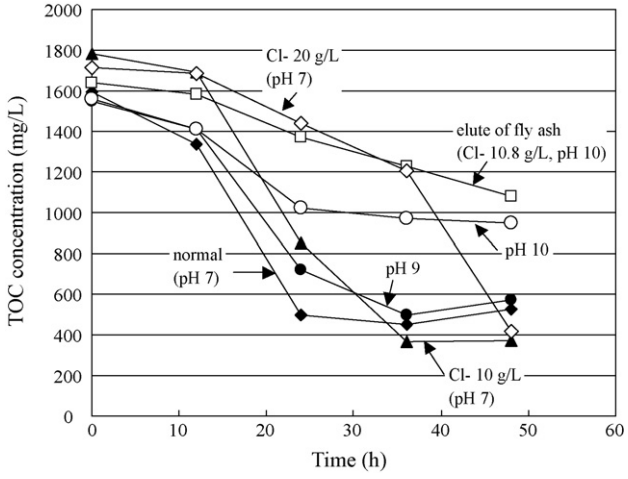

Fig. 3. Effect of chloride ion and pH on the growth of *P. boydii*.

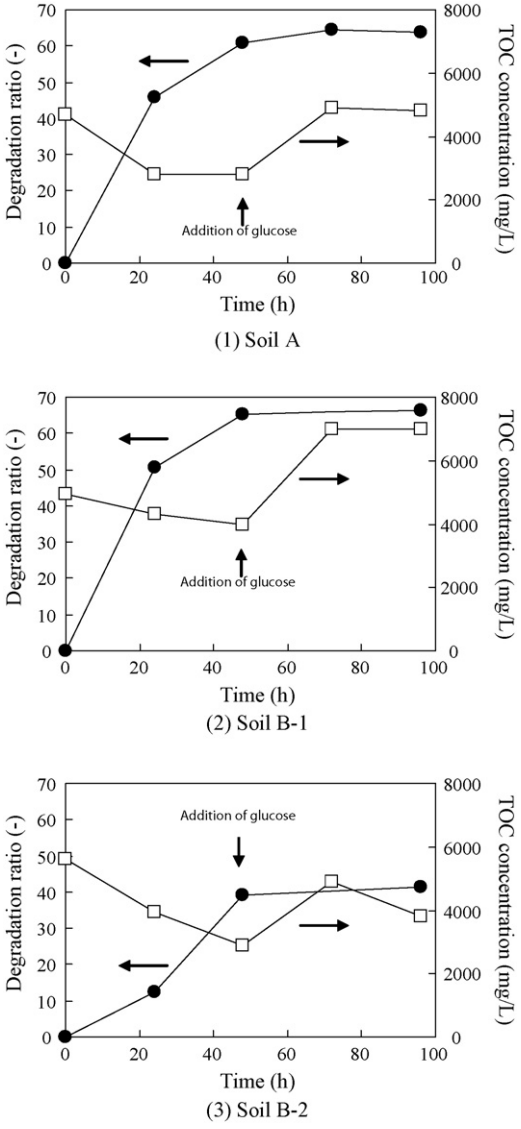

Fig. 4. Result of bioreactor tests.

With regard to the difference in the degradation ratios (the quantity of degraded dioxins) among Soil A, Soil B-1, and Soil B-2, it seemed that the difference might be related to the elution rate of dioxins from solid to liquid phases. Since Soil A was contaminated by mist containing very high concentrations of dioxins, most of dioxins might exist on the surface of soil particle. On the other hands, Soils B-1 and B-2 were contaminated with fly ash. In general, it is hard to extract all dioxins from fly ash, because dioxins exist within particles of fly ash, not only on the surface. Therefore, the elution rate might be very different, depending on the mechanism that caused the contamination.

Fig. 5 shows the time profile of 2,3,7,8-congeners for PCDDs and PCDFs in the case of Soil A. Each congener was decreased at almost the same rate, which suggests that *P. boydii* can degrade tetra to octachlorodibenzo-*p*-dioxins and furans without specificity. In the case of B-1 and B-2, similar trends in the time profile of the 2,3,7,8-congeners were observed. The reason why the degradation rates of the 2,3,7,8-congners were almost the same is discussed below.

In general, highly chlorinated dibenzo-*p*-dioxins are difficult for bacteria to degrade under aerobic condition. It is well known that they are dechlorinated under anaerobic condition by bacteria. For example, Barkovski and Adriaens [16] had conducted many studies on the dechlorination of dioxins by bacteria under anaerobic conditions, which showed that OCDD was dechlorinated to monochlorodibenzo-*p*-dioxin by anaerobic bacteria. However, in the case of fungi, as *P. boydii* in this study, there are a lot of reports showing that fungi degrade dioxins under aerobic conditions. Valli et al. [6] proposed a model for the degradation pathway of 2,3-dichlorodibenzo-*p*-dioxin by detection of chlorocatechol and trace trihydroxy benzene. Takada et al. [7] also found that 2,3,7,8-congeners were degraded by the white rot fungus at almost the same rate and detected 4,5-dichlorocatechol and tetrachlorocatechol during the degradation of 2,3,7,8-tetra- and octachlorodibenzo-*p*-dioxin. In addition, Nakamiya et al. [12] revealed that mono- to trichloro-catechol and cis-muconates were detected during degradation of OCDD. These experimental results were all obtained under aerobic conditions using fungi, as being done in this study. This fact shows that fungi degrade highly chlorinated dibenzo-*p*-dioxins through oxidation pathway under aerobic conditions.

Although a degradation pathway of dioxins by *P. boydii* is not identified completely, it is possible to consider that *P. boydii* could degrade highly chlorinated dioxins through dechlorination process, as well as, through oxidation process. That is why the degradation rates of the 2,3,7,8-congners were almost the same.

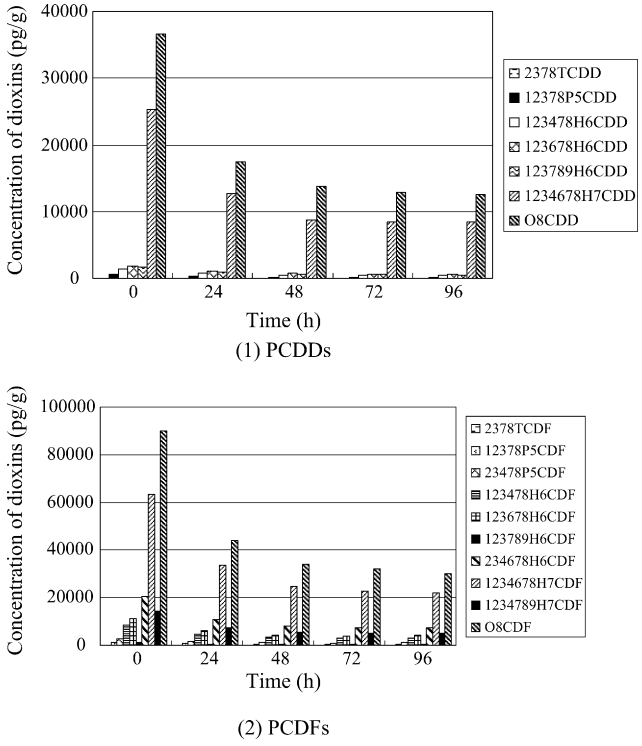

Fig. 5. Time profile of 2,3,7,8-congeners for PCDDs and PCDFs in soil A.

3.4. Solvent extraction test as post-treatment

As shown in Fig. 4, *P. boydii* could not lower the dioxins level to the environmental standard (1000 pg-TEQ/g). For example, 2860 pg-TEQ/g of dioxins were remained in the soil A. When biological methods such as the bioreactor are applied to practical use, we have to consider a combination of physicochemical methods in order to ensure the quality of treated soil.

Since our earlier study revealed that 80% ethanol (78.3 °C and agitation for 1 min) extracted almost all dioxins from soil A [17], we tried to extract dioxins from fly ash, which was used for preparing soil B-1, by using ethanol, isopropanol, and citric acid. The result is shown in Fig. 6, indicating that ethanol could not extract any dioxins from the fly ash, although its extraction ratio from soil A was very high. The solvents isopropanol (80%) and citric acid (20%) extracted dioxins from the fly ash more effectively than the other solvents, although the extraction ratios were low.

In the ideal situation, a hydrophobic solvent such as toluene or *n*-hexane should be used for effective extraction of dioxins from solid phase. However, considering the safety, handling and cost, it is preferred not to use a hydrophobic solvent such as toluene. Therefore, we decided to use moderately hydrophilic solvents as shown in Fig. 6.

The reason why isopropanol could extract dioxins better than ethanol may be because isopropanol is more hydrophobic than ethanol. On the other hand, citric acid could extract relatively large amount of dioxins from fly ash. This may be because citric acid seemed to dissolve scale, such as calcium, on the surface

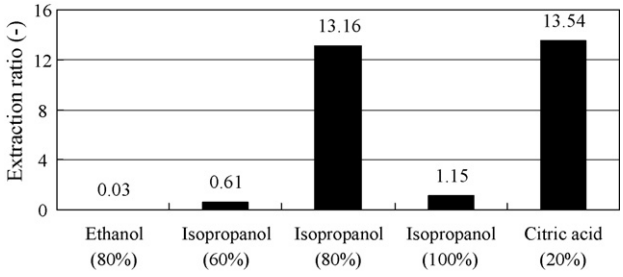

Fig. 6. Extraction ratio of dioxins from fly ash.

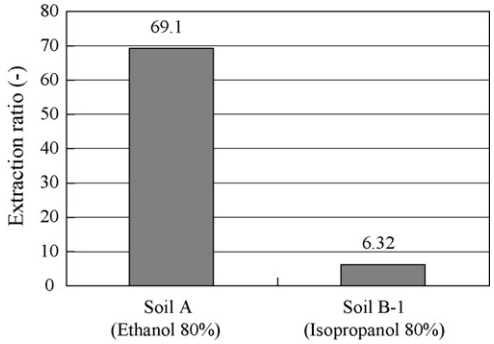

Fig. 7. Extraction ratios of dioxins from Soil A and B-l treated by bioreactor test.

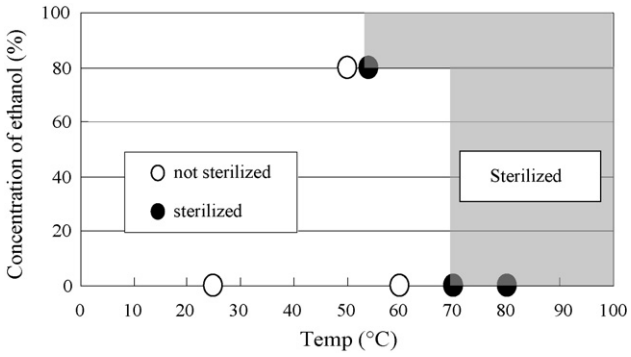

Fig. 9. Result of sterilizing *P. boydii* in the soil (case c).

of fly ash. Comparing isopropanol and citric acid, isopropanol has advantages with regard to the re-use of solvent and its sterilizing effect in the post-treatment process that will be described later. Consequently, we selected isopropanol as the solvent for extraction of dioxins from fly ash.

Fig. 7 shows the extraction ratios of dioxins from Soils A and B-1 after the bioreactor treatment. Note that the experiment for soil B-2 was not conducted. With regard to Soil A, the extraction ratio was 69.1%, which is lower than what we expected. This may be either because ethanol could not extract dioxins adsorbed into microorganism or the dioxins concentration in soils after the bioreactor treatment was relatively low, resulting in the low extraction ratio. On the other hand, as we expected, the extraction ratio of dioxins from soil B-1 after bioreactor treatment was very low. It is not easy to extract dioxins from soils with fly ash, such as soil B-1.

Although the extraction ratios were lower than what we expected, both the bioreactor and solvent extraction as post-treatment did remove dioxins from the soil A, reducing the levels from 7310 pg-TEQ/g to 580 pg-TEQ/g, resulting a total removal ratio of 92%, which is lower than the Japanese environmental quality standard in soil, i.e., dioxins level of 1000 pg-TEQ/g.

3.5. Sterilizing conditions

In the above mentioned (case a) *P. boydii*, which was separated from the medium, was sterilized by 70% ethanol at 25 °C. However, *P. boydii* in the medium (case b) was not sterilized at the same conditions, as shown in Fig. 8. This might be because

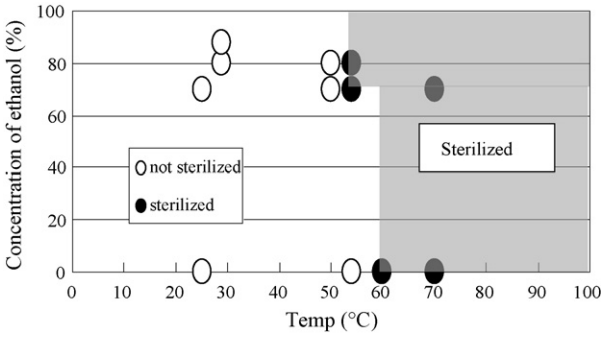

Fig. 8. Results of sterilizing *P. boydii* in the medium (case b).

the organic matter in the medium repressed the sterilizing ability of ethanol. It was found that *P. boydii* in the medium was sterilized completely at temperatures of over 60 °C in the case of no ethanol, or at 54 °C when the concentrations of ethanol are over 70%.

On the other hand, in case c, *P. boydii* in soil was not sterilized completely at 60 °C, but was sterilized over 70 °C, as shown in Fig. 9. When the concentration of ethanol was 80%, *P. boydii* was found to be sterilized at 54 °C. Since the effect of ethanol on sterilization of *P. boydii* was small, it is concluded that the sterilizing condition for *P. boydii* in soils should be at temperatures of over 70 °C for 2 h, whether or not ethanol is used in the process. Further study is needed to reduce the required time for sterilizing.

3.6. Conceptual feasible design of bioreactor system for dioxin-contaminated soil

We developed a conceptual feasible design of bioreactor system for treatment of dioxin-contaminated soil, as shown in Fig. 10, which is based on the above experimental results. The contaminated soil is firstly sieved to remove large particles (such as rocks, plant roots and so on) that should not be in the bioreactor. The sieved soils are then adjusted to slurry with water content of 70% by adding the described medium, including glucose and the other nutrients. If the chloride ion concentration is high in the slurry, chloride ion should be removed from the contaminated soil by flushing with water as pretreatment to reduce the chloride ion concentration to less than 10 mg/L in the slurry. After adding *P. boydii*, the bioreactor is operated for at least 48 h under controlled pH condition (less than pH 9). Then, the solid and liquid phases are separated. The solid phase is submitted to the post-treatment process. If the remaining dioxins are higher than the target value, solvent extraction is used as a post-treatment. It is noted that the extraction ratio is low in the case of soils with fly ash and thus, the solvent extraction process is just considered as one of the options. In the sterilizing process, the soil is heated to over 70 °C for 2 h. Finally, the treated soil is placed back to the site after checking its safety (the dioxin concentration and death of *P. boydii*).

To our knowledge, this bioreactor system is the first one being proposed as biological treatment of dioxin-contaminated soil. This system does not consume much energy, compared to the

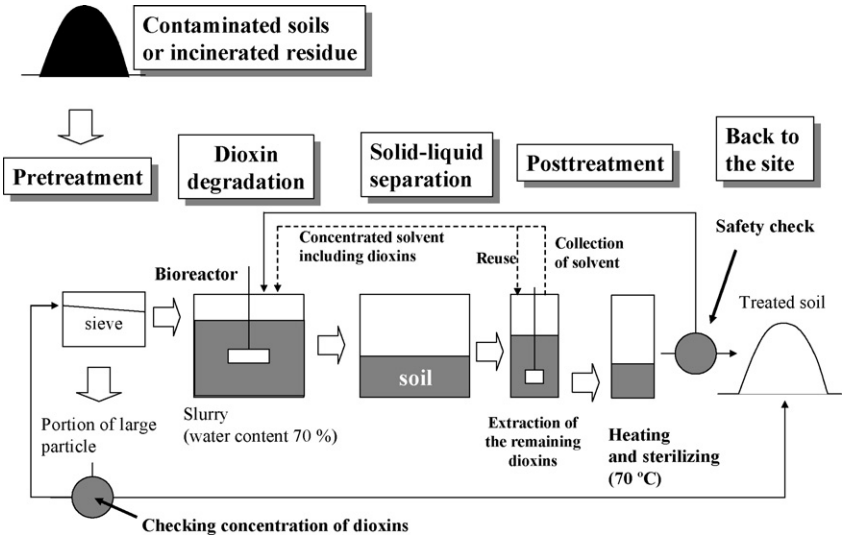

Fig. 10. Conceptual feasible design of bioreactor system for treatment of dioxin-contaminated soil.

other physicochemical treatment process such as incineration or melting. The cost is also expected to be lower than that of the physicochemical treatment process, but this might depend on the amount of contaminated soil to be treated. In the development of such biological method, the most important point was the safety of the treatment system, i.e., the safety associated with *P. boydii*. *P. boydii* is a pathogenic fungus and it is categorized as the lowest priority according to Japanese guidelines. However, infection of exposed persons is possible and so, we found effective sterilizing conditions for *P. boydii*. Therefore, our system can be applied to practical use in the field.

Although there are additional investigations and experiments to be conducted in the future, for example, development of bioreactor design methods based on kinetics of dioxins degradation by *P. boydii* and development of more effective post-treatment process, this conceptual feasible design of bioreactor system is the novel proposal for biological treatment of dioxin-contaminated soils.

4. Conclusions

The following conclusions were obtained by this study.

(1) The better growth condition for dioxin-degrading fungus, *P. boydii* was found to be chloride ion concentration of less than 10 g/L and a pH of less than 9 for treatment of dioxin-contaminated soil.
(2) The bioreactor experiments showed that the degradation ratios of dioxins in the actual dioxin-contaminated soil (Soil A) and the soil mixed with fly ash (Soils B-1 and B-2) were 60% and 40–60%, respectively.
(3) Dioxins in the actual dioxin-contaminated soil were treated from 7310 pg-TEQ/g to 580 pg-TEQ/g that is less than Japanese environmental quality standard in soil, by a combination of our bioreactor and ethanol extraction processes.
(4) Sterilizing condition of *P. boydii* required for practical use was found to be at heating condition of over 70 °C for 2 h.
(5) Consequently, a conceptual feasible design of bioreactor system for treatment of dioxin-contaminated soil was originally developed based on our experimental results.

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