REMEDIATION OF DIOXIN-CONTAMINATED SOIL WITH COMBINATION OF BIOFUEL CROPS AND WHITE ROT FUNGUS

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Abstract

Some biofuel crops and a white rot fungus, *Pleurotus ostreatus*, were primarily used to develop crop-microbial combination systems for enhancing remediation of dioxins-contaminated soil. Four biofuel crops, wheat (*Triticum spp.* L.）, barley (*Hordeum vulgare* L.), maize (*Zea mays* L.) and sunflower (*Helianthus annuus* L.) were used in this study, and the experiment was conducted with pot culture. The results showed that the crop-*Pleurotus ostreatus* combinations promoted biomass yield of the crops, increased the number of soil fungi and total microorganisms in the rhizosphere, and enhanced removal of dioxins in the contaminated soil. The results suggest that combination of white-rot fungi with suitable biofuel crops was possibly a profitable and practical choice for enhancing remediation of soils contaminated with organic pollutants.

Introduction

Soil contamination with various organic compounds such as dioxins and agrochemicals has been a worldwide concern over the last decades. Phytoremediation is an emerging technology that uses plants to cleanup pollutants in soils, which has been shown by many positive results as a most environmental friendly and economical remediation method for contaminated soils[1,2]. Phytoremediation to date indicates that it is applicable to a wide range of organic contaminants, such as petroleum hydrocarbons, pesticides, chlorinated solvents, dioxins, and surfactants[1, 3]. However, the practical application of phytoremediation has been limited because of its low efficiency and long remediation period. Another key factor limited the practical application is that although phytoremediation is a low cost technology, it needs cost year by year within the remediation period, which makes the owner of the contaminated sites no profit during the remediation.

White rot fungus is well known to degrade various persistent organic pollutants, and bioremediation of soil using white-rot fungus has been extensively reported [4-6]. On the other hand, in recent years, there is a global expansion in production of biofuels. Currently, many countries are making new investments in biofuel production capacity. The main biofuel crops include sugar and starch crops such as sugar cane, barley, wheat, rye, sweet potatoes, sweet sorghum, molasses, and cassava for bioethanol production, and a variety of oil crops such as rapeseed, sunflower, soybean for biodiesel production [7,8].

In order to enhance phytoremediation efficiency of soil organic contaminants, we developed plant-microbe combination systems, in which white rot fungus and plants were combined. Results showed that microbial activities, plant growth as well as the remediation efficiency were promoted [9]. Recently, our interests focus on developing new phytoremediation systems with combination of biofuel crops and white-rot-fungi, aiming to develop rational ways for both remediation and profitable utilization of contaminated soils. In this study, white rot fungus, *Pleurotus ostreatus*, was selected and combined with some biofuel crops for enhancing remediation of dioxin-contaminated soil, and the effects of these crop-fungus combination systems on plant biomass, soil microbial population, and remediation efficiency were assessed.

Materials and methods

The dioxin-contaminated soil used in the experiment was from a waste incineration facility in Japan. Two winter biofuel crops, wheat (*Triticum spp.* L.） and barley (*Hordeum vulgare* L.), and two summer biofuel crops, maize (*Zea mays* L.) and sunflower (*Helianthus annuus* L.) were selected to use in this study. Germination and growth of these crops were made in 1/10000a Wagner's pots filled with the dioxin-contaminated soil or with a general farm soil as control treatment to test crop growth. The wheat and barley were sown in November 2007, and harvested in June 2008. Crop and soil sample in each pot were then collected for analysis, and the soil left were returned to the pot. When wheat and barley were harvested, maize and sunflower were then immediately grown, and harvested with crop and soil sampling in November 2008. The experiment was conducted in the field of the Center for Environmental Science in Saitama with natural climatic condition.

The lignin degradation white rot fungus, *Pleurotus ostreatus*, which was isolated from eatable mushroom and having a strong ability to degrade organic compounds[10], was used in this study. *Pleurotus ostreatus* was cultivated in a liquid medium based on the method described by Chagas and Durrant[11]. The crop seedlings were given rhizosphere inoculation or non-inoculation of white rot fungus 30 days after emergence. No fungus inoculation made to the farm soil. Rhizosphere inoculation was made by adding 50ml of the liquid fungal culture to each pot every two weeks. The corresponding control treatments were made by adding 50 ml autoclaved fungal liquid medium. In addition, two other control treatments, soil only and soil with liquid fungal culture were also conducted.

At the day of crop harvest, three pots in each treatment were randomly removed for soil and crop sampling. The above-ground part of the crops were collected, and dried in a drying oven for measuring the dry biomass. Microbial number of total bacteria and fungi in the soil were measured using the spread plate method. The microbial number was expressed as the number of colony forming units (cfu/g). For dioxin analysis, 10 g air dried and thoroughly mixed soil was Soxhlet extracted with toluene for 24 hours. Then, after adding ^{13}C-labeled internal standards, the extracts were cleaned up according to procedures published by the Ministry of the Environment of Japan with minor modification. The last fraction was concentrated and spiked with two

^{13}C-labeled PCDD/Fs and four ^{13}C–labeled co-PCBs internal standards for HRGC/HRMS analysis.

Results and discussion

Germination rate, seedling emergence rate and growth of wheat and barley

The germination rate and seedling emergence rate are shown in Fig. 1. The germination rate and seedling emergence rate in the farm soil had the same value, which was 97% for wheat and 98% for barley. However, the germination rate and seedling emergence rate in the contaminated soil were much lower than that in the arable soil. Without fungus inoculation, the germination rate and seedling emergence rate in the contaminated soil were 47% and 22% respectively for wheat, and 73% and 67% respectively for barley. Fungus inoculation to wheat (WM) improved 20% to the germination rate and 10% to the seedling emergency rate. Anyway, fungus inoculation to barley (BM) improved 9% to the germination rate, but nearly no effect on the seedling emergency rate.

Total above-ground biomass of wheat (W) and barley (B) in the contaminated soil were much lower than that in the farm soil (NW, NB), as shown in Fig.1. Compared to crops in the general farm soil (NW, NB), the contaminated soil had an above-ground biomass of 42% for W, 69% for WM, 49% for B and 62% for BM. Therefore, *Pleurotus ostreatus* inoculation to the crops resulted in a biomass increase of 25% for wheat (W) and 13% for barley (B). These results indicated that although the contaminated soil had worse influence to wheat and barley germination and growth, *Pleurotus ostreatus* inoculation promoted the crop growth in the contaminated soil.

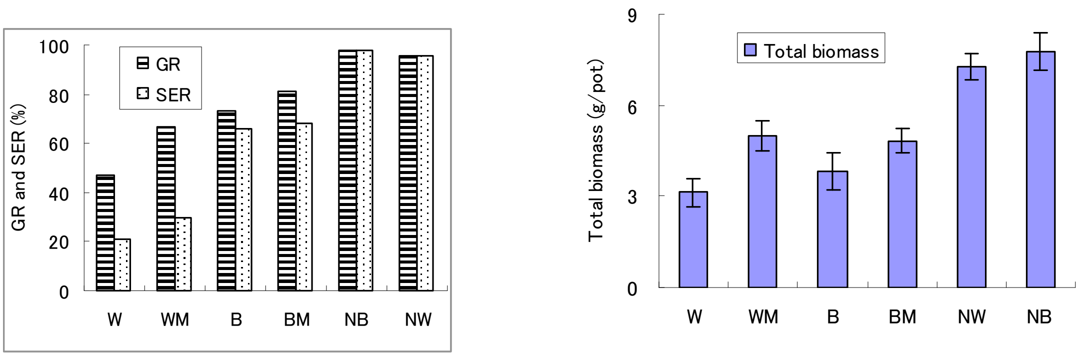

Fig.1 Germination rate (GR), Seeding emergency rate (SER), and above-ground biomass in different treatments (W: wheat; M: *Pleurotus ostreatus*; N: general farm soil; B: Barley)

Growth of maize and sunflower

Phytoremdiation is a technology to improve the contaminated soil by consecutive cultivation of plants. In order to elucidate the remediation effect with consecutive crop cultivation, wheat or sunflower was grown in each pot after wheat and barley were harvested. Maize and sunflower in the contaminated soil had less biomass than that in the farm soil (NWSF, NBSF, NWC, NBC). However, as shown in Fig.2, for maize and sunflower the biomass difference between the contaminated soil and farm soil were clearly smaller compared to that for wheat and barley (Fig.1). Moreover, crop biomass in treatments with *Pleurotus ostreatus* inoculation (WMSFM、BMSFM、WMCM、BMCM) increased 15-40% compared to the respective control treatments without inoculation (WSF, BSF, WC, BC). Maize generally had higher above-ground biomass than sunflower. The results showed that quality of the contaminated soil for crop production was improved by the crop cultivation and the inoculation of *Pleurotus ostreatus*.

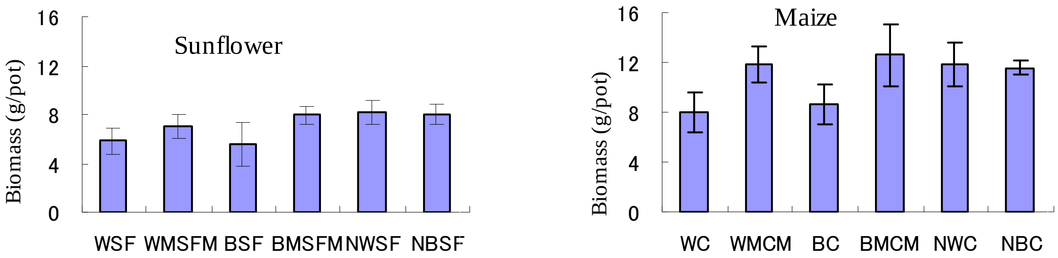

Fig.2 Above-ground biomass for sunflower and maize after wheat and barley
(W: wheat; M: *Pleurotus ostreatus*; SF: sunflower; B: barley; C: maize)

Number of soil microorganism

As shown in Fig.3, the number of total microbes and fungi in the contaminated soil under treatments with crop growing (W, B, WM, BM) were much larger compared to contaminated soil only (S) or contaminated soil with *Pleurotus ostreatus* (SM). Increase of soil microorganism was also found in the treatments of wheat and barley with *Pleurotus ostreatus* inoculation (WM, BM) compared to those without inoculation (W, B). The results indicated that crop cultivation promoted the growth of soil microorganism, and *Pleurotus ostreatus* inoculation gave a further promotion to soil microorganism growth. Our results are comparative with the results of other studies[12,13].

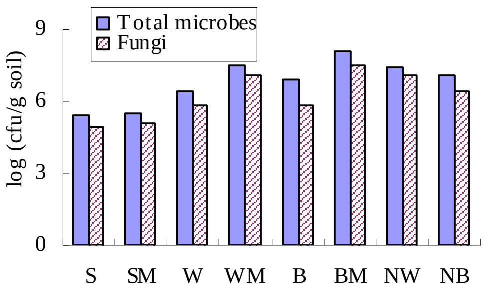

Fig.3 Number of soil microbes in various treatments
(S:contaminatd soil only; M: *Pleurotus ostreatus*; W: wheat; B: Barley; N: general farm soil)

Actually, researchers have described this increase in soil microorganism numbers in the root zone as a general rhizosphere effect. Plant roots not only supply organic nutrients and energy through exudates, but also can improve the soil porosity and air condition, thus provide oxidation-reduction microenvironment that is optimal for the growth of soil microorganisms. Therefore, a general rhizosphere effect results in increased microbial biomass, which is important to promote the activities of microbe biodegradation of organic pollutants.

The principal mechanisms of phytoremediation involve rhizosphere degradation through plant root secretion and stimulation of soil microbes, plant uptake, accumulation, stabilization, and volatilization. Thus, healthy crop growth and high soil microbial activities in contaminated soils are two most important factors governing the success of phytoremediation. So, besides remediation rate, crop growth and soil microbial population were also invested in this study.

Remediation of soil dioxins

Dioxins concentrations of the pot soil are shown in Fig. 4 after a six-month remediation with application of wheat, barley and *Pleurotus ostreatus*. Compared to the original contaminated soil (OS), the cropped treatments had the comparable removal rates ranging from 11% to 35%. The barley with *Pleurotus ostreatus* (BM) treatment was found to have the highest removal rate, followed by wheat with *Pleurotus ostreatus* (WM). Without *Pleurotus ostreatus* inoculation, wheat and barley had a similar removal rate. No dioxins reduction was found in the treatments of contaminated soil only (S) or contaminated soil with *Pleurotus ostreatus* (SM), indicating that *Pleurotus ostreatus* inoculation without cropping could not remove dioxins in the soil.

In addition, further reduction in dioxins concentration was found in maize and sunflower experimental series, which carried out after the wheat and barley were harvested (Fig.5). BMCM and BMSFM had a removal rate of 45% and 42%, respectively, based on the dioxin concentrations in the original contaminated soil (OS). WMCM and WMSFM had a similar removal rate around 17%.

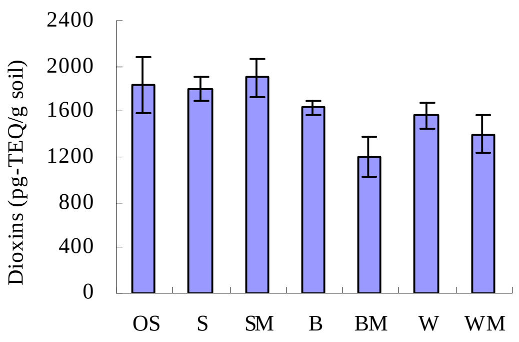

Fig.4 Soil dioxin concentrations after remediation using wheat and barley for six months

(OS: original contaminated soil; S: contaminated soil only; M: *Pleurotus ostreatus*; B: barley; W: wheat)

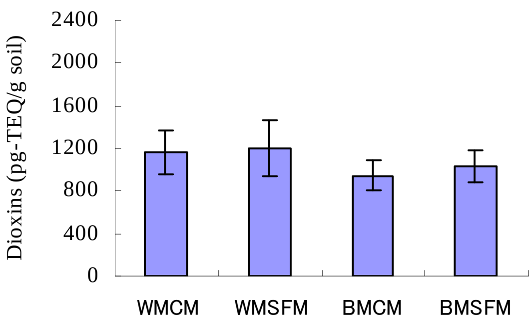

Fig.5 Soil dioxin concentrations after remediation for five months by maize and sunflower with *Pleurotus ostreatus* followed wheat and barley

(W: wheat; M: *Pleurotus ostreatus*; C: Maize; B: barley; SF: sunflower)

These results indicated that *Pleurotus ostreatus* inoculation to the biofuel crops led to greater remediation of soil dioxins compared to those without inoculation. The removal of persistent organic pollutants by phytoremediation of contaminated soils has been widely studied in recent years. The underlying mechanisms are considered to be the rhizosphere biodegradation, plant uptake, photodegradation, volatilization, root adsorption, and incorporation into soil organic materials[13-15]. In the enhanced removal of dioxins by the biofuel-fungus combination shown in this study, possibly some of these mechanisms are involved.

Conclusions

The results showed that growing wheat, barley, maize and sunflower with *Pleurotus ostreatus* had beneficial effects on promoting the growth of both crops and soil microorganisms, as well as on remediation efficiency of the dioxin contaminated soil. As crops growing in the contaminated soils can be used for production of biofuels, biofuel crop based phytoremediation possibly bring profit for the owner of the soil contaminated sites. This would suggest that biofuel crops and *Pleurotus ostreatus* combination is possibly a practically profitable measure for enhancing remediation of dioxin-contaminated soils. Our further study would be try to understand the mechanisms involved in the efficacy of the biofuel crop-white rot fungus combination systems, and to carry out field experiments for establish profitable and efficient phytoremediation systems for contaminated soils.

Acknowledgements

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