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Enhanced Polychlorinated Biphenyl Removal in a Switchgrass Rhizosphere by Bioaugmentation with *Burkholderia xenovorans* LB400

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Abstract

Phytoremediation makes use of plants and associated microorganisms to clean up soils and sediments contaminated with inorganic and organic pollutants. In this study, switchgrass (*Panicum virgatum*) was used to test for its efficiency in improving the removal of three specific polychlorinated biphenyl (PCB) congeners (PCB 52, 77 and 153) in soil microcosms. The congeners were chosen for their ubiquity, toxicity, and recalcitrance. After 24 weeks of incubation, loss of $39.9 \pm 0.41\%$ of total PCB molar mass was observed in switchgrass treated soil, significantly higher than in unplanted soil ($29.5 \pm 3.4\%$) ($p < 0.05$). The improved PCB removal in switchgrass treated soils could be explained by phytoextraction processes and enhanced microbial activity in the rhizosphere. Bioaugmentation with *Burkholderia xenovorans* LB400 was performed to further enhance aerobic PCB degradation. The presence of LB400 was associated with improved degradation of PCB 52, but not PCB 77 or PCB 153. Increased abundances of *bphA* (a functional gene that codes for a subunit of PCB-degrading biphenyl dioxygenase in bacteria) and its transcript were observed after bioaugmentation. The highest total PCB removal was observed in switchgrass treated soil with LB400 bioaugmentation ($47.3 \pm 1.22\%$), and the presence of switchgrass facilitated LB400 survival in the soil. Overall, our results suggest the combined use of phytoremediation and bioaugmentation could be an efficient and sustainable strategy to eliminate recalcitrant PCB congeners and remediate PCB-contaminated soil.

Keywords

PCBs; switchgrass; phytoextraction; *Burkholderia xenovorans* LB400; biphenyl dioxygenase gene

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1. Introduction

Polychlorinated biphenyls (PCBs) are a group of synthetic chemicals that were widely used as heat transfer fluids during the last century. The production and commercial use of PCBs were prohibited by the EPA in 1979 because they were found to be toxic and carcinogenic (Longnecker et al., 1997; Ross, 2004). Nevertheless, because of their high stability and hydrophobicity, PCBs persist in the environment and accumulate through the food web, posing a potential risk to public health and ecosystems (Ross, 2004). An estimated 1.3 million tons of PCBs were produced worldwide between the 1930s and the mid-1980s, and the current total global PCB burden in background soils (i.e. soils not considered contaminated) is estimated to be 21,000 tons (Abraham et al., 2002; Meijer et al., 2003). Conventional remediation strategies such as soil excavation and the following incineration or landfilling are usually expensive and can be considered unsustainable (Amend and Lederman, 1992). Phytoremediation, the use of plants and associated microbes to remove and detoxify PCBs, represents a potentially cost effective and less disruptive approach to clean up PCB contaminated soils and sediments (Van Aken et al., 2010).

The presence of specific plant species is known to enhance the PCB removal efficiency in soils and sediments (Campanella et al., 2002; Chekol et al., 2004; Ionescu et al., 2009; Slater et al., 2011; Smith et al., 2007). Plants can take up PCBs from soil and convert lower chlorinated congeners into hydroxylated products (Ficko et al., 2010; Greenwood et al., 2011; Ku erová et al., 2000; Lee and Fletcher, 1992; Rezek et al., 2007; Zhai et al., 2010). Also, plants contribute to PCB biodegradation by providing a favorable environment for PCB-degrading microorganisms and can thus increase PCB-degrading populations in comparison to unplanted soil (de Cárcer et al., 2007; Leigh et al., 2006; Slater et al., 2011; Uhlík et al., 2009). Plants not only increase soil permeability and oxygen diffusion in the rhizosphere, but also release organic exudates as inducers, surfactants and microbial growth factors thereby stimulating and facilitating microbial PCB biodegradation in the root zone (Gilbert and Crowley, 1997; Hernandez et al., 1997; Leigh et al., 2002; Van Aken et al., 2010). For example, augmentation of soil with plant-derived compounds such as carvone and salicylic acid assisted aerobic PCB degraders and increased PCB removal (Singer et al., 2000; Singer et al., 2003).

Microorganisms are known to degrade PCBs via two general processes: aerobic oxidation with biphenyl ring cleavage and anaerobic reductive dechlorination (Adrian et al., 2009; Borja et al., 2005; Gibson and Parales, 2000; Pieper, 2005; Wiegel and Wu, 2000). Most aerobic PCB-degraders employ the upper biphenyl degradation pathway, with biphenyl 2,3-dioxygenase (BphA) catalyzing the initial step, transforming susceptible PCB congeners into the corresponding *cis*-dihydrodiol (Furukawa, 1994). Under anaerobic conditions, some members of the phylum *Chloroflexi* use certain PCB congeners as electron acceptors and transform them into less chlorinated congeners (Adrian et al., 2009; Fagervold et al., 2007; Fagervold et al., 2005; Wiegel and Wu, 2000). Some efficient microbial PCB-degrading isolates, such as *Burkholderia xenovorans* strain LB400, *Arthrobacter* sp. strain B1B, *Ralstonia eutropha* H850, *Dehalococoides chlorocoercia* DF1 have been introduced to contaminated soils and sediments in efforts to improve PCB removal (Payne et al., 2013; Payne et al., 2011; Petri et al., 2011; Singer et al., 2000).

In this study, we monitored the removal of three selected PCB congeners (PCB 52, PCB 77 and PCB 153) in switchgrass-planted soil microcosms and attempt to improve PCB degradation by introducing biphenyl-grown *Burkholderia xenovorans* strain LB400 into the rhizosphere of selected microcosms. The PCB congeners used here were chosen because they represent bottlenecks in environmental PCB degradation (Bedard et al., 1986). PCB 52 and 153 are both *ortho*-chlorinated, while PCB 77 has a dioxin-like structure, and is considered one of the most toxic congeners (Van den Berg et al., 2006). All three congeners are quite ubiquitous in the environment (Meggo et al., 2013). A combination of PCB congener analysis and molecular microbial analysis were employed to elucidate the mechanism of PCB removal in the soil reactors.

2. Materials and Methods

2.1. Soil reactor set up

The soil microcosm set-up procedure has been described previously (Meggo et al., 2013). Briefly, PCB-free soil from the village of Middle Amana in Iowa, USA, was passed through a 60 mesh sieve before experiments. The soil was spiked with a mixture of PCB 52, PCB 77 and PCB 153 (99% pure) (Accustandard Inc., New Haven, CT) at the concentration of 500 ng g⁻¹ each. This level of PCB contamination has been reported in soils and sediments previously (Martinez and Hornbuckle, 2011; Van Metre and Mahler, 2005). In this study we investigated the possibility of using switchgrass to clean up PCB contamination that is below the dredging requirement (50 ppm) but still poses a potential risk to human and environmental health. PCB congeners were dissolved in hexane before adding to the soil. A quartering technique was used to homogenize soil. The soil was divided into four quadrants on a quartering canvas, and 20 diagonal trajectories were used to mix the soil components. The contaminated soil was aged for two months at 25°C in sealed tubs to allow PCBs sequestration into the soil matrix and thereby better represent field conditions.

Plastic containers (33.8 cm × 21.6 cm × 21.9 cm) and lids with aluminum foil covers were used for soil reactor set-up. Each reactor was filled with 2500 g sieved and homogenized soil. The spiked soil reactors were planted with switchgrass (*Panicum virgatum*) seeds (Adams-Briscoe Seed Co., Jackson, GA) (Figure S1). Switchgrass was grown at 25°C in a plant growth chamber under a 16 hour light/8 hour dark photo period (light intensity of 200 mmol m⁻² s⁻¹ and 60% humidity). Each treatment was performed to one PCB-spiked soil reactor. An unplanted reactor and a switchgrass planted reactor filled with clean soil were used as controls. A 5 ml sterile syringe with the narrow tip removed was used to take vertical core samples randomly from the reactors. The core samples were homogenized and subjected to PCB congener analysis and microbial analysis. Soil samples were taken at 12 week and 24 week for PCB congener analysis. Plant samples (roots and shoots) were collected at the end of experiment. PCB concentration measurements for soil and plant samples were performed in triplicate. For microbial analysis, soil samples were taken from each microcosm every four weeks starting at week 12.

2.2. *Burkholderia xenovorans* strain LB400 bioaugmentation

Bioaugmentation with *Burkholderia xenovorans* strain LB400 was conducted to one switchgrass-planted reactor and one unplanted reactor once every month. Prior to bioaugmentation strain LB400 was grown in K1 medium on solid biphenyl (1 g) as sole carbon and energy source until exponential phase (OD_{600} 0.6–0.8) (Zaitsev and Karasevich, 1985). Cells were harvested by centrifugation ($5000 \times g$, 15 min), washed once with sterile K1 medium, resuspended in K1 medium and inoculated into reactors (approximately 10^9 CFU per g soil). Autoclaved LB400 was added to a switchgrass planted reactor as a control.

The abundance of cultivable aerobic biphenyl-degrading bacteria was estimated by CFU enumeration one day after each bioaugmentation. Soil samples (1 g) were mixed with 9 ml of 0.9% sodium chloride solution and shaken for 1 h at 225 rpm on a platform shaker (New Brunswick Scientific, Pittsburgh, PA) at 25°C. Serially diluted supernatant was spread on K1 agar plates with biphenyl crystals provided on the lid of an inverted petriplate as sole carbon and energy source. CFUs were enumerated after 72 h of incubation at 30°C.

2.3. PCB extraction and quantification

The PCB extraction procedure has been previously described (Meggo et al., 2013). Briefly, for PCB extraction, 1:1 hexane/acetone mixture (3 ml/g) was added to homogenized soil or plant material (5 g) and sonicated for 1 h. Before sonication, surrogate standards including PCB 14 (3,5-dichlorobiphenyl), deuterated PCB 65 (2,3,5,6-tetrachlorobiphenyl) and PCB 166 (2,3,4,4',5,6-hexachlorobiphenyl) (Cambridge Isotope Laboratories, Inc.) were added into the samples (50 ng of each surrogate) to account for any loss during the extraction. Surrogate recoveries were $103 \pm 17.2\%$ (PCB 14), $97.9 \pm 15.2\%$ (PCB 65) and $97.7 \pm 17.7\%$ (PCB 166).

The sonicated mixture was centrifuged at $1500 \times g$ for 5 min, after which the supernatant was transferred to another sterile vial. The precipitates were subjected to a second extraction. The combined supernatants of the first and second extraction were evaporated to dryness using rotary evaporation and then dissolved in hexane. Double extraction with concentrated sulfuric acid and hexane was performed to remove of lipids and other polar substances. This hexane extract was concentrated to approximately 0.5 ml under a gentle nitrogen stream. The concentrate was eluted with 10 ml of hexane through a filter consisting of 0.1 g of silica (70–230 mesh, Fisher Scientific, Inc.), 0.1 g of anhydrous sodium sulfate and 0.9 g silica gel acidified with H_2SO_4 (silica: H_2SO_4 = 2:1).

The PCB congener analysis procedure was previously described (Hu et al., 2010). Briefly, the concentrated extracts were spiked with the internal standard containing 100 ng of PCB 204 (2,2',3,4,4',5,6,6'-octachlorobiphenyl). The samples were analyzed for PCB congeners using a gas chromatograph with mass selective detection (GC-MS/MS) modified from the EPA method 1668A (U.S.EPA 1999). The quantification of PCBs was performed by an Agilent 6890N gas chromatograph with an Agilent 7683 series auto sampler coupled to a Waters Micromass Quattro micro GC mass spectrometer (Milford, MA, USA) operating under electron impact (EI) positive mode at 70 eV and multiple reaction monitoring (MRM), and the trap current was 200 μA . The retention windows were defined by PCB parent/

daughter ion pairs from mono- to deca- homologs which were 188/152, 222/152.10, 255.96/186, 291.92/222, 325.88/255.90, 359.84/289.90, 393.80/323.90, 427.76/357.80, 461.72/391.83, 497.68/427.70, respectively.

2.4. DNA and RNA extraction, reverse transcription, and quantitative PCR

Total RNA and DNA were extracted in replicate immediately after sampling from 2 g soil using the MoBio RNA PowerSoil Total RNA Isolation Kit (Mobio, Carlsbad, CA) and RNA PowerSoil DNA Elution Accessory Kit (Mobio, Carlsbad, CA) and stored at -80°C prior to further analysis. Before quantitative PCR (qPCR), RNA samples were subjected to contaminating DNA removal, RNA clean-up and reverse transcription. Contaminating DNA was removed by DNase I treatment according to the manufacturer's instructions (Biolab, Ipswich, MA). Each 100 μl DNase I treatment reaction contained 30 μl RNA and 20U DNase I. RNA was then purified with the RNeasy Mini Kit (Qiagen, Germantown, MD) and reversed-transcribed to cDNA by SuperScript II Reverse Transcriptase (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. Each 80 μl reverse transcription reaction contained 40 μl purified RNA, 10mM dNTP mix, and 3 μg random primers.

To evaluate the RNA recovery efficiency during DNase I treatment, RNA purification and reverse transcription, 1 ng of luciferase control mRNA (Promega, Madison, WI) was added to RNA sample before DNase I treatment. After reverse transcription, luciferase cDNA was quantified by qPCR with the ref primer set (Table S1) as described previously (Johnson et al., 2005). The RNA recovery efficiency averaged $14.2\% \pm 11.5\%$ among all RNA samples processed in this study.

The abundances of total bacteria, aerobic PCB-degrading bacteria, and *Burkholderia xenovorans* strain LB400 were estimated by qPCR using the bacterial 16S rRNA gene primer set 16SU f/r (Nadkarni et al., 2002), *bphA* primer set *bphA* 463f/674r (Petri et al., 2011), and LB400 16S–23S rRNA internal transcribed spacers (ITS) primer set LB400 84f/278r (Norini et al., 2013), respectively (Table S1). Each 25 μl qPCR reaction contained 12.5 μl Power SYBR Green PCR Master Mix (Invitrogen, Carlsbad, CA), and variable amounts of primers and templates (Table S2). Bovine serum albumin (0.5 μg) was added to relieve possible PCR inhibition (Kreader, 1996). PCR conditions were as follows: 10 min at 95°C , followed by 40 cycles at 95°C (15 s) and 60°C (1 min) followed by a dissociation step. All qPCR was performed in triplicate with an ABI 7000 Sequence Detection System (Applied Biosystem, Grand Island, NY) and fluorescence data was analyzed by ABI 7000 System SDS Software (Applied Biosystems, Grand Island, NY). With each primer set, the target gene was not detected in no template (DI water) controls (Ct value > 35). Additional qPCR information, such as primer concentrations, template concentrations, qPCR linear range, qPCR efficiency range of the standard curves, and Y-intercepts are provided in Table S2, in accordance with MIQE guidelines (Bustin et al., 2009).

For luciferase mRNA qPCR, the standard DNA template was PCR products amplified from luciferase DNA with primer set ref ST (Table S1). For the bacterial 16S rRNA gene, the standard DNA template was prepared from PCR products amplified from *Burkholderia xenovorans* strain LB400 with primer set 8F/1492R (Klappenbach et al., 2000). For *bphA*, the standard DNA template was the LB400 *bphA* (amplified with the 463f/674r primer set)

cloned into the pCR 2.1-TOPO vector. For LB400 16S–23S rRNA ITS gene, the standard DNA template was the LB400 16S–23S rRNA ITS gene (amplified with the LB400 84f/278r primer set) cloned into the pCR 2.1-TOPO vector.

2.5. Statistical analyses

PCB congener data was analyzed with an independent sample t test using R. Total bacterial 16S rRNA gene abundance, *bphA* (gene and transcript) abundance and LB400 16S–23S rRNA ITS abundance were analyzed with a two factor analysis of variance after log transformation using R (Rieu and Powers, 2009).

3. Results and Discussion

3.1. Enhanced degradation of specific PCB congeners in soil with switchgrass treatment

Significantly higher removal of PCB parent congeners (PCB 52, 77, 153) was achieved in the switchgrass-treated soil than in the unplanted soil after 24 weeks of incubation ($p < 0.05$) (Figure 1). The presence of switchgrass enhanced PCB 52 removal more than the other two congeners (16.7%), while PCB 77 and PCB 153 removal was improved by 7.9% and 9.6%, respectively. Overall, $39.9 \pm 0.4\%$ of the total PCB molar mass loss was observed in switchgrass treated soil after 24 weeks of incubation, compared to $29.5 \pm 3.4\%$ in unplanted soil (Figure 1). Switchgrass has been observed previously to enhance PCB removal in soil and promote microbial activity in the rhizosphere (Chekol et al., 2004; Dzantor et al., 2000; Meggo et al., 2013). The increased PCB removal efficiency by switchgrass observed here is comparable to previous reports, although in those studies different plants were used and the soils were contaminated by PCB commercial mixtures rather than with specific congeners (Ding et al., 2009; Ionescu et al., 2009; Secher et al., 2013; Smith et al., 2007). In this study, addition of specific congeners allowed a focus on the variation in removals of congeners with differing chlorine positions.

The enhanced PCB removal described above could be explained by rhizosphere biostimulation, rhizofiltration, phytoextraction, phytotransformation and phytovolatilization (Van Aken et al., 2010). We investigated a potential phytoextraction mechanism by quantifying total PCBs in switchgrass roots and shoots. At the end of the 24 week experiment, we found 4.3–7.3% of total PCBs in switchgrass roots, and 0.11–0.24% in shoots, suggesting that plant extraction processes were active as PCB removal mechanisms (Table 1). All three parent congeners and some transformation products were detected in switchgrass roots and shoots. Especially, the detection of PCB 153 in switchgrass shoots demonstrated its capability to take up and translocate highly chlorinated PCB congeners. Volatilization and deposition is excluded as a possible source of PCBs in the shoots because the shoot PCB concentration from the PCB-spiked reactor (9.30 ± 0.92 ng/g) is much higher than that from the blank reactor without PCB spiking (0.59 ± 0.21 ng/g) (Table S3). The uptake of PCBs by switchgrass and other plants has been reported (Ficko et al., 2010; Greenwood et al., 2011; Liu et al., 2009), although in some studies the effect of phytoextraction in removing PCBs from soil was considered negligible because of the low percentage of total PCB mass observed in plant roots and shoots ($< 1\%$ of total PCBs) (Di Gregorio et al., 2013; Huesemann et al., 2009). In this study, the fraction of total PCB mass

in switchgrass was higher than previous studies, probably because of the high plant biomass for each switchgrass treated soil microcosm (560 ± 51 g root sand 360 ± 41 g shoots per soil microcosm). On the other hand, phytovolatilization and phytotransformation were not considered to significantly affect PCB removal, given the relatively low PCB concentrations in switchgrass shoots. In a previous study it was also shown that hydroxylated metabolites were not detected in switchgrass hydroponically exposed to PCBs, suggesting that PCB transformation in switchgrass was not active (Liu et al., 2009).

Biostimulation of microbial PCB degradation is another mechanism potentially contributing to enhanced PCB removal in the rhizosphere soil. The occurrence of aerobic microbial PCB degradation in both unplanted and switchgrass treated soils was verified by the decrease in total PCB molar mass (Figure 1A). It is estimated that the enhanced microbial activity in the rhizosphere was responsible for the 7.1% lower molar mass recovery in the switchgrass-treated reactor than that in the unplanted reactor ($p < 0.05$) (Table 1). However, the recoveries of PCB 77 and 153 in the switchgrass treated reactor are not significantly different from those in the unplanted reactor, suggesting microbial degradation of PCB 77 and 153 was not significantly improved with the presence of switchgrass (Table 1). PCB 52 appears to be the most susceptible to aerobic degradation by indigenous microorganisms. We propose that the dioxin-like structure of PCB 77 renders it relatively more toxic than other tetrachlorinated biphenyls. PCB 153 is the most recalcitrant probably because of its chlorine substituents at the 2,3,5,2',3',5' - positions, which make the congener unsuitable for the oxidation at either the 2,3- or 3,4-position (Arnett et al., 2000; Mondello et al., 1997). The high chlorine content of PCB 153 also results in high hydrophobicity and reduces its accessibility to enzymes. The bulkiness of the chlorine atoms may also prevent access to the enzyme's active site (Bedard et al., 1986).

3.2. Molecular biology analyses of unplanted and switchgrass planted soils

We estimated the abundance of total bacteria, biphenyl dioxygenase genes (*bphA*) and *bphA* transcripts as an independent means of tracking aerobic microbial PCB biodegradation during the experiment. Total bacterial abundance (as estimated by the abundance of bacterial 16S rRNA genes) was significantly higher in switchgrass reactors than in unplanted reactors ($p < 0.05$, Figure 3A). We suspect that the switchgrass serves to improve oxygen diffusion and soil permeability (Singer et al., 2003). Switchgrass root exudates could also increase the microbial population in the rhizosphere by serving as a carbon and energy source (Kamath et al., 2004).

Detecting *bphA* in both unplanted and switchgrass-treated soils indicates that the indigenous soil microbial community harbored the potential for aerobic PCB degradation. In the presence of switchgrass, *bphA* abundance was about 20 times higher ($p < 0.05$, Figure 3B). However, *bphA* transcripts in both switchgrass planted and unplanted reactors were below our RT-qPCR quantification limit (40 copies/qPCR reaction), suggesting that *bphA* expression was not promoted by the presence of switchgrass. It is possible that the presence of other carbon sources in the rhizosphere, such as naturally-occurring organic carbon and organic plant exudates, made PCBs less favorable as substrates for indigenous microorganisms and subsequently inhibited *bphA* expression (Kamath et al., 2004; Parnell et

al., 2010; Rentz et al., 2004). Soil extract may also inhibit *bphA* expression (Master and Mohn, 2001). Low levels of *bphA* expression would also manifest as low microbial PCB degradation in the switchgrass rhizosphere, which supports the PCB congener data presented above.

3.3. Enhanced microbial PCB degradation in soil with LB400 bioaugmentation

Thus, to further enhance aerobic degradation of PCB congeners in the rhizosphere, biphenyl-grown *Burkholderia xenovorans* strain LB400 was introduced to the soil (at a loading of approximately 10^9 CFU per g soil). LB400 is an effective aerobic PCB-degrader, capable of degrading a broad spectrum of PCB congeners including those used in this study (PCB 52, 77, 153) (Bedard et al., 1986; Bopp, 1986). With LB400 bioaugmentation, total PCB molar mass loss increased to $39.4 \pm 1.4\%$ and $47.3 \pm 1.2\%$ in the unplanted and switchgrass-treated soil, respectively, after 24 weeks incubation, suggesting enhanced aerobic PCB degradation (Figure 1). Removal of PCB 52, 77 and 153 was not significantly improved in the control switchgrass reactor which was amended with autoclaved LB400 (Figure 1). This suggests that the bioaugmented LB400 biomass is not simply acting as a fertilizer for the plant.

Specifically, PCB 52 removal appeared to be most improved by LB400 bioaugmentation, achieving 43.7% and 24.9% more removal in unplanted and switchgrass-treated soils, respectively ($p < 0.05$). PCB 153 removal in switchgrass-treated soil with bioaugmentation increased by 3.3% as compared to soil with only switchgrass treatment ($p < 0.05$). In unplanted soil with bioaugmentation, PCB 77 and 153 removal was not improved (Figure 1). This observation is consistent with previous LB400 PCB biodegradation studies, where LB400 displayed a stronger capability to degrade PCB 52 than PCB 77 and 153 (Bedard et al., 1986; Bopp, 1986; Gibson et al., 1993; Rein et al., 2007). Compared with PCB 52, PCB 77 and 153 have higher K_{ow} values and lower water solubilities (Van Noort et al., 2010). Although LB400 could degrade PCB 77 and 153 in resting cell assays and the biphenyl dioxygenase from LB400 is capable of oxidation at both the 2,3- and 3,4-position of the biphenyl ring, in the soil microcosms, the two congeners may be sorbed strongly to soil particles and thus are less bioavailable (Bedard et al., 1986; Haddock et al., 1995).

3.4. Molecular biology analyses of unplanted, switchgrass planted, and LB400 bioaugmented soils

The enhanced PCB removal in bioaugmented soil was associated with elevated abundance of *bphA* and its transcripts, suggesting that aerobic PCB degradation was actively occurring (Figures 3 and 4). In bioaugmented soil, *bphA* abundance increased throughout incubation time, and was two orders of magnitude higher than in reactors without bioaugmentation ($p < 0.05$, Figure 3B, 3D). The transcripts of *bphA* were detected ($3.17 \times 10^5 - 8.90 \times 10^5$ transcripts per g soil in unplanted soil with bioaugmentation, $4.73 \times 10^4 - 4.14 \times 10^6$ transcripts per g soil in switchgrass treated soil with bioaugmentation), suggesting successful *bphA* expression (Figure 4).

Bioaugmentation did not significantly increase the total microbial abundance ($p > 0.05$, Figure 3A, 3C). Yet the reactor with autoclaved LB400 injection displayed greater total 16S

rRNA gene abundance than the reactor with live LB400 bioaugmentation ($p < 0.05$, Figure 3A), probably because that debris from the autoclaved LB400 cells was used as carbon and energy source by indigenous microorganisms.

The survival of bioaugmented bacteria is crucial to successful bioaugmentation and contaminant removal (Bouchez et al., 2000). To improve LB400 survival and keep a relatively stable LB400 population, we performed bioaugmentation once a month. The survival of LB400 after bioaugmentation was estimated by quantifying biphenyl-degrading bacteria with CFU enumeration. After bioaugmentation, the biphenyl-degrading bacterial abundance increased (4.7×10^8 – 1.9×10^9 CFU per g soil for unplanted reactor, 2.1×10^8 – 2.3×10^9 CFU per g soil for switchgrass planted reactor). Yet this number decreased to the level of approximately 10^7 CFU per g soil after four weeks, prior to the next bioaugmentation event (Figure S2). The decrease may result from the competition with indigenous microbial community members and a possibly stressful environment caused by PCBs (Kim et al., 2001; Parnell et al., 2006; Van Veen et al., 1997). The LB400 16–23S internal transcribed spacer (ITS) region was quantified approximately two weeks after bioaugmentation, and ranged from 1.16×10^7 to 4.15×10^8 copies per g unplanted soil, and 9.61×10^7 to 7.04×10^8 copies per g switchgrass treated soil (Figure 5). An accumulation of ITSs was observed throughout the 24 week experiment, suggesting that after each bioaugmentation, a percentage of LB400 adapted to the soil environment and survived alongside indigenous microorganisms (Figure 5). The support of plants to bioaugmentation has been reported (Juhanson et al., 2009; Secher et al., 2013; Tam and Wong, 2008). Switchgrass facilitated LB400 survival possibly by improving aeration and providing root exudates as carbon and energy sources. The root system can help spread LB400 through the PCB contaminated soil. Furthermore, switchgrass may increase the bioavailability of PCB molecules by releasing organic compounds as surfactants (LeFevre et al., 2013).

4. Conclusions

In this study, switchgrass improved the removal of a three congener mixture of PCB 52, 77 and 153, likely by phytoextraction and microbial biostimulation mechanisms. PCB 52 was most susceptible to microbial degradation, and its removal was improved greater in switchgrass-planted and LB400 bioaugmented reactors than PCB 77 and 153. LB400 abundance was greater in the presence of switchgrass as revealed by the LB400 16–23S ITS gene quantification. Also, the most PCB removal and the highest *bphA* and transcript abundances were observed in bioaugmented soil with switchgrass treatment after 24 weeks, indicating switchgrass not only benefited LB400 survival, but also facilitated PCB removal by LB400. The combination of phytoremediation and bioaugmentation offers an efficient and environmental-friendly strategy to eliminate recalcitrant PCB congeners and remediate PCB contamination in the environment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Switchgrass improved PCB 52, 77 and 153 removal from soil by both plant and microbial activity.
- PCB 52 removal in planted and LB400-augmented reactors was greater than PCB 77 and 153 removal.
- Increased *bphA* abundance and expression was noted in LB400-augmented reactors.
- Switchgrass presence appeared to improve LB400 survival and facilitated increased PCB removal.

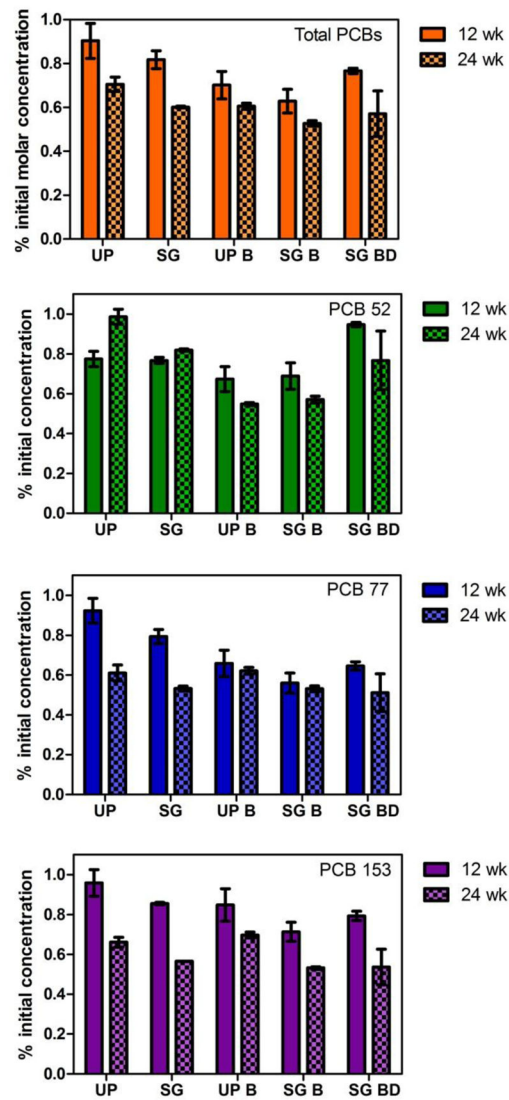


Figure 1.

The percentage of initial molar concentrations for total PCB (orange), PCB 52 (green), PCB 77 (blue), and PCB 153 (purple) after 12 weeks and 24 weeks incubation in unplanted soil (UP), switchgrass treated soil (SG), unplanted with bioaugmentation soil (UP B), switchgrass treated with bioaugmentation soil (SG B), and switchgrass treated with bioaugmentation with dead LB400 soil (SG BD). Error bars indicate the standard deviation of three soil subsamples from the same reactor.

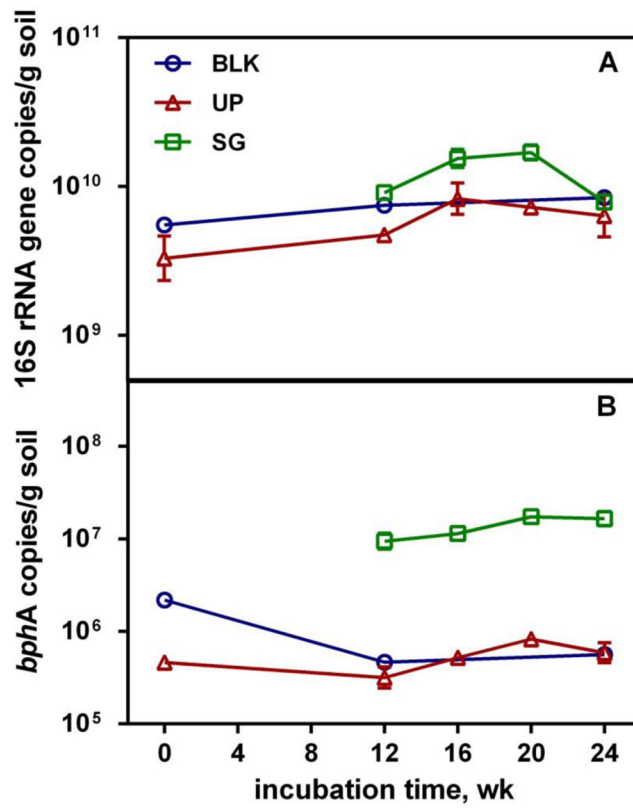


Figure 2. qPCR analysis of (A) bacterial 16S rRNA genes and (B) *bphA* abundance with time in blank (BLK), PCB spiked and unplanted soil (UP), PCB spiked and switchgrass treated soil (SG). Error bars indicate the range of two soil subsamples from the same reactor.

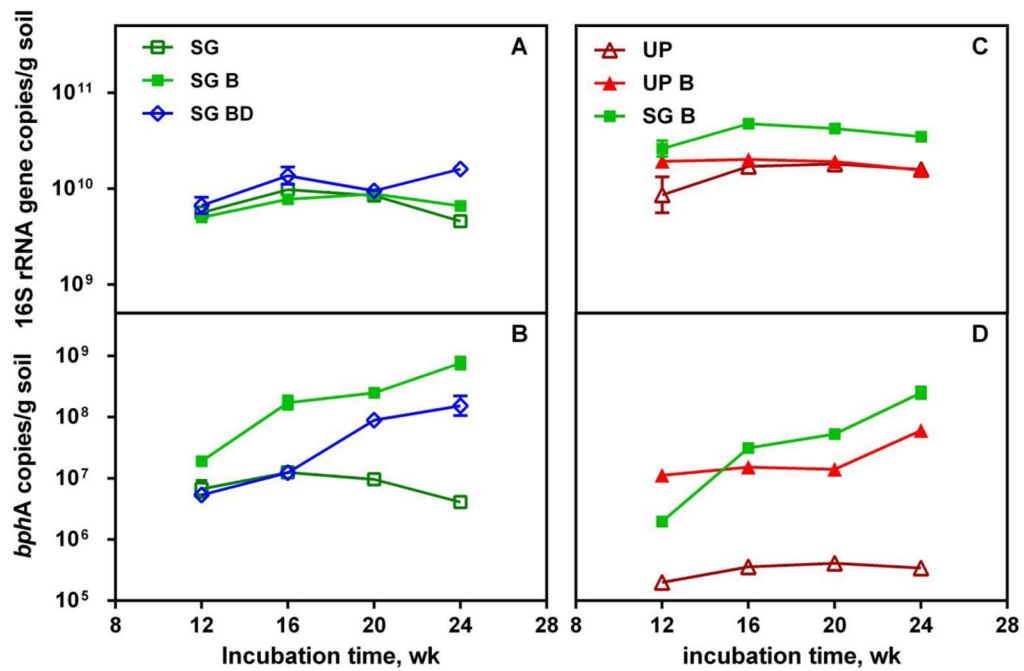


Figure 3. qPCR analysis of bacterial 16S rRNA gene (A) and *bphA* (B) abundance with time in switchgrass treated soil (SG), switchgrass treated soil with bioaugmentation (SG B), switchgrass treated soil with autoclaved LB400 bioaugmentation (SG BD); and bacterial 16S rRNA gene (C) and *bphA* (D) in unplanted soil (UP), unplanted soil with LB400 bioaugmentation (UP B), switchgrass treated soil with bioaugmentation (SG B). Error bars indicate the range of two soil subsamples from the same reactor.

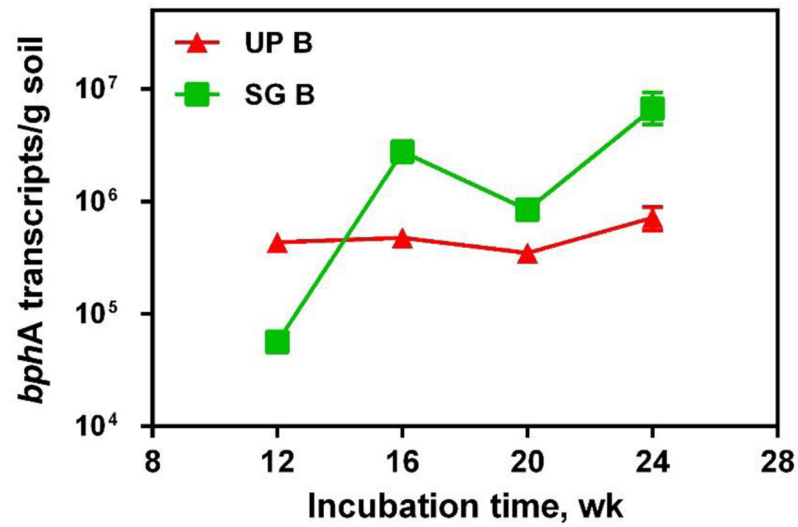


Figure 4. qPCR analysis of *bphA* transcripts with time in unplanted soil with bioaugmentation (UP B), and switchgrass treated soil with bioaugmentation (SG B). Error bars indicate the range of two soil subsamples from the same reactor.

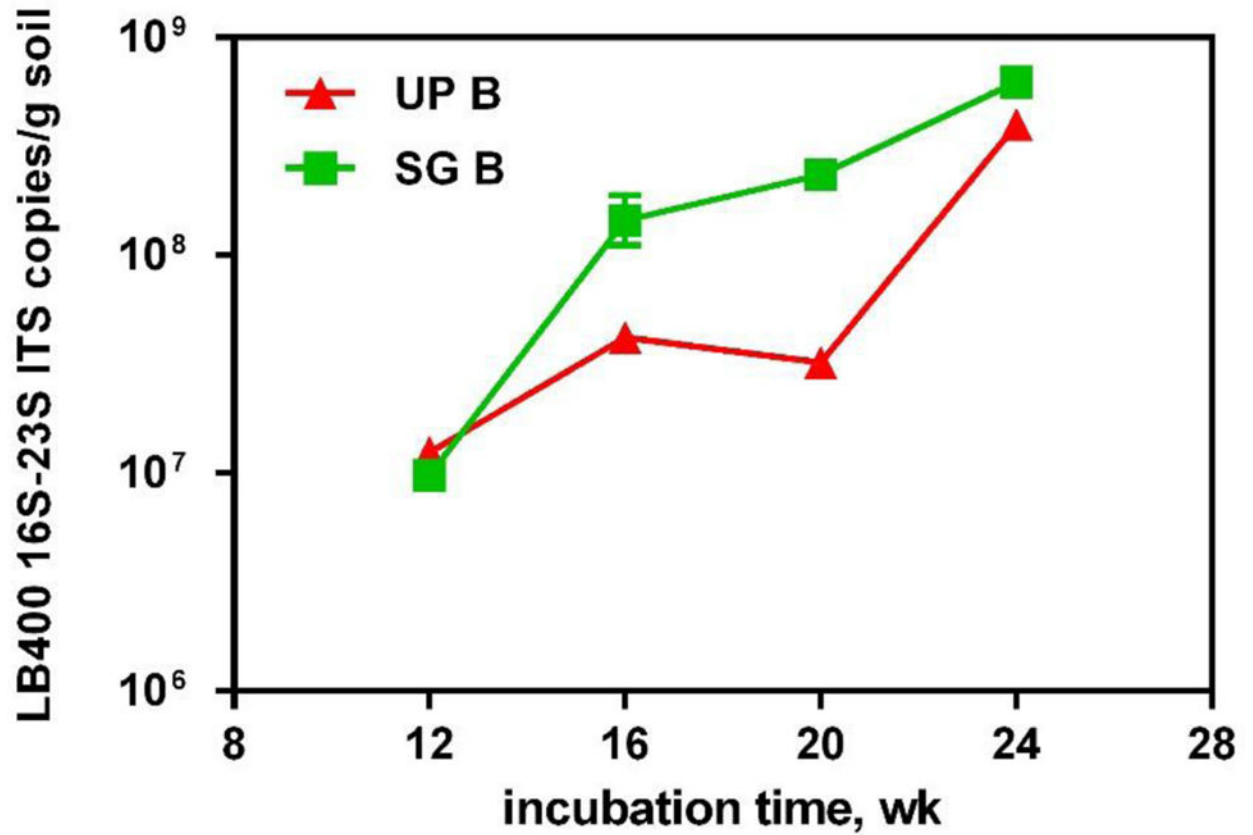


Figure 5. qPCR analysis of LB400 16S–23S ITS abundance with time in unplanted soil with bioaugmentation (UP B), and switchgrass treated soil with bioaugmentation (SG B). Error bars indicate the range of two soil subsamples from the same reactor.

Molar mass balance of PCBs in unplanted soil (UP), switchgrass treated soil (SG), unplanted with bioaugmentation soil (UP B), switchgrass treated with bioaugmentation soil (SG B), and switchgrass treated with bioaugmentation with dead LB400 soil (SG BD) after 24 weeks of incubation. Results were the reported as mean \pm standard deviation (n=3).

Table 1

	PCB fraction in soils	PCB fraction in roots	PCB fraction in shoots	Total recovery
UP	70.6 \pm 4.1%	-	-	70.6 \pm 4.1%
SG	60.1 \pm 2.0%	7.3 \pm 0.3%	0.11 \pm 0.01%	67.5 \pm 2.3%
Overall	60.6 \pm 2.5%	-	-	60.6 \pm 2.5%
UP B	52.7 \pm 2.1%	4.3 \pm 0.2%	0.24 \pm 0.03%	57.3 \pm 2.3%
SG B	57.1 \pm 10.6%	-	-	57.1 \pm 10.6%
SG BD	98.6 \pm 4.8%	-	-	98.6 \pm 4.8%
UP	81.9 \pm 2.4%	9.4 \pm 0.3%	0.17 \pm 0.01%	91.4 \pm 2.7%
SG	54.8 \pm 1.8%	-	-	54.8 \pm 1.8%
Overall	57.0 \pm 2.4%	4.7 \pm 0.2%	0.40 \pm 0.05%	62.2 \pm 2.5%
UP B	76.7 \pm 14.9%	-	-	76.7 \pm 14.9%
SG B	61.0 \pm 4.9%	-	-	61.0 \pm 4.9%
SG BD	53.2 \pm 2.8%	6.0 \pm 0.3%	0.06 \pm 0.01%	59.2 \pm 3.0%
UP	62.1 \pm 3.4%	-	-	62.1 \pm 3.4%
Overall	53.1 \pm 2.9%	3.8 \pm 0.3%	0.16 \pm 0.02%	57.1 \pm 3.1%
UP B	51.1 \pm 9.7%	-	-	51.1 \pm 9.7%
SG B	66.1 \pm 4.2%	-	-	66.1 \pm 4.2%
SG BD	56.5 \pm 2.9%	8.2 \pm 0.5%	0.11 \pm 0.02%	64.8 \pm 3.4%
UP	69.7 \pm 3.9%	-	-	69.7 \pm 3.9%
Overall	53.2 \pm 2.8%	5.0 \pm 0.3%	0.17 \pm 0.02%	58.4 \pm 3.1%
UP B	53.7 \pm 9.4%	-	-	53.7 \pm 9.4%
SG B				
SG BD				