

The Potentials for Bioremediation of Polychlorinated Biphenyls in Effluents from a Large Advanced Wastewater Treatment Plant

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Background/Objectives. This study involved an evaluation of the potential for bioremediation of polychlorinated biphenyls (PCBs) in the effluents from a large municipal wastewater treatment plant. It was focused on the presence of PCBs in two types of effluents: the continuous effluent present during dry weather conditions and the intermittently present effluent that was present during wet weather due to incoming stormwater. The annual discharge of PCBs for both types of effluent was calculated based on a five-year dataset (2011-2015). The average number of chlorine per biphenyl for the detected PCB congeners showed a 19% difference between the two types of effluent, which indicated a potential for organohalide respiration of PCBs during the continuous treatment. This was further supported by polymerase chain reaction (PCR) using 348F/884R primer set.

Approach/Activities. In this study, 10 PCB homologs and 209 PCB congeners were evaluated in effluent discharge during normal treatment and in case of stormwater overflow during a five-year study period (2011-2015). Extraction of PCBs from the samples was performed using solid-phase extraction (SPE) or a continuous liquid/liquid extraction (CLLE). All 209 PCB congeners were analyzed at a certified laboratory by using EPA method 1668B. The collection of effluent samples from continuous treatment occurred approximately 11 times per year, while collection of grab samples from the stormwater overflow took place 6-7 times per year over the five year study period. Missing data points between the sampling events were estimated based on the existing sampling data. The calculation of the total PCB mass distribution and contribution from PCB homologs was based on the concentration from the analyzed samples and converted into mass by multiplying with the effluent flowrate.

DNA was extracted from 10 locations at the WWTP: primary sedimentation tank (PST), nitri/denitrification reactor (NDR), primary effluent (PE), anaerobic digestion reactor (digested biosolids: DB), final product biosolids (FPB), nitri/denitrification sedimentation (NDS), secondary reactor (SR), secondary sedimentation tank (SST), centrifuge pre-dewatering (liquid and biosolid:LB), east primary influent (EPI). DNA was extracted using the MoBIO PowerSoil DNA Isolation Kits (Qiagen Inc., Germantown, USA). The extracted DNA from the nine samples was analyzed on the nano-drop (Thermo Fisher Scientific, Waltham, USA) to measure the concentration of the DNA and the purity. Polymerase chain reaction (PCR) was performed by using DreamTaq Green PCR Master Mix (Thermo Fisher Scientific, Waltham, USA) and primers set 348F/884R specific for the 16S rRNA gene targeting putative PCB-dechlorinating *Chloroflexi*. Polymerase chain reaction (PCR) in this study was used iQ SYBR green supermix (Bio-Rad Laboratories) and 348F/884R primer set specific for the 16S rRNA gene of a putative PCB-dechlorinating *Chloroflexi*. Each PCR reaction was conducted by using 12.5 ul of iQ SYBR green supermix, 8.5 μ L nuclease-free water, 1 μ L forward and reverse primers, and 2 μ L of sample DNA. The PCR products were evaluated via gel electrophoresis and bands were compared to the amplicon length for the positive control (536 bp) and a molecular ladder (Genprice Inc., San Jose, USA).

Results/Lessons Learned. The results from this study showed that the continuous effluent contributed the major PCB discharge (80.7%-94.7%) of total PCB discharge from both intermittent effluent and continuous effluent over the five years period. A significantly ($p < 0.01$)

larger contribution (1821.4 g) came from the continuous effluent discharge compared to the stormwater overflow (260.3 g) thus showing that the sources of PCBs in the effluent originate from the wastewater and not from the stormwater contribution in the combined sewer system.

The distribution of PCB homologs for the two types of effluent showed that tri-, tetra-, penta-, hexa-, and hepta- chlorinated PCBs were the most abundant congeners in the stormwater overflow with penta- and hexa- chlorinated congeners having the highest abundance. The contributions from these five homologs ranged on average for 49.5%-58.1 % of the total annual PCBs. For the continuous effluent, di-, tri-, tetra-, penta-, and hexa- chlorinated congeners were the most abundant. Here, tetra- and penta- chlorinated congeners accounted for annual mass discharge ranging from 47.8%- 51.1% of the total PCBs over the five-year period.

The average number of chlorine/biphenyl for 209 PCB congeners from the continuous effluent was 3.83-4.03 over a five-year study period, which was 19% lower than that of the intermittent effluent (4.80 to 4.93). This difference of an average number of chlorine/biphenyl indicated that a PCB organohalide respiration process might occur during normal wastewater treatment. This was mainly attributed to the mass of tetra- and penta- chlorinated congeners that accounted for 47.8%-57.8% of the total annual PCBs over five years for the continuous effluent. In contrast, penta- and hexa- chlorinated congeners had an increased abundance in the intermittent effluent with 49.5%-58.2% of total annual PCBs from 2011 to 2015.

Six of 10 samples (i.e., PST, PE, NDS, SR, SS and EPI) contained detectable concentrations of DNA. In addition, four PCR-amplified 16S rRNA gene products of these 10 samples showed the expected size (536 bp) from 10 locations of the WWTP when the genomic DNA was targeted with primers 348F and 884R. These results showed that putative PCB-dechlorinating *Chloroflexi* such as *Dehalococcoides* species might exist in the wastewater samples and play important roles in dechlorination of PCBs. It should be noted that the exhibiting bands of the PCR amplicon in this study need to be extracted and sequenced to compare the sequence similarity to determine the affiliation with the *Dehalococcoides* clusters. However, the discussion of that is beyond the scope of this study.