Effect of Environmental Factors on Dechlorination of Polychlorinated Biphenyls

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1. INTRODUCTION

Polychlorinated biphenyls (PCBs) have been found to persist in many lake and river sediments. In the past, extensive studies were performed to understand the PCBs dechlorination by indigenous microorganisms in the contaminated river, lake or esturine sediments [1-3]. Environmental parameters are important in determining the growth and metabolic performance of the microorganisms in natural ecosystems and therefore need to be clearly understood [4]. Temperature, oxygen, redox potential, nutrients, pH, inoculum size and bioavailability are major rate limiting environmental factors in the biodegradation of environmental pollutants [5]. Additionally, sediment type, concentration and degree of PCBs, partition co-efficient, availability of primary nutrients, co-contaminants and electron donors also play a major role in determining the fate and efficacy of dechlorination and biodegradation [3,6]. The impact of chlorine substitution on PCB-degradation was reported by Bedard and Haberi [7].

A self-immobilized anaerobic granulated consortium developed in our laboratory was found to dechlorinate PCBs under methanogenic conditions [8]. In this investigation, our focus was to study the effect of temperature, oxygen and substrates on the PCB-dechlorinating anaerobic microbial granules under sediment free conditions.

2. METHODOLOGY

Experimental Set-up and PCB Extraction. All experimental procedures and sampling were performed under anoxic conditions (95% N_2 and 5% H_2). Dechlorination studies were performed in 158 ml serum vials (Wheaton Scientific, Millville, NJ) containing 45 ml phosphate buffered basal (PBB) medium sealed with Teflon-coated rubber stoppers and aluminum seals. The PBB medium contained (g/L): NaCl, 0.5; NH_4Cl, 0.5; $MgCl_2$. $6H_2O$, 0.2; $CaCl_2$. $2H_20$, 0.1; resazurin, 0.02 and 10 ml of trace element solution. The PBB medium was reduced with Na_2S (2.5%, w/v) and the pH was adjusted with phosphate buffer (15% K_2HPO_4 and 29% KH_2PO_4) before inoculation. Selected defined PCB congeners were spiked with a glass Hamilton syringe into the experimental bottles before inoculation. The PCB congeners 23456-chlorobiphenyl (CB) and 2346-CB were dissolved

(approximately 20 mg/ml stock) in acetone. The anaerobic serum vials were inoculated in an anaerobic glove box (Coy Manufacturing Co., Ann Arbor, MI). Each bottle was inoculated with 5 ml of microbial granules (approximately 0.1 g dry weight). The headspace of each vial was purged with nitrogen gas. Periodically, 2-4 ml aliquots were sampled in glass tubes, sealed with Teflon-coated stoppers and PCBs were extracted as described below. PCBs were extracted with acetone that contained octachloronapthalene, (1.6 ppm) as an internal standard. This was followed by the extraction with a mixture of acetone and hexane (1:9 v/v) as per the method described by Quensen *et al.* [3].

3. RESULTS AND DISCUSSIONS

Temperature: A batch study was conducted to determine the impact of temperature (4, 12, 30 and 37° C) on the rates of dechlorination of the defined PCB congener, 2346-CB. Metabolic performance of the microorganisms varied with the temperature. Figure 1 exhibits the dechlorination of 2346-CB by the granules at different temperatures. Except at 4° C, substantial dechlorination was observed at all three temperatures, i.e. 12, 30 and 37° C. Dechlorination at 4° C was very minimal. In 32 weeks, only 18 μM of 2346-CB was dechlorinated at 4° C. Rates of biodegradation generally decrease with the decrease in temperature. The higher dechlorination rate of 2346-CB was observed at 30° C followed by 12 and 37° C. In 24 weeks, about 5, 50, 100 and 25% of spiked congener was dechlorinated at 4, 12, 30 and 37° C respectively. A temperature of 37° C appeared to inhibit the dechlorinating activity of some of the microorganisms in the granules. These results indicate applicability of the microbial granules even at temperatures that are lower than the ambient temperature. Similar temperature effects were studied between 4 to 66° C with a pond sediment [9] and rates of dechlorination were optimal at 30° C and slower (marginal) at 4° C.

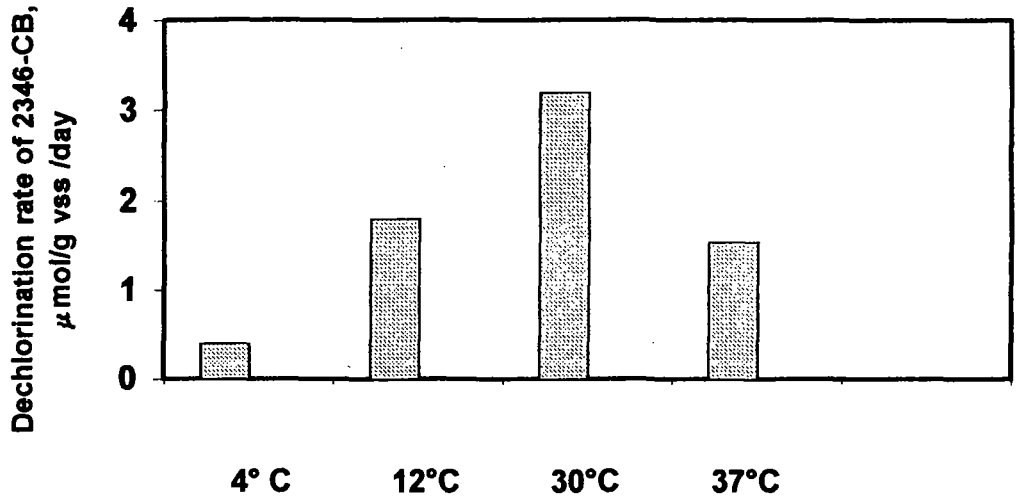

Figure 1 ***Effect of Temperature on Dechlorinating Performance of the Anaerobic Microbial Granules***

Oxygen: To determine the effect of initial oxygenated conditions in the medium, dechlorination of 23456-CB by the anaerobic granules was examined under three conditions including anaerobic, semi-anaerobic and aerobic. These three conditions were established by the methods of media preparation, i.e. aerobic, anaerobic (gassed with N_2 during media preparation and reduced with sulfide) and semi-anaerobic (media gassed with N_2 but no addition of sulfide).

Dechlorination of 23456-CB was observed under all three conditions, however, the rates of dechlorination differed (Figure 2). Almost 60% of 23456-CB was transformed in 8 weeks in the aerobically prepared medium, whereas about 80% of dechlorination was observed in the semi-anaerobic medium. Under strict anaerobic conditions, the 23456-CB was completely dechlorinated in 8 weeks, however, it took almost 16 weeks for complete dechlorination under both semi-anaerobic and aerobic conditions. The delay was probably due to the time required in consumption of initial oxygen in the medium. The dechlorination rate of 23456-CB was 6.11, 5.01 and 5 μM / g vss / day under anaerobic, semi-anaerobic and aerobic conditions, respectively (Figure 2). In this study, the oxygen exposure affected the rate of initial dechlorination, but not the extent and pattern of dechlorination of the anaerobic microbial granules.

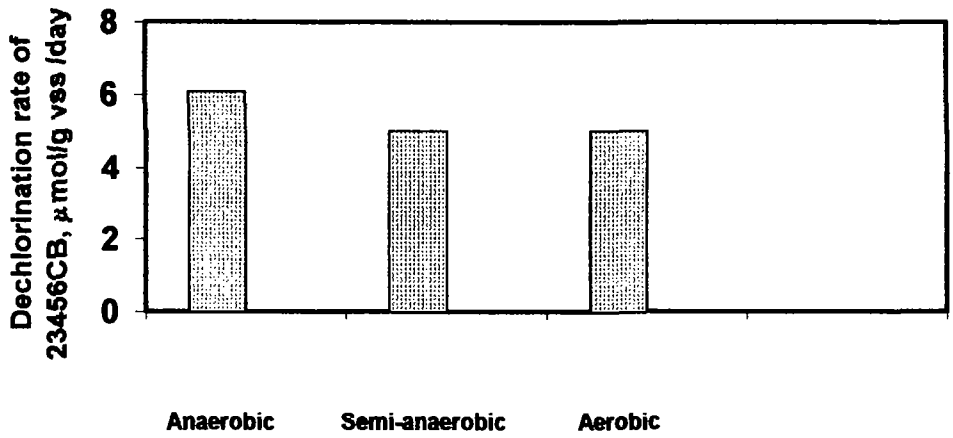

Figure 2 *Effect of Oxygen on Dechlorinating Performance of the Anaerobic Microbial Granules*

Nutrients: A suitable substrate is important for enhancing the rate of microbial activity. We therefore conducted studies using different nutrients such as glucose plus methanol, avicel and wood powder to determine the effect on the dechlorination performance of the granules. Dechlorination of 23456-CB was observed in all of the treatments that received nutrient amendments (Figure 3). Relative to the wood powder, amendments with the mixture of glucose plus methanol, and avicel supported enhanced dechlorination. The 23456-CB was completely dechlorinated in 24 and 36 weeks in the presence of glucose plus methanol and avicel, respectively. In the case of wood powder, approximately 90% of 23456-CB was dechlorinated in 36 weeks. In the absence of nutrient

supplementation, dechlorination of 23456-CB was minimal (18 mM) even in 36 weeks. The minimal dechlorination was supported probably by the substrates carried over with the granules from the reactor. In all of these cases, 23456-CB was dechlorinated to 2346-CB and 246-CB by removal of two *meta* chlorines. The dechlorination products, 2346-CB and 246-CB decreased with an increase in the incubation period. The rate of dechlorination of 23456-CB in the presence of glucose plus methanol was almost similar to avicel (Figure 3). However, the substrate did not alter the pattern and extent of dechlorination suggesting the dechlorinating mechanisms of the microbial populations is not substrate specific.

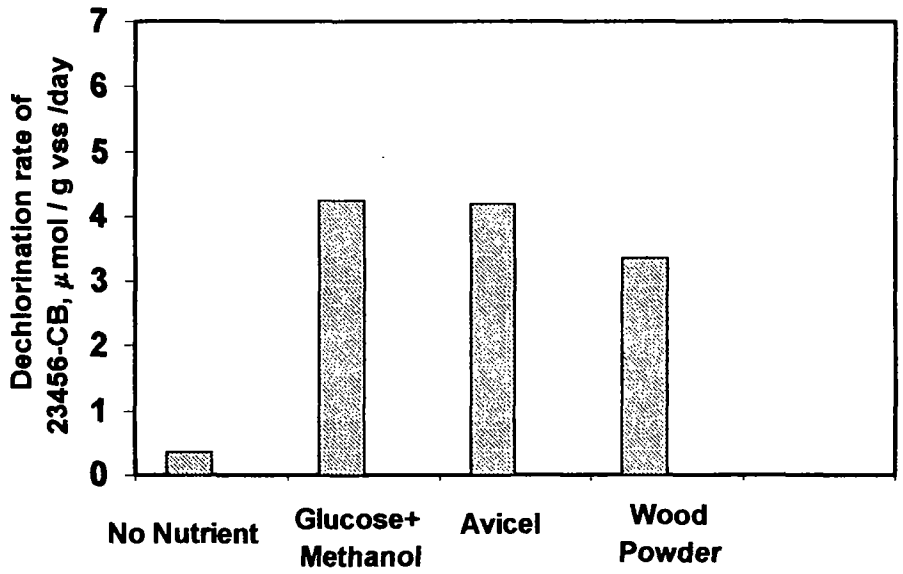

Figure 3 *Effect of Nutrients on Dechlorinating Performance of the Anaerobic Microbial Granules*

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