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Short communication

Biocontainment of polychlorinated biphenyls (PCBs) on flat concrete surfaces by microbial carbonate precipitation

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ABSTRACT

In this study, a biosealant obtained from microbial carbonate precipitation (MCP) was evaluated as an alternative to an epoxy-coating system. A bacterium *Sporosarcina pasteurii* strain ATCC 11859, which metabolizes urea and precipitates calcite in a calcium-rich environment, was used in this study to generate the biosealant on a PCB-contaminated concrete surface. Concrete cylinders measuring 3 in (76.2 mm) by 6 in (152.4 mm) were made in accordance with ASTM C33 and C192 and used for this purpose. The PCB, urea, Ca^{2+}, and bacterial cell concentrations were set at 10 ppm, 666 mM, 250 mM, and about 2.1×10^8 cells mL^{-1}, respectively. The results indicate that the biosealed surfaces reduced water permeability by 1–5 orders of magnitude, and had a high resistance to carbonation. Since the MCP biosealant is thermally stable under temperatures of up to 840 °C, the high temperatures that normally exist in the surrounding equipment, which may contain PCB-based fluids, have no effect on the biosealed surfaces. Consequently, there is greater potential to obtain a stronger, coherent, and durable surface by MCP. No measurable amount of PCBs was detected in the permeating water, indicating that the leaching water, if any, will have a minimum impact on the surrounding environment.

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1. Introduction

Concrete surfaces are commonly contaminated with PCBs when oil spills or leaks from motor equipment such as machinery containing PCB-based oils. Methods to remove PCBs from contaminated surfaces include physical and chemical methods such as sandblasting, shot blasting, scarification, scabbing, hydro blasting, solvent washing, and degradation. In addition, concrete surfaces are often encapsulated with one or more layers of epoxy coatings to act as a physical barrier to prevent PCBs from leaching out or contaminating workers when complete PCB removal is not technically achievable, especially in confined areas. However, epoxy coatings can be ineffective due to "bleedback" (the resurfacing of oils and PCBs from concrete after cleaning) caused by elevated temperatures induced by heating. Epoxy resins degrade at temperatures above 177 °C (350 °F) (Morena, 1988), and high temperatures lower the density of the oil, preventing or damaging the epoxy-concrete adhesion (Pizarro et al., 2002). Poor bonding due to the presence of free oil on the concrete surface may also cause the coating system to fail.

In this study, we investigated the potential of bio-sealant obtained from microbial carbonate precipitation (MCP) as an alternative to epoxy coatings to confine PCBs on concrete surfaces. MCP can be induced by natural microbial metabolic processes such as photosynthesis (McConnaughey and Whelan, 1997), urea hydrolysis (Fujita et al., 2000; Hammes et al., 2003; Dick et al., 2006; De Muynck et al., 2007a; De Muynck et al., 2007b; Ercole et al., 2007), and sulfate reduction (Castanier et al., 1999; Knorre and Krumbein, 2000; Hammes et al., 2003). The negative surface charge of most microbial cells makes them ideal crystal nucleation sites for divalent cations in aquatic environments (Ferris et al., 1986, 1987; Schultze-Lam et al., 1996; Stocks-Fischer et al., 1999; Ramachandran et al., 2001). The accumulation of these divalent cations on bacterial cell surfaces promotes carbonate precipitation, which ultimately entombs the bacteria and forms a hard monolith. Consequently, MCP has been used to mitigate several engineering problems such as crack repair in concrete (Bang et al., 2001; Ramachandran et al., 2001; Bachmeier et al., 2002; DeJong et al., 2006), sand consolidation (Ferris and Stehmeier, 1992; Gollapudi et al., 1995; Stocks-Fischer et al., 1999; Nemati and Voordouw, 2003), repairing calcareous monuments (Le Metayer-Levrel et al., 1999; Tiano et al., 1999, 2006; Rodriguez-Navarro et al., 2003; De Belie et al., 2006; Dick et al., 2006; Jimenez-Lopez et al., 2008), concrete compressive strength improvement (Bang et al., 2001;

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Ramachandran et al., 2001; Ghosh et al., 2005; Jonkers et al., 2010), concrete durability improvement (De Muynck et al., 2007a; De Muynck et al., 2007b), selective plugging for enhanced oil recovery (Gollapudi et al., 1995), wastewater treatment (Hammes et al., 2003), and soil improvement (Whiffin et al., 2007; Ivanov and Chu, 2008; DeJong et al., 2010).

Facultative ureolytic bacteria such as *Sporosarcina pasteurii* and *Bacillus sphaericus* have been studied extensively (Fujita et al., 2000; Hammes et al., 2003; Dick et al., 2006; De Muynck et al., 2007a; De Muynck et al., 2007b; Ercole et al., 2007), especially their ability to precipitate calcite through the enzymatic hydrolysis of urea. The microbial urease enzyme hydrolyzes urea to produce dissolved ammonium, dissolved inorganic carbon, and CO_2, and the ammonia released in the surroundings subsequently increases pH, leading to the accumulation of insoluble $CaCO_3$ in a calcium-rich environment. Quantitatively, 1 mol of urea is hydrolyzed intracellularly to 2 mol of ammonium (Eqs. 1 and 2).

$$CO(NH_2)_2 + 2H_2O \xrightarrow{Urease} 2NH_4^+ + CO_3^{2-} \quad (1)$$

$$CO(NH_2)_2 + 2H_2O + Ca^{2+} \xrightarrow{Urease} 2NH_4^+ + CaCO_3\downarrow \quad (2)$$

These reactions occur under the influence of natural environmental factors that control the activity of the urease enzyme. These factors include the type of bacteria, bacteria cell concentration, temperature, urea concentration, calcium concentration, ionic strength, and the pH of the media, all of which may have a significant impact on MCP and must be carefully considered when designing carbonate deposition experiments. The bacteria should possess high ureolytic efficiency, alkalophilic (optimum growth rate occurs at pH around 9, and no growth at all around pH 6.5), be non-pathogenic, and be able to deposit calcite homogeneously on the substratum. The bacteria should also have a high negative zeta-potential (Dick et al., 2006; De Muynck et al., 2007a; De Muynck et al., 2007b) to promote adhesion and surface colonization and to produce enormous amounts of urease enzyme in the presence of high concentrations of ammonium (Kaltwasser et al., 1972; Friedrich and Magasanik, 1977) to enhance the rate of ureolysis and MCP (Nemati and Voordouw, 2003).

The objective of this research is to use the optimum conditions determined by Okwadha and Li (2010) and the urease enzyme supplied by the soil bacteria *S. pasteurii* strain ATCC 11859 to deposit a biosealant on a PCB-contaminated concrete surface. The durability of this surface was determined by water permeability and resistance to carbonation tests.

2. Materials and methods

2.1. Stock culture

S. pasteurii strain ATCC 11859, (Manassas, VA) was grown at 30 °C for 72 h with agitation in brain heart infusion broth (BHI). After growth, cells were platted in an agar plate to determine their viability and storage.

2.2. Culture medium

The culture medium consisted of 3 g of BHI broth, 10 g of ammonium chloride, and 2.1 g of sodium bicarbonate per liter of distilled water. Urea was added to the mixture and the pH adjusted to 6.5 using 1N HCl before adding $CaCl_2$ to avoid premature $CaCO_3$ precipitation. The mixture was then autoclaved at 121 °C for 20 min.

2.3. Bacterial cell concentration

The bacterial cell concentration of about 10^8 cells mL^{-1} was obtained by dilution using ultrapure water and quantified by measuring the absorbance (optical density) of the suspension using a Spectronic Genesys 5 Spectrophotometer (Thermo Electron Corporation, Madison, WI) at 600 nm wavelength. The concentration of cells suspended in the stock culture was estimated by the expression

$$Y = 8.59x10^7.Z^{1.3627} \quad (3)$$

(Ramachandran et al., 2001), where Z is reading at OD_{600}, and Y is the concentration of cells mL^{-1}.

2.4. Biocontainment experimental set-up

Concrete specimens were made in accordance with ASTM C33, 2003 and C192, 2007. The mix design for concrete materials was selected from the We Energies Coal Combustion Handbook, 2nd edition page 56, Tables 4–2 (Ramme and Tharaniyil, 2004). Plastic straws were incorporated during casting to mimic cracks; however, most of these artificial cracks were blocked at the bottom by mortar and were not easy to open. The artificial cracks were first filled with sand, and a cylindrical ring was fitted at the top of the concrete sample to act as a reservoir for bacteria stock culture and the culture medium (Fig. 1a). PCBs were sprayed on the surface and allowed to stand overnight.

The culture medium was prepared as described in the previous section and the pH was adjusted to 6.5 before adding Ca^{2+} to prevent premature calcite precipitation. The mixture was then autoclaved at 121 °C for 20 min, allowed to cool to room temperature (25 °C), then poured into a burette. The stock culture was grown in BHI broth at 30 °C with agitation for 72 h to 2.1×10^8 cells mL^{-1}. 10 mL of the stock culture and equal amount of the culture medium were poured into the cylinder without agitation. The culture medium was allowed to drip into the cylinder continuously from the burette. The experiment was done in triplicate. The stock culture and the culture medium were replenished after 3 d (72 h). The experiment was allowed to proceed for four more days to enable more $CaCO_3$ deposition (Fig. 1b). On the seventh day, sand was sprinkled on the biosealant (Fig. 1c) to increase friction and allowed to dry at room temperature.

2.5. Permeability test

The permeability test evaluated the effectiveness of the biosealant surface at preventing PCB ingression into the concrete slab matrix. Constant head permeability (ASTM D5084 Method F, 2003) test on the control and the specimen samples were performed by Giles Engineering Associates (Waukesha, WI). The specimens were coated with a thin layer of silicon vacuum grease to prevent sidewall leakage due to irregular sidewalls by introducing a vacuum seal between the specimen sidewall and the permeameter cell membrane (Bowders et al., 2002, 2003). All tests were done in triplicate with a back pressure of 55 psi, mean hydraulic gradient of 13.8 cm, and maximum consolidation pressure of 5, 10, and 20 psi using water as the permeating fluid.

2.6. Amount of PCBs in the permeating water

Chemical analysis of PCBs in the permeating water was performed by Pace Analytical Services, Inc. (Green Bay, WI) in accordance with the National Environmental Laboratory Accreditation Conference (NELAC) standards, and prepared and analyzed in