Journal of Environmental Management 92 (2011) 2860–2864

Contents lists available at ScienceDirect

Journal of Environmental Management

journal homepage: www.elsevier.com/locate/jenvman

Short communication

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

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A R T I C L E I N F O

Article history:
Received 6 April 2011
Received in revised form
23 May 2011
Accepted 28 May 2011
Available online 21 June 2011

Keywords:
Microbial carbonate precipitation
Biosealant
Urea hydrolysis
Ureolytic bacteria
Permeability
Carbonation

A B S T R A C T

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.

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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0301-4797/$ – see front matter Published by Elsevier Ltd.
doi:10.1016/j.jenvman.2011.05.029

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

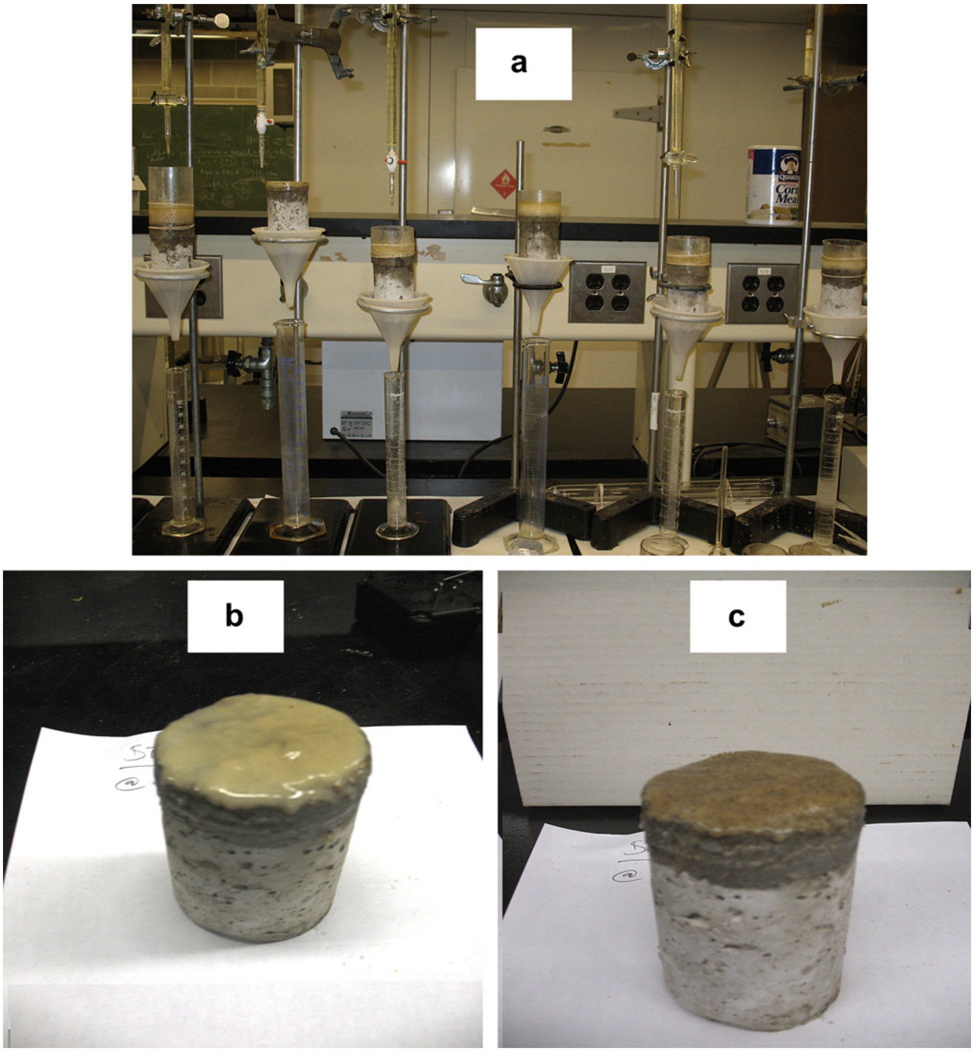

Fig. 1. $CaCO_3$ (biosealant) deposition experimental set-up on concrete cylinders. Laboratory experimental set-up (a), after $CaCO_3$ deposition (b), and after sprinkling sand on the deposited $CaCO_3$ (c).

accordance with EPA Method 8082, 2007 with a reporting limit of 5 ng L^{-1}. The results are shown in Table 1.

2.7. *Carbonation test*

A carbonation test (using phenolphthalein indicator) was performed to determine the durability of the concrete surface. Carbonation occurs when the products of concrete hydration especially calcium hydroxide, calcium silicate hydrates, and calcium aluminate hydrates react with carbonic acid. These carbonation reactions cause the high pH of concrete to dramatically drop from about 12.5 to about 8.5–9, resulting into dusty weak surfaces; therefore, a strong concrete surface treatment should improve its resistance to carbonation (Basheer et al., 1997).

Table 1
Concentration of PCBs in the permeating water by EPA Method 8082.

Concrete Cylinder Sample Number	Artificial cracking condition	Permeability ($cm\ s^{-1}$)		PCB concentration in the permeating water (ppm)
		Before biosealing	After biosealing	
A	No crack	7.97×10^{-3}	3.26×10^{-8}	ND[a]
B	One crack	9.20×10^{-3}	1.55×10^{-5}	ND
C	Two cracks	7.33×10^{-3}	7.35×10^{-4}	ND

[a] None detected.

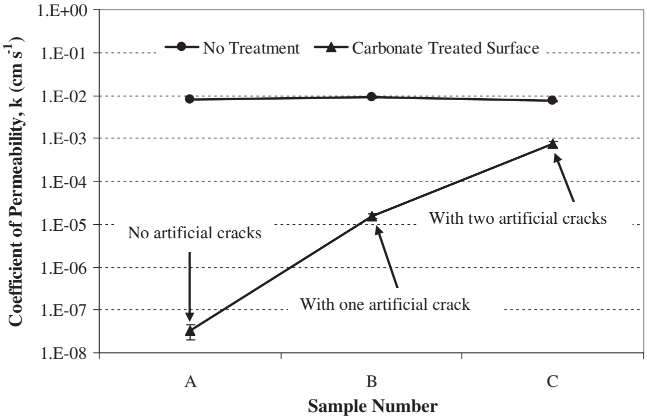

Fig. 2. Mean permeability test results before and after the carbonate biosealant application, and with and without artificial cracks.

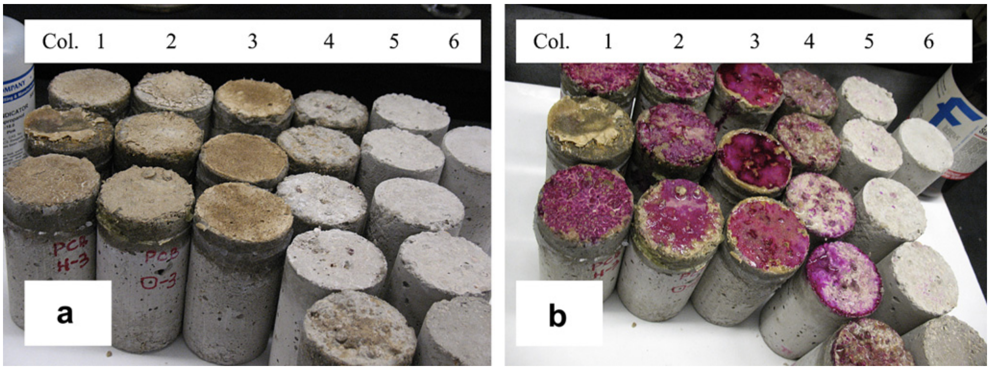

Fig. 3. Carbonation test on the PCB-contaminated concrete cylindrical surfaces after calcium carbonate biosealant treatment. Columns are numbered from left to right. Columns 1, 2, 3, and 4 were biosealed whereas columns 5 and 6 were not. Columns 1, 2, and 3 were left indoors whereas columns 4, 5, and 6 were subjected to ambient environmental conditions. Before phenolphthalein addition (a), and after phenolphthalein application (b).

3. Results and discussion

3.1. Permeability test results

The results of the permeability test are presented in Fig. 2. The permeability of the carbonate treated samples were 3.26 $\times 10^{-8}$ cm s^{-1} (without artificial cracks), 1.55 $\times 10^{-5}$ cm s^{-1} (with one artificial crack), and 7.35 $\times 10^{-4}$ cm s^{-1} (with two artificial cracks) whereas the control samples had permeabilities of 7.97 $\times 10^{-3}$, 9.20 $\times 10^{-3}$, and 7.33 $\times 10^{-3}$ cm s^{-1}, all without artificial cracks. These results indicate that the carbonate treatment reduced the permeability of the surface between 1 and 5 orders of magnitude compared with the untreated (control) surface. Consequently, both the lateral and vertical movement of PCB-based oil will have an insignificant impact on the surroundings.

3.2. Amount of PCBs in the permeating water

The results of PCBs concentration is shown in Table 1. No PCBs were detected in the permeating water. The low coefficient of permeability obtained in all the specimens could have contributed to the adherence and adsorption of PCBs on the concrete matrix, allowing the polar water molecules to displace the oil as it percolates out of the matrix.

3.3. Carbonation test results

Fig. 3 shows carbonation test results. Fig. 3a and b show the concrete cylinders before and after the addition of phenolphthalein indicator, respectively. Columns 1, 2, 3, and 4 contain cylinders whose surfaces were biosealed, whereas columns 5 and 6 were not. All cylinders in columns 4, 5, and 6 were placed outside into the ambient environmental conditions for three months. All other cylinders were left indoors at room temperature. All the biosealed surfaces show a pink color when treated with phenolphthalein indicator, indicating a pH range of between 8.3 and 9. These results show that carbonation reactions were suppressed by the carbonate biosealant; however, the phenolphthalein indicator was colorless on the untreated surfaces, indicating that carbonation reactions occurred. In addition, the column 4 cylinders subjected to the ambient environmental conditions exhibited little carbonation reaction because their surfaces were slightly pink; this is not an impediment because the biosealant surface treatment will be confined to PCB spills indoors away from harsh ambient environmental conditions.

4. Conclusions

Our results show that the biosealant deposited by MCP on the concrete surface reduced permeability by 1–5 orders of magnitude, indicating the potential of MCP at encapsulating and confining PCB in-situ by forming a very strong durable surface barrier with a very high resistance to carbonation suitable for indoor application. The low permeability results obtained in this experiment are also a measure of durability of the concrete because the loss of fines and the subsequent increase in porosity in the concrete matrix will be suppressed and the concrete slab will maintain its functional framework. The impact on the environment will also be reduced because the PCBs are confined in the concrete matrix. Another advantage of the biosealant is that $CaCO_3$ and calcite have very high thermal stability, decomposing at 840 °C and 825 °C, respectively, compared with epoxy resin adhesives that degrade at only 177 °C. This indicates the prevailing temperature at these equipment installations will not affect the performance of the biosealant. Consequently, the biosealant will provide a safe working environment while the equipment is still in service.

Acknowledgments

The authors thank Dr. Bruce Ramme of We Energies, Environmental Department - Land Quality division, for providing funds and technical support for this research.

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