

**BIOCONTAINMENT OF PCBs ON FLAT
CONCRETE SURFACES AND COPRECIPITATION
OF PCBs AND METALS IN BOILER CHEMICAL
CLEANING WASTEWATER BY MICROBIAL
CARBONATE PRECIPITATION**

by

George D.O. Okwadha

A Dissertation Submitted in
Partial Fulfillment of the
Requirements for the Degree of

Doctor of Philosophy

in Engineering

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ABSTRACT

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by

George D.O. Okwadha

The University of Wisconsin-Milwaukee, 2010
Under Supervision of Dr. Jin Li

Some industrial equipment such as electric transformers and capacitors are located in vaults and substations on flat concrete surfaces. Due to accidents, normal routine maintenance or replacements, these equipments may spill oil which may contain PCBs on these surfaces. These spills must be cleaned up in accordance with United States Environmental Protection Agency's (USEPA) guidelines. Current cleanup operations are abiotic in nature, encompassing both physical and chemical methods. These methods generate enormous amounts of impacted wastewater and solid wastes which have to be disposed of carefully at a cost. In addition, some chemicals used are harmful to both humans and other organisms. Epoxy coatings have been used to encapsulate PCBs on concrete surfaces. However, these coating systems can be ineffective because the adhesion with concrete is easily damaged by elevated temperatures causing failure of the coating system.

The use of biosealant obtained from microbial carbonate precipitation (MCP) on PCB-contaminated concrete surfaces was studied as a possible alternative to epoxy

coatings. *Sporosarcina pasteurii* strain ATCC 11859 was used for this purpose. This bacterium metabolizes urea, and in a calcium-rich environment precipitates calcite which deposits on the surface.

This research therefore exploits this unique microbial activity to determine optimum conditions for MCP, and uses these conditions to lay a biosealant on the Polychlorinated Biphenyls (PCBs) contaminated concrete surface. An investigation into the possibility of solid-phase capture (by co-precipitation) of PCBs and metals in boiler chemical cleaning wastewater (BCCW) was also explored. The results indicate that the presence of bacteria and bacterial cell concentration have a significant influence in MCP and the rate of urea hydrolysis. At 25 mM/L Ca^{2+} concentration, increasing bacteria cell concentration from 10^6 to 10^8 cells/mL increases the CaCO_3 precipitated and CO_2 sequestered by over 30%. However, when Ca^{2+} concentration is increased 10-fold to 250 mM/L Ca^{2+} , the CaCO_3 precipitated and CO_2 sequestered increased by more than 100% irrespective of urea concentration. This result shows that the amount of CaCO_3 precipitated is determined by the concentration of the Ca^{2+} ions present. Reduction in the coefficient of water permeability by 1-5 orders of magnitude and high resistance to carbonation was also exhibited by the biosealed surfaces indicating a greater potential for obtaining a stronger coherent impermeable durable surface by MCP.

Coprecipitation results have shown that the PCBs and BCCW had little effect on the activity of the urease enzyme. Consequently, the hexane-based PCBs and the metals in the BCCW were effectively coprecipitated. However, no coprecipitation occurred in the oil-based PCBs. Chemical and Energy Dispersive X-ray (EDX)

analyses on the coprecipitated solids confirmed the precipitation of metals in the BCCW. However, the ASTM Method D3987 test to determine the leaching potential of the coprecipitated metals was inconclusive, and more research needs to be done in this area.



5/4/10

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To you all, may the Almighty God bless you as you navigate the minefield of life everyday.

1.0 Introduction

Polychlorinated biphenyls (PCBs) are a class of organic compounds with 1 to 10 chlorine atoms attached to biphenyl (Fig.1) with a general formula of $C_{12}H_{10-x}Cl_x$. Theoretically, there are 209 individual isomers or congeners produced anthropogenically in the environment with a wide range of individual applications in heat transfer fluids, dielectric fluid, hydraulic fluids, flame-retardants, plasticizers, solvent extenders, and organic diluents. PCBs are hydrophobic, lipophilic, with high chemical stability, low transformation rate, and therefore are recalcitrant to biodegradation (Kamei et al., 2006). Due to these properties, PCBs persist in the environment, and bioaccumulate in the food chain.

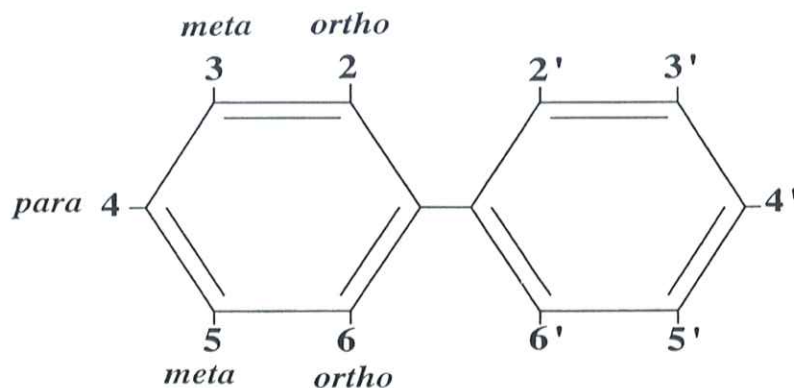


Fig. 1: Basic structure of PCB

The toxicity and general resistance to biodegradability of individual congeners is generally directly correlated with the number of chlorines substituted on the molecule (De et al.2006). Moreover, the position of the chlorine atoms on the phenol rings also influences the toxicity and persistence of the individual congeners. For example,

congeners having chlorine substitutions at one or more of the *ortho* positions of either phenol ring tend to be particularly resistant to biological breakdown. Likewise, congeners with one or more chlorines at the *meta* positions of either ring are classified as coplanar, and are more toxic relative to the other noncoplanar PCBs. The more highly chlorinated (5 or more chlorines) PCB congeners; Aroclors for example, are cometabolized under a broad variety of anaerobic conditions. Their formulations are identified by four-digit numbers, the first two indicating the number of carbons in the biphenyl ring and the last two indicating the percent chlorine by weight in the molecule. Aroclors 1254 and 1260 most commonly used in dielectric fluids are the least amenable to biodegradation, and thus makes bioremediation of such sites difficult.

PCB contamination may occur due to spills especially from transformer oil when accidentally damaged or when being replaced. In addition, leaks and spills may occur in industrial facilities where PCB-bearing organic liquids were employed (Pizzarro et al. 2002). Due to their hydrophobicity and lipophilicity, major portions of PCBs released into the aquatic environment are adsorbed onto sediments (humic acids) or rest as sludge (oil-water mixtures) on the bottom of rivers, lakes and oceans. Through mechanical disturbances including sediment dwelling organisms, wind and rainwater, PCBs subsequently enter food chains, and finally end up in the tissue of nearly all terrestrial and marine plants and animals, fish, mammals, fish- and non-fish-eating birds and humans, where it may bioaccumulate to harmful concentrations. It has been reported that PCBs are absorbed by humans and other animals through the skin, the lungs, and the gastrointestinal track (Wiegel et al., 2000). Once inside the body, they are transported through the blood stream to the liver, various muscles and adipose tissue where they

accumulate. Research shows that PCBs cause a variety of adverse health effects depending on the route of exposure, age, sex, and area of the body where PCBs are concentrated. Studies done on animals show conclusive evidence that PCBs are toxic and carcinogenic. Some of the health concerns include endocrine disruption, impairment of immune system, reproductive system, nervous system (causing dizziness, headaches, depression, nervousness, and fatigue) and endocrine system. Others are liver, stomach, and thyroid gland injuries, immunosuppression, tumor promotion, and behavioral alterations. PCBs are passed from mother to the fetus through placental blood and to the body via breast milk (Tharakan et al. 2006). PCBs can affect the productivity of phytoplankton and the composition of phytoplankton communities. Phytoplankton is the primary food source of all sea organisms and a major source of oxygen in the atmosphere (Borja et al. 2005). These factors, combined with growing awareness of the fragility of the environment have resulted in the urgency in restricting and limiting the use of PCBs. In addition, PCB spill sites are thoroughly evaluated after remediation operations to ensure that the USEPA set limit ($<10\mu\text{g}/100\text{cm}^2$) is met and the metabolites of remediation are not harmful to the environment.

1.1 Current remediation practice

The current PCB remediation methods are abiotic in nature, encompassing both physical and chemical methods. Physical methods involve cutting away the contaminated surface and treating both the wastewater and the solid waste generated. They include sandblasting, shot blasting, scarifying, and scabbling (Mitchell and Scadden, 2001).

Sandblasting and shot blasting are the most commonly used techniques where PCB contamination is limited to the upper 2 inches of a porous media such as concrete. Sandblasting involves blasting fine-grained, abrasive sand onto the PCB-contaminated surface to strip away surface coatings and remove porous material below. Shot blasting involves shooting varying sizes of metal shot against the surface, and is more effective at bulk material removal. The shot is recovered in the process using a specially fitted vacuum system that separates the shot from PCB-contaminated residue.

Scarifying and scabbling are more applicable where PCBs extend 4 to 20 inches into the porous material. Scarifiers contain a helical rotating cutting tool that is attached to a tractor or large mobile roller and used to remove a layer of concrete. Scabblers use small, high-pressure pistons to sequentially break up the concrete. Scabblers are generally smaller than scarifiers and have a lower concrete removal rate, but scabblers are more adaptable to indoor environments. Both devices are able to shave off from 1/16 inch to 1/8 inch of concrete per pass.

Hydroblasting uses high pressure water (between 1,000 psi and 60,000 psi) to wash a PCB-contaminated concrete surface. The wastewater is then collected, treated, and disposed. Hydroblasting can be effective for removing paint and coating layers. Under very high pressure, it can also be used to cut and remove concrete, but is generally less effective and results in more wastewater than other available methods. Hydrovacuuming involves using high pressure water to blast the concrete surface and vacuum-cleaning the debris in one step. It is very effective and leaves a very clean surface.

Chemical methods are designed to decontaminate the PCB-contaminated surface. They include solvent washing, degradation and encapsulation. Solvent washing uses

kerosene, diesel, terpene hydrocarbons and chemicals such as TechXtract® (Bonem and Borah, 1995) to reduce the concentration of PCB in a concrete surface. However, PCB must be 5% soluble in the selected solvent for it to be effective. Self-stripping polymers painted onto the concrete surface to remove PCB have also been used. These polymers flake off once dry, taking the PCB with it. An alkali metal-polyethylene glycolate mixture (Glycolate dehalogenation) can also be applied directly onto the contaminated concrete surface for in situ degradation of PCBs. Encapsulation has also been used as a physical barrier when complete PCB removal is not technically achievable (Pizarro et al. 2002). It involves painting the concrete surface with one or more epoxy-coatings systems to confine PCB in place and prevent it from leaching out.

1.2 Drawbacks

All these abiotic methods are expensive and generate enormous amounts of both wastewater and solid waste which must be treated before disposal. Some of the methods involve the use of chemicals. These chemicals degrade with age, and require several applications from time to time, and may be harmful to humans through physical contact and ingestion. Any spill from these chemicals would contribute to environmental pollution (De Belie et al. 2006). In addition, epoxy coatings are less effective due to “bleedback” (resurfacing of oils and PCBs from concrete after cleaning) caused by elevated temperatures induced by heating. 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 failure of the

coating system. Epoxy resins are also degraded at temperatures above 177°C (350°F) (Morena, 1988).

2.0 Bioremediation

Another way to clean up PCBs is to use biological processes (bioremediation). Bioremediation is the use of microorganisms or microbial processes to degrade or breakdown toxic substances especially xenobiotics to products which are less toxic to the environment, humans, and other organisms. Bioremediation is often less expensive, and can eliminate wastes permanently. Bioremediation technologies can be done both ex-situ and in-situ. Ex-situ technologies involve the physical removal of the contaminated material, whereas in-situ involves treatment of the contaminated material in place with minimal site disruption. Successful bioremediation depends on correct identification of the right microbes with high degradation potential, and putting them in the right place with favorable environmental factors to degrade the contaminants (Boopathy, 2000). The right microbes are bacteria or fungi, which have the physiological and metabolic capacities to degrade the contaminants. However, cleaned sites need to be re-evaluated to make sure that toxic metabolites from microbial metabolism are not left in-situ (Boopathy, 2000, Ganey et al., 2005).

Bioremediation may be divided broadly into biodegradation and biocontainment. Biodegradation is the breaking down of highly chlorinated biphenyls to less chlorinated molecules, which can then be removed from the environment easily. Biocontainment is the in-situ introduction of a physical barrier between contaminants and the environment through microbial deposition of calcium carbonate. Microbial carbonate precipitation (MCP) occurs as a byproduct of common microbial metabolic processes, such as photosynthesis, urea hydrolysis, and sulfate reduction (Knorre and Krumbein, 2000;

Castanier et al. 1999; Hammes et al. 2003). MCP can be referred to as bacterial carbonatogenesis (Metayer-Levrel, et al., 1999, Castanier et al., 1999), biocalcification (Zamarreno et al., 2009), or biomineralization (Konhauser et al., 2008, Phoenix and Konhauser, 2008). However, another basic advantage of MCP is its ability to sequester atmospheric CO₂ through calcium carbonate formation (Ferris et al. 1994, Rodriguez-Navarro et al. 2003, Manning, 2008).

2.1 Biocontainment using MCP

Organic and inorganic coatings have been used in the past in conventional surface treatments. However, environmental concerns from these volatile compounds have shifted focus to biotechnology. The participation of numerous diverse microbial species in biodeposition of CaCO₃ to act as coatings and sealants have been studied and documented. Calcite precipitation is a function of cell concentration, ionic strength, and pH in the surrounding aqueous environment (Ramachandran et al. 2001, Ramakrishnan et al., 2001, 2002).

2.1.1 MCP by bacterial surface charge

Several studies have indicated that microorganisms whose net cell surface charge is negative are capable of removing divalent cations including Ca²⁺ and Mg²⁺ from the environment, by binding them onto their cell surfaces, thereby making microorganisms ideal crystal nucleation sites (Schultze-Lam, et al. 1996, Ferris et al. 1986, 1987, Stocks-

Fisher et al. 1999, Day et al. 2003, Ramachandran et al. 2001, Bang et al. 2001a, Hontoria and Sanchez-Blanco, 2006). During this process, the microorganisms may plug the pores in the substrata not only by acting as small particles but also by adhering to the available surfaces through extracellular organic compounds (Ramachandran et al. 2001, Ramakrishnan et al., 2001, 2002) and finally get entombed within the carbonate precipitate. The following equations summarize possible biochemical reactions in an aqueous environment to precipitate CaCO_3 at the bacterial cell surface that serves as a nucleation site.



2.1.2 MCP by Photosynthetic organisms

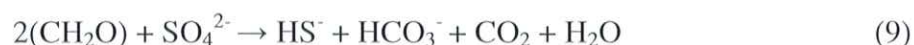
The most common form of MCP in aquatic environments is caused by photosynthetic organisms (McConnaughey and Whelan, 1997). The metabolic processes of algae and cyanobacteria utilize dissolved CO_2 (Equation 4), which is in equilibrium with HCO_3^- and CO_3^{2-} (Equation 5). The removal of CO_2 induces a shift in this equilibrium resulting in an increase in pH (Equation 6) (Ehrlich, 1998). When this reaction occurs in calcium rich environment, calcium carbonate is precipitated (Equation 7) (Hammes and Verstraete, 2002).





2.1.3 MCP by heterotrophic organisms

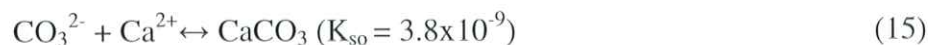
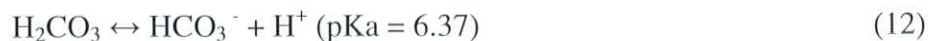
Heterotrophic organisms can precipitate calcite by the production of carbonate or bicarbonate and modification of the environment to favor precipitation (Castanier et al. 1999). The abiotic dissolution of gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) (Equation 8) provides an environment that is rich in both sulfate and calcium ions. In the presence of organic matter and absence of oxygen, sulfate reducing bacteria (SRB) can reduce sulfate to hydrogen sulfide (H_2S) and release HCO_3^- (Equation 9) (Castanier et al. 1999; Ehrlich, 1998; Wright, 1999). If H_2S degasses from the environment, an increase in pH occurs which favors CaCO_3 precipitation (Equation 7) (Castanier et al. 1999).



2.1.4 MCP by Urea hydrolysis

Microorganisms involved in the nitrogen cycle through ammonification of amino acids, nitrate reduction and hydrolysis of urea can induce MCP of which urea hydrolysis is the simplest and most important process in biocontainment of contaminants. Ureolytic

bacteria especially *Bacillus pasteurii* and *Bacillus sphaericus* have generated a lot of interest in this area, and have been studied extensively (De Muynck et al. 2007, Ercole et al. 2007, Dick et al. 2006, Hammes et al. 2003, Day et al. 2003, Fujita et al. 2000). These aerobic bacteria are able to precipitate calcite through the enzymatic hydrolysis of urea. The microbial urease enzyme hydrolyzes urea to produce dissolved ammonium (NH_4^+), dissolved inorganic carbon (DIC) and CO_2 , and the ammonia released in the surroundings subsequently increases pH, leading to accumulation of insoluble CaCO_3 (Day et al. 2003) in a calcium rich environment. Quantitatively, one mole of urea is hydrolyzed intracellularly to one mole of ammonia and one mole of carbamate (equation 10), which spontaneously hydrolyzes to form an additional one mole of ammonia and carbonic acid (equation 11). These products subsequently equilibrate in water to form bicarbonate and 2 moles of ammonium and hydroxide ions (equations 12 and 13). The latter give rise to a pH increase, which in turn can shift the bicarbonate equilibrium, resulting in the formation of carbonate ions (equation 14). These ions then precipitate as CaCO_3 in the presence of soluble calcium ions (equation 15) (Castanier et al. 1999, Hammes et al. 2003).



As these reactions proceed, the carbonate crystals continue to grow, coating, filling, and forming bridging links (Fig.2) in the voids between sand grains (or other porous media). The result is a robustly cemented dense block of material (Hontaria and Sanchez-Blanco, 2006), which is impermeable with high compressive strength, and is resistant to deterioration due to freezing and thawing (Ramachandran et al. 2001, Ramakrishnan et al., 2002). Similar results have been reported using anaerobic thermophilic bacteria belonging to the *Shewanella* species (Ghosh et al., 2005).

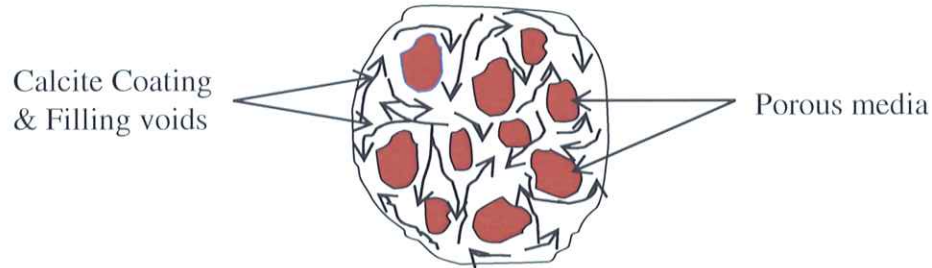


Fig. 2: Calcium carbonate coating and filling in voids between porous media grains

Microbial pathways are very complicated to be precisely predicted, and results reported by various researchers are hardly duplicated. However, the simplicity of MCP by urea hydrolysis has made it attractive to researchers especially its application in bioremediation because the rate and quantity of the carbonate precipitated can be easily controlled (De Muynck et al. 2010). In addition, using ureolytic bacteria to cause an increase in pH is additionally preferable to direct addition of a basic solution because the gradual hydrolysis of urea is likely to promote a wider spatial distribution of calcite precipitation in the subsurface than the direct addition of base, which in contrast is likely to cause immediate precipitation only in the borehole (Ferris et al. 2003).

2.2 Some reported applications of MCP

2.2.1 Crack repair in rocks and concrete

The quest for new, sustainable and environmental friendly methods to solve engineering problems has come of age and is going strong. MCP has been used to enhance oil recovery in oil reservoirs by plugging cracks in adjacent rocks which allow ground water ingress in the pumping area (DeJong et al. 2006, Cacchio et al. 2003, Stocks-Fisher et al. 1999, Gollapudi et al. 1995). De Muynck et al. 2007a and b, used *Bacillus sphaericus* in the presence of CaCl_2 (as a calcium source) to improve concrete durability by precipitating a dense and coherent layer of calcite on the concrete surface. This microbiologically induced calcium carbonate precipitation and deposition (or bioclogging) resulted in a decrease in capillary water uptake and permeability towards gas (Ivanov and Chu, 2008). Although *Bacillus pasteurii* and *Bacillus sphaericus* are both facultative, their bacteriogenic CaCO_3 precipitation activity is only intense near the surface where oxygen is abundant (Ramachandran et al. 2001, Bang et al. 2001, Bachmeier et al. 2002).

2.2.2 Monuments restoration

Dick et al. (2006) used *Bacillus sphaericus* and *Bacillus lentus* to restore a deteriorated Enville limestone. The results showed that both *Bacillus* strains were able to produce a carbonate layer that could reduce capillary water absorption on the treated

stone. However, the amount of CaCO_3 precipitated was different. MCP has also been used to clean, protect and consolidate porous ornamental limestone (Tiano et al. 1999, 2006, Rodriguez-Navarro et al. 2003, Dick et al. 2006, De Belie et al. 2006, Jimenez-Lopez et al. 2008) by forming a heterogeneous organic-inorganic bond on the limestone surface. Le Metayer-Levrel et al. (1999) used *Bacillus* spp. as a biocalcifying agent for protection of three types of limestone with different pore sizes. The results showed that the biocalcin deposited on the stone surface was similar to the limestone substrate. This could be because biocalcification uses natural microbial mediation which follows the same natural processes that formed many limestones (Le Metayer-Levrel et al. 1999). Cappitelli et al. (2007) compared the use of sulfate reducing bacteria (*Desulfovibrio vulgaris* ATCC 29579) and the traditional chemical technology in removing black crusts (containing gypsum) on marble of the Milan Cathedral (Italy). The results showed that the biological treatment not only resulted in more homogeneous removal of the surface deposits, it also preserved the patina noble under the black crust. In this regard, the microbial technology acted both as a cleaning treatment and a conservation treatment. Whereas both methods converted gypsum to calcite, the chemical technology also formed the undesirable sodium sulfate on the marble surface.

2.2.3 Compressive strength and durability improvement

Day et al. (2003) and Bang et al. (2001) used *Bacillus pasteurii* immobilized in hydrophilic polyurethane (PU), lime, silica fume and fly ash in concrete crack remediation (Day et al., 2003, Bang et al., 2001a, 2001b, Ramakrishnan et al., 2002). The

results indicated that microbiologically-enhanced crack remediation has the potential in cementing concrete and other structural cracks as well as providing a significant increase in compressive strength, and resistance to deterioration due to freezing and thawing cycles. It was also observed that PU polymer was the most effective as compared with other immobilization techniques. Moreover, encapsulated microbial cells retain high metabolic activities, and are protected from the high pH of concrete and adverse environmental conditions. In addition, PU serves as additional nucleation sites for calcite crystals (Bang et al. 2001b). Ghosh et al. (2005), Ramachandran et al. (2001), Ramakrishnan et al. (2001), and Jonkers et al. (2010) successfully used *Bacillus pasteurii* to make a bacteria-concrete mixture to improve compressive strength and durability of concrete. The ability of ureolytic bacteria to produce endospores enabled them to endure the highly alkaline pH (12.5) of fresh concrete without encapsulation. This has enabled ureolytic bacteria to be used as a self-healing agent for the development of sustainable concrete (Jonkers et al. 2010, Ramakrishnan, 2007).

2.2.4 Consolidation of soil and sand columns

Gollapudi et al. (1995), Stocks-Fischer et al. (1999) and DeJong et al. (2006) used *Bacillus pasteurii* in sand column plugging experiments, and observed that MCP was so effective that it needed only a few days to reduce the permeability of sand columns to zero. Nemati et al. (2003) observed that an increase in urease concentration enhances CaCO_3 precipitation and hence plugging of unconsolidated porous media, and consequently leads to a significant decrease in permeability. However, at low

concentrations of the enzyme (0.03g/l urease), an increase in temperature from 22 to 30°C is required to enhance the extent of bacteriogenic mineral plugging. In the geotechnical engineering field, Harkes et al. (2010) used BioGrout to fix and homogeneously distribute bacterial activity within sand. This biocementation technique enhanced MCP, and in the process promoted ground reinforcement by improving soil strength and stiffness of sandy soils with minimal reduction of permeability (Whiffin et al. 2007, Ivanov and Chu, 2008).

2.2.5 Coprecipitation of divalent metals and radionuclides

MCP has also been investigated for the solid-phase capture and immobilization of divalent metals and radionuclide contaminants such as $^{90}\text{Sr}^{2+}$, UO_2^{2+} , and Co^{2+} in groundwater aquifers (Fujita et al. 2000 and Warren et al. 2001). Warren et al. (2001) reported that 95% and 30% of $^{90}\text{Sr}^{2+}$ and UO_2^{2+} was captured respectively, reducing both their radioactivity and chance of migration downstream. The divalent Sr^{2+} ion in particular substituted Ca^{2+} readily in a CaCO_3 crystal lattice, precipitating out as $^{90}\text{SrCO}_3$. Mitchell and Ferris (2006) have also reported that, higher bacterial concentrations are needed during ureolysis to generate larger and less soluble carbonate crystals which would promote co-precipitation of metals and radionuclides in contaminated aquifers. Ions with similar radius and same charge as the replaced ion should be incorporated more efficiently in a crystal lattice than those with different size and charge. Consequently, cations larger than Ca^{2+} (ionic radius 1.00Å) should partition weakly in calcite because they are too large to occupy octahedral sites in the calcite lattice (Curti, 1999). However,

this was not found to be true (Curti, 1999, Warren et al., 2001). Curti, 1999 then concluded that differences in ionic charge between substituted and substituting ion do not hinder coprecipitation. The partition coefficient of Sr^{2+} (ionic radius 1.12 Å) and Ra^{2+} (ionic radius 1.48 Å) in calcite are in the range of 0.03-0.09 and 0.003-0.053 respectively. The partition coefficient of Ra^{2+} in calcite falls within the range for Sr^{2+} . From this analogy, it can be hypothesized that isomorphous substitution of Ra^{2+} is possible in the calcite crystal lattice. In addition, MCP have also been used in calcium removal from wastewater (Hammes et al. 2003). These positive attributes of MCP are possible because urea hydrolyzing bacteria are widespread in the environment (Fujita et al., 2000), and an in situ remediation scheme based on urea hydrolysis is not likely to require the introduction of foreign microorganisms. The subsurface hydrolysis of urea offers the additional benefit of producing ammonium which can be exchanged for sorbed metals on aquifer solids, which enhances their capture by coprecipitation in calcite (Fujita et al., 2004).

2.3 Important factors affecting MCP and the derivation of the factorial experiments

2.3.1 Type of Bacteria

The bacterial species to be used for calcite precipitation should be able to possess high ureolytic efficiency, alkalophilic (optimum growth rate occurs at pH around 9, and no growth at all around pH 6.5), deposit calcite homogeneously on the substratum, and a very high negative zeta (ξ)-potential (Dick et al. 2006, De Muynck et al. 2007). Zeta

potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle (bacteria). Colloids with high ξ -potential (negative or positive) are electrically stable and stay dispersed whereas low ξ -potential colloids tend to adhere together and flocculate. This reduces their potential to colonize a surface. Conversely, due to the positive ξ -potential of the substratum, the bacteria with a high negative ξ -potential would have a high adhesion and surface colonization. This promotes the formation of a good biofilm and production of abundant extracellular polymeric substances (EPS), which traps and concentrates Ca^{2+} ions on the substratum. As well as meeting the needs for biocementation, the bacteria must also meet the needs for safe environmental application. It must be non-pathogenic, non-genetically modified, and not contain any transferable elements that may increase the pathogenicity of environmental strains which might be detrimental to other living organisms both directly and indirectly.

During the biocementation reaction, the urease enzyme hydrolyzes urea releasing two ammonium molecules. Researchers have observed that the generation of high concentrations of ammonium suppresses urease activity (Kaltwasser et al 1972a, Friedrich and Magasanik, 1977) in some bacteria. Consequently, the bacteria with the ability to produce urease enzyme steadily in the presence of high concentrations of ammonium would be the right choice for biocementation. *Sporosarcina pasteurii* and *Bacillus sphaericus* meet this condition.

2.3.2 Bacteria cell concentration

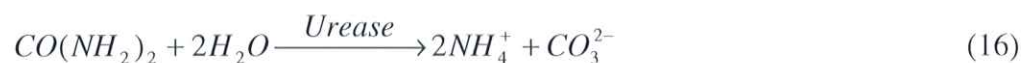
The rate of urea hydrolysis (ureolysis) is directly proportional to bacteria cell concentration. High bacteria cell concentration would provide enormous amounts of urease enzyme for ureolysis. Nemati and Voordouw (2003) reported that an increase in urease concentration enhances the rate of MCP. However, at low urease concentration, an increase in temperature enhances MCP. In addition, at high concentrations of urease enzyme, temperature increase has insignificant role in enhancing MCP.

2.3.3 Temperature

Urease-catalyzed ureolysis like any other enzymatic reaction is temperature dependent. However, the optimum temperature ranges from 20°C to 37°C depending on environmental conditions and concentrations of other reactants in the system. Ferris et al. (2003) reported that increasing the temperature from 15°C to 20°C increased the rate of ureolysis, k_{urea} 5 times (from 0.18 to 0.91 d⁻¹) and 10 times greater than k_{urea} at 10°C (0.09d⁻¹). Nemati and Voordouw (2003), and Mitchell and Ferris (2005), observed a similar trend. It can therefore be emphasized that increasing temperature within the optimum range enhances the rate of ureolysis.

2.3.4 Urea concentration

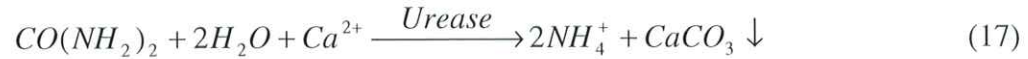
Ureolytic bacteria use urea as a food source. An increase in urea concentration would definitely increase urease activity and rate of urea hydrolysis. Moreover, urea hydrolysis generate CO_3^{2-} at a 1:1 molar ratio (equation 16), and any increase in urea concentration would increase the CO_3^{2-} concentration with a subsequent increase in MCP.



However, an increase in urea concentration should only be done to the limits of economic advantage. Nemati and Voordouw (2003) established that increasing urea concentration beyond 36g/L had no effect in enhancing the rate of MCP.

2.3.5 Ca^{2+} ions concentration

A rich Ca^{2+} source is required for MCP. Stoichiometrically, the carbonate precipitate and the Ca^{2+} source at a 1:1 molar ratio (equation 17). An increase in Ca^{2+} concentration would increase MCP because it enhances urease activity (Hammes et al. 2003, Nemati and Voordouw, 2003). In addition, since Ca^{2+} is not likely utilized by microbial metabolic processes, it would accumulate outside the cell where it would be readily available for MCP (Silver et al. 1975). However, Nemati and Voordouw (2003) reported that increasing Ca^{2+} concentration beyond 90g/L does not improve MCP.



2.3.6 Ionic Strength

Ionic strength is the concentration of free ions in solution. It is closely related to the concentration of electrolytes. It is higher if full ionization took place and/or when some ions have more charge, and lower if incomplete ionization takes place or when the ions are monovalent. It can be expressed as:

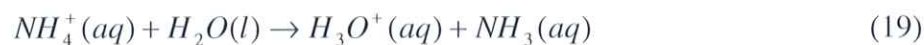
$$I = \frac{1}{2} \sum_{i=1}^n C_i Z_i^2 \quad (18)$$

(Hunter, 1981), where C and Z are molar concentration and ionic charge respectively. Ionic charge influences enzymatic reactions like temperature and concentration. In transport in porous media, the total interaction energy needed by microbial particles to adhere and attach themselves to solid surfaces as explained by the classical Derjaguin-Landau-Verwey-Overbeek (DLVO) theory, is composed of the repulsive electrostatic forces and the attractive Van Der Waals forces. High ionic strength increases electrical double layer (EDL) compression by decreasing EDL repulsive forces leaving attractive Van Der Waals' forces to dominate, and in the process promotes bacterial adhesion and attachment to the substratum (Faibish et al. 1998, Foppen et al. 2006). Increase in ionic strength from 0.1 to 1.0 may increase the equilibrium constant for ammonia speciation from 9.3 to 9.4 (Martell and Smith, 1974).

2.3.7 pH of the medium

A pH increase is an indication of urea hydrolysis, and is an important property of alkalophiles (optimum growth at pH 9.25 and no growth below pH 6.5). pH also determines which ammonia species exist and at what concentration. The pH at which NH_3 and NH_4^+ exist in a 50:50 ratio is about 9.25 ($\text{pK}_a \sim 9.25$). This relationship may be derived from the chemistry of buffer solutions involving ammonium ion as follows:

Ammonium ionizes in water as



The equilibrium constant for ammonium ion, K_a may be expressed as

$$K_a = \frac{[\text{H}_3\text{O}^+][\text{NH}_3]}{[\text{NH}_4^+]} \quad (20)$$

Then,
$$\frac{[\text{H}_3\text{O}^+]}{K_a} = \frac{[\text{NH}_4^+]}{[\text{NH}_3]} \quad (21)$$

And
$$10^{-\text{pH}} \times 10^{\text{pK}_a} = \frac{[\text{NH}_4^+]}{[\text{NH}_3]} \quad (22)$$

The equilibrium constant for the ammonium ion, K_a is 5.6×10^{-10} , and pKa is ~ 9.25 .

$$\text{Finally, } \frac{[NH_4^+]}{[NH_3]} = 10^{9.25-pH} \quad (23)$$

When pH values are known, the ammonia species can be computed by equation 23 and then may be plotted as in Fig. 3.

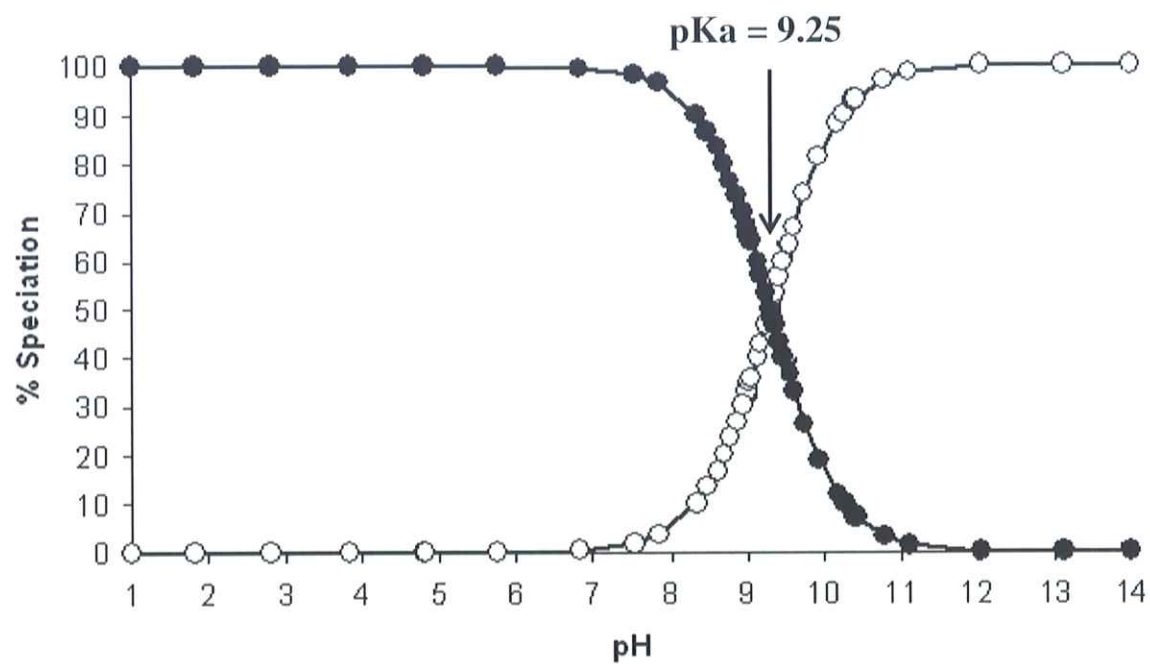


Fig. 3: Variation of chemical speciation of NH_3 (○) and NH_4^+ (●) with pH. pKa of ammonia (9.25) is the pH where the ammonia species exist in a 50:50 ratio.

2.3.8 Biofilm

A biofilm may be defined as a gelatinous extracellular polymeric substance (EPS) of biological origin containing a consortium of microbial cells such as bacteria, fungi, and algae attached to biotic or abiotic surface (Decho 2000a, Ballance et al., 2001, Singh et al., 2006). Biofilm formation occurs in response to an environmental stress (defense), quest for nutrients (colonization), and utilization of cooperative benefits (community) (Jefferson et al., 2004, Luttge et al., 2005, Bennett et al., 2001). Several authors have reported that in order to decide when to form a biofilm, bacteria continually assess their population densities by using an interbacterial communication mechanism called quorum sensing (QS) (Achal et al. 2009, Decho, 2010, Blackwell, 2005, Costerton et al., 2005, Hastings and Greenberg, 1999). When a sufficient number of bacteria (a “quorum”) are present (Fig.4), they alter gene expression to carry out processes that necessitate the cooperation of a large number of cells. Quorum sensing therefore, is a cell-to-cell signaling mechanism that refers to the ability of bacteria to communicate and coordinate behavior through signaling molecules (Singh et al., 2006, Reading and Sperandio, 2006) in aqueous and solid surfaces. It occurs in both gram-positive and gram-negative bacteria (Reading and Sperandio, 2006, Perbal, 2003, Costerton et al., 2005). Current research suggests that QS differs from one bacterial species to another to enable each one of them to preferentially colonize its substrate efficiently (Stanley and Lazazzera, 2004). In addition, the structure of a mature biofilm in all bacterial species depends on nutrient availability and QS (Stanley and Lazazzera, 2004, Bollinger et al., 2001). Effective bioremediation therefore depends on the ability of bacteria to colonize a surface and form

biofilm. The biofilm EPS provides protection to the bacteria, enhances bacterial cell adhesion to a surface, traps and concentrates calcium ions and captures the produced CaCO_3 (Achal et al. 2009, Decho, 2010, Hammes et al. 2003).

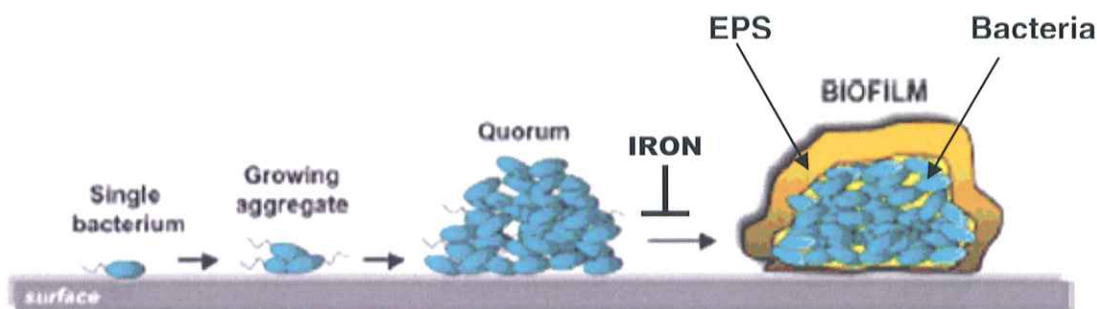


Fig. 4: Schematic of the Bacterial-Biofilm Formation Process and its Inhibition by High Concentrations of Iron. The Biofilm is depicted as a cut-away image. (Blackwell, 2005)

2.3.9 Mineral composition of the substratum

Bacteria attach themselves to mineral surfaces to extract and utilize phosphates and metal oxides in varied amounts (Rogers et al. 1998, Luttge et al., 2005, Rogers and Bennett, 2004). Iron is needed by bacteria for growth (Blackwell, 2005, Appenzeller et al., 2005, Banin et al., 2005, Berlutti et al., 2005, Rogers and Bennett, 2004, Lunsdorf et al., 1997, Banfield et al., 1999). However, excess iron blocks biofilm formation and disrupts preexisting biofilm (Blackwell, 2005, Banin et al., 2005, Weinberg, 2004). Certain metals and metal oxides inhibit biofilm formation. For example, aluminum forms complexes with organic materials such as humic acids and EPS (Ballance et al., 2001). When present as Al^{3+} cation, aluminum forms stable Al^{3+} -Adenosine Triphosphate (ATP)

complexes. Aluminum also forms irreversible complexes with polyphosphates of nucleic acids, thus interfering with the accuracy of DNA repair processes in microorganisms (Lunsdorf et al., 1997), and blocks uptake of iron in *Bacillus megaterium* mutants. Aluminum-bearing silicates containing greater than 1.2% Al_2O_3 is less colonized by most microorganisms (Rogers, 2004). Consequently, aluminum is toxic to most microorganisms (Roberts, 2004, Lunsdorf et al. 1997, Ballance et al. 2001). However, toxicity of metallic ions is bacteria specific (Lehtola et al., 2004). Generally, microorganisms will preferentially colonize those mineral surfaces that offer beneficial nutrients and avoid those that contain potentially toxic elements (Roberts, 2004).

3.0 Objectives of the research

This chapter outlines the primary objectives of this research, and the systematic approach to finding solutions to the problems.

The primary objectives of this research are to:

- (1) Determine the optimum conditions for MCP using *Sporosarcina pasteurii* strain ATCC 11859.
- (2) Use the optimum conditions to deposit a biosealant (calcite) on a PCB-contaminated concrete surface
- (3) Evaluate the performance and durability of the biosealant
- (4) Investigate the possibility of solid-phase capture (by co-precipitation) of the divalent metals in boiler chemical cleaning wastewater (BCCW).

3.1 Systematic Approach

This section describes the equipment and materials needed for this research in order to meet the outlined objectives.

3.1.1 Concrete making

A 2.4 in. diameter by 4 in. high concrete cylinder mold was used for this research. This specimen size was chosen to conform to the allowable size of the permeability testing equipment. The mix design for concrete materials was selected from We Energies Coal Combustion Handbook, 2nd edition page 56, Table 4-2 (Ramme and Tharaniyil, 2004). It comprised of 341 lbs cement, 100 lbs fly ash, 273 lbs water, 1610 lbs sand and 1810 lbs 3/8 in. coarse aggregates. No air entrainment was done. Concrete mixing, casting and curing were performed in accordance with ASTM C33 and C192.

3.1.2 Determination of the optimum conditions for MCP

This involved acquiring the bacteria (*S. pasteurii* strain ATCC 11859, American Type Culture Collection, (ATCC), Manassas, VA), making broth medium (brain heart infusion, Fisher Scientific, Pittsburgh, PA) for growing the bacteria, disinfecting the broth in an autoclave (Sterilmatic Autoclave, Market Forge Co., Everett, MA), growing the stock culture at 30°C for 72 hours with agitation, plating the bacteria in an agar plate to confirm their viability and storage, making and autoclaving the culture medium, varying and determining the bacteria cell concentration by dilution and spectrophotometer (Spectronic Genesys 5, Thermo Electron Corporation, Madison, WI) respectively.

A pH meter (AB 15 accumet® Basic, Fisher Scientific, Pittsburgh, PA) and ammonia electrode (Cole Parmer, Vernon Hills, IL) was used to measure pH and

dissolved ammonium. They were calibrated periodically as recommended by the manufacturers.

Factorial experiments were performed at room temperature (25°C), and in triplicate. Bacterial cell concentration, Ca^{2+} concentration, and urea concentration were varied. Ionic strength would remain constant at each experimental combination.

From the factorial experiments,

- Rate of urea hydrolysis was determined
- Urea replenishment time was determined
- Optimum condition for MCP was selected (shortest time to equilibrium pH with high CaCO_3 precipitation)
- X-ray diffraction analysis (XRD) was used to identify the minerals and quantify the deposited CaCO_3
- Scanning Electron Microscopy (SEM) and Energy dispersive X-ray (EDX) analyses were used to observe the morphology of the particles and identify the chemical elements in the precipitate.

3.1.3 Biosealant deposition on a PCB-contaminated concrete surface

This involves designing the equipment for the experiment, acquiring PCB samples (Oil-based PCB and Hexane-based PCB), and growing the bacteria on the concrete cylinder surface to deposit the biosealant. The following tests were performed on the biosealed concrete surface:

- Constant head permeability (ASTM D5084 Method F) test on the control and specimen samples was performed by Giles Engineering Associates (Waukesha, WI). This test is designed to check the performance of the biosealant. 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).
- Carbonation test (using phenolphthalein indicator) to determine the durability of the concrete surface was performed. 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 to 9 resulting into dusty weak surfaces. A strong concrete surface treatment therefore should improve its resistance to carbonation (Basheer et al. 1997).
- XRD was used to identify the minerals and quantify the CaCO_3 deposited, SEM and EDX were used to observe particle morphology and identify the elements in the biosealant respectively.

3.2 Coprecipitation factorial experiments

Oil-based PCB, hexane-based PCB and BCCW were used for this exercise. Bacterial cell concentration was varied. pH was measured and compared with the control.

A 1:1 pH variation with the control would indicate that these chemicals do not suppress ureolysis. Coprecipitation was checked by XRD and chemical analysis.

4.0 Determination of optimum conditions for MCP

4.1 Introduction

The factors that affect MCP as outlined in chapter 2 were carefully considered when designing the factorial experiments. This is because too much or too little of any of the requirements directly affect the outcome of these experiments. Qualitative analysis therefore, disqualifies the notion that more is good, and instead embraces optimized quantities that take into consideration economic advantage while at the same time providing quality results.

The main objective of this research is to determine the optimum conditions for urease catalyzed MCP. The urease enzyme will be supplied by the soil bacteria *Sporosarcina pasteurii* strain ATCC 11859, and these conditions will be determined by the factorial experiments. The factorial experiments were designed based on the important factors that affect MCP as previously outlined.

4.2 Materials and Methods

4.2.1 Stock culture

Sporosarcina pasteurii strain ATCC 11859, (American Type Culture Collection (ATCC), Manassas, VA) was grown at 30°C for 72 hours with agitation in brain heart

infusion broth. After growth, cells were plated in an agar plate to confirm their viability and storage.

4.2.2 Culture Medium

The culture medium consisted of 3 g of brain heart infusion broth, 10 g of ammonium chloride, and 2.1 g of sodium bicarbonate (Fisher Scientific, Pittsburgh, PA) per liter of distilled water. A varied amount of urea was added to the mixture and pH adjusted to 6.5 using 1N HCL (Fisher Scientific, Pittsburgh, PA) before addition of a varied amount of CaCl_2 (Fisher Scientific, Pittsburgh, PA) to avoid premature CaCO_3 precipitation. The mixture was then autoclaved at 121°C for 20 minutes.

4.2.3 Calibration of the pH electrode

The pH meter (accumet® AB 15, Fisher Scientific, Pittsburgh, PA) was calibrated with pH 4, 7, and 10 buffer solutions in accordance with the manufacturer's requirements. When the calibration slope of the pH meter is in the range 90-102%, 'good electrode' will be displayed, and the electrode is good for use. It is good to check this slope periodically to ensure accurate results.

4.2.4 Calibration of the ammonia electrode

A Cole-Parmer gas-sensing electrode (Cole Parmer, Vernon, IL) was used. The electrode was calibrated weekly with a mixture of NH_3 standard solution and 10M NaOH ionic strength adjuster (ISA) against the manufacturer's calibration graph supplied. It is recommended by the manufacturer that the change in potential observed when concentration changes by a factor of 10 should be 56 ± 3 mV. Using this slope, a calibration graph was drawn (fig. 5).

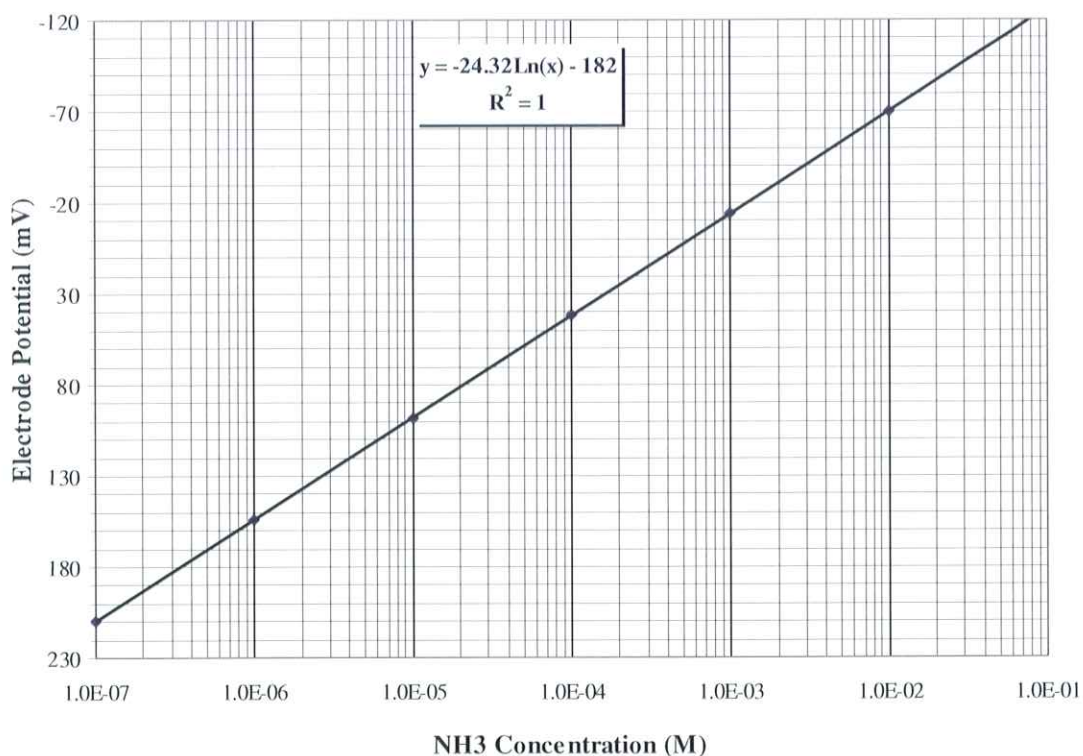


Fig. 5: Typical Cole-Parmer Gas-sensing ammonia electrode calibration graph

From the calibration graph (Fig. 5) slope equation, a reading in millivolts was converted to ammonia concentration in moles per liter as:

$$E_p (mV) = -24.329(\ln[NH_3]) - 182 \quad (24)$$

$$\text{Then } [NH_3] = e^{\frac{(-182-E_p)}{24.32}} \quad (25)$$

Dissolved ammonium concentration, $[NH_4^+]$ can be computed from equation 23.

4.2.5 Factorial experimental Design

Factorial experiments were designed based on the important factors that affect MCP (Table 1). Bacterial cell concentration was varied from 10^6 to 10^8 cells/mL by dilution using ultrapure water (UPW) (Milli-Q® Gradient, Molsheim, France) and quantified by measuring the absorbance (optical density) of the suspension using Spectronic Genesys 5 Spectrophotometer (Thermo Electron Corporation, Madison, WI) at 600nm wavelength. The concentration of cells suspended in the stock culture was estimated by the expression

$$Y = 8.59 \times 10^7 x Z^{1.3627} \quad (26)$$

(Ramachandran et al. 2001) where Z is reading at OD₆₀₀, and Y is the concentration of cells per mL.

For each test, 20 mL of the culture medium was mixed with 10 mL of the stock culture in a beaker, and the mixture stirred slowly using a magnetic stirrer. A pH meter (accumet® AB 15, Fisher Scientific, Pittsburgh, PA) and ammonia electrode (Cole Parmer, Vernon Hills, IL) were then dipped into the solution in succession to measure pH and ammonia concentration of the mixture. Measurements were done after 15 minutes, 30 minutes, 1 hour, 3 hours, 6 hours, 12 hours, 24 hours, and every 24 hours for 7 days. All experiments were done in triplicate.

4.2.6 Estimation of urea replenishment time

In order to determine urea replenishment time, the rate of urea hydrolysis was first determined. The determination of k_{urea} is based on the assumption that ureolytic reactions follow first order, and may be expressed as:

$$\frac{d[urea]}{dt} = -k_{urea}[urea] \quad (27)$$

Integrating
$$\int \frac{d[urea]}{[urea]} = \int -k_{urea} .dt \quad (28)$$

Gives
$$\ln[urea] = -k_{urea} .t + C \quad (29)$$

Applying boundary conditions, at $t = 0$, $C = \ln[urea]_0$

Therefore,
$$\ln[urea]_t = -k_{urea} .t + \ln[urea]_0 \quad (30)$$

Manipulating equation 30 gives

$$\ln[urea]_t - \ln[urea]_0 = -k_{urea} \cdot t \quad (31)$$

Then

$$\ln \frac{[urea]_t}{[urea]_0} = -k_{urea} \cdot t \quad (32)$$

And

$$\frac{[urea]_t}{[urea]_0} = e^{-k_{urea} \cdot t} \quad (33)$$

Finally

$$[urea]_t = [urea]_0 e^{-k_{urea} \cdot t} \quad (34)$$

where $[urea]_t$ is the concentration of urea remaining at time t , and $[urea]_0$ is the initial urea concentration at $t = 0$. From urea hydrolysis reaction stoichiometry (equation 16), the amount of urea hydrolyzed at a time t is equivalent to one-half of the corresponding dissolved ammonium $[NH_4^+]_t$. Therefore, the concentration of urea remaining at time t may be expressed by the mass balance expression:

$$[urea]_t = [urea]_0 - \frac{1}{2}[NH_4^+]_t \quad (35)$$

Combining equations 34 and 35 gives

$$[NH_4^+]_t = 2[urea]_0(1 - e^{-k_{urea} \cdot t}) \quad (36)$$

Equation 36 can then be used to compute the rate of urea hydrolysis k_{urea} for each and every factorial experiment since $[NH_4^+]_t$ and the initial urea concentrations $[urea]_0$ are known.

4.3 Results and discussion

4.3.1 pH changes and NH_4^+ generated

Fig. 6 show pH changes and ammonium generated during urea hydrolysis. The time taken for the experiments to reach equilibrium pH decreased with increase in bacteria cell concentration (Figures 6A, 6C and 6E). There is a significant difference between the control and the inoculated experiment (Figures 6A and 6B), indicating the great influence urease enzyme has on urea hydrolysis. Ammonium production was generally constant at about 0.09M (Figures 6B, 6D and 6F).

4.3.2 Rate of urea hydrolysis, k_{urea}

Table 1 shows results from the factorial experiments. Bacterial cell concentration and Ca^{2+} concentration were varied by an order of magnitude from 2.5 mM/L to 250 mM/L and 10^6 cells/mL to 10^8 cells/mL for Ca^{2+} and bacteria respectively. However, urea concentration was varied by a factor of 2, from 333 mM/L to 666 mM/L. The k_{urea} values increased consistently with increase in bacteria cell concentration irrespective of initial urea concentration. These results show that k_{urea} is controlled much by the bacteria cell concentration. It can also be noticed that k_{urea} result from combination C1U1 (0.770

d^{-1}) with the bacteria cell concentration of 5.5×10^6 cells/mL was lower than $0.91 d^{-1}$ and $0.99 d^{-1}$ (at 5.9×10^6 cells/mL) obtained by Ferris et al (2003) and Mitchell and Ferris (2005) (using strontium contaminated artificial groundwater (AGW)) respectively, but slightly higher than $0.73 d^{-1}$ obtained by Mitchell and Ferris (2005) using AGW without strontium contamination (Table 2).

The mean k_{urea} values obtained by bacteria cell concentrations 10^6 (B1), 10^7 (B2) and 10^8 (B3) cells/mL were statistically compared at 95% confidence limit. The results indicate that k_{urea} values obtained by B1 and B2 were not statistically different (p-value = 0.153). However, both B1 and B2 k_{urea} values were statistically different from those obtained by B3 (p-value = 0.001 and 0.017 respectively). Although individual k_{urea} values obtained by B3 were not statistically different (stdev = 0.07) as compared with B1 (stdev = 0.11) and B2 (stdev = 0.10) for all the factorial experiments, the amount of the carbonate precipitated and the speed at which the precipitation took place were significantly different, and subsequently formed the basis for acceptance or rejection of a combination. After carefully analyzing k_{urea} values and the amount of $CaCO_3$ precipitated from Table 1 and Fig. 7, combination C2U2 with the highest bacteria concentration (B3 = 2.3×10^8 cells/mL) was chosen as the optimum condition for MCP.

Table 1. Mean k_{urea} , mean mass of CaCO_3 precipitated and mean mass of CO_2 sequestered during the factorial experiments using *Sporosarcina pasteurii* strain ATCC 11859 to determine the optimum conditions for MCP. C1: 25 mM/L Ca^{2+} , C2: 250 mM/L Ca^{2+} , and C3: 2.5 mM/L Ca^{2+} , U1: 333 mM/L Urea, U2: 666 mM/L Urea. Mean mass of CaCO_3 is \pm standard deviation.

Combination	Bacteria cell concentration (cells/mL)	Mean k_{urea} /day	Mean mass of CaCO_3 precipitated (mg)	Mean mass of CO_2 consumed (mg)
C1U1	B1 5.5×10^6	0.770	37.3 ± 0.14	16.4
	B2 7.4×10^7	0.838	39.2 ± 22.2	17.2
	B3 3.1×10^8	0.912	49.9 ± 7.4	22.0
C2U1	B1 8.9×10^6	0.780	30.2 ± 0.16	13.3
	B2 7.2×10^7	0.852	53.5 ± 16.8	23.5
	B3 2.9×10^8	0.924	66.7 ± 6.26	29.3
C3U1	B1 8.4×10^6	0.768	-	-
	B2 7.1×10^7	0.840	-	-
	B3 2.7×10^8	0.931	-	-
C1U2	B1 8.2×10^6	0.768	43.6 ± 9.35	19.2
	B2 8.1×10^7	0.804	53.2 ± 16.6	23.4
	B3 3.1×10^8	0.922	56.8 ± 28.9	25.0
C2U2	B1 8.5×10^6	0.778	44.6 ± 1.98	19.6
	B2 7.5×10^7	0.835	66.4 ± 19.2	29.2
	B3 2.3×10^8	0.924	91.1 ± 9.1	40.1
C3U2	B1 8.7×10^6	0.778	-	-
	B2 8.2×10^7	0.840	-	-
	B3 2.7×10^8	0.902	-	-

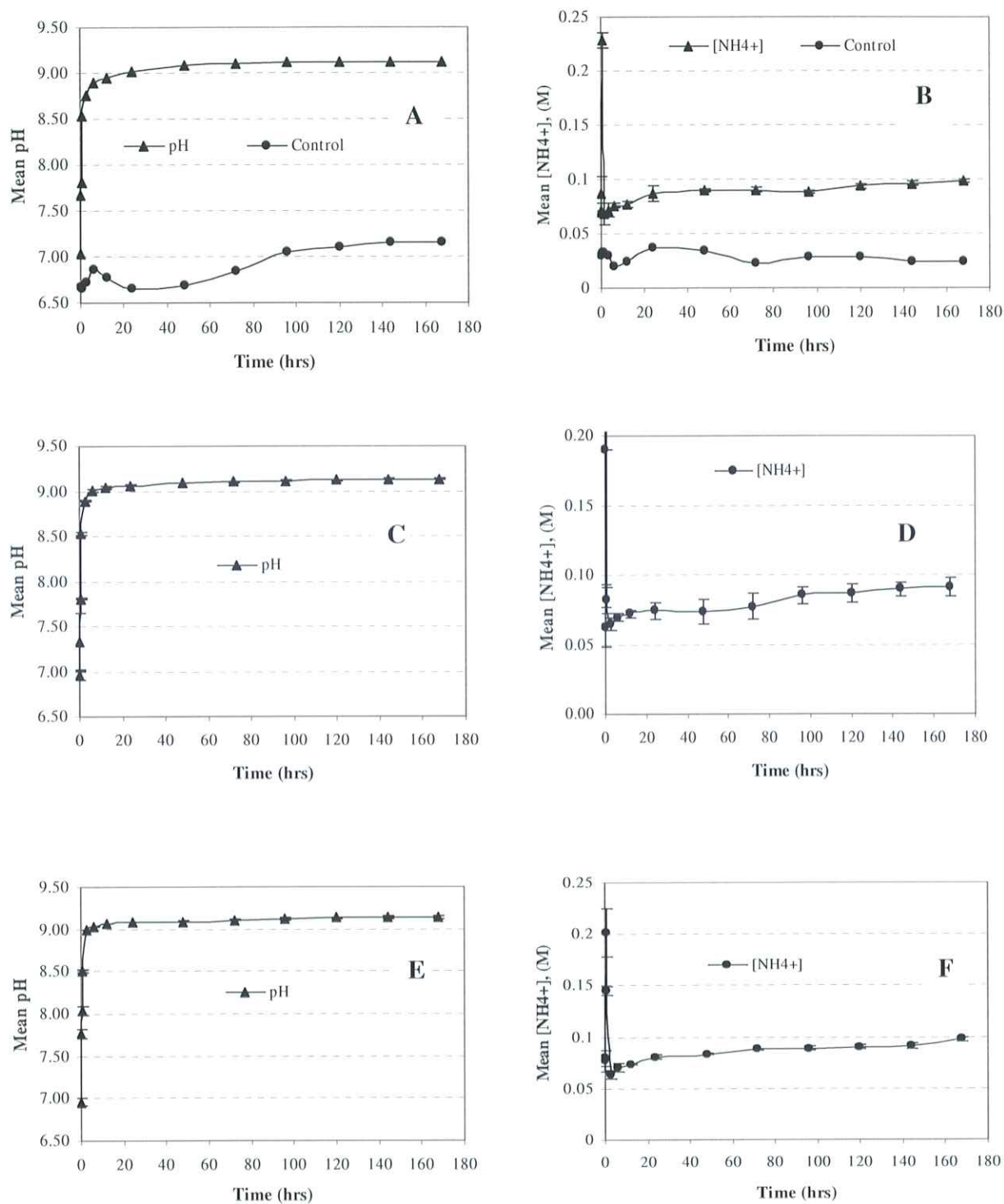


Fig. 6: Graphical representation of changes in mean pH and NH_4^+ generated during urea hydrolysis. A and B: at 8.5×10^6 cells/mL, C and D: at 7.5×10^7 cells/mL, E and F: at 2.3×10^8 cells/mL. A takes longer time to reach pH 9 than C and E.

Table 2. Summary of rate of ureolysis (k_{urea}) values obtained with the AGW* (5.5×10^6 cells/mL), AGW and Strontium contaminated AGW (5.9×10^6 cells/mL).

Temperature °C	Mixture	Ferris et al., 2003 k_{urea} (day ⁻¹)	Mitchell and Ferris, 2005 k_{urea} (day ⁻¹)	This Research k_{urea} (day ⁻¹)
10	AGW	0.09	0.069	
10	AGW + Sr		0.073	
15	AGW	0.18	0.064	
15	AGW + Sr		0.068	
20	AGW	0.91	0.730	
20	AGW + Sr		0.990	
25	AGW*			0.770

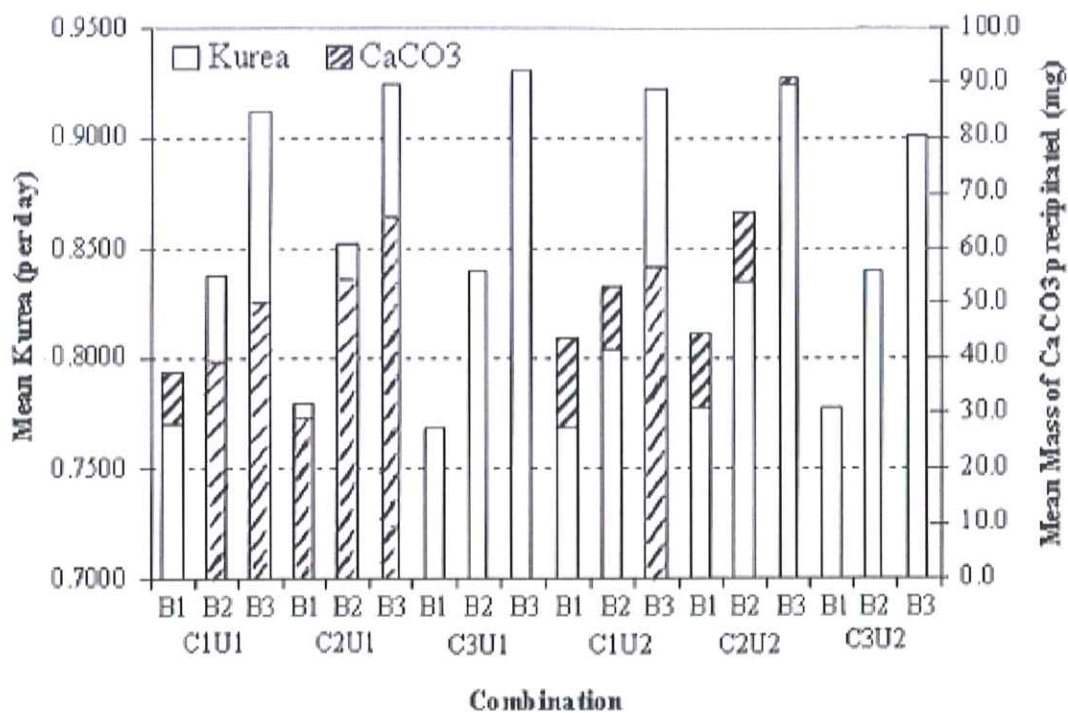


Fig. 7: Mean mass of CaCO₃ precipitated at various combinations and bacteria cell concentrations, and corresponding mean k_{urea} values obtained. Bacteria cell concentration B1, B2, and B3 for each combination are listed in Table 1.

4.3.3 Estimation of urea replenishment time

Fig. 8 shows the variation of bacteria cell concentration with the rate of urea hydrolysis. This result indicates a linear relationship ($R^2 = 0.9048$) between the bacteria cell concentration and rate of urea hydrolysis. Using this relationship, the rate of urea hydrolysis can be estimated when bacteria cell concentration is known and under the same conditions. It is also worth noting that the mean k_{urea} values obtained by 333 mM/L urea and 666 mM/L urea are in approximately 1:1 ratio (Fig. 9). Consequently, a lower amount of urea would be needed for ureolysis so long as it is enough to sustain the bacteria. However, it would be economical to replenish both bacteria and urea after approximately 80 hours (Fig. 10).

4.3.4 Estimation of the amount of CaCO_3 precipitated and CO_2 sequestered

At the end of the experiments (7 days), the carbonate precipitate was vacuum-filtered through a $0.20 \mu\text{m}$ filter paper of known mass, and then allowed to dry in air inside a petri dish at room temperature for 4 days before being weighed. The mass of carbonate deposited was then determined by difference. The results presented in Table 1 and Fig. 7 indicate that the CaCO_3 precipitated and CO_2 sequestered increase monotonically with bacteria cell concentration. From ureolysis and CaCO_3 formation stoichiometry, hydrolyzing one mole of urea sequesters one mole of CO_2 . Consequently, the amount of CO_2 sequestered is directly proportional to the amount of CaCO_3 precipitated by MCP (Table 1). In addition, at the same bacteria cell concentration, increasing urea and Ca^{2+} concentrations increases the amount of carbonate

precipitated. At 25 mM/L Ca^{2+} concentration, increasing bacteria cell concentration from 10^6 to 10^8 cells/mL increases the CaCO_3 precipitated and CO_2 sequestered by over 30%. However, when Ca^{2+} concentration is increased 10-fold to 250 mM/L Ca^{2+} , the CaCO_3 precipitated and CO_2 sequestered increased by more than 100% irrespective of urea concentration. This result indicates that the amount of CaCO_3 precipitated and CO_2 sequestered depend more on the Ca^{2+} concentration than the amount of urea.

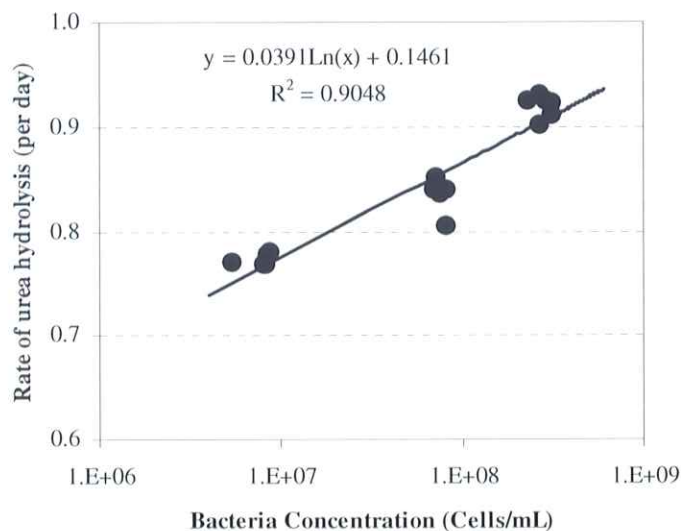


Fig. 8. Variation of bacteria cell concentration (cells/mL) with mean rate of urea hydrolysis (k_{urea}).

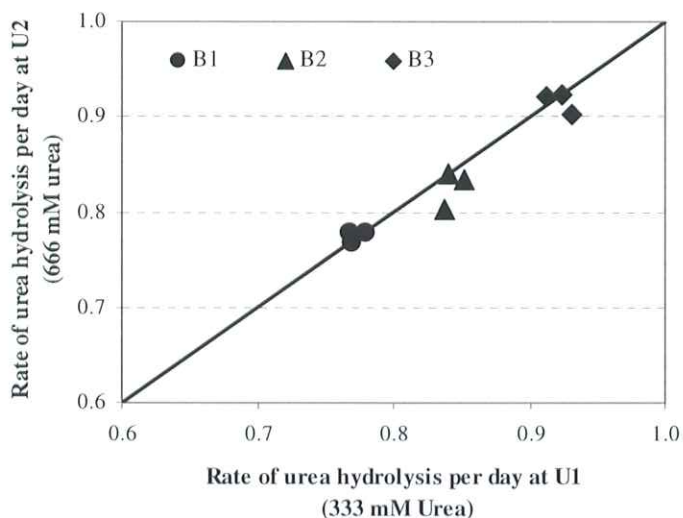


Fig. 9. A comparison between mean rate of urea hydrolysis (k_{urea}) obtained by U1 (333 mM/L Urea) and U2 (666 mM/L Urea) at bacteria cell concentrations B1 (10^6 cells/mL), B2 (10^7 cells/mL) and B3 (10^8 cells/mL).

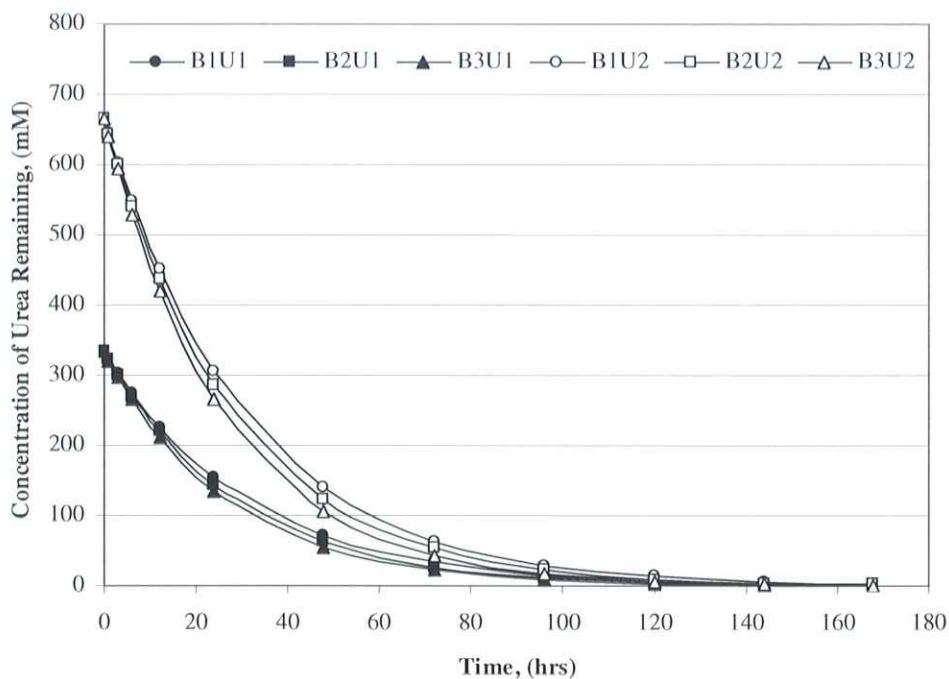


Fig. 10. Estimation of the concentration of urea remaining at any given time using *S. pasteurii* strain ATCC 11859. B1, B2 and B3 are bacteria cell concentrations at 5.5×10^6 , 7.4×10^7 and 3.1×10^8 cells/mL respectively for combination C1U1, and 8.5×10^6 , 7.5×10^7 , and 2.3×10^8 cells/mL respectively for combination C2U2. U1 and U2 are initial urea concentrations at 333 mM/L and 666 mM/L respectively.

4.3.5 XRD, SEM and EDX analysis

Fig. 11 shows X-ray Diffraction (XRD) (A), Scanning Electron Microscopy (SEM) (B), and Energy Dispersive X-Ray (EDX) (C) analysis of the precipitated calcium carbonate powder. The CaCO_3 precipitate is composed of calcite and vaterite crystals (Fig. 11A) but predominantly calcite with a rhombohedra crystalline structure (Fig. 11B). The EDX (Fig. 11C) peaks show that the elemental composition of the precipitate is mostly calcium, carbon and oxygen. This is further evidence that the precipitate formed is calcium carbonate.

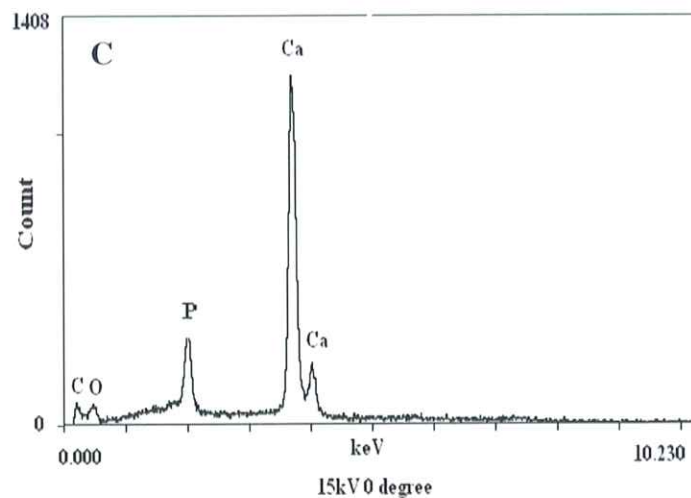
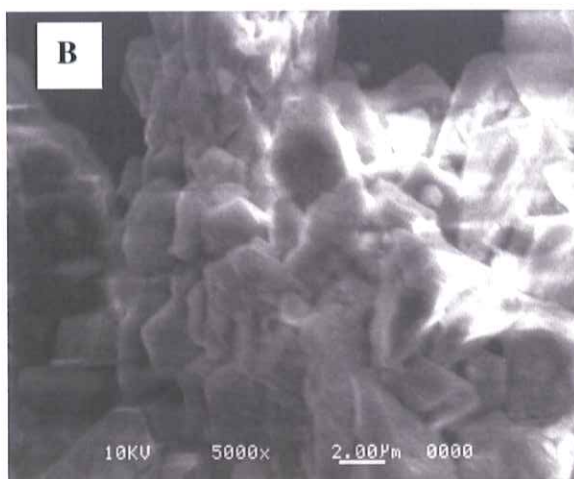
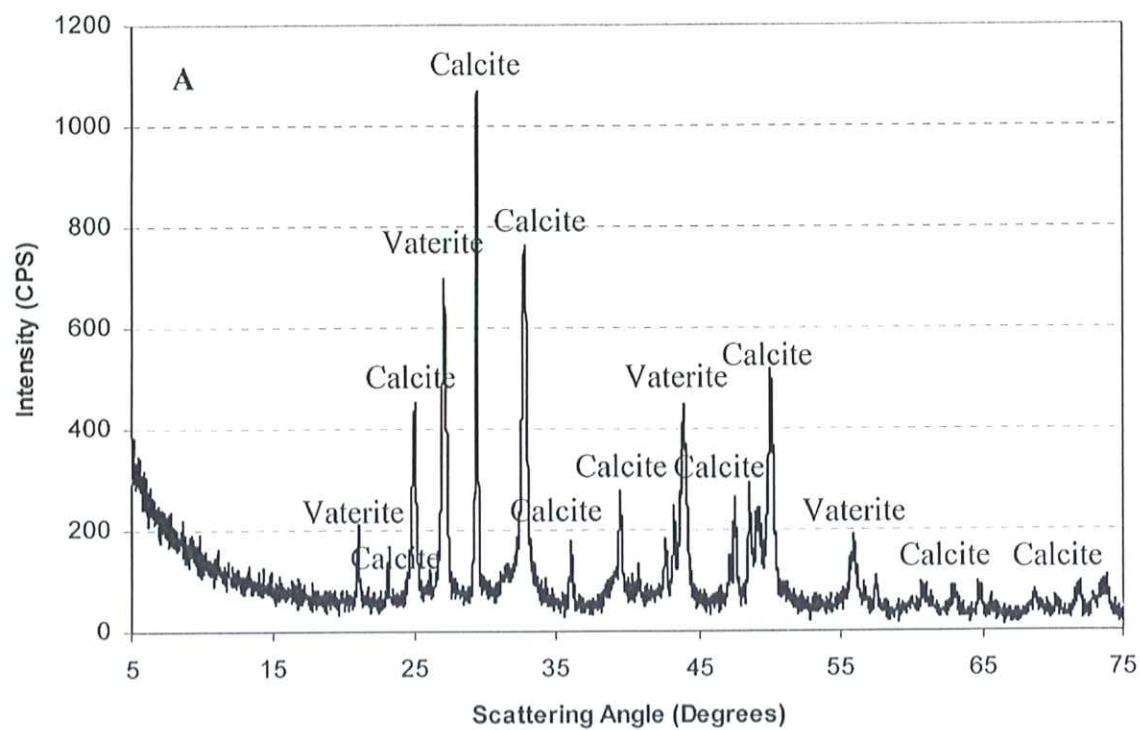


Fig. 11. The X-Ray Diffraction (XRD) at a continuous scanning rate of $2^\circ/\text{minute}$ (A), Scanning Electron Microscopy (SEM) (B) at 5000X magnification, and Energy Dispersive X-Ray (EDX) (C) analysis of the precipitated calcium carbonate powder.

4.4 Conclusions

The rate of urea hydrolysis, k_{urea} and the amount of CaCO_3 precipitated formed the basis of selecting the optimum conditions for MCP. Our results indicate that k_{urea} is dependant more on the bacteria cell concentration than initial urea concentration so long as there is enough urea to sustain the bacteria. The bacteria cell concentration, initial urea concentration and Ca^{2+} concentration all influence the amount of CaCO_3 precipitated and CO_2 sequestered. However, increasing the Ca^{2+} concentration 10-fold doubles the amount of CaCO_3 precipitated and CO_2 sequestered irrespective of initial urea concentration. Consequently, combination C2U2 at the highest bacteria cell concentration ($B3 = 2.3 \times 10^8$ cells/mL) gave the highest CaCO_3 deposited, and thus the optimum condition for MCP for this work. These results also indicate that more CaCO_3 would be precipitated with higher bacteria cell concentration, Ca^{2+} and urea so long as these quantities are within their economic advantage. X-Ray Diffraction, Scanning Electron Microscopy and Energy Dispersive X-Ray analysis confirmed that the precipitate formed was CaCO_3 , and composed of predominantly calcite crystals with little vaterite crystals.

5.0 Biocontainment Experiments

5.1 Introduction

As was mentioned in chapter 1, the use of abiotic methods to mitigate PCB spills on concrete surfaces is not cost effective because physical methods generate enormous amounts of solid waste and chemical methods use some chemicals which may have detrimental effects both on human beings and the environment. For example, epoxy resin adhesives are the main source of occupational asthma (Mayo Clinic, 2010). Moreover, when used as surface coatings, epoxy sealants need to be reapplied periodically because of their fast deterioration by environmental factors (degrades at 177°C). In addition, a lot of money is also spent on contract disposal because of safety risk, disposal costs, and haul distance to Toxic Substance Control Act (TSCA) waste handling facilities and landfills. In this section, the use of MCP as a biosealant is proposed. It is hypothesized that MCP would promote a wider spatial distribution of calcite precipitation on the concrete surface than the direct addition of base, and the biosealant would form a coherent, less permeable and a thermally stable (Calcite and CaCO_3 decompose at 825°C and 840°C respectively) durable surface.

5.2 Materials and Methods

5.2.1 Experimental Setup

Concrete specimens were made in accordance with ASTM C33 and C192. 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 up. The artificial cracks were first filled with sand. 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 12). PCB was sprayed on the surface and allowed to stand overnight. The culture medium was prepared at optimum concentration (C2U2: 250 mM/L Ca^{2+} and 666 mM/L urea), and pH adjusted before adding Ca^{2+} to prevent premature calcite precipitation. The mixture was then autoclaved at 121°C for 20 minutes, allowed to cool to room temperature (25°C) then poured into a burette. The stock culture was grown in brain heart infusion broth at 30°C with agitation for 72 hours to an OD_{600} of at least 1.90 (2.1×10^8 cells/mL). 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 (Fig. 12). The stock culture and the culture medium were replenished after 3 days (72 hours). The experiment was allowed to proceed for 4 more days. On the seventh day, sand was sprinkled on the biosealant (Fig. 13) to increase friction, and allowed to dry at room temperature.

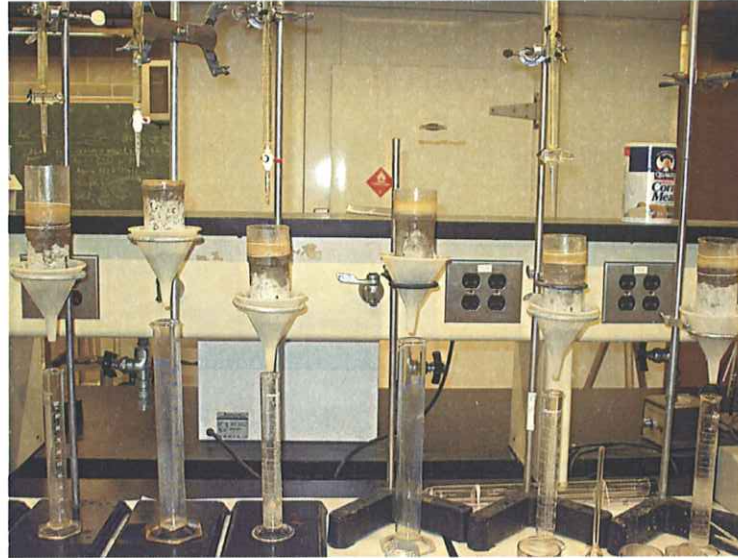


Fig. 12: Biosealant deposition experimental set up on the concrete cylinder surface

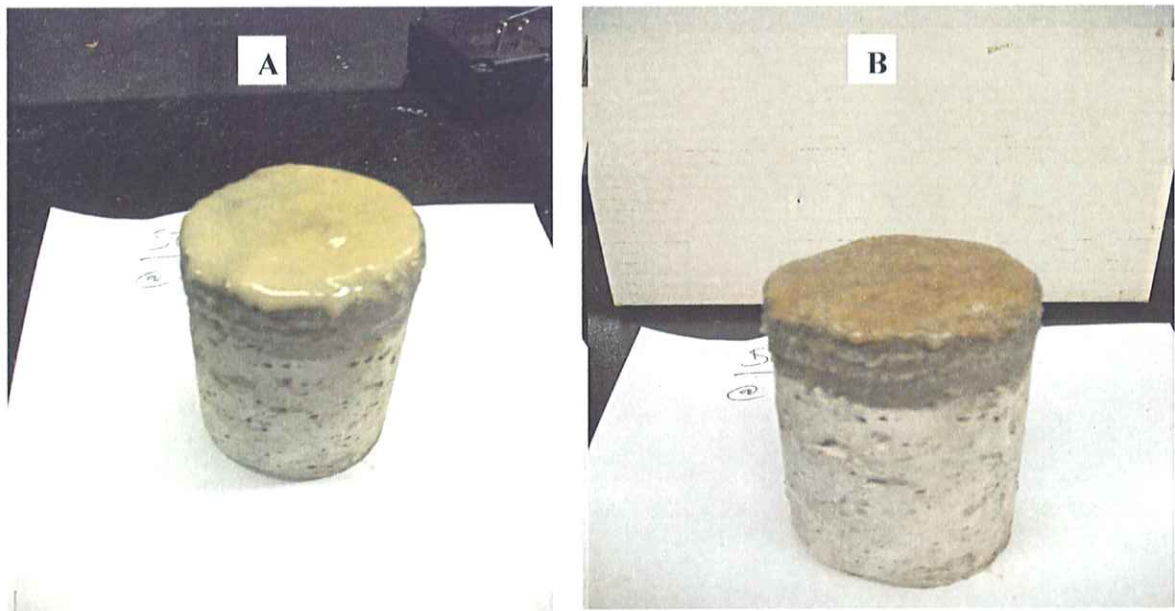


Fig. 13: CaCO_3 biosealant deposited on the concrete surface. A: Without sand and B: After sprinkling sand.

5.2.2 Permeability Test

As was outlined in chapter 3, the permeability test was proposed to evaluate the effectiveness of the biosealant surface at preventing PCB ingress into the concrete slab matrix. Constant head permeability (ASTM D5084 Method F) test on the control and the specimen samples were performed at 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 (using water as the permeating fluid) with a back pressure of 55 psi, mean hydraulic gradient of 13.8 cm, and maximum consolidation pressure of 5, 10, and 20 psi.

5.2.3 Carbonation Test

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 to 9 resulting in a dusty weak surfaces. A strong concrete surface treatment therefore should improve its resistance to carbonation (Basheer et al. 1997).

5.3 Results and Discussion

5.3.1 Permeability test results

The results of the permeability test are presented in Fig. 14. The permeability of the carbonate treated samples were 3.26×10^{-8} cm/s (without artificial cracks), 1.55×10^{-5} cm/s (with one artificial crack), and 7.35×10^{-4} cm/s (with two artificial cracks) whereas the control samples had permeabilities of 7.97×10^{-3} , 9.20×10^{-3} , and 7.33×10^{-3} cm/s, all without artificial cracks. These results indicate that the carbonate treatment reduced the permeability of the surface by between 1 to 5 orders of magnitude as compared to the untreated (control) surface. Consequently, both lateral and vertical movement of oil-based PCB would have insignificant impact on the surroundings.

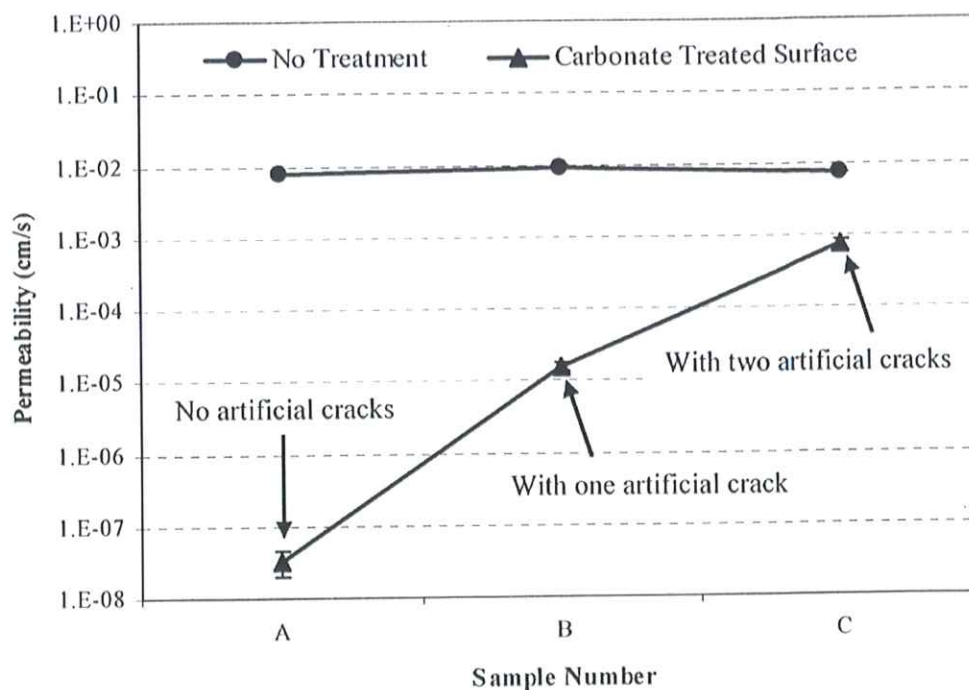


Fig. 14: Mean Constant head permeability (ASTM D5084 Method F) test results before and after the carbonate biosealant application.

5.3.2 Carbonation test results

Fig. 15 shows carbonation test results. Figs. 15A and 15B 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 the pink color when they were 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 turned 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 but an indication that the biosealant surface treatment is effective in encapsulating PCB spills indoors away from the harsh ambient environmental conditions.

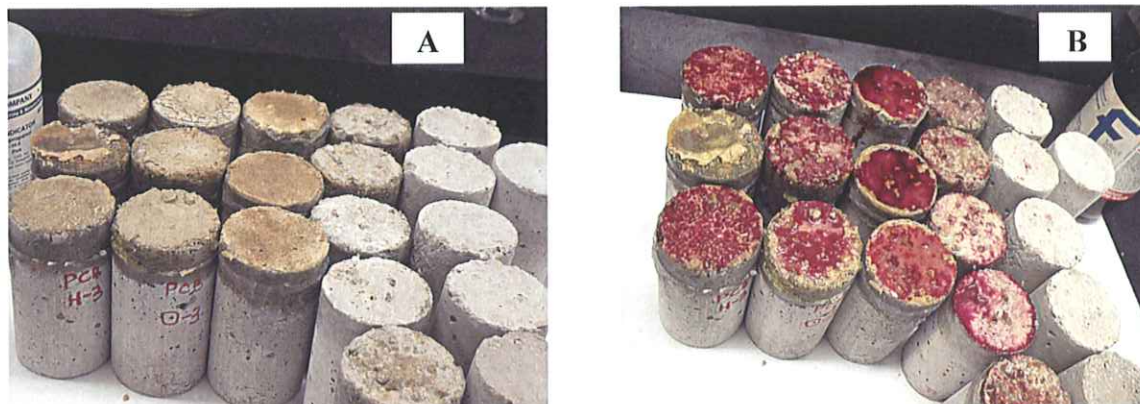


Fig. 15: Carbonation test on the PCB contaminated concrete cylindrical surfaces before and after calcium carbonate biosealant treatment. Columns are numbered from left to right. Columns 1, 2, 3, and 4 were biosealed whereas columns 5 and 6 were not. Columns 1, 2, and 3 were left indoors whereas columns 4, 5, and 6 were subjected to ambient environmental conditions outside for 3 months. A: Before phenolphthalein addition, and B: After phenolphthalein application.

5.4 Conclusions

The reduction of the coefficient of permeability by 1-5 orders of magnitude exhibited by the biosealed surfaces is an indication of the effective performance potential of MCP at confining PCB in situ. The result is also an indication that migration of PCBs from the concrete surface would be significantly reduced if water is the permeating fluid, and on the basis of the concrete mix design used in this experiment. The low permeability results obtained in this experiment is also a measure of durability of the concrete because loss of fines and subsequent increase in porosity in the concrete matrix would be suppressed, and the concrete slab would maintain its functional framework. Another advantage of the biosealant is that CaCO_3 and calcite have very high thermal stability, decomposing at 840°C and 825°C respectively as compared to epoxy resin adhesives which degrades at only 177°C . This indicates that the prevailing temperatures at these

equipment installations would not have effect on the performance of the biosealant. Together with the high resistance to carbonation exhibited by the biosealant surfaces, our experiments indicate that MCP is capable of forming a strong durable surface suitable for indoor applications.

6.0 Coprecipitation of PCBs and metals

6.1 Introduction

As was mentioned in chapter 2, the use of MCP in the solid phase capture of common groundwater inorganic contaminants by coprecipitation takes advantage of the equilibrium conditions with respect to calcite solubility that favors the recrystallization of mostly divalent ions although there is evidence that trivalent and tetravalent ions may also be recrystallized (Curti, 1999). The divalent metallic ions are sorbed onto the surface of calcite and immobilized into a monolithic material. These metallic ions are toxic to the environment especially when they get into contact with surface and/or ground water or when ingested by animals. Coprecipitation therefore, would not only make water safe but also remove some metallic ions from the environment. Calcite has been selected for coprecipitation experiments because it forms ubiquitously in near surface environments, and would play a key role in many other repository situations. It is therefore worth investigating what role microbial calcite precipitation may play in mitigating PCBs and boiler chemical cleaning waste water (BCCW).

6.1.1 Boiler Chemical Cleaning Wastewater (BCCW)

Boiler chemical cleaning wastewater (BCCW) is obtained when chelating solutions such as inhibited hydrochloric acid, ammonium bromate, ammoniated ethylenediamine tetraacetic acid (EDTA), ammoniated citric acid and hydroxyacetic-

fomic acid (HFA) are used to remove scales and deposits of iron oxides, metallic copper, and other impurities in boiler tube surfaces. This is essential to improve heat transfer efficiency of these tubes. BCCW therefore contains large amounts of dissolved and suspended metals of iron, copper, and chelating agents. Chromium, magnesium, nickel, zinc and organic compounds are also present (see the Chemical Hazard Evaluation System (CHES) sheet in Appendix B).

BCCW is considered a hazardous material because the concentration of most of its chemical constituents exceeds water quality disposal standards, and most wastewater treatment plants would not handle them. Wisconsin Department of Natural Resources (WDNR) and USEPA consider BCCW hazardous when chromium concentration is more than 5 mg/L. However, when chromium concentration is less than 5 mg/L, some coal-fired utilities without selective catalytic reduction (SCR) for nitrogen oxides (NO_x) control, evaporate it in the boiler. We Energies has applied for an exemption from the WDNR to exclude BCCW from hazardous material classification because the chromium in the BCCW is exclusively trivalent chromium, and the reducing atmosphere in the boiler tubes cannot allow the hexavalent chromium to form. These conditions qualify BCCW for exclusion in accordance with Wisconsin Administrative Code NR 661.04(2) (f) (1) (a) to (c). But even if this exclusion is granted, wastewater utilities may still not accept BCCW. Utilities therefore have a need for other cost-effective methods for its handling and disposal.

6.2 Materials and Methods

6.2.1 Effect of contaminants on urease enzyme

In order to investigate coprecipitation of inorganic constituents in BCCW by MCP, the effect of these constituents on the bacteria must first be investigated. To do this, 20 mL of the culture medium (at optimum conditions) was mixed with 10 mL of varied amounts of bacteria (7.5×10^6 , 5.2×10^7 , and 2.3×10^8 cells/mL). 10 mL of the contaminant was added to the mixture, and pH measured under agitation at room temperature. A bacterial control was also set up without addition of the contaminants. All experiments were done in triplicate, and run concurrently. A plot of pH values in bacterial treatments as a function of pH values in the bacterial control was made (Fig. 16). A 1:1 slope of the graph would indicate no toxic effect of the contaminants on the bacteria.

6.2.2 Chemical analysis

Chemical analysis of the CaCO_3 precipitate and the solution (Table 4) was performed by Pace Analytical Services, Inc. (Green Bay, WI) in accordance with the National Environmental Laboratory Accreditation Conference (NELAC) standards to determine if coprecipitation occurred. Energy Dispersive X-ray analysis (Fig. 17) was also performed to confirm the elemental constituents of the carbonate precipitate.

6.2.3 Leaching test

Leaching tests were performed in accordance with the ASTM D3987 (using distilled de-ionized water as the leaching solution) on the solid material to determine the mobility of the coprecipitated metals. A known weight of the solid material was shaken for 18 hours in de-ionized water followed by the separation of the aqueous phase for total elemental analysis. The results are shown in Table 5.

6.3 Results and discussion

Fig. 16 shows pH values in bacterial treatments as a function of pH values in bacterial control. The results show that the bacterial activity is less affected by hexane-based PCB than oil-based PCB (Fig. 16B & 16C). In addition, higher bacteria cell concentration does not improve the bacterial activity in both the PCB media (Fig. 16C). However, the BCCW does not negatively affect the bacteria (Fig. 16), and higher bacteria cell concentration exhibits higher pH values indicating greater bacterial activity.

Tables 3 and 4 show PCBs and BCCW coprecipitation results respectively. The oil-based PCB was stratified into three distinct layers of oil underlain by a water-like liquid and solids at the bottom, whereas the hexane-based PCB was homogeneously a water-like liquid. The oil layer retained 98%, 54% and 66% of PCBs using bacteria cell concentrations B1, B2, and B3 respectively. The PCBs concentration in the water-like liquid was slightly more than 2% for bacteria cell concentrations B1 and B2, and less than 1% for bacteria cell concentration B3. In the solid phase, 44% and 33% of PCBs was

retained using bacteria cell concentrations B2 and B3 respectively. No solids were recovered using bacteria cell concentration B1. This result shows that more PCBs were retained in the oil layer than in the solid layer indicating that MCP is not an effective method for coprecipitation of oil-based PCBs. Like in the oil-based PCB, the hexane-based PCB was stratified into two distinct layers of water-like liquid underlain by the solid layer. The solid layer retained 98.5%, 99.8% and 99.8% of PCBs using bacteria cell concentrations B1, B2, and B3 respectively. These results indicate that coprecipitation of hexane-based PCBs might have occurred using these bacteria cell concentrations. However, since hexane is very volatile (boiling point 68.7°C and vapor pressure 16.0 – 25.3 kPa), it might have easily evaporated leaving the PCBs trapped by the carbonate solids. More research needs to be done to confirm this coprecipitation hypothesis.

Table 3: Coprecipitation of PCBs by MCP using bacteria cell concentrations B1 (10^6 cells/mL), B2 (10^7 cells/mL) and B3 (10^8 cells/mL) at optimum conditions (250 mM/L Ca^{2+} and 666 mM/L urea). All concentrations are in $\mu\text{g/L}$.

PCB Media	Layer Tested	Bacteria Concentration					
		B1		B2		B3	
		Conc. in Liquid	Conc. in Solid	Conc. in Liquid	Conc. in Solid	Conc. in Liquid	Conc. in Solid
Oil-based	Oil	2500		2500	2050	6250	3160
	Water	58.5		67.6		81.5	
Hexane-based	Water	6.2	407	6.9	2800	6.0	3490

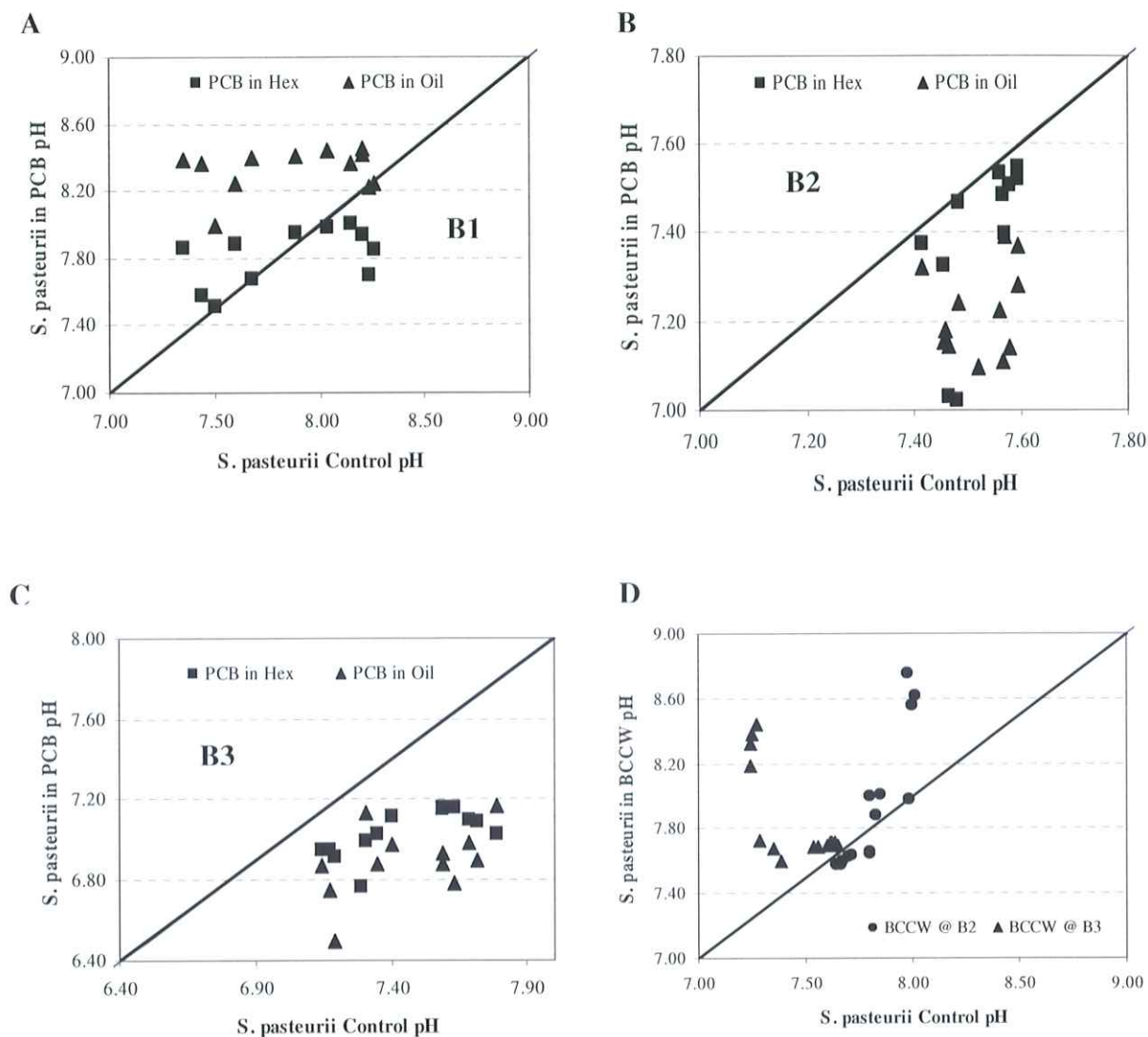


Fig. 16: Mean pH values in bacterial treatments as a function of mean pH values in bacterial control. All experiments were done concurrently and in triplicate. Bacterial cell concentrations were (B1) 5.7×10^6 , (B2) 7.4×10^7 , (B3) 1.4×10^8 , 6.4×10^7 , and 1.4×10^8 cells/mL for A, B, C, D (B2 & B3) respectively.

Fig. 16D shows bacterial activities in terms of pH values in both bacteria-BCCW mixture at bacteria cell concentrations B2 and B3, and bacterial control. The results show that the addition of BCCW enhances bacterial activity.

Table 4 shows the chemical analysis results of the stratified portions of the bacteria-BCCW mixture. Both the liquid and solid portions were analyzed for barium, calcium, chromium, copper, iron, magnesium, manganese, nickel, and zinc. These nine elements were chosen based on their measurable quantities in the original liquid. Over 93% and 85% of the chemical constituents were retained in the solid calcite layer using bacteria cell concentrations B2 and B3 respectively. This result shows that MCP successfully coprecipitated the analyzed chemical constituents at the bacteria cell concentrations used in this experiment, reducing chromium concentration to less than 2 mg/L well below the toxicity regulatory level. However, the concentrations of all these chemical constituents are still well above the Water Quality-Based Effluent Limits (NR 105) and Technology-Based Effluent Limits (NR 290.12). In addition, EDX analysis spectrum (Fig. 17) on the solids confirmed the presence of the coprecipitated elements in the solid matrix.

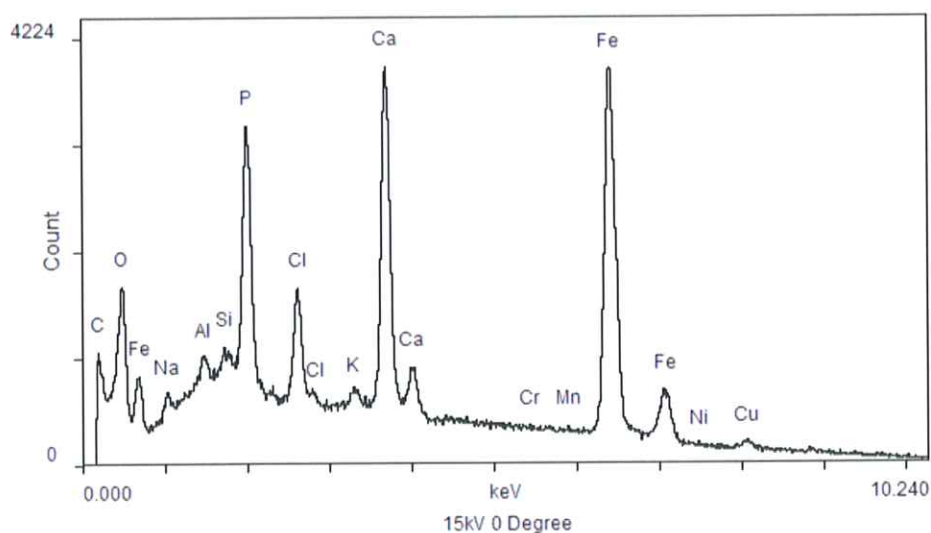


Fig. 17. Elemental composition of the coprecipitated solids by Energy Dispersive X-ray (EDX) Analysis.

Table 4: Coprecipitation of the chemical elements in the boiler chemical cleaning waste water (BCCW) by MCP using bacteria cell concentrations B2 (10^7 cells/mL) and B3 (10^8 cells/mL) at optimum conditions (250 mM/L Ca^{2+} and 666 mM/L urea). All concentrations are in mg/L.

Parameter	Bacteria Concentration, B2			Bacteria Concentration, B3		
	Conc.in the Liquid	Conc.in the solids	% coprec	Conc. in the Liquid	Conc. in the solids	% coprec
Ba	0.109	124	99.9	0.141	134	99.9
Ca	3330	58900	94.6	4600	74600	94.2
Cr	1.59	29.4	94.9	1.76	19.8	91.8
Cu	9.36	145	93.9	10.5	102	90.7
Fe	720	162000	99.6	820	148000	99.4
Mg	6.65	123	94.9	7.06	83.3	92.2
Mn	13	237	94.8	14.7	155	91.3
Ni	10	132	93.0	11.4	68	85.6
Zn	1.56	25.7	94.3	1.92	19.7	91.1

Statistical analysis on the coprecipitation of these metals by bacteria cell concentrations B2 and B3 show no significant difference at 95% confidence limit (p-value = 0.1464).

Table 5 and Appendix A (C) show the concentration of metals in the coprecipitated solid material after extraction using ASTM Method D3987. The means of all results obtained by both the bacteria and the control were not significantly different at 95% confident limit (p-values greater than 0.05). Of the 8 regulated metals in drinking water, only barium and chromium concentrations were determined as explained elsewhere in this section. These concentrations were significantly lower than the TCLP limits of 100 mg/l and 5 mg/l for barium and chromium respectively. However, these results are inconclusive because most of the metal concentrations are inconsistent with their respective control concentrations.

Table 5: Concentrations of the metals in the leachate extracted by the ASTM Method D3987 from the coprecipitated solids using de-ionized water as the leaching solution. The bacterial cell concentrations used were: B2 (10^7 cells/mL) and B3 (10^8 cells/mL). No bacterial cells were used in the Control.

Parameter	Concentrations of the metals in the Leachate (mg/L)		
	B2 (cells/mL)	B3 (cells/mL)	Control (No cells)
Ba	Less than 0.0094	Less than 0.0094	0.0100
Ca	350.00	290.00	400.00
Cr	0.28	0.30	0.37
Cu	0.33	0.27	0.26
Fe	100.00	140.00	120.00
Mg	1.20	1.10	0.85
Mn	2.00	2.00	3.10
Ni	1.40	1.40	1.20
Zn	0.40	0.24	0.28

6.4 Conclusions

Our experimental results have shown that the PCBs and BCCW had little effect on the activity of the urease enzyme. However, the carbonate effectively coprecipitated the hexane-based PCB and the metals in the BCCW. No coprecipitation occurred in the oil-based PCB. Chemical and EDX analyses on the coprecipitated solids confirmed the precipitation of metals in the BCCW. However, the ASTM Method D3987 test to determine the leaching potential of the coprecipitated metals was inconclusive. In addition, the results obtained by both the bacteria and the control were not significantly different at 95% confidence limit.

7.0 General Conclusions

This research has demonstrated that a higher concentration of both bacterial cells and Ca^{2+} ions are required to achieve the optimal conditions for MCP so long as there is enough urea to sustain the bacteria. However, the amount of calcium and urea required must be within their economic advantage because increasing the amounts of urea and calcium beyond 36 g/L and 90 g/L respectively do not enhance the rate of MCP and the amount of carbonate precipitated.

Our results have also demonstrated that the biosealant deposited by MCP on the concrete surface reduces the permeability by 1-5 orders of magnitude, and also forms a very strong durable surface barrier with a very high resistance to carbonation suitable for indoor application away from the ambient environmental conditions. Another advantage of the biosealant is that CaCO_3 and calcite have very high thermal stability, decomposing at 840°C and 825°C respectively as compared to epoxy resin adhesives which degrades at only 177°C. This indicates that the prevailing temperatures at these equipment installations would not have effect on the performance of the biosealant. Consequently, the biosealant would provide a safe working environment while the equipment is still in service.

In addition, MCP has the potential to coprecipitate hexane-based PCBs and metals in BCCW. However, more research needs to be done to evaluate the leaching potential of these metals from the calcite crystal lattice.

8.0 References

1. Achal, V., Mukherjee, A., Basu, P.C., and Sudhakara Reddy, M., Strain improvement of *Sporosarcina pasteurii* for enhanced urease and calcite production, *J. Ind. Microbiol. Biotechnol.* 2009, 36, 981-988.
2. Appenzeller, B.M.R., Yanez, C., Jorand, F., Block, J-C., Advantage provided by iron for *Escherichia coli* growth and cultivability in drinking water, *Appl. Environ Microbiol.*, 2005, 71(9), 5621-5623.
3. ASTM Standard C192 (2007), Standard Practice for making and curing concrete test specimens in the laboratory, ASTM International, West Conshohocken, PA.
4. ASTM Standard C33 (2003), Standard Specification for concrete aggregates, ASTM International, West Conshohocken, PA.
5. ASTM Standard D3987 (2006), Standard Test Method for Shake Extraction of Solid Waste with Water, ASTM International, West Conshohocken, PA.
6. ASTM Standard D5084 Method F (2003), Standard Test Methods for Measurement of Hydraulic Conductivity of Saturated Porous Materials Using a Flexible Wall Permeameter, ASTM International, West Conshohocken, PA.
7. Ballance, S., Phillips, P.J., McCrohan, C.R., Powell, J.J., Jugdaohsingh, R., and White, K.N., Influence of sediment biofilm on the behaviour of aluminum and its bioavailability to the snail *Lymnaea stagnalis* in neutral freshwater, *Can. J. Fish. Aquat. Sci.*, 2001, 58, 1708-1715.
8. Banfield, J.F., Barker, W.W., Welch, S.A., and Tauton, A., Biological impact of mineral dissolution: Application of the lichen model to understand mineral weathering in the rhizosphere, *Proc. Natl. Acad. Sci. USA.*, 1999, 96, 3404-3411.
9. Bang, S.S., and Ramakrishnan, V., Microbiologically-enhanced crack remediation (MECR), *Proceedings of the International Symposium on Industrial Application of Microbial Genomes, Taegu, Korea, 2001a*.
10. Bang, S.S., Galinat, J.K., and Ramakrishnan, V., Calcite precipitation induced by polyurethane-immobilized *Bacillus pasteurii*, *Enzyme and Microbial Technology*, 2001b, 28(4-5), 404-409.

11. Banin, E., Vasil, M.L., and Greenberg, P., Iron and *Pseudomonas aeruginosa* biofilm formation, *PNAS*, 2005, 102(31), 11076-11081.
12. Basheer, P.A.M., Basheer, L., Cleland, D.J., and Long, E., Surface treatments for concrete: Assessment methods and reported performance, *Construction and Building Materials*, 1997, 11(7-8), 413-429.
13. Bennett, P.C., Rogers, J.R., Choi, W.J., and Hiebert, F.K., Silicates, silicate weathering, and microbial ecology, *Geomicrobiology Journal*, 2001, 18, 3-19.
14. Berlutti, F., Morea, C., Battistoni, A., Sarli, S., Cipriani, P., Superti, F., Ammendolia, M.G., and Valenti, P., Iron availability influences aggregation, biofilm, adhesion and invasion of *Pseudomonas aeruginosa* and *Burkholderia cenocepacia*, *Int. J. Immunopathol. Pharmacol.*, 2005, 18(4), 661-670.
15. Blackwell, H.E., Bacterial Crowd Control with iron, *Chemistry and Biology*, 2005, 12, 721-723.
16. Bollinger, N., Hassett, D.J., Iglewski, B.H., Costerton, J.W., and McDermitt, T.R., Gene expression in *Pseudomonas aeruginosa*: Evidence of iron override effects on Quorum sensing and biofilm-specific gene regulation, *Journal of Bacteriology*, 2001, 183(6), 1990-1996.
17. Bonem, M.W., and Borah, R.E., Extraction Chemistry Goes Deep to Grab PCBs, *Environmental Protection*, 1995.
18. Boopathy, R. Factors limiting bioremediation technologies, *Bioresource Technology*, 2000, 74, 63-67.
19. Borja, J., Taleon, D.M., Auresenia, J., and Gallardo, S. Polychlorinated biphenyls and their biodegradation. *Process Biochemistry*, 2005, 40, 1999-2013.
20. Bowders, J.J., Loehr, J.E., Neupane, D., and Bouazza, A., Construction Quality Control for Asphalt concrete hydraulic barriers, *Journal of Geotechnical and Geoenvironmental Engineering*, 2003, 129(3), 219-223.
21. Bowders, J.J., Neupane, D., and Loehr, J.E., Sidewall Leakage in Hydraulic Conductivity Testing of Asphalt Concrete Specimens, *Geotechnical Testing Journal*, 2002, 25(2), 210-214.
22. Cacchio, P., Ercole, C., Cappuccio, G., and Lepidi A., Calcium Carbonate Precipitation by bacterial strains isolated from a limestone cave and from a loamy soil, *Geomicrobiology Journal*, 2003, 20, 85-98.

23. Cappitelli, F., Toniolo, L., Sansonetti, A., Gulotta, D., Ranalli, G., Zanardini, E., and Sorlini, C., Advantages of Using Microbial Technology over Traditional Chemical Technology in Removal of Black Crusts from Stone Surfaces of Historical Monuments, *Appl. Environ Microbiol.*, 2007, 73(17), 5671-5675.
24. Castanier, S., Le Metayer-Levrel, G., and Perthuisot, J-P., Ca-carbonates precipitation and limestone genesis-the microbiogeologist point of view, *Sedimentary Geology*, 1999, 126, 9-23.
25. Costerton, J.W., Montanaro, and Arciola, C.R., Biofilm in implant infections: Its production and regulation, *The International Journal of Artificial Organs*, 2005, 28(11), 1062-1068.
26. Curti, E., Coprecipitation of radionuclides with calcite: estimation of partition coefficients based on a review of laboratory investigations and geochemical data, *Applied Geochemistry*, 1999, 14, 433-445.
27. Day, J.L., Ramakrishnan, V., and Bang, S.S., Microbiologically induced sealant for concrete crack remediation, *In proceedings of the 16th ASCE Engineering Mechanics Conference, Seattle, WA, July 16-18, 2003.*
28. De Muynck, W., Cox, K., and De Belie, N., Bacterial carbonate precipitation reduces the permeability of cementitious materials, *Sustainable Construction Materials and Technologies*, 2007a, 411-416.
29. De Muynck, W., De Belie, N., and Verstraete, W., Improvement of concrete durability with the aid of bacteria, *Proceedings of the First International Conference on Self Healing Materials, Noordwijk aan Zee, The Netherlands, April 18-20, 2007b.*
30. De Muynck, W., De Belie, N., and Verstraete, W., Microbial carbonate precipitation in construction materials: A review. *Ecological Engineering*, 2010, 36(2), 118-136.
31. De, J., Ramaiah, N., Sarkar, A. Aerobic degradation of highly chlorinated polychlorobiphenyls by marine bacterium, *Pseudomonas* CH07, *World J. Microbiol Biotechnol.* 2006, 22, 1321-1327.
32. Decho, A.D., Microbial biofilms in intertidal systems: An overview. *Cont. Shelf Res.*, 2000a, 20, 1257-1273.
33. Decho, A.W., Overview of biopolymer-induced mineralization: What goes on in biofilms? *Ecological Engineering* 2010, 36(2), 137-144.

34. DeJong, J.T., Fritzges, M.B. and Nusslein, K., Microbially induced cementation to control sand response to undrained shear, *J. Geotech. Geoenviron. Eng.*, 2006, 132(11), 1381-1392.
35. DeJong, J.T., Mortensen, B.M., Martinez, B.C., and Nelson, D.C., Bio-mediated soil improvement, *Ecological Engineering*, 2010, 36(2), 197-210.
36. Dick, J., De Windt, W., De Graef, B., Saveyn, H., Van der Meeren, P., De Belie, N., and Verstraete, W., Bio-deposition of a calcium carbonate layer on degraded limestone by *Bacillus* species, *Biodegradation*, 2006, 17, 357-367.
37. Ehrlich, H.L., Geomicrobiology: its significance for geology. *Earth-Sci. Rev.*, 1998, 45, 45-60.
38. Ercole, C., Cacchio, P., Botta, A.L., Centi, V., and Lepidi, A., Bacterially induced mineralization of calcium carbonate: The role of exopolysaccharides and capsular polysaccharides, *Microscopy and Microanalysis*, 2007, 13, 42-50.
39. Faibish, R.S., Elimelech, M., and Cohen, Y., Effect of interparticle electrostatic double layer interactions on permeable flux decline in crossflow membrane filtration of colloidal suspensions: An experimental investigation, *J. Colloid Interface Sci.* 1998, 204, 77-86.
40. Ferris, F.G., Beveridge, T.J., and Fyfe, W.S., Iron-silica crystallite nucleation by bacteria in a geothermal sediment, *Nature*, 1986, 320, 609-611.
41. Ferris, F.G., Fyfe, W.S., and Beveridge, T.J., Bacteria as nucleation sites for authigenic minerals in metal-contaminated lake sediment, *Chem. Geol.* 1987, 63, 225-232.
42. Ferris, F.G., Wiese, R.G., and Fyfe, W.S., Precipitation of carbonate minerals by microorganisms: Implications of silicate weathering and the global carbon dioxide budget, *Geomicrobiology Journal*, 1994, 12, 1-13.
43. Ferris, F.G., Phoenix, V., Fujita, Y., and Smith, R.W., Kinetics of calcite precipitation induced by ureolytic bacteria at 10 to 20°C in artificial groundwater, *Geochimica et Cosmochimica Acta*, 2003, 67(8), 1701-1722.
44. Foppen, J.W.A., and Schijven, J.F., Evaluation of data from the literature on the transport and survival of *Escherichia coli* and thermotolerant coliforms in aquifers under saturated conditions, *Water Research*, 2006, 40, 401-426.
45. Friedrich, B., and Magasanik, B., Urease of *Klebsiella aerogenes*: Control of its synthesis by glutamine synthetase, *Journal of Bacteriology*, 1977, 28, 313-322.

46. Fujita, Y., Ferris, F.G., Lawson, R.D., Colwell, F.S., Smith, R.W., Calcium carbonate precipitation by ureolytic subsurface bacteria, *Geomicrobiology Journal*, 2000, 17, 305-318.
47. Fujita, Y., Redden, G.D., Ingram, J.C., Cortez, M.M., Ferris, F.G., and Smith, R.W., Strontium incorporation into calcite generated by bacterial ureolysis, *Geochimica et Cosmochimica Acta*, 2004, 68(15), 3261-3270.
48. Ganey, P.E., and Boyd, S.A. An approach to evaluation of the effect of bioremediation on biological activity of environmental contaminants: Dechlorination of polychlorinated biphenyls, *Environmental Health Perspectives*, 2005, 113(2), 180-185.
49. Ghosh, P., Mandal, S., Chattopadhyay, B.D., and Pal, S., Use of microorganism to improve the strength of cement mortar, *Cement and Concrete Research*, 2005, 35, 1980-1983.
50. Hammes, F., and Verstraete, W., Key roles of pH and calcium metabolism in microbial carbonate precipitation, *Re/Views in Environmental Science & Bio/Technology*, 2002, 1, 3-7.
51. Hammes, F., Boon, N., De Villiers, J., Verstraete, W., Siciliano, S.D., Strain-specific ureolytic microbial calcium carbonate precipitation, *Appl. Environ Microbiol.*, 2003, 69(8), 4901-4909.
52. Harkes, M.P., Van Paassen, L.A., Booster, J.L., Whiffin, V.S., and Van Loosdrecht, M.C.M., Fixation and distribution of bacterial activity in sand to induce carbonate precipitation for ground reinforcement, *Ecological Engineering*, 2010, 36(2), 112-117.
53. Hastings, J.W., and Greensberg, E.P., Quorum sensing: the explanation of a curious phenomenon reveals a common characteristic of bacteria, *Journal of Bacteriology*, 1999, 181(9), 2667-2668.
54. Hontaria, E., and Sanchez-Blanco, M.,: D2.4.4.1. SoA report: Ground injections, *Technology Innovation in Underground Construction*, 2006, 22-51.
55. Hunter, R.J. (1981). "Zeta potential in colloid science: Principles and applications." *Academic press, London*.
56. Ivanov, V. and Chu, J., Applications of microorganisms to geotechnical engineering for bioclogging and biocementation of soil in situ, *Rev. Environ. Sci. Biotechnol.* 2008, 7(2), 139-153.

57. Jefferson, K.K. What drives bacteria to produce a biofilm? *FEMS Microbiology Letters*, 2004, 236, 163-173.
58. Jimenez-Lopez, C., Jroundi, F., Pascolini, C., Rodriguez-Navarro, C., Pinar-Larrubia, G., Rodriguez-Gallego, M., and Gonzalez-Munoz, M.T., Consolidation of quarry calcarenite by calcium carbonate precipitation induced by bacteria activated among the microbiota inhabiting the stone, *International Biodeterioration and biodegradation*, 2008, 62, 352-363.
59. Jonkers, H.M., Thijssen, A., Muyzer, G., Copuroglu, O., Schlangen, E., Application of bacteria as self-healing agen for the development of sustainable concrete, *Ecological Engineering*, 2010, 36(2), 230-235.
60. Kaltwasser, H., Kramer, J., and Conger, W.R., Control of urease formation in certain aerobic bacteria, *Archiv fur Mikrobiologie*, 1972a, 81-178-196.
61. Kamei, I., Sonoki, S., Haraguchi, K., Kondo, R. Fungal bioconversion of toxic polychlorinated biphenyls by white-rot fungus, *Phlebia brevispora*. *Appl. Microbial Biotechnol.*, 2006, 73, 932-940.
62. Knorre, H., Krumbein, W., Bacterial calcification, *In R.E. Riding and S.M. Awramik (ed.), Microbial Sediments. Springer-Verlag, Berlin, Germany, 2000, 25-31.*
63. Konhauser, K.O., Lalonde, S.V. and Phoenix, V.R., Bacterial biomineralization: Where to from here? *Geobiology*, 2008, 6, 298—302.
64. Lehtola, M.J., Juhna, T., Miettinen, I.T., Vartiainen, T., Martikainen, P.J., Formation of biofilms in drinking water distribution networks, a case study in two cities in Finland and Latvia, *J. Ind. Microbiol. Biotechnol.*, 2004, 31, 489-494.
65. Lunsdorf, H., Brummer, I., Timmis, K.N., and Wagner-Dobler, I., Metal sensitivity to in situ microcolonies in biofilms of the Elbe River, *Journal of Bacteriology*, 1997, 179, 31-40.
66. Luttge, A., Zhang, L., and Nealson, K.H., Mineral surfaces and their implications for microbial attachment: Results from Monte Carlo Simulations and Direct surface observations, *American Journal of Science*, 2005, 305, 766-790.
67. Manning, D.A.C., Biological enhancement of soil carbonate precipitation: passive removal of atmospheric CO₂, *Mineralogical Magazine*, 2008, 72(2), 639-649.

68. Martell, A., and Smith, R., Critical Stability Constants, *Plenum Press, New York, 1974.*
69. Mayo Clinic. Occupational Asthma. ([http://www.mayoclinic.com/health/occupational-asthma/DS00591/DSECTION = risk-factors](http://www.mayoclinic.com/health/occupational-asthma/DS00591/DSECTION=risk-factors)). Accessed on April 28, 2010.
70. McConnaughey, T.A., and Whelan, J.F., Calcification generates protons for nutrient and bicarbonate uptake, *Earth Science Reviews, 1997, 42, 95-117.*
71. Mitchell, A.C. and Ferris, F.G., The coprecipitation of Sr into calcite precipitates induced by bacterial ureolysis in artificial groundwater: Temperature and kinetics dependence, *Geochimica et Cosmochimica Acta, 2005, 69(17), 4199-4210.*
72. Mitchell, A.C. and Ferris, F.G., The influence of *Bacillus pasteurii* on the nucleation and growth of calcium carbonate, *Geomicrobiology Journal, 2006, 23, 213-226.*
73. Mitchell, S.J., and Scadden, R.A., PCB Decontamination Methods for Achieving TSCA Compliance During Facility Decommissioning Projects, *In proceedings of the NDIA 27th Environmental Symposium and Exhibition, Austin, Texas, April 25, 2001.*
74. Morena, J.J. Advanced Composite Mold Making. New York: Van Nostrand Reinhold Co. inc. 1988, 124-125, ISBN 0-142-26414-3.
75. Nemati, M., and Voordouw, G., Modification of porous media permeability using calcium carbonate produced enzymatically in situ, *Enzyme and Microbial Technology, 2003, 33, 635-642.*
76. Perbal, B., Communication is the key, *Cell Communication and Signaling, 2003, 1, 1-4.*
77. Phoenix, V.R. and Konhauser, K.O., Benefits of bacterial biomineralization, *Geobiology, 2008, 6, 303-308.*
78. Pizarro, G.E.L., Dzombak, D.A., and Smith, J.R., Evaluation of Cleaning and Coating Techniques for PCB-Contaminated Concrete, *Environmental Progress, 2002, 21(1), 47-56.*
79. Ramachandran, S.K., Ramakrishnan, V., and Bang, S.S., Remediation of concrete using microorganisms, *ACI Materials Journal, 2001, 98(1), 3-9.*

80. Ramakrishnan, V., Bang, S.S., Ramesh, K.P., and Mulukutla, S., Performance of bacterial cement mortar, *Proceedings of the 24th International Conference on Concrete Microscopy*, 2002.
81. Ramakrishnan, V., Ramesh, K.P., and Bang, S.S., Bacterial Concrete, *Proceedings of SPIE 2000, Symposium on Smart Materials and Mems*, 2001, 4234, 168-176.
82. Ramme, B.W and Tharaniyil, M.P., Coal Combustion Products Utilization Handbook, *Second Edition*, 2004, A We Energies Publication.
83. Reading, N.C., and Sperandio, V., Quorum sensing: the many languages of bacteria, *FEMS Microbiology Letters*, 2006, 254, 1-11.
84. Roberts, J.A., Inhibition and enhancement of microbial surface colonization: the role of silicate composition, *Chemical Geology*, 2004, 212, 313-327.
85. Rogers, J.R., and Bennett, P.C., Mineral stimulation of subsurface microorganisms: release of limiting nutrients from silicates, *Chemical Geology*, 2004, 203, 91-108.
86. Rogers, J.R., Bennett, P.C., and Choi, W.J., Feldspars as a source of nutrients for microorganisms, *American Mineralogist*, 1998, 83, 1532-1540.
87. Schultze-Lam, S., Fortin, D., and Beveridge, T.J., Mineralization of bacterial surfaces, *Chemical Geology*, 1996, 132, 171-181.
88. Silver, S., Toth, K., and Scribner, H. (1975). "Facilitated Transport of Calcium by Cells and Subcellular Membranes of *Bacillus subtilis* and *Escherichia coli*." *Journal of Bacteriology*, 122(3), 880-885.
89. Singh, R., Paul, D., and Jain, R.K., Biofilms: implications in bioremediation, *TRENDS in Microbiology*, 2006, 14(9), 389-397.
90. Stanley, N.R., and Lazazzera, B.A., Environmental signals and regulatory pathways that influence biofilm formation, *Molecular Microbiology*, 2004, 52(4), 917-924.
91. Stocks-Fischer, S., Galinat, J.K., and Bang, S.S., Microbiological precipitation of CaCO₃, *Soil Biol. Biochem.*, 1999, 31, 1563-1571.
92. Tharakan, J., Tomlinson, D., Addagada, A., Shafagati, A. Biotransformation of PCBs in contaminated sludge: Potential for novel biological technologies, *Eng. Life Sci.* 2006, 6(1), 43-50.

93. Tiano, P., Biagiotti, L., and Mastromei, G., Bacterially bio-mediated calcite precipitation for monumental stones conservation: methods of evaluation, *J. Microbiol. Methods*, 1999, 36, 139-145.
94. Tiano, P., Cantisani, E., Sutherland, I., and Paget, J.M., Biomediated reinforcement of weathered calcareous stones, *Journal of cultural Heritage*, 2006, 7, 49-55.
95. United States Department of Natural Resources, Wisconsin Administrative Code Chapter NR 105. *Surface Water Quality Criteria and Secondary Values for Toxic Substances, NR 105*, 2004, No. 578.
96. United States Department of Natural Resources, Wisconsin Administrative Code Chapter NR 661. *Hazardous Waste Identification and Listing, NR 661.04*, 2007, No. 615.
97. Weinberg, E.D., Suppression of bacterial biofilm formation by iron limitation, *Medical Hypotheses*, 2004, 63, 863-865.
98. Whiffin, V.S., Van Paassen, L.A., and Harkes, M.P., Microbial Carbonate Precipitation as a soil improvement technique, *Geomicrobiology Journal*, 2007, 24, 417-423.
99. Zachara, J.M., Cowan, C.E., and Resch, C.T., Sorption of divalent metals on calcite, *Geochimica et Cosmochimica Acta*, 1991, 55, 1549-1562.
100. Zamarreno, D.V., May, E. and Inkpen, R., Influence of environmental temperature on biocalcification by non-sporing freshwater bacteria, *Geomicrobiology Journal*, 2009, 26, 298-309.

APPENDIX A

STATISTICAL ANALYSIS (HYPOTHESIS TESTING)

Null hypothesis, $H_0: \mu_1 = \mu_2$

Alternative hypothesis, $H_1: \mu_1 \neq \mu_2$

Data size, n

At 95 % confidence limit, $\alpha = 0.05$

Test statistic is t-distribution ($\sigma_1^2 \neq \sigma_2^2$ and are unknown)

Test Statistic,
$$t_o^* = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}$$

Degree of Freedom,

$$v = \frac{\left(\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}\right)^2}{\left(\frac{S_1^2}{n_1}\right)^2 * \frac{1}{n_1 - 1} + \left(\frac{S_2^2}{n_2}\right)^2 * \frac{1}{n_2 - 1}}$$

Where t_o^* is the test statistic, v is the degree of freedom for the distribution, \bar{X}_1 and \bar{X}_2 , and S_1 and S_2 are means and standard deviations respectively of the distributions to be compared.

Confidence Interval:
$$\bar{X}_1 - \bar{X}_2 - t_{0.05,v}^* \sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}} \leq \mu_1 - \mu_2 \leq \bar{X}_1 - \bar{X}_2 + t_{0.05,v}^* \sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}$$

- Decision Conditions: (1) Reject $H_0: \mu_1 = \mu_2$ if $t_o^* > t_{0.025,v}$ or if $t_o^* < -t_{0.025,v}$
- (2) Means are not different if the 95% CI includes zero
- (3) Do not reject H_0 if the p-value is greater than 0.05 at 95% Confidence limit.

(A) THE EFFECT OF BACTERIA CELL CONCENTRATION ON THE RATE OF UREA HYDROLYSIS

Test No	k_{urea} (per day)		
	B1 (cells/mL)	B2 (cells/mL)	B3 (cells/mL)
1	1.1112	0.8352	0.9216
2	0.6240	0.9120	0.9360
3	0.9360	0.7920	0.8880
4	0.9024	0.9744	0.9432
5	0.6696	0.7680	0.9552
6	0.9048	0.8880	0.8832
7	0.6864	1.0632	1.0680
8	0.8616	0.7128	0.9312
9	0.8206	0.9864	0.8592
10	0.7776	0.7776	1.0296
11	0.8904	0.8400	0.8544
12	0.6960	0.7992	0.9600
13	0.7848	0.8016	0.9696
14	0.7920	0.9144	0.8496
15	0.7560	0.8160	1.0032
16	0.7896	0.8256	1.0440
17	0.7800	0.7680	0.8712
18	0.7656	1.0392	0.8560
Mean	0.8083	0.8619	0.9346
Stdev	0.11481442	0.10003	0.06877

Variable	B1 vs B2	B1 vs B3	B2 vs B3
\bar{X}_1	0.8083	0.8083	0.8619
\bar{X}_2	0.8619	0.9346	0.9346
S_1	0.1148	0.1148	0.1000
S_2	0.1000	0.0688	0.0688
n	18	18	18
t_{0^*}	-1.4934	-4.0038	-2.5409
\bar{Y}	33.3738	27.8070	30.1356
$t_{0.025,v}$	2.0420	2.0480	2.0420
p-value	0.1448	0.0004	0.0165
Lower Conf. Interval	-0.1269	-0.1909	-0.1311
Upper Conf. Interval	0.0197	-0.0617	-0.0143
Mean Different?	NO	YES	YES
Reject Ho?	NO	YES	YES

(B) **PERCENTAGE COPRECIPITATION OF CHEMICALS AT BACTERIA CELL CONCENTRATIONS B2 (10^7 cells/mL) AND B3 (10^8 cells/mL)**

Parameter	% Coprec. @ B2	% Coprec. @ B3
Ba	99.9	99.9
Ca	94.6	94.2
Cr	94.9	91.8
Cu	93.9	90.7
Fe	99.6	99.4
Mg	94.9	92.2
Mn	94.8	91.3
Ni	93.0	85.6
Zn	94.3	91.1
Mean	95.5	92.9
Stdev	2.4602	4.45209

Variable	B2 vs B3
Mean % prec. (B2), \bar{X}_1	95.5444
Mean % prec. (B3), \bar{X}_2	92.9111
Standard dev. (B2), S_1	2.4602
Standard dev. (B3), S_2	4.4521
Data Size, $n_1 = n_2$	9
Test Statistic, t_0^*	1.5531
Degree of Freedom, v	12.4692
$t_{0.025, v}$	2.1701
p-value	0.1464
Lower Conf. Interval	-1.0461
Upper Conf. Interval	6.3128
Mean Different?	NO
Reject H_0 ?	NO

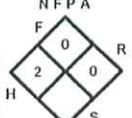

(C) LEACHING OF THE METALS FROM THE COPRECIPITATED SOLIDS (ASTM D3987 EXTRACT)

Parameter	Concentrations of the Leachate (mg/L)		
	B2 (cells/mL)	B3 (cells/mL)	Control (No cells)
Ba	0.0094	0.0094	0.0100
Ca	350.00	290.00	400.00
Cr	0.28	0.30	0.37
Cu	0.33	0.27	0.26
Fe	100.00	140.00	120.00
Mg	1.20	1.10	.85
Mn	2.00	2.00	3.10
Ni	1.40	1.40	1.20
Zn	0.40	0.24	0.28
Mean	50.6244	48.3688	58.4522
Stdev	116.9628	101.6437	134.0066

Variable	B2 vs B3	B2 vs Control	B3 vs Control
Mean Conc. (B2), \bar{X}_1	50.6244	50.6244	48.3688
Mean Conc. (B3), \bar{X}_2	48.3688	58.4522	58.4522
Mean Conc. (Control), \bar{X}_3	58.4522	48.3688	50.6244
Stdev (B2), S_1	116.9628	116.9628	101.6437
Stdev (B3), S_2	101.6437	134.0066	134.0066
Stdev (Control), S_3	134.0066	101.6437	116.9628
Data Size, n	9	9	9
t_0^*	0.0437	-0.1320	-0.1799
v	15.6947	15.7128	14.9160
$t_{0.025,v}$	2.1234	2.1232	2.1319
p-value	0.9657	0.8966	0.8595
Lower Conf. Interval	-107.4231	-133.7130	-129.6076
Upper Conf. Interval	111.9343	118.0574	109.4408
Mean Different?	NO	NO	NO
Reject Ho?	NO	NO	NO

APPENDIX B

BOILER CHEMICAL CLEANING WASTEWATER (BCCW) CHES SHEET

	 Wisconsin Energy Corporation C.H.E.S. - Chemical Hazard Evaluation System PRODUCT DATA SHEET	CHES#: 16754 REVISION: 03 DATE: 01/19/07 SUPERCEDES: 1/19/2007 <small>15-07-02</small>
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I General Information

Trade Name:	BOILER CHEM - CLEANING LIQUID	
Chemical Name:	Mixture	
Manufacturer:	We Energies 333 W. Everett St. Milwaukee, WI 53201	Information Phone No.: 414-221-2345 Emergency Phone: 262-542-1440

II Ingredients

CHEMICAL	%	CAS	TLV	PEL	STEL	CEIL	TRI
EDTA (Tetraammonium Salt)	0.04 - 1	22473-78-5					<input type="checkbox"/>
EDTA <small>(Chelated w/Fe, Cu, Ca, Mg, & Trace metals)</small>	0.2 - 5	60-00-4					<input type="checkbox"/>
AMMONIUM SALTS (As Sulfate, Chloride, and Carbonate)	0.1 - 1.5	N/A					<input type="checkbox"/>
AMMONIUM HYDROXIDE <small>(Dissociation product)</small>	0.02 - 0.5	1336-21-6					<input type="checkbox"/>
IRON <small>(Chelated w/EDTA)</small>	50 - 15000 ppm	7439-89-6					<input type="checkbox"/>
COPPER <small>(Chelated w/EDTA)</small>	5 - 500 ppm	7440-50-8	0.2 mg/m3	0.1 mg/m3			<input type="checkbox"/>
CHROMIUM <small>(Chelated w/EDTA)</small>	0.2 - 25 ppm	7440-47-3	0.5 mg/m3	1 mg/m3			<input type="checkbox"/>
MAGNESIUM <small>(Chelated w/EDTA)</small>	5 - 500 ppm	7439-95-4					<input type="checkbox"/>
TRACE METALS <small>(Chelated w/EDTA)</small>	5 - 500 ppm						<input type="checkbox"/>
HEAVY AROMATIC NAPHTHA	0.2 - 15 ppm	64742-94-5					<input type="checkbox"/>
WATER	93 - 99.9	7732-18-5					<input type="checkbox"/>

III Health Hazard Data

Routes/Effects of Acute Overexposure

Skin	May cause slight irritation to the skin. Dried product may cause irritation to the skin.
Eyes	May cause mild irritation and a burning sensation. Particles from dried material may cause irritation.
Inhalation	Little hazard produced by normal operations in open or well ventilated areas. Nose and throat irritation. Dust from dried material may be hazardous to your health with irritation to the nose/throat, chills, fever, and dryness in throat - metal fume fever.
Ingestion	Harmful if swallowed. Mild irritation of throat and G.I. tract.

Chronic Overexposure Effects	Warning Properties
Chronic overexposure unlikely. Lung damage. Cancer (IARC/NTP/OSHA).	Eye, nose, and/or throat irritation. Skin redness or burning. Coughing.

First Aid	
Skin	Wash with mild soap and water.
Eyes	Flush with large amount of water for at least 15 minutes -- Seek medical attention.
Inhalation	Remove to fresh air -- Seek medical help promptly -- Use artificial respiration, if necessary.
Ingestion	Give milk or water - DO NOT induce vomiting - Seek medical attention promptly.

IV Exposure Controls / Personal Protection

Engineering Controls
Local exhaust if uncontrolled dust/mist/vapor present.

Personal Protective Equipment

Eyes/Face Faceshield or goggles shall be worn at all times.
Skin Rubber apron. Rubber gloves shall be worn at all times.
Respiratory Lot No. 742-8046/5988 (Green) Ammonia cartridge if needed with full/half respirator.
Special Clothing or Equipment Wash after use/handling; eye wash/shower access. Change/wash contaminated clothing before reuse.

V Fire/Explosion Data

Flash Point (PMCC) NONE
Auto Ignition N/A

Extinguishing Media

Carbon Dioxide, Dry Chemical

VI Physical Data

Bolling Point	210 F	98.9 C	Appearance	Red to grey colored liquid
Melting Point	30 F	-1.1 C	Odor	Ammonia
Vapor Density	N/A		Vapor Pressure (mmHg)	N/A
Specific Gravity	1.01 - 1.06		Percent Volatile	< 0.5
Bulk Density	N/A		pH	9 - 9.6
Solubility (H2O)	Complete		Corrosivity on Metal	Brass

VII Reactivity**Chemical Stability**

Stable.

Hazardous Polymerization

May not occur.

Incompatibility

Reactive metals such as aluminum. Strong oxidizers.

Hazardous Decomposition

Ammonia, hydrogen.

VIII Environmental/Handling/Storage**Spill Or Leak Procedure**

Collect and dispose of per environmental. Prevent from entering drains or waterways. Soak up with absorbent.

Storage Procedures

Store away from strong oxidizers. Store between 32 F - 120 F. Do not store in aluminum, nickel, zinc or copper containers. Label all unlabeled containers with a CHES label (L/N 138-2010).

Is This Product Listed Or Does It Contain Any Chemical Listed For The Following:

- Disposal Of Product Or Any Residue A Hazardous Waste? No
Hazardous Waste Code:
- An Extremely Hazardous Substance Under Emergency Planning And Community Right-To-Know?
Substance Name: Threshold Planning Quantity (lbs.):
No N/A
- An EPA Hazardous Substance Requiring Spill Reporting?
Listed Substance: Reportable Quantity (lbs.):
AMMONIUM HYDROXIDE 1000
CHROMIUM 5000
COPPER 5000
EDTA 5000
- An OSHA Hazardous Chemical? Chemical Name: CHROMIUM
COPPER

Does It Contain Any Materials Regulated As Hazardous Materials Or Hazardous Substances By The Department Transportation?

Proper Shipping Name: Environmentally hazardous substances, liquid, n.o.s.
Hazard Class: 9 : Packaging Group III
UN/NA Code: UN3082 Quantity Required for Placarding Optional >1
Labels Required: 9

IX Other

This information was based upon current scientific literature. Information may be developed from time to time which may render this document incorrect; therefore, the Wisconsin Energy Corporation nor any of its subsidiaries makes any warranties to its agents, employees, or contractors as to the applicability of this data to the user's intended purpose.

APPENDIX C

COPRECIPITATION LABORATORY TEST RESULTS

To: Bruce Ramme
PSB Annex A231



From: Laboratory Services Division
PSB Annex A070
WDNR Cert # 241329000

Report Date: Monday, February 8, 2010

The following are the analytical results for the sample(s) received by Laboratory Services on 1/28/10 :

Sample Description: B2 + BCCW Liquid
Sample ID: AD19169 Serial/Impact ID:
Sample Collector: OKWADA Sample Collection Date: 1/28/10 Collection Time: 00:00

<u>Parameter</u>	<u>Result</u>	<u>MDL</u>	<u>Units</u>	<u>Result</u>	<u>Analysis</u>	<u>Analysis</u>	<u>Analysis</u>
				<u>Flag</u>	<u>Method</u>	<u>Date</u>	<u>Analyst</u>
Sendout Analysis	See attached report					2/1/10	020

Sample Comments:

If there are any questions concerning this report, please contact Dave Kollakowsky at (414) 221-2835.

To: Bruce Ramme
PSB Annex A231



From: Laboratory Services Division
PSB Annex A070
WDNR Cert # 241329000

Report Date: Monday, February 8, 2010

The following are the analytical results for the sample(s) received by Laboratory Services on 1/28/10 :

Sample Description: B3 + BCCW Liquid
Sample ID: AD19170
Sample Collector: OKWADA
Serial/Impact ID:
Sample Collection Date: 1/28/10
Collection Time: 00:00

<u>Parameter</u>	<u>Result</u>	<u>MDL</u>	<u>Units</u>	<u>Result</u> <u>Flag</u>	<u>Analysis</u> <u>Method</u>	<u>Analysis</u> <u>Date</u>	<u>Analyst</u>
Sendout Analysis	See attached report					2/1/10	020

Sample Comments:

If there are any questions concerning this report, please contact

Dave Kollakowsky at (414) 221-2835.

To: Bruce Ramme
PSB Annex A231



From: Laboratory Services Division
PSB Annex A070
WDNR Cert # 241329000

Report Date: Monday, February 8, 2010

The following are the analytical results for the sample(s) received by Laboratory Services on 1/28/10 :

Sample Description: BCCW + B2 A&B Solid
Sample ID: AD19171 Serial/Impact ID:
Sample Collector: OKWADA Sample Collection Date: 1/28/10 Collection Time: 00:00

<u>Parameter</u>	<u>Result</u>	<u>MDL</u>	<u>Units</u>	<u>Result</u>	<u>Analysis</u>	<u>Analysis</u>	<u>Analysis</u>
				<u>Flag</u>	<u>Method</u>	<u>Date</u>	<u>Analyst</u>
Sendout Analysis	See attached report					2/1/10	020

Sample Comments:

If there are any questions concerning this report, please contact Dave Kolakowsky at (414) 221-2835.

To: Bruce Rammie
PSB Annex A231



From: Laboratory Services Division
PSB Annex A070
WDNR Cert # 241329000

Report Date: Monday, February 8, 2010

The following are the analytical results for the sample(s) received by Laboratory Services on 1/28/10 :

Sample Description: BCCW + B3 A&B Solid
Sample ID: AD19172 Serial/Impact ID:
Sample Collector: OKWADA Sample Collection Date: 1/28/10 Collection Time: 00:00

<u>Parameter</u>	<u>Result</u>	<u>MDL</u>	<u>Units</u>	<u>Result</u>	<u>Analysis</u>	<u>Analysis</u>	<u>Analysis</u>
				<u>Flag</u>	<u>Method</u>	<u>Date</u>	<u>Analyst</u>
Sendout Analysis	See attached report					2/1/10	020

Sample Comments:

If there are any questions concerning this report, please contact

Dave Kollakowsky at (414) 221-2835.



Pace Analytical Services, Inc.
1241 Bellevue Street - Suite 9
Green Bay, WI 54302
(920)469-2436

February 05, 2010

David Kollakowsky
We Energies
PO Box 2179
Room P129
Milwaukee, WI 532012179

RE: Project: 1302398 BCCW METALS
Pace Project No.: 4028016

Dear David Kollakowsky:

Enclosed are the analytical results for sample(s) received by the laboratory on January 30, 2010. The results relate only to the samples included in this report. Results reported herein conform to the most current NELAC standards, where applicable, unless otherwise narrated in the body of the report.

If you have any questions concerning this report, please feel free to contact me.

Sincerely,

Brian Basten

brian.basten@pacelabs.com
Project Manager

Enclosures

REPORT OF LABORATORY ANALYSIS

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CERTIFICATIONS

Project: 1302398 BCCW METALS
Pace Project No.: 4028016

Green Bay Certification IDs

California Certification #: 09268CA
Florida/NELAP Certification #: E87948
Illinois Certification #: 200050
Kentucky Certification #: 82
Louisiana Certification #: D4-168
Minnesota Certification #: 055-999-334
New York Certification #: 11887

New York Certification #: 11888
North Carolina Certification #: 503
North Dakota Certification #: R-150
South Carolina Certification #: 83006001
Wisconsin Certification #: 405132750
Wisconsin DATCP Certification #: 105-444
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SAMPLE SUMMARY

Project: 1302398 BCCW METALS
Pace Project No.: 4028016

Lab ID	Sample ID	Matrix	Date Collected	Date Received
4028016001	AD1916S LIQUID	Water	01/27/10 00:00	01/30/10 08:05
4028016002	AD19170 LIQUID	Water	01/27/10 00:00	01/30/10 08:05
4028016003	AD19171 SOLID	Solid	01/27/10 00:00	01/30/10 08:05
4028016004	AD19172 SOLID	Solid	01/27/10 00:00	01/30/10 08:05

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SAMPLE ANALYTE COUNT

Project: 1302398 BCCW METALS
 Pace Project No.: 4028015

Lab ID	Sample ID	Method	Analysts	Analytes Reported
4028016001	AD19169 LIQUID	EPA 6010	DLB	9
4028016002	AD19170 LIQUID	EPA 6010	DLB	9
4028016003	AD19171 SOLID	EPA 6010	DLB	9
4028016004	AD19172 SOLID	EPA 6010	DLB	9

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ANALYTICAL RESULTS

Project: 1302398 BCCW METALS
Pace Project No.: 4028016

Sample: AD19169 LIQUID Lab ID: 4028016001 Collected: 01/27/10 00:00 Received: 01/30/10 08:05 Matrix: Water

Parameters	Results	Units	LOQ	LOD	DF	Prepared	Analyzed	CAS No.	Qual
Analytical Method: EPA 6010 Preparation Method: EPA 3010									
Barium	109	ug/L	25.0	0.90	1	02/01/10 13:30	02/02/10 18:09	7440-39-3	
Calcium	3330000	ug/L	10000	608	10	02/01/10 13:30	02/04/10 11:33	7440-70-2	
Chromium	1590	ug/L	25.0	1.6	1	02/01/10 13:30	02/02/10 18:09	7440-47-3	
Copper	9360	ug/L	50.0	1.4	1	02/01/10 13:30	02/02/10 18:09	7440-50-8	
Iron	720000	ug/L	5000	198	10	02/01/10 13:30	02/04/10 11:33	7439-89-6	
Magnesium	6650J	ug/L	10000	669	10	02/01/10 13:30	02/04/10 11:33	7439-95-4	
Manganese	13000	ug/L	25.0	0.45	1	02/01/10 13:30	02/02/10 18:09	7439-96-5	
Nickel	10000	ug/L	50.0	1.1	1	02/01/10 13:30	02/02/10 18:09	7440-02-0	
Zinc	1560	ug/L	200	10.0	1	02/01/10 13:30	02/02/10 18:09	7440-66-6	

Sample: AD19170 LIQUID Lab ID: 4028016002 Collected: 01/27/10 00:00 Received: 01/30/10 08:05 Matrix: Water

Parameters	Results	Units	LOQ	LOD	DF	Prepared	Analyzed	CAS No.	Qual
Analytical Method: EPA 6010 Preparation Method: EPA 3010									
Barium	141	ug/L	25.0	0.90	1	02/01/10 13:30	02/02/10 18:14	7440-39-3	
Calcium	4600000	ug/L	10000	608	10	02/01/10 13:30	02/04/10 11:45	7440-70-2	
Chromium	1760	ug/L	25.0	1.6	1	02/01/10 13:30	02/02/10 18:14	7440-47-3	
Copper	10500	ug/L	50.0	1.4	1	02/01/10 13:30	02/02/10 18:14	7440-50-8	
Iron	820000	ug/L	5000	198	10	02/01/10 13:30	02/04/10 11:45	7439-89-6	
Magnesium	7060J	ug/L	10000	669	10	02/01/10 13:30	02/04/10 11:45	7439-95-4	
Manganese	14700	ug/L	25.0	0.45	1	02/01/10 13:30	02/02/10 18:14	7439-96-5	
Nickel	11400	ug/L	50.0	1.1	1	02/01/10 13:30	02/02/10 18:14	7440-02-0	
Zinc	1920	ug/L	200	10.0	1	02/01/10 13:30	02/02/10 18:14	7440-66-6	

Sample: AD19171 SOLID Lab ID: 4028016003 Collected: 01/27/10 00:00 Received: 01/30/10 08:05 Matrix: Solid
Results reported on a "wet-weight" basis

Parameters	Results	Units	LOQ	LOD	DF	Prepared	Analyzed	CAS No.	Qual
Analytical Method: EPA 6010 Preparation Method: EPA 3050									
Barium	124	mg/kg	2.7	0.15	5	02/01/10 12:10	02/01/10 17:29	7440-39-3	
Calcium	58900	mg/kg	108	13.7	5	02/01/10 12:10	02/01/10 17:29	7440-70-2	
Chromium	23.4	mg/kg	2.7	0.26	5	02/01/10 12:10	02/01/10 17:29	7440-47-3	
Copper	145	mg/kg	5.4	0.15	5	02/01/10 12:10	02/01/10 17:29	7440-50-8	
Iron	162000	mg/kg	54.2	10.7	5	02/01/10 12:10	02/01/10 17:29	7439-89-6	
Magnesium	123	mg/kg	108	7.6	5	02/01/10 12:10	02/01/10 17:29	7439-95-4	
Manganese	237	mg/kg	2.7	0.34	5	02/01/10 12:10	02/01/10 17:29	7439-96-5	
Nickel	132	mg/kg	5.4	0.22	5	02/01/10 12:10	02/01/10 17:29	7440-02-0	
Zinc	25.7	mg/kg	21.7	1.1	5	02/01/10 12:10	02/01/10 17:29	7440-66-6	

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ANALYTICAL RESULTS

Project: 1302398 BCCW METALS

Pace Project No.: 4028016

Sample: AD13172 SOLID Lab ID: 4028016004 Collected: 01/27/10 00:00 Received: 01/30/10 08:05 Matrix: Solid

Results reported on a "wet-weight" basis

Parameters	Results	Units	LOQ	LOD	DF	Prepared	Analyzed	CAS No.	Qual
6010 MET ICP									
Analytical Method: EPA 6010 Preparation Method: EPA 3050									
Barium	134	mg/kg	2.6	0.15	5	02/01/10 12:10	02/01/10 17:33	7440-39-3	
Calcium	74600	mg/kg	105	13.3	5	02/01/10 12:10	02/01/10 17:33	7440-70-2	
Chromium	19.8	mg/kg	2.6	0.25	5	02/01/10 12:10	02/01/10 17:33	7440-47-3	
Copper	102	mg/kg	5.3	0.14	5	02/01/10 12:10	02/01/10 17:33	7440-50-8	
Iron	148000	mg/kg	52.5	10.3	5	02/01/10 12:10	02/01/10 17:33	7439-89-6	
Magnesium	83.3J	mg/kg	105	7.4	5	02/01/10 12:10	02/01/10 17:33	7439-95-4	
Manganese	155	mg/kg	2.6	0.33	5	02/01/10 12:10	02/01/10 17:33	7439-96-5	
Nickel	68.0	mg/kg	5.3	0.22	5	02/01/10 12:10	02/01/10 17:33	7440-02-0	
Zinc	19.7J	mg/kg	21.0	1.0	5	02/01/10 12:10	02/01/10 17:33	7440-66-6	

Date: 02/05/2010 01:32 PM

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QUALITY CONTROL DATA

Project: 1302398 BCCW METALS
 Pace Project No.: 4026016

QC Batch:	MPPR/3541	Analysis Method:	EPA 6010
QC Batch Method:	EPA 3050	Analysis Description:	6010 MET
Associated Lab Samples:	4028016003, 4028016004		

METHOD BLANK: 260974 Matrix: Solid
 Associated Lab Samples: 4028016003, 4028016004

Parameter	Units	Blank Result	Reporting Limit	Analyzed	Qualifiers
Barium	mg/kg	<0.028	0.50	02/01/10 16:06	
Calcium	mg/kg	2.7J	20.0	02/01/10 16:06	
Chromium	mg/kg	0.11J	0.50	02/01/10 16:06	
Copper	mg/kg	<0.028	1.0	02/01/10 16:06	
Iron	mg/kg	2.8J	10.0	02/01/10 16:06	
Magnesium	mg/kg	<1.4	20.0	02/01/10 16:06	
Manganese	mg/kg	<0.062	0.50	02/01/10 16:06	
Nickel	mg/kg	<0.041	1.0	02/01/10 16:06	
Zinc	mg/kg	0.27J	4.0	02/01/10 16:06	

LABORATORY CONTROL SAMPLE: 260975

Parameter	Units	Spike Conc.	LCS Result	LCS % Rec	% Rec Limits	Qualifiers
Barium	mg/kg	50	46.1	92	80-120	
Calcium	mg/kg	500	501	100	80-120	
Chromium	mg/kg	50	48.0	96	80-120	
Copper	mg/kg	50	45.2	90	80-120	
Iron	mg/kg	500	493	99	80-120	
Magnesium	mg/kg	500	475	95	80-120	
Manganese	mg/kg	50	48.8	98	80-120	
Nickel	mg/kg	50	49.0	98	80-120	
Zinc	mg/kg	50	51.8	104	80-120	

MATRIX SPIKE & MATRIX SPIKE DUPLICATE: 260976 260977

Parameter	Units	4027980001		MS		MSD		% Rec	% Rec	% Rec Limits	Max RPD	Qual
		Result	Spike Conc.	Spike Conc.	Result	Result	% Rec					
Barium	mg/kg	65.1	64.4	63.6	119	120	84	86	75-125	9	20	
Calcium	mg/kg	43100	644	636	42300	33800	-122	-1470	75-125	22	20	P6,R1
Chromium	mg/kg	11.1	64.4	63.6	72.1	71.8	95	95	75-125	4	20	
Copper	mg/kg	39.7	64.4	63.6	93.9	104	84	101	75-125	10	20	
Iron	mg/kg	32000	644	636	26300	26700	-584	-628	75-125	2	20	P6
Magnesium	mg/kg	23200	644	636	23300	15100	19	-1280	75-125	43	20	P6,R1
Manganese	mg/kg	446	64.4	63.6	445	373	-2	-114	75-125	17	20	P6
Nickel	mg/kg	19.1	64.4	63.6	81.4	78.7	97	94	75-125	3	20	
Zinc	mg/kg	138	64.4	63.6	189	178	80	64	75-125	6	20	M0

Date: 02/05/2010 01:32 PM

REPORT OF LABORATORY ANALYSIS

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QUALITY CONTROL DATA

Project: 1302398 BCCW METALS
Pace Project No.: 4028016

QC Batch: MPRP/3545 Analysis Method: EPA 6010
QC Batch Method: EPA 3010 Analysis Description: 6010 MET
Associated Lab Samples: 4028016001, 4028016002

METHOD BLANK: 261047 Matrix: Water
Associated Lab Samples: 4028016001, 4028016002

Parameter	Units	Blank Result	Reporting Limit	Analyzed	Qualifiers
Barium	ug/L	0.22J	5.0	02/02/10 17:26	
Calcium	ug/L	<12.2	200	02/02/10 17:26	
Chromium	ug/L	0.74J	5.0	02/02/10 17:26	
Copper	ug/L	<0.29	10.0	02/02/10 17:26	
Iron	ug/L	6.7J	100	02/04/10 11:13	
Magnesium	ug/L	27.5J	200	02/04/10 11:13	
Manganese	ug/L	0.093J	5.0	02/02/10 17:26	
Nickel	ug/L	<0.23	10.0	02/02/10 17:26	
Zinc	ug/L	<2.0	40.0	02/02/10 17:26	

LABORATORY CONTROL SAMPLE: 261048

Parameter	Units	Spike Conc.	LCS Result	LCS % Rec	% Rec Limits	Qualifiers
Barium	ug/L	500	485	97	80-120	
Calcium	ug/L	5000	4670	93	80-120	
Chromium	ug/L	500	483	97	80-120	
Copper	ug/L	500	466	93	80-120	
Iron	ug/L	5000	5080	102	80-120	
Magnesium	ug/L	5000	4860	97	80-120	
Manganese	ug/L	500	474	95	80-120	
Nickel	ug/L	500	470	94	80-120	
Zinc	ug/L	500	474	95	80-120	

MATRIX SPIKE & MATRIX SPIKE DUPLICATE: 261049 261050

Parameter	Units	4028003001 Result	MS		MSD		MS % Rec	MSD % Rec	% Rec Limits	Max RPD	Qual
			Spike Conc.	MS Result	MSD Result	MSD Result					
Barium	ug/L	<0.18	500	473	489	95	98	75-125	3	20	
Calcium	ug/L	18.0J	5000	4510	4670	90	93	75-125	3	20	
Chromium	ug/L	<0.32	500	476	493	95	99	75-125	4	20	
Copper	ug/L	28.0	500	492	515	93	97	75-125	4	20	
Iron	ug/L	22.3J	5000	5010	5220	100	104	75-125	4	20	
Magnesium	ug/L	<13.4	5000	4710	4910	94	98	75-125	4	20	
Manganese	ug/L	0.25J	500	469	487	94	97	75-125	4	20	
Nickel	ug/L	<0.23	500	454	480	91	95	75-125	6	20	
Zinc	ug/L	8.1J	500	472	494	93	97	75-125	5	20	

Date: 02/05/2010 01:32 PM

REPORT OF LABORATORY ANALYSIS

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QUALIFIERS

Project: 1302398 BCCW METALS
 Pace Project No.: 4028015

DEFINITIONS

DF - Dilution Factor, if reported, represents the factor applied to the reported data due to changes in sample preparation, dilution of the sample aliquot, or moisture content.

ND - Not Detected at or above adjusted reporting limit.

J - Estimated concentration above the adjusted method detection limit and below the adjusted reporting limit.

MDL - Adjusted Method Detection Limit.

S - Surrogate

1,2-Diphenylhydrazine (8270 listed analyte) decomposes to Azobenzene.

Consistent with EPA guidelines, unrounded data are displayed and have been used to calculate % recovery and RPD values.

LCS(D) - Laboratory Control Sample (Duplicate)

MS(D) - Matrix Spike (Duplicate)

DUP - Sample Duplicate

RPD - Relative Percent Difference

NC - Not Calculable.

Pace Analytical is NELAP accredited. Contact your Pace PM for the current list of accredited analytes.

U - Indicates the compound was analyzed for, but not detected.

ANALYTE QUALIFIERS

M0	Matrix spike recovery and/or matrix spike duplicate recovery was outside laboratory control limits.
p6	Matrix spike recovery was outside laboratory control limits due to a parent sample concentration notably higher than the spike level.
R1	RPD value was outside control limits.

To: Bruce Rammie
PSB Annex A231



From: Laboratory Services Division
PSB Annex A070
WDNR Cert # 241329000

Report Date: Thursday, February 4, 2010

The following are the analytical results for the sample(s) received by Laboratory Services on 1/28/10 :

Sample Description: B2 + PCB-Oil A Solid
Sample ID: AD19187 **Serial/Impact ID:**
Sample Collector: OKWADA **Sample Collection Date:** 1/27/10 **Collection Time:** 00:00

<u>Parameter</u>	<u>Result</u>	<u>MDL</u>	<u>Units</u>	<u>Result</u>	<u>Analysis</u>	<u>Analysis</u>	<u>Analyst</u>
				<u>Flag</u>	<u>Method</u>	<u>Date</u>	
Sendout Analysis	See attached report					2/1/10	020

Sample Comments:

If there are any questions concerning this report, please contact Dave Kollakowsky at (414) 221-2835.

To: Bruce Rammie
PSB Annex A231



From: Laboratory Services Division
PSB Annex A070
WDNR Cert # 241329000

Report Date: Thursday, February 4, 2010

The following are the analytical results for the sample(s) received by Laboratory Services on 1/28/10 :

Sample Description: B1 + PCB-Oil B Solid
Sample ID: AD19188 Serial Impact ID:
Sample Collector: OKWADA Sample Collection Date: 1/27/10 Collection Time: 00:00

<u>Parameter</u>	<u>Result</u>	<u>MDL</u>	<u>Units</u>	<u>Result</u> <u>Flag</u>	<u>Analysis</u> <u>Method</u>	<u>Analysis</u> <u>Date</u>	<u>Analyst</u>
Sendout Analysis	See attached report					2/1/10	020

Sample Comments:

If there are any questions concerning this report, please contact Dave Kollinkowsky at (414) 221-2835.

To: Bruce Ramme
PSB Annex A331



From: Laboratory Services Division
PSB Annex A070
WDNR Cert # 241329000

Report Date: Thursday, February 4, 2010

The following are the analytical results for the sample(s) received by Laboratory Services on 1/28/10 :

Sample Description: B3 + PCB-Oil A Solid
Sample ID: AD19189 Serial/Impact ID:
Sample Collector: OKWADA Sample Collection Date: 1/27/10 Collection Time: 00:00

<u>Parameter</u>	<u>Result</u>	<u>MDL</u>	<u>Units</u>	<u>Result</u>	<u>Analysis</u>	<u>Analysis</u>	<u>Analyst</u>
				<u>Flag</u>	<u>Method</u>	<u>Date</u>	
Sendout Analysis	See attached report					2/1/10	020

Sample Comments:

If there are any questions concerning this report, please contact

Dave Kollakowsky at (414) 221-2835.

To: Bruce Ramme
PSB Annex A231



From: Laboratory Services Division
PSB Annex A070
WDNR Cert # 241329000

Report Date: Thursday, February 4, 2010

The following are the analytical results for the sample(s) received by Laboratory Services on 1/28/10 :

Sample Description: B3 + PCB-Oil B Solid
 Sample ID: AD19190 Serial/Impact ID:
 Sample Collector: OKWADA Sample Collection Date: 1/27/10 Collection Time: 00:00

<u>Parameter</u>	<u>Result</u>	<u>MDL</u>	<u>Units</u>	<u>Result</u>	<u>Analysis</u>	<u>Analysis</u>	<u>Analysis</u>
				<u>Flag</u>	<u>Method</u>	<u>Date</u>	<u>Analyst</u>
Sendout Analysis	See attached report					2/1/10	020

Sample Comments:

If there are any questions concerning this report, please contact Dave Kollakowsky at (414) 221-2835.

To: Bruce Ramme
PSB Annex A231



From: Laboratory Services Division
PSB Annex A070
WDNR Cert # 241329000

Report Date: Thursday, February 4, 2010

The following are the analytical results for the sample(s) received by Laboratory Services on 1/28/10 :

Sample Description: B1 + PCB in Hexane Solid
Sample ID: AD19191 Serial Impact ID:
Sample Collector: OKWADA Sample Collection Date: 1/27/10 Collection Time: 00:00

<u>Parameter</u>	<u>Result</u>	<u>MDL</u>	<u>Units</u>	<u>Result</u>	<u>Analysis</u>	<u>Analysis</u>	<u>Analysis</u>
				<u>Flag</u>	<u>Method</u>	<u>Date</u>	<u>Analyst</u>
Sendout Analysis	See attached report					2/1/10	020

Sample Comments:

If there are any questions concerning this report, please contact Dave Kollakowsky at (414) 221-2835.

To: Bruce Ramme
PSB Annex A231



From: Laboratory Services Division
PSB Annex A070
WDNR Cert # 241329000

Report Date: Thursday, February 4, 2010

The following are the analytical results for the sample(s) received by Laboratory Services on 1/28/10 :

Sample Description: B1 + PCB in Hexane Solid
Sample ID: AD19192 Serial Impact ID:
Sample Collector: OKWADA Sample Collection Date: 1/27/10 Collection Time: 00:00

<u>Parameter</u>	<u>Result</u>	<u>MDL</u>	<u>Units</u>	<u>Result</u>	<u>Analysis</u>	<u>Analysis</u>	<u>Analysis</u>
				<u>Flag</u>	<u>Method</u>	<u>Date</u>	<u>Analyst</u>
Sendout Analysis	See attached report					2/1/10	020

Sample Comments:

If there are any questions concerning this report, please contact Dave Kollakowsky at (414) 221-2835.

To: Bruce Ramme
PSB Annex A231



From: Laboratory Services Division
PSB Annex A070
WDNR Cert # 241329000

Report Date: Thursday, February 4, 2010

The following are the analytical results for the sample(s) received by Laboratory Services on 1/28/10 :

Sample Description: B3 + PCB in Hexane Solid
Sample ID: AD19193 Serial/Impact ID:
Sample Collector: OKWADA Sample Collection Date: 1/27/10 Collection Time: 00:00

<u>Parameter</u>	<u>Result</u>	<u>MDL</u>	<u>Units</u>	<u>Result</u>	<u>Analysis</u>	<u>Analysis</u>	<u>Analysis</u>
				<u>Flag</u>	<u>Method</u>	<u>Date</u>	<u>Analyst</u>
Sediment Analysis	See attached report					2/1/10	020

Sample Comments:

If there are any questions concerning this report, please contact

Dave Kolinkowsky at (414) 221-2835.



Pace Analytical Services, Inc.
1241 Bellevue Street - Suite 9
Green Bay, WI 54302
(920)489-2436

February 03, 2010

David Kollakowsky
We Energies
PO Box 2179
Room P129
Milwaukee, WI 532012179

RE: Project: 1302398 BECW SOLIDS
Pace Project No.: 4028014

Dear David Kollakowsky:

Enclosed are the analytical results for sample(s) received by the laboratory on January 30, 2010. The results relate only to the samples included in this report. Results reported herein conform to the most current NELAC standards, where applicable, unless otherwise narrated in the body of the report.

If you have any questions concerning this report, please feel free to contact me.

Sincerely,

A handwritten signature in black ink, appearing to read "Brian Basten".

Brian Basten

brian.basten@pacelabs.com
Project Manager

Enclosures

REPORT OF LABORATORY ANALYSIS

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CERTIFICATIONS

Project: 1302398 BECIW SOLIDS
Pace Project No.: 4028014

Green Bay Certification IDs

California Certification #: 09268CA
Florida/NE LAP Certification #: E87948
Illinois Certification #: 200050
Kentucky Certification #: 82
Louisiana Certification #: D4168
Minnesota Certification #: 055-999-334
New York Certification #: 11887

New York Certification #: 11888
North Carolina Certification #: 503
North Dakota Certification #: R-150
South Carolina Certification #: 83006001
Wisconsin Certification #: 405132750
Wisconsin DATCP Certification #: 105-444
1241 Bellevue Street Green Bay, WI 54302

REPORT OF LABORATORY ANALYSIS

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SAMPLE SUMMARY

Project: 1302398 BECW SOLIDS
Pace Project No.: 4028014

Lab ID	Sample ID	Matrix	Date Collected	Date Received
4028014001	AD19187	Solid	01/27/10 00:00	01/30/10 08:05
4028014002	AD19188	Solid	01/27/10 00:00	01/30/10 08:05
4028014003	AD19189	Solid	01/27/10 00:00	01/30/10 08:05
4028014004	AD19190	Solid	01/27/10 00:00	01/30/10 08:05
4028014005	AD19191	Solid	01/27/10 00:00	01/30/10 08:05
4028014006	AD19192	Solid	01/27/10 00:00	01/30/10 08:05
4028014007	AD19193	Solid	01/27/10 00:00	01/30/10 08:05

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SAMPLE ANALYTE COUNT

Project: 1302398 BECW SOLIDS
Pace Project No.: 4026014

Lab ID	Sample ID	Method	Analysts	Analytes Reported
4028014001	AD13187	EPA 8082	CAH	10
4028014002	AD13188	EPA 8082	CAH	10
4028014003	AD13189	EPA 8082	CAH	10
4028014004	AD13190	EPA 8082	CAH	10
4028014005	AD13191	EPA 8082	CAH	10
4028014006	AD13192	EPA 8082	CAH	10
4028014007	AD13193	EPA 8082	CAH	10

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ANALYTICAL RESULTS

Project: 1302398 BECW SOLIDS
Pace Project No.: 4028014

Sample: AD19187 Lab ID: 4028014001 Collected: 01/27/10 00:00 Received: 01/30/10 08:05 Matrix: Solid

Results reported on a "wet-weight" basis

Parameters	Results	Units	LOQ	LOD	DF	Prepared	Analyzed	CAS No.	Qual
8082 GCS PCB Analytical Method: EPA 8082 Preparation Method: EPA 3541									
PCB-1016 (Aroclor 1016)	<1130	ug/kg	4760	1130	1	02/01/10 13:58	02/02/10 16:48	12674-11-2	
PCB-1221 (Aroclor 1221)	<1130	ug/kg	4760	1130	1	02/01/10 13:58	02/02/10 16:48	11104-28-2	
PCB-1232 (Aroclor 1232)	<1130	ug/kg	4760	1130	1	02/01/10 13:58	02/02/10 16:48	11141-16-5	
PCB-1242 (Aroclor 1242)	<1130	ug/kg	4760	1130	1	02/01/10 13:58	02/02/10 16:48	53469-21-9	
PCB-1248 (Aroclor 1248)	<1130	ug/kg	4760	1130	1	02/01/10 13:58	02/02/10 16:48	12672-29-6	
PCB-1254 (Aroclor 1254)	<1130	ug/kg	4760	1130	1	02/01/10 13:58	02/02/10 16:48	11097-69-1	
PCB-1260 (Aroclor 1260)	<1130	ug/kg	4760	1130	1	02/01/10 13:58	02/02/10 16:48	11096-82-5	
PCB, Total	<1130	ug/kg	4760	1130	1	02/01/10 13:58	02/02/10 16:48	1336-36-3	
Tetrachloro-m-xylene (S)	62	%	50-137		1	02/01/10 13:58	02/02/10 16:48	877-09-8	
Decachlorobiphenyl (S)	74	%	56-130		1	02/01/10 13:58	02/02/10 16:48	2051-24-3	

Sample: AD19188 Lab ID: 4028014002 Collected: 01/27/10 00:00 Received: 01/30/10 08:05 Matrix: Solid

Results reported on a "wet-weight" basis

Parameters	Results	Units	LOQ	LOD	DF	Prepared	Analyzed	CAS No.	Qual
8082 GCS PCB Analytical Method: EPA 8082 Preparation Method: EPA 3541									
PCB-1016 (Aroclor 1016)	<738	ug/kg	3120	738	1	02/01/10 13:58	02/02/10 17:05	12674-11-2	
PCB-1221 (Aroclor 1221)	<738	ug/kg	3120	738	1	02/01/10 13:58	02/02/10 17:05	11104-28-2	
PCB-1232 (Aroclor 1232)	<738	ug/kg	3120	738	1	02/01/10 13:58	02/02/10 17:05	11141-16-5	
PCB-1242 (Aroclor 1242)	1230J	ug/kg	3120	738	1	02/01/10 13:58	02/02/10 17:05	53469-21-9	
PCB-1248 (Aroclor 1248)	<738	ug/kg	3120	738	1	02/01/10 13:58	02/02/10 17:05	12672-29-6	
PCB-1254 (Aroclor 1254)	<738	ug/kg	3120	738	1	02/01/10 13:58	02/02/10 17:05	11097-69-1	
PCB-1260 (Aroclor 1260)	816J	ug/kg	3120	738	1	02/01/10 13:58	02/02/10 17:05	11096-82-5	
PCB, Total	2050J	ug/kg	3120	738	1	02/01/10 13:58	02/02/10 17:05	1336-36-3	
Tetrachloro-m-xylene (S)	61	%	50-137		1	02/01/10 13:58	02/02/10 17:05	877-09-8	
Decachlorobiphenyl (S)	69	%	56-130		1	02/01/10 13:58	02/02/10 17:05	2051-24-3	

Sample: AD19189 Lab ID: 4028014003 Collected: 01/27/10 00:00 Received: 01/30/10 08:05 Matrix: Solid

Results reported on a "wet-weight" basis

Parameters	Results	Units	LOQ	LOD	DF	Prepared	Analyzed	CAS No.	Qual
8082 GCS PCB Analytical Method: EPA 8082 Preparation Method: EPA 3541									
PCB-1016 (Aroclor 1016)	<251	ug/kg	1060	251	1	02/01/10 13:58	02/02/10 17:23	12674-11-2	
PCB-1221 (Aroclor 1221)	<251	ug/kg	1060	251	1	02/01/10 13:58	02/02/10 17:23	11104-28-2	
PCB-1232 (Aroclor 1232)	<251	ug/kg	1060	251	1	02/01/10 13:58	02/02/10 17:23	11141-16-5	
PCB-1242 (Aroclor 1242)	883J	ug/kg	1060	251	1	02/01/10 13:58	02/02/10 17:23	53469-21-9	
PCB-1248 (Aroclor 1248)	<251	ug/kg	1060	251	1	02/01/10 13:58	02/02/10 17:23	12672-29-6	
PCB-1254 (Aroclor 1254)	505J	ug/kg	1060	251	1	02/01/10 13:58	02/02/10 17:23	11097-69-1	
PCB-1260 (Aroclor 1260)	774J	ug/kg	1060	251	1	02/01/10 13:58	02/02/10 17:23	11096-82-5	
PCB, Total	2170	ug/kg	1060	251	1	02/01/10 13:58	02/02/10 17:23	1336-36-3	

Date: 02/03/2010 03:17 PM

REPORT OF LABORATORY ANALYSIS

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ANALYTICAL RESULTS

Project: 1302398 BECW SOLIDS
Pace Project No.: 4028014

Sample: AD19189 Lab ID: 4028014003 Collected: 01/27/10 00:00 Received: 01/30/10 08:05 Matrix: Solid
Results reported on a "wet-weight" basis

Parameters	Results	Units	LOQ	LOD	DF	Prepared	Analyzed	CAS No.	Qual
8082 GCS PCB Analytical Method: EPA 8082 Preparation Method: EPA 3541									
Tetrachloro-m-xylene (S)	56 %		50-137		1	02/01/10 13:58	02/02/10 17:23	877-09-8	
Decachlorobiphenyl (S)	59 %		56-130		1	02/01/10 13:58	02/02/10 17:23	2051-24-3	

Sample: AD19190 Lab ID: 4028014004 Collected: 01/27/10 00:00 Received: 01/30/10 08:05 Matrix: Solid
Results reported on a "wet-weight" basis

Parameters	Results	Units	LOQ	LOD	DF	Prepared	Analyzed	CAS No.	Qual
8082 GCS PCB Analytical Method: EPA 8082 Preparation Method: EPA 3541									
PCB-1016 (Aroclor 1016)	<269 ug/kg		1140	269	1	02/01/10 13:58	02/02/10 17:40	12674-11-2	
PCB-1221 (Aroclor 1221)	<269 ug/kg		1140	269	1	02/01/10 13:58	02/02/10 17:40	11104-28-2	
PCB-1232 (Aroclor 1232)	<269 ug/kg		1140	269	1	02/01/10 13:58	02/02/10 17:40	11141-16-5	
PCB-1242 (Aroclor 1242)	1350 ug/kg		1140	269	1	02/01/10 13:58	02/02/10 17:40	53469-21-9	
PCB-1248 (Aroclor 1248)	<269 ug/kg		1140	269	1	02/01/10 13:58	02/02/10 17:40	12672-29-6	
PCB-1254 (Aroclor 1254)	846J ug/kg		1140	269	1	02/01/10 13:58	02/02/10 17:40	11097-69-1	
PCB-1260 (Aroclor 1260)	371J ug/kg		1140	269	1	02/01/10 13:58	02/02/10 17:40	11096-82-5	
PCB, Total	3160 ug/kg		1140	269	1	02/01/10 13:58	02/02/10 17:40	1336-36-3	
Tetrachloro-m-xylene (S)	50 %		50-137		1	02/01/10 13:58	02/02/10 17:40	877-09-8	
Decachlorobiphenyl (S)	55 %		56-130		1	02/01/10 13:58	02/02/10 17:40	2051-24-3	

Sample: AD19191 Lab ID: 4028014005 Collected: 01/27/10 00:00 Received: 01/30/10 08:05 Matrix: Solid
Results reported on a "wet-weight" basis

Parameters	Results	Units	LOQ	LOD	DF	Prepared	Analyzed	CAS No.	Qual
8082 GCS PCB Analytical Method: EPA 8082 Preparation Method: EPA 3541									
PCB-1016 (Aroclor 1016)	<407 ug/kg		1720	407	1	02/01/10 13:58	02/02/10 17:58	12674-11-2	
PCB-1221 (Aroclor 1221)	<407 ug/kg		1720	407	1	02/01/10 13:58	02/02/10 17:58	11104-28-2	
PCB-1232 (Aroclor 1232)	<407 ug/kg		1720	407	1	02/01/10 13:58	02/02/10 17:58	11141-16-5	
PCB-1242 (Aroclor 1242)	<407 ug/kg		1720	407	1	02/01/10 13:58	02/02/10 17:58	53469-21-9	
PCB-1248 (Aroclor 1248)	<407 ug/kg		1720	407	1	02/01/10 13:58	02/02/10 17:58	12672-29-6	
PCB-1254 (Aroclor 1254)	<407 ug/kg		1720	407	1	02/01/10 13:58	02/02/10 17:58	11097-69-1	
PCB-1260 (Aroclor 1260)	<407 ug/kg		1720	407	1	02/01/10 13:58	02/02/10 17:58	11096-82-5	
PCB, Total	<407 ug/kg		1720	407	1	02/01/10 13:58	02/02/10 17:58	1336-36-3	
Tetrachloro-m-xylene (S)	77 %		50-137		1	02/01/10 13:58	02/02/10 17:58	877-09-8	
Decachlorobiphenyl (S)	84 %		56-130		1	02/01/10 13:58	02/02/10 17:58	2051-24-3	

Date: 02/03/2010 03:17 PM

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ANALYTICAL RESULTS

Project: 1302398 BECW SOLIDS
Pace Project No.: 4028014

Sample: AD19192 Lab ID: 4029014006 Collected: 01/27/10 00:00 Received: 01/30/10 08:05 Matrix: Solid

Results reported on a "wet-weight" basis

Parameters	Results	Units	LOQ	LOD	DF	Prepared	Analyzed	CAS No.	Qual
8082 GCS PCB									
Analytical Method: EPA 8082 Preparation Method: EPA 3541									
PCB-1016 (Aroclor 1016)	<348	ug/kg	1470	348	1	02/01/10 13:58	02/02/10 18:15	12674-11-2	
PCB-1221 (Aroclor 1221)	<348	ug/kg	1470	348	1	02/01/10 13:58	02/02/10 18:15	11104-28-2	
PCB-1232 (Aroclor 1232)	<348	ug/kg	1470	348	1	02/01/10 13:58	02/02/10 18:15	11141-16-5	
PCB-1242 (Aroclor 1242)	<348	ug/kg	1470	348	1	02/01/10 13:58	02/02/10 18:15	53469-21-9	
PCB-1248 (Aroclor 1248)	<348	ug/kg	1470	348	1	02/01/10 13:58	02/02/10 18:15	12672-29-6	
PCB-1254 (Aroclor 1254)	<348	ug/kg	1470	348	1	02/01/10 13:58	02/02/10 18:15	11097-69-1	
PCB-1260 (Aroclor 1260)	2800	ug/kg	1470	348	1	02/01/10 13:58	02/02/10 18:15	11096-82-5	
PCB, Total	2800	ug/kg	1470	348	1	02/01/10 13:58	02/02/10 18:15	1336-36-3	
Tetrachloro-m-xylene (S)	75	%	50-137		1	02/01/10 13:58	02/02/10 18:15	877-09-8	
Decachlorobiphenyl (S)	85	%	56-130		1	02/01/10 13:58	02/02/10 18:15	2051-24-3	

Sample: AD19193 Lab ID: 4029014007 Collected: 01/27/10 00:00 Received: 01/30/10 08:05 Matrix: Solid

Results reported on a "wet-weight" basis

Parameters	Results	Units	LOQ	LOD	DF	Prepared	Analyzed	CAS No.	Qual
8082 GCS PCB									
Analytical Method: EPA 8082 Preparation Method: EPA 3541									
PCB-1016 (Aroclor 1016)	<211	ug/kg	893	211	1	02/01/10 13:58	02/02/10 18:33	12674-11-2	
PCB-1221 (Aroclor 1221)	<211	ug/kg	893	211	1	02/01/10 13:58	02/02/10 18:33	11104-28-2	
PCB-1232 (Aroclor 1232)	<211	ug/kg	893	211	1	02/01/10 13:58	02/02/10 18:33	11141-16-5	
PCB-1242 (Aroclor 1242)	<211	ug/kg	893	211	1	02/01/10 13:58	02/02/10 18:33	53469-21-9	
PCB-1248 (Aroclor 1248)	<211	ug/kg	893	211	1	02/01/10 13:58	02/02/10 18:33	12672-29-6	
PCB-1254 (Aroclor 1254)	<211	ug/kg	893	211	1	02/01/10 13:58	02/02/10 18:33	11097-69-1	
PCB-1260 (Aroclor 1260)	3490	ug/kg	893	211	1	02/01/10 13:58	02/02/10 18:33	11096-82-5	
PCB, Total	3490	ug/kg	893	211	1	02/01/10 13:58	02/02/10 18:33	1336-36-3	
Tetrachloro-m-xylene (S)	75	%	50-137		1	02/01/10 13:58	02/02/10 18:33	877-09-8	
Decachlorobiphenyl (S)	87	%	56-130		1	02/01/10 13:58	02/02/10 18:33	2051-24-3	

Date: 02/03/2010 03:17 PM

REPORT OF LABORATORY ANALYSIS

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QUALITY CONTROL DATA

Project: 1302398 BECW SOLIDS
Pace Project No.: 4028014

QC Batch: OEXT/5647 Analysis Method: EPA 8082
QC Batch Method: EPA 3541 Analysis Description: 8082 GCS PCB
Associated Lab Samples: 4028014001, 4028014002, 4028014003, 4028014004, 4028014005, 4028014006, 4028014007

METHOD BLANK: 261063 Matrix: Solid
Associated Lab Samples: 4028014001, 4028014002, 4028014003, 4028014004, 4028014005, 4028014006, 4028014007

Parameter	Units	Blank Result	Reporting Limit	Analyzed	Qualifiers
PCB-1016 (Aroclor 1016)	ug/kg	<23.6	100	02/02/10 15:55	
PCB-1221 (Aroclor 1221)	ug/kg	<23.6	100	02/02/10 15:55	
PCB-1232 (Aroclor 1232)	ug/kg	<23.6	100	02/02/10 15:55	
PCB-1242 (Aroclor 1242)	ug/kg	<23.6	100	02/02/10 15:55	
PCB-1248 (Aroclor 1248)	ug/kg	<23.6	100	02/02/10 15:55	
PCB-1254 (Aroclor 1254)	ug/kg	<23.6	100	02/02/10 15:55	
PCB-1260 (Aroclor 1260)	ug/kg	<23.6	100	02/02/10 15:55	
Decachlorobiphenyl (S)	%	80	56-130	02/02/10 15:55	
Tetrachloro-m-xylene (S)	%	77	50-137	02/02/10 15:55	

Parameter	Units	261064		261065		% Rec Limits	RPD	Max RPD	Qualifiers
		Spike Conc.	LCS Result	LCSD Result	LCS % Rec				
PCB-1016 (Aroclor 1016)	ug/kg		<23.6	<23.6					
PCB-1221 (Aroclor 1221)	ug/kg		<23.6	<23.6					
PCB-1232 (Aroclor 1232)	ug/kg		<23.6	<23.6					
PCB-1242 (Aroclor 1242)	ug/kg		<23.6	<23.6					
PCB-1248 (Aroclor 1248)	ug/kg		<23.6	<23.6					
PCB-1254 (Aroclor 1254)	ug/kg		<23.6	<23.6					
PCB-1260 (Aroclor 1260)	ug/kg		<23.6	<23.6					
Decachlorobiphenyl (S)	%	500	396	398	79	80	53-109	.7	
Tetrachloro-m-xylene (S)	%				83	84	56-130		
					80	76	50-137		

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QUALIFIERS

Project: 1302398 BECW SOLIDS
Pace Project No.: 4028014

DEFINITIONS

DF - Dilution Factor. If reported, represents the factor applied to the reported data due to changes in sample preparation, dilution of the sample aliquot, or moisture content.

ND - Not Detected at or above adjusted reporting limit.

J - Estimated concentration above the adjusted method detection limit and below the adjusted reporting limit.

MDL - Adjusted Method Detection Limit.

S - Surrogate

1,2-Diphenylhydrazine (8270 listed analyte) decomposes to Azobenzene.

Consistent with EPA guidelines, unrounded data are displayed and have been used to calculate % recovery and RPD values.

LCS(D) - Laboratory Control Sample (Duplicate)

MS(D) - Matrix Spike (Duplicate)

DUP - Sample Duplicate

RPD - Relative Percent Difference

NC - Not Calculable

Pace Analytical is NELAP accredited. Contact your Pace PM for the current list of accredited analytes.

U - Indicates the compound was analyzed for, but not detected.

BATCH QUALIFIERS

Batch: GCSV3949

[MS] A matrix spike/matrix spike duplicate was not performed for this batch due to insufficient sample volume.





Pace Analytical Services, Inc.
1241 Bellevue Street - Suite 9
Green Bay, WI 54302
(920)469-2436

February 11, 2010

David Kollakowsky
We Energies
PO Box 2179
Room P129
Milwaukee, WI 532012179

RE: Project: 1302398 BCCW LIQUIDS
Pace Project No.: 4028015

Dear David Kollakowsky:

Enclosed are the analytical results for sample(s) received by the laboratory on January 30, 2010. The results relate only to the samples included in this report. Results reported herein conform to the most current NELAC standards, where applicable, unless otherwise narrated in the body of the report.

If you have any questions concerning this report, please feel free to contact me.

Sincerely,

A handwritten signature in black ink, appearing to read "S. Mieczko".

Steven Mieczko for
Brian Basten
brian.basten@pacelabs.com
Project Manager

Enclosures

REPORT OF LABORATORY ANALYSIS

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CERTIFICATIONS

Project: 1302398 BCCW LIQUIDS
Pace Project No.: 4028015

Minnesota Certification IDs

Alaska Certification #: UST-078
Arizona Certification #: AZ-0014
California Certification #: 01155CA
Florida/NELAP Certification #: E87605
Illinois Certification #: 200011
Iowa Certification #: 368
Kansas Certification #: E-10167
Louisiana Certification #: 03086
Louisiana Certification #: LA080009
Maine Certification #: 2007029
Michigan DEQ Certification #: 9909
Minnesota Certification #: 027-053-137

Montana Certification #: MT CERT0092
New Jersey Certification #: MN-002
New York Certification #: 11647
North Carolina Certification #: 530
North Dakota Certification #: R-036
Oregon Certification #: MN200001
Pennsylvania Certification #: 68-00563
Tennessee Certification #: 02818
Washington Certification #: C754
Wisconsin Certification #: 999407970
1700 Elm Street SE, Suite 200 Minneapolis, MN 55414

Green Bay Certification IDs

California Certification #: 09268CA
Florida/NELAP Certification #: E87948
Illinois Certification #: 200050
Kentucky Certification #: 82
Louisiana Certification #: 04168
Minnesota Certification #: 055-999-334
New York Certification #: 11887

New York Certification #: 11888
North Carolina Certification #: 503
North Dakota Certification #: R-150
South Carolina Certification #: 83005001
Wisconsin Certification #: 405132750
Wisconsin DATCP Certification #: 105-444
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SAMPLE SUMMARY

Project: 1302398 BCCW LIQUIDS
 Pace Project No.: 4028015

Lab ID	Sample ID	Matrix	Date Collected	Date Received
4028015001	AD19194	Water	01/27/10 00:00	01/30/10 08:05
4028015002	AD19195	Water	01/27/10 00:00	01/30/10 08:05
4028015003	AD19196	Water	01/27/10 00:00	01/30/10 08:05
4028015004	AD19197-OIL LAYER	Water	01/27/10 00:00	01/30/10 08:05
4028015005	AD19198-OIL LAYER	Water	01/27/10 00:00	01/30/10 08:05
4028015006	AD19199-OIL LAYER	Water	01/27/10 00:00	01/30/10 08:05
4028015007	AD19200-WATER LAYER	Water	01/27/10 00:00	01/30/10 08:05
4028015008	AD19201-WATER LAYER	Water	01/27/10 00:00	01/30/10 08:05
4028015009	AD19202-WATER LAYER	Water	01/27/10 00:00	01/30/10 08:05

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SAMPLE ANALYTE COUNT

Project: 1302398 BCCW LIQUIDS
 Pace Project No.: 4028015

Lab ID	Sample ID	Method	Analysts	Analytes Reported	Laboratory
4028015001	AD13134	EPA 8082	CAH	10	PASI-G
4028015002	AD13135	EPA 8082	CAH	10	PASI-G
4028015003	AD13136	EPA 8082	CAH	10	PASI-G
4028015004	AD13137-OIL LAYER	EPA 8082	KL1	11	PASI-M
4028015005	AD13138-OIL LAYER	EPA 8082	KL1	11	PASI-M
4028015006	AD13139-OIL LAYER	EPA 8082	KL1	11	PASI-M
4028015007	AD13200-WATER LAYER	EPA 8082	CAH	10	PASI-G
4028015008	AD13201-WATER LAYER	EPA 8082	CAH	10	PASI-G
4028015009	AD13202-WATER LAYER	EPA 8082	CAH	10	PASI-G

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ANALYTICAL RESULTS

Project: 1302398 BCCW LIQUIDS
Pace Project No.: 4028015

Sample: AD13134		Lab ID: 4028015001	Collected: 01/27/10 00:00	Received: 01/30/10 08:05	Matrix: Water				
Parameters	Results	Units	LOQ	LOD	DF	Prepared	Analyzed	CAS No.	Qual
8082 GCS PCB Analytical Method: EPA 8082 Preparation Method: EPA 3510									
PCB-1016 (Aroclor 1016)	<6.2 ug/L		25.6	6.2	1	02/03/10 13:00	02/05/10 01:58	12674-11-2	
PCB-1221 (Aroclor 1221)	<6.2 ug/L		25.6	6.2	1	02/03/10 13:00	02/05/10 01:58	11104-28-2	
PCB-1232 (Aroclor 1232)	<6.2 ug/L		25.6	6.2	1	02/03/10 13:00	02/05/10 01:58	11141-16-5	
PCB-1242 (Aroclor 1242)	<6.2 ug/L		25.6	6.2	1	02/03/10 13:00	02/05/10 01:58	53469-21-9	
PCB-1248 (Aroclor 1248)	<6.2 ug/L		25.6	6.2	1	02/03/10 13:00	02/05/10 01:58	12672-29-6	
PCB-1254 (Aroclor 1254)	<6.2 ug/L		25.6	6.2	1	02/03/10 13:00	02/05/10 01:58	11097-69-1	
PCB-1260 (Aroclor 1260)	<6.2 ug/L		25.6	6.2	1	02/03/10 13:00	02/05/10 01:58	11096-82-5	
PCB, Total	<6.2 ug/L		25.6	6.2	1	02/03/10 13:00	02/05/10 01:58	1336-36-3	
Tetrachloro-m-xylene (S)	83 %		51-130		1	02/03/10 13:00	02/05/10 01:58	877-09-8	
Decachlorobiphenyl (S)	86 %		18-150		1	02/03/10 13:00	02/05/10 01:58	2051-24-3	

Sample: AD13135		Lab ID: 4028015002	Collected: 01/27/10 00:00	Received: 01/30/10 08:05	Matrix: Water				
Parameters	Results	Units	LOQ	LOD	DF	Prepared	Analyzed	CAS No.	Qual
8082 GCS PCB Analytical Method: EPA 8082 Preparation Method: EPA 3510									
PCB-1016 (Aroclor 1016)	<4.9 ug/L		20.4	4.9	1	02/03/10 13:00	02/05/10 02:16	12674-11-2	
PCB-1221 (Aroclor 1221)	<4.9 ug/L		20.4	4.9	1	02/03/10 13:00	02/05/10 02:16	11104-28-2	
PCB-1232 (Aroclor 1232)	<4.9 ug/L		20.4	4.9	1	02/03/10 13:00	02/05/10 02:16	11141-16-5	
PCB-1242 (Aroclor 1242)	<4.9 ug/L		20.4	4.9	1	02/03/10 13:00	02/05/10 02:16	53469-21-9	
PCB-1248 (Aroclor 1248)	<4.9 ug/L		20.4	4.9	1	02/03/10 13:00	02/05/10 02:16	12672-29-6	
PCB-1254 (Aroclor 1254)	<4.9 ug/L		20.4	4.9	1	02/03/10 13:00	02/05/10 02:16	11097-69-1	
PCB-1260 (Aroclor 1260)	6.3J ug/L		20.4	4.9	1	02/03/10 13:00	02/05/10 02:16	11096-82-5	
PCB, Total	6.3J ug/L		20.4	4.9	1	02/03/10 13:00	02/05/10 02:16	1336-36-3	
Tetrachloro-m-xylene (S)	73 %		51-130		1	02/03/10 13:00	02/05/10 02:16	877-09-8	
Decachlorobiphenyl (S)	71 %		18-150		1	02/03/10 13:00	02/05/10 02:16	2051-24-3	

Sample: AD13136		Lab ID: 4028015003	Collected: 01/27/10 00:00	Received: 01/30/10 08:05	Matrix: Water				
Parameters	Results	Units	LOQ	LOD	DF	Prepared	Analyzed	CAS No.	Qual
8082 GCS PCB Analytical Method: EPA 8082 Preparation Method: EPA 3510									
PCB-1016 (Aroclor 1016)	<6.0 ug/L		25.0	6.0	1	02/03/10 13:00	02/05/10 02:33	12674-11-2	
PCB-1221 (Aroclor 1221)	<6.0 ug/L		25.0	6.0	1	02/03/10 13:00	02/05/10 02:33	11104-28-2	
PCB-1232 (Aroclor 1232)	<6.0 ug/L		25.0	6.0	1	02/03/10 13:00	02/05/10 02:33	11141-16-5	
PCB-1242 (Aroclor 1242)	<6.0 ug/L		25.0	6.0	1	02/03/10 13:00	02/05/10 02:33	53469-21-9	
PCB-1248 (Aroclor 1248)	<6.0 ug/L		25.0	6.0	1	02/03/10 13:00	02/05/10 02:33	12672-29-6	
PCB-1254 (Aroclor 1254)	<6.0 ug/L		25.0	6.0	1	02/03/10 13:00	02/05/10 02:33	11097-69-1	
PCB-1260 (Aroclor 1260)	<6.0 ug/L		25.0	6.0	1	02/03/10 13:00	02/05/10 02:33	11096-82-5	
PCB, Total	<6.0 ug/L		25.0	6.0	1	02/03/10 13:00	02/05/10 02:33	1336-36-3	
Tetrachloro-m-xylene (S)	83 %		51-130		1	02/03/10 13:00	02/05/10 02:33	877-09-8	
Decachlorobiphenyl (S)	71 %		18-150		1	02/03/10 13:00	02/05/10 02:33	2051-24-3	

Date: 02/11/2010 09:07 AM

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ANALYTICAL RESULTS

Project: 1302398 BCCW LIQUIDS
Pace Project No.: 4028015

Sample: AD19197-OIL LAYER Lab ID: 4028015004 Collected: 01/27/10 00:00 Received: 01/30/10 08:05 Matrix: Water

Parameters	Results	Units	LOQ	LOD	DF	Prepared	Analyzed	CAS No.	Qual
8082 GCS PCB Oil		Analytical Method: EPA 8062							
PCB-1016 (Aroclor 1016)	<2500 ug/kg		5000	2500	1	02/04/10 17:01	02/09/10 21:44	12674-11-2	
PCB-1221 (Aroclor 1221)	<2500 ug/kg		5000	2500	1	02/04/10 17:01	02/09/10 21:44	11104-28-2	
PCB-1232 (Aroclor 1232)	<2500 ug/kg		5000	2500	1	02/04/10 17:01	02/09/10 21:44	11141-16-5	
PCB-1242 (Aroclor 1242)	<2500 ug/kg		5000	2500	1	02/04/10 17:01	02/09/10 21:44	53469-21-9	
PCB-1248 (Aroclor 1248)	<2500 ug/kg		5000	2500	1	02/04/10 17:01	02/09/10 21:44	12672-29-6	
PCB-1254 (Aroclor 1254)	<2500 ug/kg		5000	2500	1	02/04/10 17:01	02/09/10 21:44	11097-69-1	
PCB-1260 (Aroclor 1260)	<2500 ug/kg		5000	2500	1	02/04/10 17:01	02/09/10 21:44	11096-82-5	
PCB-1262 (Aroclor 1262)	<2500 ug/kg		5000	2500	1	02/04/10 17:01	02/09/10 21:44	37324-23-5	
PCB-1268 (Aroclor 1268)	<2500 ug/kg		5000	2500	1	02/04/10 17:01	02/09/10 21:44	11100-14-4	
Tetrachloro-m-xylene (S)	81 %		72-150		1	02/04/10 17:01	02/09/10 21:44	877-09-8	
Decachlorobiphenyl (S)	81 %		42-150		1	02/04/10 17:01	02/09/10 21:44	2051-24-3	

Sample: AD19198-OIL LAYER Lab ID: 4028015005 Collected: 01/27/10 00:00 Received: 01/30/10 08:05 Matrix: Water

Parameters	Results	Units	LOQ	LOD	DF	Prepared	Analyzed	CAS No.	Qual
8082 GCS PCB Oil		Analytical Method: EPA 8062							
PCB-1016 (Aroclor 1016)	<2500 ug/kg		5000	2500	1	02/04/10 17:01	02/09/10 22:00	12674-11-2	
PCB-1221 (Aroclor 1221)	<2500 ug/kg		5000	2500	1	02/04/10 17:01	02/09/10 22:00	11104-28-2	
PCB-1232 (Aroclor 1232)	<2500 ug/kg		5000	2500	1	02/04/10 17:01	02/09/10 22:00	11141-16-5	
PCB-1242 (Aroclor 1242)	<2500 ug/kg		5000	2500	1	02/04/10 17:01	02/09/10 22:00	53469-21-9	
PCB-1248 (Aroclor 1248)	<2500 ug/kg		5000	2500	1	02/04/10 17:01	02/09/10 22:00	12672-29-6	
PCB-1254 (Aroclor 1254)	<2500 ug/kg		5000	2500	1	02/04/10 17:01	02/09/10 22:00	11097-69-1	
PCB-1260 (Aroclor 1260)	<2500 ug/kg		5000	2500	1	02/04/10 17:01	02/09/10 22:00	11096-82-5	
PCB-1262 (Aroclor 1262)	<2500 ug/kg		5000	2500	1	02/04/10 17:01	02/09/10 22:00	37324-23-5	
PCB-1268 (Aroclor 1268)	<2500 ug/kg		5000	2500	1	02/04/10 17:01	02/09/10 22:00	11100-14-4	
Tetrachloro-m-xylene (S)	83 %		72-150		1	02/04/10 17:01	02/09/10 22:00	877-09-8	
Decachlorobiphenyl (S)	83 %		42-150		1	02/04/10 17:01	02/09/10 22:00	2051-24-3	

Sample: AD19199-OIL LAYER Lab ID: 4028015006 Collected: 01/27/10 00:00 Received: 01/30/10 08:05 Matrix: Water

Parameters	Results	Units	LOQ	LOD	DF	Prepared	Analyzed	CAS No.	Qual
8082 GCS PCB Oil		Analytical Method: EPA 8062							
PCB-1016 (Aroclor 1016)	<2500 ug/kg		5000	2500	1	02/04/10 17:01	02/09/10 22:16	12674-11-2	
PCB-1221 (Aroclor 1221)	<2500 ug/kg		5000	2500	1	02/04/10 17:01	02/09/10 22:16	11104-28-2	
PCB-1232 (Aroclor 1232)	<2500 ug/kg		5000	2500	1	02/04/10 17:01	02/09/10 22:16	11141-16-5	
PCB-1242 (Aroclor 1242)	<2500 ug/kg		5000	2500	1	02/04/10 17:01	02/09/10 22:16	53469-21-9	
PCB-1248 (Aroclor 1248)	<2500 ug/kg		5000	2500	1	02/04/10 17:01	02/09/10 22:16	12672-29-6	
PCB-1254 (Aroclor 1254)	<2500 ug/kg		5000	2500	1	02/04/10 17:01	02/09/10 22:16	11097-69-1	
PCB-1260 (Aroclor 1260)	6250 ug/kg		5000	2500	1	02/04/10 17:01	02/09/10 22:16	11096-82-5	
PCB-1262 (Aroclor 1262)	<2500 ug/kg		5000	2500	1	02/04/10 17:01	02/09/10 22:16	37324-23-5	

Date: 02/11/2010 09:07 AM

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ANALYTICAL RESULTS

Project: 1302398 BCCW LIQUIDS
Pace Project No.: 4028015

Sample: AD19199-OIL LAYER Lab ID: 4028015006 Collected: 01/27/10 00:00 Received: 01/30/10 08:05 Matrix: Water

Parameters	Results	Units	LOQ	LOD	DF	Prepared	Analyzed	CAS No.	Qual
8082 GCS PCB Oil Analytical Method: EPA 8082									
PCB-1268 (Aroclor 1268)	<2500	ug/kg	5000	2500	1	02/04/10 17:01	02/09/10 22:16	11100-14-4	
Tetrachloro-m-xylene (S)	82	%	72-150		1	02/04/10 17:01	02/09/10 22:16	877-09-8	
Decachlorobiphenyl (S)	89	%	42-150		1	02/04/10 17:01	02/09/10 22:16	2051-24-3	

Sample: AD19200-WATER LAYER Lab ID: 4028015007 Collected: 01/27/10 00:00 Received: 01/30/10 08:05 Matrix: Water

Parameters	Results	Units	LOQ	LOD	DF	Prepared	Analyzed	CAS No.	Qual
8082 GCS PCB Analytical Method: EPA 8082 Preparation Method: EPA 3510									
PCB-1016 (Aroclor 1016)	<6.9	ug/L	28.6	6.9	1	02/03/10 13:00	02/05/10 02:51	12674-11-2	
PCB-1221 (Aroclor 1221)	<6.9	ug/L	28.6	6.9	1	02/03/10 13:00	02/05/10 02:51	11104-28-2	
PCB-1232 (Aroclor 1232)	<6.9	ug/L	28.6	6.9	1	02/03/10 13:00	02/05/10 02:51	11141-16-5	
PCB-1242 (Aroclor 1242)	30.5	ug/L	28.6	6.9	1	02/03/10 13:00	02/05/10 02:51	53469-21-9	
PCB-1248 (Aroclor 1248)	<6.9	ug/L	28.6	6.9	1	02/03/10 13:00	02/05/10 02:51	12672-29-6	
PCB-1254 (Aroclor 1254)	<6.9	ug/L	28.6	6.9	1	02/03/10 13:00	02/05/10 02:51	11097-69-1	
PCB-1260 (Aroclor 1260)	28.0	ug/L	28.6	6.9	1	02/03/10 13:00	02/05/10 02:51	11096-82-5	
PCB, Total	58.5	ug/L	28.6	6.9	1	02/03/10 13:00	02/05/10 02:51	1336-36-3	
Tetrachloro-m-xylene (S)	71	%	51-130		1	02/03/10 13:00	02/05/10 02:51	877-09-8	
Decachlorobiphenyl (S)	68	%	18-150		1	02/03/10 13:00	02/05/10 02:51	2051-24-3	

Sample: AD19201-WATER LAYER Lab ID: 4028015008 Collected: 01/27/10 00:00 Received: 01/30/10 08:05 Matrix: Water

Parameters	Results	Units	LOQ	LOD	DF	Prepared	Analyzed	CAS No.	Qual
8082 GCS PCB Analytical Method: EPA 8082 Preparation Method: EPA 3510									
PCB-1016 (Aroclor 1016)	<7.3	ug/L	30.3	7.3	1	02/03/10 13:00	02/05/10 03:08	12674-11-2	
PCB-1221 (Aroclor 1221)	<7.3	ug/L	30.3	7.3	1	02/03/10 13:00	02/05/10 03:08	11104-28-2	
PCB-1232 (Aroclor 1232)	<7.3	ug/L	30.3	7.3	1	02/03/10 13:00	02/05/10 03:08	11141-16-5	
PCB-1242 (Aroclor 1242)	35.4	ug/L	30.3	7.3	1	02/03/10 13:00	02/05/10 03:08	53469-21-9	
PCB-1248 (Aroclor 1248)	<7.3	ug/L	30.3	7.3	1	02/03/10 13:00	02/05/10 03:08	12672-29-6	
PCB-1254 (Aroclor 1254)	<7.3	ug/L	30.3	7.3	1	02/03/10 13:00	02/05/10 03:08	11097-69-1	
PCB-1260 (Aroclor 1260)	32.2	ug/L	30.3	7.3	1	02/03/10 13:00	02/05/10 03:08	11096-82-5	
PCB, Total	67.6	ug/L	30.3	7.3	1	02/03/10 13:00	02/05/10 03:08	1336-36-3	
Tetrachloro-m-xylene (S)	71	%	51-130		1	02/03/10 13:00	02/05/10 03:08	877-09-8	
Decachlorobiphenyl (S)	70	%	18-150		1	02/03/10 13:00	02/05/10 03:08	2051-24-3	

Date: 02/11/2010 09:07 AM

REPORT OF LABORATORY ANALYSIS

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Pace Analytical Services, Inc.
1241 Bellevue Street - Suite 9
Green Bay, WI 54302
(920)469-2436

ANALYTICAL RESULTS

Project: 1302398 BCCW LIQUIDS
Pace Project No.: 4028015

Sample: AD19202-WATER LAYER Lab ID: 4028015009 Collected: 01/27/10 00:00 Received: 01/30/10 08:05 Matrix: Water

Parameters	Results	Units	LOQ	LOD	DF	Prepared	Analyzed	CAS No.	Qual
8082 GCS PCB									
Analytical Method: EPA 8082 Preparation Method: EPA 3510									
PCB-1016 (Aroclor 1016)	<6.9 ug/L		28.6	6.9	1	02/03/10 13:00	02/05/10 03:26	12674-11-2	
PCB-1221 (Aroclor 1221)	<6.9 ug/L		28.6	6.9	1	02/03/10 13:00	02/05/10 03:26	11104-28-2	
PCB-1232 (Aroclor 1232)	<6.9 ug/L		28.6	6.9	1	02/03/10 13:00	02/05/10 03:26	11141-16-5	
PCB-1242 (Aroclor 1242)	42.9 ug/L		28.6	6.9	1	02/03/10 13:00	02/05/10 03:26	53469-21-9	
PCB-1248 (Aroclor 1248)	<6.9 ug/L		28.6	6.9	1	02/03/10 13:00	02/05/10 03:26	12672-29-6	
PCB-1254 (Aroclor 1254)	<6.9 ug/L		28.6	6.9	1	02/03/10 13:00	02/05/10 03:26	11097-69-1	
PCB-1260 (Aroclor 1260)	38.6 ug/L		28.6	6.9	1	02/03/10 13:00	02/05/10 03:26	11096-82-5	
PCB, Total	81.5 ug/L		28.6	6.9	1	02/03/10 13:00	02/05/10 03:26	1336-36-3	
Tetrachloro-m-xylene (S)	70 %		51-130		1	02/03/10 13:00	02/05/10 03:26	877-09-8	
Decachlorobiphenyl (S)	67 %		18-150		1	02/03/10 13:00	02/05/10 03:26	2051-24-3	

Date: 02/11/2010 09:07 AM

REPORT OF LABORATORY ANALYSIS

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1241 Bellevue Street - Suite 9
Green Bay, WI 54302
(920)469-2426

QUALITY CONTROL DATA

Project: 1302398 BCCW LIQUIDS
Pace Project No.: 4028015

QC Batch: GCSV12327 Analysis Method: EPA 8082
QC Batch Method: EPA 8082 Analysis Description: 8082 GCS PCB Oil
Associated Lab Samples: 4028015004, 4028015005, 4028015006

METHOD BLANK: 744753 Matrix: Oil

Associated Lab Samples: 4028015004, 4028015005, 4028015006

Parameter	Units	Blank Result	Reporting Limit	Analyzed	Qualifiers
PCB-1016 (Aroclor 1016)	ug/kg	<2500	5000	02/09/10 20:08	
PCB-1221 (Aroclor 1221)	ug/kg	<2500	5000	02/09/10 20:08	
PCB-1232 (Aroclor 1232)	ug/kg	<2500	5000	02/09/10 20:08	
PCB-1242 (Aroclor 1242)	ug/kg	<2500	5000	02/09/10 20:08	
PCB-1248 (Aroclor 1248)	ug/kg	<2500	5000	02/09/10 20:08	
PCB-1254 (Aroclor 1254)	ug/kg	<2500	5000	02/09/10 20:08	
PCB-1260 (Aroclor 1260)	ug/kg	<2500	5000	02/09/10 20:08	
PCB-1262 (Aroclor 1262)	ug/kg	<2500	5000	02/09/10 20:08	
PCB-1268 (Aroclor 1268)	ug/kg	<2500	5000	02/09/10 20:08	
Decachlorobiphenyl (S)	%	107	42-150	02/09/10 20:08	
Tetrachloro-m-xylene (S)	%	120	72-150	02/09/10 20:08	

LABORATORY CONTROL SAMPLE: 744754

Parameter	Units	Spike Conc.	LCS Result	LCS % Rec	% Rec Limits	Qualifiers
PCB-1016 (Aroclor 1016)	ug/kg	100000	118000	118	75-134	
PCB-1260 (Aroclor 1260)	ug/kg	100000	114000	114	75-138	
Decachlorobiphenyl (S)	%			114	42-150	
Tetrachloro-m-xylene (S)	%			123	72-150	

MATRIX SPIKE & MATRIX SPIKE DUPLICATE: 744755 744756

Parameter	Units	10121728001 Result	MS		MSD		MS % Rec	MSD % Rec	% Rec Limits	Max RPD	Qual
			Spike Conc.	Result	Spike Conc.	Result					
PCB-1016 (Aroclor 1016)	ug/kg	ND	100000	100000	103000	102000	103	102	45-150	1	30
PCB-1260 (Aroclor 1260)	ug/kg	ND	100000	100000	76300	78300	76	78	40-150	3	30
Decachlorobiphenyl (S)	%						78	79	42-150		
Tetrachloro-m-xylene (S)	%						114	115	72-150		

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1241 Bellevue Street - Suite 9
Green Bay, WI 54302
(920)469-2436

QUALITY CONTROL DATA

Project: 1302398 BCCW LIQUIDS
Pace Project No.: 4028015

QC Batch: OEXT/6662 Analysis Method: EPA 8082
QC Batch Method: EPA 3510 Analysis Description: 8082 GCS PCB
Associated Lab Samples: 4028015001, 4028015002, 4028015003, 4028015007, 4028015008, 4028015009

METHOD BLANK: 261663 Matrix: Water
Associated Lab Samples: 4028015001, 4028015002, 4028015003, 4028015007, 4028015008, 4028015009

Parameter	Units	Blank Result	Reporting Limit	Analyzed	Qualifiers
PCB-1016 (Aroclor 1016)	ug/L	<-0.24	1.0	02/05/10 01:05	
PCB-1221 (Aroclor 1221)	ug/L	<-0.24	1.0	02/05/10 01:05	
PCB-1232 (Aroclor 1232)	ug/L	<-0.24	1.0	02/05/10 01:05	
PCB-1242 (Aroclor 1242)	ug/L	<-0.24	1.0	02/05/10 01:05	
PCB-1248 (Aroclor 1248)	ug/L	<-0.24	1.0	02/05/10 01:05	
PCB-1254 (Aroclor 1254)	ug/L	<-0.24	1.0	02/05/10 01:05	
PCB-1260 (Aroclor 1260)	ug/L	<-0.24	1.0	02/05/10 01:05	
Decachlorobiphenyl (S)	%	88	18-150	02/05/10 01:05	
Tetrachloro-m-xylene (S)	%	73	51-130	02/05/10 01:05	

Parameter	Units	261664		261665		LCS % Rec	LCSD % Rec	% Rec Limits	RPD	Max RPD	Qualifiers
		Spike Conc.	LCS Result	LCSD Result	LCS % Rec						
PCB-1016 (Aroclor 1016)	ug/L		<-0.24	<-0.24						20	
PCB-1221 (Aroclor 1221)	ug/L		<-0.24	<-0.24						20	
PCB-1232 (Aroclor 1232)	ug/L		<-0.24	<-0.24						20	
PCB-1242 (Aroclor 1242)	ug/L		<-0.24	<-0.24						20	
PCB-1248 (Aroclor 1248)	ug/L		<-0.24	<-0.24						20	
PCB-1254 (Aroclor 1254)	ug/L		<-0.24	<-0.24						20	
PCB-1260 (Aroclor 1260)	ug/L		<-0.24	<-0.24						20	
Decachlorobiphenyl (S)	%	5	4.6	4.5	92	91	62-130	1		20	
Tetrachloro-m-xylene (S)	%				92	93	18-150				
					63	65	51-130				

Date: 02/11/2010 09:07 AM

REPORT OF LABORATORY ANALYSIS

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1241 Bellevue Street - Suite 9
Green Bay, WI 54302
(920)469-2436

QUALIFIERS

Project: 1302398 BCCW LIQUIDS
Pace Project No.: 4028015

DEFINITIONS

DF - Dilution Factor, if reported, represents the factor applied to the reported data due to changes in sample preparation, dilution of the sample aliquot, or moisture content.

ND - Not Detected at or above adjusted reporting limit.

J - Estimated concentration above the adjusted method detection limit and below the adjusted reporting limit.

MDL - Adjusted Method Detection Limit.

S - Surrogate

1,2-Diphenylhydrazine (8270 listed analyte) decomposes to Azobenzene.

Consistent with EPA guidelines, unrounded data are displayed and have been used to calculate % recovery and RPD values.

LCS(D) - Laboratory Control Sample (Duplicate)

MS(D) - Matrix Spike (Duplicate)

DUP - Sample Duplicate

RPD - Relative Percent Difference

NC - Not Calculable.

Pace Analytical is NELAP accredited. Contact your Pace PM for the current list of accredited analytes.

U - Indicates the compound was analyzed for, but not detected.

LABORATORIES

PASI-G Pace Analytical Services - Green Bay

PASI-M Pace Analytical Services - Minneapolis

BATCH QUALIFIERS

Batch: GCSV3958

[M5] A matrix spike/matrix spike duplicate was not performed for this batch due to insufficient sample volume.



APPENDIX D

(A) PERMEABILITY TEST RESULTS FOR CONTROL SAMPLES

Giles Engineering Associates, Inc.

GEOTECHNICAL, ENVIRONMENTAL AND CONSTRUCTION MATERIALS CONSULTANTS
 N8 W22350 JOHNSON DRIVE, SUITE A1 / WAUKESHA, WI 53186 / (262) 544-0118 / FAX: (262) 549-5868

REPORT OF FLEXIBLE WALL PERMEABILITY TEST (ASTM D 5084)

CLIENT: UW-Milwaukee Dept. of Civil Eng.
 PO Box 784 EMS Room 562-C/O Dr. Jin Li
 Milwaukee, Wisconsin 53201784

PROJECT: Biocontainment Research
 Milwaukee, WI

DATE: March 05, 2009

PROJECT NO.: 1M-0902023

SAMPLE PREPARATION

Height (in): 4.09	Maximum Effective Consolidation (psi): 5.0
Diameter (in): 2.35	Minimum Effective Consolidation (psi): 4.0
Area (sq in): 4.34	Back Pressure (psi): 55.0
Membrane Type: Latex	B Parameter (%): 0.94
Hydraulic Gradient: 13.5	Permeant Fluid: Deaired water

TEST DATA

Date	Clock	Time (min)	Burette Change		Flow (mL)	Permeability (cm/s)
			lower	upper		
2/27/09	10:43	1	90.0000	91.0000	90.5000	7.94E-03
2/27/09	10:45	1	91.0000	91.0000	91.0000	7.98E-03
2/27/09	10:47	1	91.5000	92.0000	91.7500	8.05E-03
2/27/09	10:49	1	91.0000	92.0000	91.5000	8.02E-03
Time Weighted Average Hydraulic Conductivity, K (cm/s): 8.00E-03						

SAMPLE CHARACTERISTICS

% W	Dd (pcf)	P200	% Clay	LL	PL	PI	Max. Dd (pcf)	Optimum % W

<u>Material Description</u>	<u>Sample Location and Depth</u>
Concrete Cylinder No. 1	
Date Received: 2/14/09 Sample No.: A09044A	

Reviewing Engineer: Charles S. Gresser, P.E.

Giles Engineering Associates, Inc.
 GEOTECHNICAL, ENVIRONMENTAL AND CONSTRUCTION MATERIALS CONSULTANTS
 N8 W22350 JOHNSON DRIVE, SUITE A1 / WAUKESHA, WI 53186 / (262) 544-0118 / FAX: (262) 549-5868

REPORT OF FLEXIBLE WALL PERMEABILITY TEST (ASTM D 5084)

CLIENT: UW-Milwaukee Dept. of Civil Eng. PROJECT: Biocontainment Research
 PO Box 794 EMS Room 562-C/O Dr. Jin Li
 Milwaukee, Wisconsin 53201794 Milwaukee, WI

DATE: March 05, 2009

PROJECT NO.: 1M-0902023

SAMPLE PREPARATION

Height (in): 4.09	Maximum Effective Consolidation (psi): 10.0
Diameter (in): 2.35	Minimum Effective Consolidation (psi): 9.0
Area (sq in): 4.34	Back Pressure (psi): 55.0
Membrane Type: Latex	B Parameter (%): 0.94
Hydraulic Gradient: 13.5	Permeant Fluid: Deaired water

TEST DATA

Date	Clock	Time (min)	Burette Change		Flow (mL)	Permeability (cm/s)
			lower	upper		
2/27/09	10:55	1	91.0000	91.0000	91.0000	7.98E-03
2/27/09	10:57	1	91.0000	91.0000	91.0000	7.98E-03
2/27/09	10:59	1	91.0000	92.0000	91.5000	8.02E-03
2/27/09	11:01	1	91.5000	91.0000	91.2500	8.00E-03
Time Weighted Average Hydraulic Conductivity, K (cm/s): 8.00E-03						

SAMPLE CHARACTERISTICS

% W	Dd (pcf)	P200	% Clay	LL	PL	PI	Max. Dd (pcf)	Optimum % W

<u>Material Description</u>	<u>Sample Location and Depth</u>
Concrete Cylinder No. 1	
Date Received: 2/14/09 Sample No.: A09044B	

Reviewing Engineer: Charles S. Gresser, P.E.

Giles Engineering Associates, Inc.

GEOTECHNICAL, ENVIRONMENTAL AND CONSTRUCTION MATERIALS CONSULTANTS
N6 W22350 JOHNSON DRIVE, SUITE A1 / WAUKESHA, WI 53186 / (262) 544-0118 / FAX: (262) 549-5868

REPORT OF FLEXIBLE WALL PERMEABILITY TEST (ASTM D 5084)

CLIENT: UW-Milwaukee Dept. of Civil Eng. PROJECT: Biocontainment Research
PO Box 784 EMS Room 562-C/O Dr. Jin Li
Milwaukee, Wisconsin 53201784 Milwaukee, WI
DATE: March 05, 2009 PROJECT NO.: 1M-0902023

SAMPLE PREPARATION

Height (in): 4.09	Maximum Effective Consolidation (psi): 20.0
Diameter (in): 2.35	Minimum Effective Consolidation (psi): 19.0
Area (sq in): 4.34	Back Pressure (psi): 55.0
Membrane Type: Latex	B Parameter (%): 0.94
Hydraulic Gradient: 13.5	Permeant Fluid: Deaired water

TEST DATA

Date	Clock	Time (min)	Burette Change		Flow (mL)	Permeability (cm/s)
			lower	upper		
2/27/09	11:08	1	90.0000	90.0000	90.0000	7.89E-03
2/27/09	11:10	1	90.0000	90.0000	90.0000	7.89E-03
2/27/09	11:12	1	90.5000	91.0000	90.7500	7.96E-03
2/27/09	11:14	1	90.0000	91.0000	90.5000	7.94E-03
Time Weighted Average Hydraulic Conductivity, K (cm/s): 7.92E-03						

SAMPLE CHARACTERISTICS

% W	Dd (pcf)	P200	% Clay	LL	PL	PI	Max. Dd (pcf)	Optimum % W

<u>Material Description</u>	<u>Sample Location and Depth</u>
Concrete Cylinder No. 1	
Date Received: 2/14/09 Sample No.: A09044C	

Reviewing Engineer: Charles S. Gresser, P.E.

Giles Engineering Associates, Inc.

GEOTECHNICAL, ENVIRONMENTAL AND CONSTRUCTION MATERIALS CONSULTANTS
N8 W22350 JOHNSON DRIVE, SUITE A1 / WAUKESHA, WI 53186 / (262) 544-2118 / FAX: (262) 549-5668

REPORT OF FLEXIBLE WALL PERMEABILITY TEST (ASTM D 5084)

CLIENT: UW-Milwaukee Dept. of Civil Eng.
PO Box 784 EMS Room 562-C/O Dr. Jin Li
Milwaukee, Wisconsin 53201784

PROJECT: Biocontainment Research
Milwaukee, WI

DATE: March 05, 2009

PROJECT NO.: 1M-0902023

SAMPLE PREPARATION

Height (in): 4.06	Maximum Effective Consolidation (psi): 5.0
Diameter (in): 2.35	Minimum Effective Consolidation (psi): 4.0
Area (sq in): 4.34	Back Pressure (psi):
Membrane Type: Latex	B Parameter (%): 0.94
Hydraulic Gradient: 13.6	Permeant Fluid: Deaired water

TEST DATA

Date	Clock	Time (min)	Burette Change		Flow (mL)	Permeability (cm/s)
			lower	upper		
2/27/09	14:38	1	100.0000	100.0000	100.0000	8.70E-03
2/27/09	14:51	1	102.0000	101.0000	101.5000	8.84E-03
2/27/09	14:53	1	101.5000	102.0000	101.7500	8.86E-03
2/27/09	14:55	1	102.5000	102.5000	102.5000	8.92E-03
Time Weighted Average Hydraulic Conductivity, K (cm/s): 8.83E-03						

SAMPLE CHARACTERISTICS

% W	Dd (pcf)	P200	% Clay	LL	PL	PI	Max. Dd (pcf)	Optimum % W

<u>Material Description</u> Concrete Cylinder No. 2	<u>Sample Location and Depth</u>
Date Received: 2/14/09 Sample No.: A09045A	

Reviewing Engineer: Charles S. Gresser, P.E.

Giles Engineering Associates, Inc.

GEOTECHNICAL, ENVIRONMENTAL AND CONSTRUCTION MATERIALS CONSULTANTS
N8 W22350 JOHNSON DRIVE, SUITE A1 / WAUKESHA, WI 53186 / (262) 544-0118 / FAX: (262) 549-5668

REPORT OF FLEXIBLE WALL PERMEABILITY TEST (ASTM D 5084)

CLIENT: UW-Milwaukee Dept. of Civil Eng.
PO Box 784 EMS Room 562-C/O Dr. Jin Li
Milwaukee, Wisconsin 53201794

PROJECT: Biocontainment Research
Milwaukee, WI

DATE: March 05, 2009

PROJECT NO.: 1M-0902023

SAMPLE PREPARATION

Height (in): 4.06	Maximum Effective Consolidation (psi): 10.0
Diameter (in): 2.35	Minimum Effective Consolidation (psi): 9.0
Area (sq in): 4.34	Back Pressure (psi): 60.0
Membrane Type: Latex	B Parameter (%): 0.94
Hydraulic Gradient: 13.6	Permeant Fluid: Deaired water

TEST DATA

Date	Clock	Time (min)	Burette Change		Flow (mL)	Permeability (cm/s)
			lower	upper		
2/27/09	14:58	1	104.0000	106.0000	105.0000	9.14E-03
2/27/09	15:00	1	108.0000	109.0000	108.5000	9.44E-03
2/27/09	15:02	1	108.0000	109.0000	108.5000	9.44E-03
2/27/09	15:04	1	107.0000	107.0000	107.0000	9.31E-03
Time Weighted Average Hydraulic Conductivity, K (cm/s): 9.34E-03						

SAMPLE CHARACTERISTICS

% W	Dd (pcf)	P200	% Clay	LL	PL	PI	Max. Dd (pcf)	Optimum % W

<u>Material Description</u> Concrete Cylinder No. 2	<u>Sample Location and Depth</u>
Date Received: 2/14/09 Sample No.: A09045B	

Reviewing Engineer: Charles S. Gresser, P.E.

Giles Engineering Associates, Inc.

GEOTECHNICAL, ENVIRONMENTAL AND CONSTRUCTION MATERIALS CONSULTANTS
N6 W22350 JOHNSON DRIVE, SUITE A1 / WALKESHA, WI 53186 / (262) 544-0118 / FAX: (262) 549-5668

REPORT OF FLEXIBLE WALL PERMEABILITY TEST (ASTM D 5084)

CLIENT: UW-Milwaukee Dept. of Civil Eng.
PO Box 784 EMS Room 562-C/O Dr. Jin Li
Milwaukee, Wisconsin 53201784

PROJECT: Biocontainment Research
Milwaukee, WI

DATE: March 05, 2009

PROJECT NO.: 1M-0902023

SAMPLE PREPARATION

Height (in): 4.06	Maximum Effective Consolidation (psi): 20.0
Diameter (in): 2.35	Minimum Effective Consolidation (psi): 19.0
Area (sq in): 4.34	Back Pressure (psi): 60.0
Membrane Type: Latex	B Parameter (%): 0.94
Hydraulic Gradient: 13.6	Permeant Fluid: Deaired water

TEST DATA

Date	Clock	Time (min)	Burette Change		Flow (mL)	Permeability (cm/s)
			lower	upper		
2/27/09	15:08	1	112.0000	112.5000	112.2500	9.77E-03
2/27/09	15:10	1	112.0000	113.0000	112.5000	9.79E-03
2/27/09	15:12	1	112.0000	111.0000	111.5000	9.71E-03
2/27/09	15:14	1	110.0000	110.5000	110.2500	9.60E-03
Time Weighted Average Hydraulic Conductivity, K (cm/s): 9.72E-03						

SAMPLE CHARACTERISTICS

% W	Dd (pcf)	P200	% Clay	LL	PL	PI	Max. Dd (pcf)	Optimum % W

<u>Material Description</u> Concrete Cylinder No. 2	<u>Sample Location and Depth</u>
Date Received: 2/14/09 Sample No.: A09045C	

Reviewing Engineer: Charles S. Gresser, P.E.

Giles Engineering Associates, Inc.
GEOTECHNICAL, ENVIRONMENTAL AND CONSTRUCTION MATERIALS CONSULTANTS
 N8 W22350 JOHNSON DRIVE, SUITE A1 / WAUKESHA, WI 53186 / (262) 544-0118 / FAX: (262) 549-8668

REPORT OF FLEXIBLE WALL PERMEABILITY TEST (ASTM D 5084)

CLIENT: UW-Milwaukee Dept. of Civil Eng. PROJECT: Biocontainment Research
 PO Box 784 EMS Room 562-C/O Dr. Jin Li
 Milwaukee, Wisconsin 53201784 Milwaukee, WI

DATE: March 05, 2009

PROJECT NO.: 1M-0902023

SAMPLE PREPARATION

Height (in): 4.05	Maximum Effective Consolidation (psi): 10.0
Diameter (in): 2.35	Minimum Effective Consolidation (psi): 9.0
Area (sq in): 4.34	Back Pressure (psi): 60.0
Membrane Type: Latex	B Parameter (%): 0.92
Hydraulic Gradient: 13.7	Permeant Fluid: Deaired water

TEST DATA

Date	Clock	Time (min)	Burette Change		Flow (mL)	Permeability (cm/s)
			lower	upper		
3/2/09	14:05	1	84.0000	83.5000	83.7500	7.27E-03
3/2/09	14:07	1	83.5000	84.5000	84.0000	7.29E-03
3/2/09	14:09	1	82.5000	83.0000	82.7500	7.19E-03
3/2/09	14:11	1	82.5000	83.5000	83.0000	7.21E-03

Time Weighted Average Hydraulic Conductivity, K (cm/s): 7.24E-03

SAMPLE CHARACTERISTICS

% W	Dd (pcf)	P200	% Clay	LL	PL	PI	Max. Dd (pcf)	Optimum % W

<u>Material Description</u>	<u>Sample Location and Depth</u>
Concrete Cylinder No. 3	

Date Received: 2/14/09 Sample No.: A09046B	
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Reviewing Engineer: Charles S. Gresser, P.E.

(B) PERMEABILITY TEST RESULTS FOR THE TEST SAMPLES**Giles Engineering Associates, Inc.**

GEOTECHNICAL, ENVIRONMENTAL AND CONSTRUCTION MATERIALS CONSULTANTS
 N8 W22350 JOHNSON DRIVE, SUITE A1 / WALKESHA, WI 53186 / (262) 544-0118 / FAX: (262) 549-3668

REPORT OF FLEXIBLE WALL PERMEABILITY TEST (ASTM D 5084)

CLIENT: UW-Milwaukee Dept. of Civil Eng.
 PO Box 794 EMS Room 562-C/O Dr. Jin Li
 Milwaukee, Wisconsin 53201784

PROJECT: Biocontainment Research
 Milwaukee, WI

DATE: September 25, 2009

PROJECT NO.: 1M-0902023

SAMPLE PREPARATION

Height (in): 4.02	Maximum Effective Consolidation (psi): 5.0
Diameter (in): 2.35	Minimum Effective Consolidation (psi): 3.0
Area (sq in): 4.34	Back Pressure (psi): 55.0
Membrane Type: Latex	B Parameter (%): 0.94
Hydraulic Gradient: 13.8	Permeant Fluid: Deaired water

TEST DATA

Date	Clock	Time (min)	Burette Change		Flow (mL)	Permeability (cm/s)
			lower	upper		
9/16/09	11:22	111	0.1245	0.1394	0.1320	5.12E-08
9/16/09	13:11	109	0.1079	0.1148	0.1114	4.40E-08
9/16/09	13:53	42	0.0415	0.0451	0.0433	4.44E-08
9/16/09	15:12	79	0.0830	0.0779	0.0805	4.39E-08
Time Weighted Average Hydraulic Conductivity, K (cm/s): 4.64E-08						

SAMPLE CHARACTERISTICS

% W	Dd (pcf)	P200	% Clay	LL	PL	PI	Max. Dd (pcf)	Optimum % W

<u>Material Description</u>	<u>Sample Location and Depth</u>
Concrete Cylinder A-1	
Date Received: 9/15/09 Sample No.: A09475A	

Reviewing Engineer: Charles S. Gresser, P.E.

Giles Engineering Associates, Inc.

GEOTECHNICAL, ENVIRONMENTAL AND CONSTRUCTION MATERIALS CONSULTANTS
N8 W22350 JOHNSON DRIVE, SUITE A1 / WAUKESHA, WI 53186 / (262) 544-0118 / FAX: (262) 549-5668

REPORT OF FLEXIBLE WALL PERMEABILITY TEST (ASTM D 5084)

CLIENT: UW-Milwaukee Dept. of Civil Eng. PO Box 784 EMS Room 562-C/O Dr. Jin Li Milwaukee, Wisconsin 53201784	PROJECT: Biocontainment Research Milwaukee, WI
DATE: September 25, 2009	PROJECT NO.: 1M-0902023

SAMPLE PREPARATION

Height (in): 4.02	Maximum Effective Consolidation (psi): 10.0
Diameter (in): 2.35	Minimum Effective Consolidation (psi): 8.0
Area (sq in): 4.34	Back Pressure (psi): 55.0
Membrane Type: Latex	B Parameter (%): 0.94
Hydraulic Gradient: 13.8	Permeant Fluid: Deaired water

TEST DATA

Date	Clock	Time (min)	Burette Change		Flow (mL)	Permeability (cm/s)
			lower	upper		
9/17/09	9:09	63	0.0332	0.0410	0.0371	2.54E-08
9/17/09	10:04	55	0.0415	0.0369	0.0392	3.07E-08
9/17/09	12:46	162	0.0996	0.1148	0.1072	2.85E-08
9/17/09	14:02	76	0.0498	0.0492	0.0495	2.81E-08
Time Weighted Average Hydraulic Conductivity, K (cm/s): 2.82E-08						

SAMPLE CHARACTERISTICS

% W	Dd (pcf)	P200	% Clay	LL	PL	PI	Max. Dd (pcf)	Optimum % W

Material Description
Concrete Cylinder A-1

Sample Location and Depth

Date Received: 9/15/09
Sample No.: A09475B

Reviewing Engineer: Charles S. Gresser, P.E.

Giles Engineering Associates, Inc.

GEOTECHNICAL, ENVIRONMENTAL AND CONSTRUCTION MATERIALS CONSULTANTS
N6 W22350 JOHNSON DRIVE, SUITE A1 / WAUKESHA, WI 53186 / (262) 544-0118 / FAX: (262) 549-8668

REPORT OF FLEXIBLE WALL PERMEABILITY TEST (ASTM D 5084)

CLIENT: UW-Milwaukee Dept. of Civil Eng.
PO Box 784 EMS Room 562-C/O Dr. Jin Li
Milwaukee, Wisconsin 53201784

PROJECT: Biocontainment Research
Milwaukee, WI

DATE: September 25, 2009

PROJECT NO.: 1M-0902023

SAMPLE PREPARATION

Height (in): 4.02	Maximum Effective Consolidation (psi): 20.0
Diameter (in): 2.35	Minimum Effective Consolidation (psi): 18.0
Area (sq in): 4.34	Back Pressure (psi): 55.0
Membrane Type: Latex	B Parameter (%): 0.94
Hydraulic Gradient: 13.8	Permeant Fluid: Deaired water

TEST DATA

Date	Clock	Time (min)	Burette Change		Flow (mL)	Permeability (cm/s)
			lower	upper		
9/17/09	16:20	152	0.0913	0.0779	0.0846	2.40E-08
9/18/09	9:00	1000	0.5271	0.5084	0.5177	2.23E-08
9/18/09	9:54	52	0.0374	0.0328	0.0351	2.91E-08
9/18/09	12:14	140	0.0871	0.0820	0.0846	2.60E-08
9/18/09	13:43	89	0.0415	0.0574	0.0495	2.39E-08
Time Weighted Average Hydraulic Conductivity, K (cm/s): 2.32E-08						

SAMPLE CHARACTERISTICS

% W	Dd (pcf)	P200	% Clay	LL	PL	PI	Max. Dd (pcf)	Optimum % W

<u>Material Description</u> Concrete Cylinder A-1	<u>Sample Location and Depth</u>
Date Received: 9/15/09 Sample No.: A09475C	

Reviewing Engineer: Charles S. Gresser, P.E.

Giles Engineering Associates, Inc.

GEOTECHNICAL, ENVIRONMENTAL AND CONSTRUCTION MATERIALS CONSULTANTS
N8 W22350 JOHNSON DRIVE, SUITE A1 / WAUKESHA, WI 53186 / (262) 544-0118 / FAX: (262) 549-5868

REPORT OF FLEXIBLE WALL PERMEABILITY TEST (ASTM D 5084)

CLIENT: UW-Milwaukee Dept. of Civil Eng.
PO Box 794 EMS Room 562-C/O Dr. Jin Li
Milwaukee, Wisconsin 53201784

PROJECT: Biocontainment Research
Milwaukee, WI

DATE: September 25, 2009

PROJECT NO.: 1M-0902023

SAMPLE PREPARATION

Height (in): 4.10	Maximum Effective Consolidation (psi): 5.0
Diameter (in): 2.35	Minimum Effective Consolidation (psi): 3.0
Area (sq in): 4.34	Back Pressure (psi): 55.0
Membrane Type: Latex	B Parameter (%): 0.94
Hydraulic Gradient: 13.5	Permeant Fluid: Deaired water

TEST DATA

Date	Clock	Time (min)	Burette Change		Flow (mL)	Permeability (cm/s)
			lower	upper		
9/17/09	15:12	2	0.8200	0.8200	0.8200	1.80E-05
9/17/09	15:15	2	0.8364	0.8610	0.8487	1.87E-05
9/17/09	15:18	2	0.7544	0.7872	0.7708	1.69E-05
9/17/09	15:21	2	0.7708	0.7954	0.7831	1.72E-05
Time Weighted Average Hydraulic Conductivity, K (cm/s): 1.77E-05						

SAMPLE CHARACTERISTICS

% W	Dd (pcf)	P200	% Clay	LL	PL	PI	Max. Dd (pcf)	Optimum % W

<u>Material Description</u> Concrete Cylinder No. B-2	<u>Sample Location and Depth</u>
Date Received: 9/15/09 Sample No.: A09479A	

Reviewing Engineer: Charles S. Gresser, P.E.

Giles Engineering Associates, Inc.

GEOTECHNICAL, ENVIRONMENTAL AND CONSTRUCTION MATERIALS CONSULTANTS
N6 W22350 JOHNSON DRIVE, SUITE A1 / WAUKESHA, WI 53196 / (262) 544-0118 / FAX: (262) 549-5868

REPORT OF FLEXIBLE WALL PERMEABILITY TEST (ASTM D 5084)

CLIENT: UW-Milwaukee Dept. of Civil Eng.
PO Box 784 EMS Room 562-C/O Dr. Jin Li
Milwaukee, Wisconsin 53201794

PROJECT: Biocontainment Research
Milwaukee, WI

DATE: September 25, 2009

PROJECT NO.: 1M-0902023

SAMPLE PREPARATION

Height (in): 4.10	Maximum Effective Consolidation (psi): 10.0
Diameter (in): 2.35	Minimum Effective Consolidation (psi): 8.0
Area (sq in): 4.34	Back Pressure (psi): 55.0
Membrane Type: Latex	B Parameter (%): 0.94
Hydraulic Gradient: 13.5	Permeant Fluid: Deaired water

TEST DATA

Date	Clock	Time (min)	Burette Change		Flow (mL)	Permeability (cm/s)
			lower	upper		
9/17/09	15:35	2	0.6560	0.6642	0.6601	1.45E-05
9/17/09	15:38	2	0.6724	0.6806	0.6765	1.49E-05
9/17/09	15:41	2	0.6806	0.6970	0.6888	1.51E-05
9/17/09	15:44	2	0.6560	0.6642	0.6601	1.45E-05
Time Weighted Average Hydraulic Conductivity, K (cm/s): 1.48E-05						

SAMPLE CHARACTERISTICS

% W	Dd (pcf)	P200	% Clay	LL	PL	PI	Max. Dd (pcf)	Optimum % W

<p style="text-align: center;"><u>Material Description</u> Concrete Cylinder No. B-2</p>	<p style="text-align: center;"><u>Sample Location and Depth</u></p>
<p>Date Received: 9/15/09 Sample No.: A09479B</p>	

Reviewing Engineer: Charles S. Gresser, P.E.

Giles Engineering Associates, Inc.

GEOTECHNICAL, ENVIRONMENTAL AND CONSTRUCTION MATERIALS CONSULTANTS
N6 W22350 JOHNSON DRIVE, SUITE A1 / WAUKESHA, WI 53186 / (262) 544-0118 / FAX: (262) 549-5668

REPORT OF FLEXIBLE WALL PERMEABILITY TEST (ASTM D 5084)

CLIENT: UW-Milwaukee Dept. of Civil Eng.
PO Box 784 EMS Room 562-C/O Dr. Jin Li
Milwaukee, Wisconsin 53201794

PROJECT: Biocontainment Research
Milwaukee, WI

DATE: September 25, 2009

PROJECT NO.: 1M-0902023

SAMPLE PREPARATION

Height (in): 4.10	Maximum Effective Consolidation (psi): 20.0
Diameter (in): 2.35	Minimum Effective Consolidation (psi): 18.0
Area (sq in): 4.34	Back Pressure (psi): 55.0
Membrane Type: Latex	B Parameter (%): 0.94
Hydraulic Gradient: 13.5	Permeant Fluid: Deaired water

TEST DATA

Date	Clock	Time (min)	Burette Change		Flow (mL)	Permeability (cm/s)
			lower	upper		
9/17/09	15:59	2	0.6478	0.6437	0.6458	1.42E-05
9/17/09	16:02	2	0.6478	0.6478	0.6478	1.42E-05
9/17/09	16:05	2	0.6478	0.6560	0.6519	1.43E-05
9/17/09	16:08	2	0.6232	0.6314	0.6273	1.38E-05
Time Weighted Average Hydraulic Conductivity, K (cm/s): 1.41E-05						

SAMPLE CHARACTERISTICS

% W	Dd (pcf)	P200	% Clay	LL	PL	PI	Max. Dd (pcf)	Optimum % W

<u>Material Description</u>	<u>Sample Location and Depth</u>
Concrete Cylinder No. B-2	
Date Received: 9/15/09 Sample No.: A09479C	

Reviewing Engineer: Charles S. Gresser, P.E.

Giles Engineering Associates, Inc.

GEOTECHNICAL, ENVIRONMENTAL AND CONSTRUCTION MATERIALS CONSULTANTS
N8 W22350 JOHNSON DRIVE, SUITE A1 / WAUKESHA, WI 53196 / (262) 544-0118 / FAX: (262) 549-8888

REPORT OF FLEXIBLE WALL PERMEABILITY TEST (ASTM D 5084)

CLIENT: UW-Milwaukee Dept. of Civil Eng. PO Box 784 EMS Room 562-C/O Dr. Jin Li Milwaukee, Wisconsin 53201784	PROJECT: Biocontainment Research Milwaukee, WI
DATE: September 25, 2009	PROJECT NO.: 1M-0902023

SAMPLE PREPARATION

Height (in): 4.07	Maximum Effective Consolidation (psi): 5.0
Diameter (in): 2.35	Minimum Effective Consolidation (psi): 3.0
Area (sq in): 4.34	Back Pressure (psi): 55.0
Membrane Type: Latex	B Parameter (%): 0.94
Hydraulic Gradient: 13.6	Permeant Fluid: Deaired water

TEST DATA

Date	Clock	Time (min)	Burette Change		Flow (mL)	Permeability (cm/s)
			lower	upper		
9/22/09	8:24	4	59.0000	56.0000	57.5000	6.27E-04
9/22/09	8:29	4	66.0000	65.0000	65.5000	7.14E-04
9/22/09	8:34	4	62.0000	61.0000	61.5000	6.71E-04
9/22/09	8:39	4	60.5000	60.0000	60.2500	6.57E-04
Time Weighted Average Hydraulic Conductivity, K (cm/s): 6.67E-04						

SAMPLE CHARACTERISTICS

% W	Dd (pcf)	P200	% Clay	LL	PL	PI	Max. Dd (pcf)	Optimum % W

<u>Material Description</u> Concrete Cylinder No. C-3	<u>Sample Location and Depth</u>
Date Received: 9/15/09 Sample No.: A09483A	

Reviewing Engineer: Charles S. Gresser, P.E.

Giles Engineering Associates, Inc.

GEOTECHNICAL, ENVIRONMENTAL AND CONSTRUCTION MATERIALS CONSULTANTS
 N6 W22350 JOHNSON DRIVE, SUITE A1 / WAUKESHA, WI 53186 / (262) 544-0118 / FAX: (262) 549-5668

REPORT OF FLEXIBLE WALL PERMEABILITY TEST (ASTM D 5084)

CLIENT: UW-Milwaukee Dept. of Civil Eng. PROJECT: Biocontainment Research
 PO Box 784 EMS Room 562-C/O Dr. Jin Li
 Milwaukee, Wisconsin 53201794 Milwaukee, WI
 DATE: September 25, 2009 PROJECT NO.: 1M-0902023

SAMPLE PREPARATION

Height (in): 4.07	Maximum Effective Consolidation (psi): 10.0
Diameter (in): 2.35	Minimum Effective Consolidation (psi): 8.0
Area (sq in): 4.34	Back Pressure (psi): 55.0
Membrane Type: Latex	B Parameter (%): 0.94
Hydraulic Gradient: 13.6	Permeant Fluid: Deaired water

TEST DATA

Date	Clock	Time (min)	Burette Change		Flow (mL)	Permeability (cm/s)
			lower	upper		
9/22/09	9:19	4	81.5000	81.0000	81.2500	8.86E-04
9/22/09	9:24	4	81.0000	80.0000	80.5000	8.78E-04
9/22/09	9:29	4	81.0000	80.0000	80.5000	8.78E-04
9/22/09	9:34	4	80.0000	78.0000	79.0000	8.62E-04
Time Weighted Average Hydraulic Conductivity, K (cm/s): 8.76E-04						

SAMPLE CHARACTERISTICS

% W	Dd (pcf)	P200	% Clay	LL	PL	PI	Max. Dd (pcf)	Optimum % W

Material Description

Concrete Cylinder No. C-3

Sample Location and Depth

Date Received: 9/15/09

Sample No.: A09483B

Reviewing Engineer: Charles S. Gresser, P.E.

Giles Engineering Associates, Inc.

GEOTECHNICAL, ENVIRONMENTAL AND CONSTRUCTION MATERIALS CONSULTANTS
N8 W22350 JOHNSON DRIVE, SUITE A1 / WALKESHA, WI 53186 / (262) 544-0118 / FAX: (262) 549-5658

REPORT OF FLEXIBLE WALL PERMEABILITY TEST (ASTM D 5084)

CLIENT: UW-Milwaukee Dept. of Civil Eng.
PO Box 784 EMS Room 562-C/O Dr. Jin Li
Milwaukee, Wisconsin 53201784

PROJECT: Biocontainment Research
Milwaukee, WI

DATE: September 25, 2009

PROJECT NO.: 1M-0902023

SAMPLE PREPARATION

Height (in): 4.07	Maximum Effective Consolidation (psi): 20.0
Diameter (in): 2.35	Minimum Effective Consolidation (psi): 18.0
Area (sq in): 4.34	Back Pressure (psi): 55.0
Membrane Type: Latex	B Parameter (%): 0.94
Hydraulic Gradient: 13.6	Permeant Fluid: Deaired water

TEST DATA

Date	Clock	Time (min)	Burette Change		Flow (mL)	Permeability (cm/s)
			lower	upper		
9/22/09	10:41	4	67.0000	65.5000	66.2500	7.23E-04
9/22/09	10:46	4	59.0000	58.0000	58.5000	6.38E-04
9/22/09	10:51	4	60.5000	59.0000	59.7500	6.52E-04
9/22/09	10:57	5	74.0000	72.0000	73.0000	6.37E-04
Time Weighted Average Hydraulic Conductivity, K (cm/s): 6.61E-04						

SAMPLE CHARACTERISTICS

% W	Dd (pcf)	P200	% Clay	LL	PL	PI	Max. Dd (pcf)	Optimum % W

<u>Material Description</u> Concrete Cylinder No. C-3	<u>Sample Location and Depth</u>
Date Received: 9/15/09 Sample No.: A09483C	

Reviewing Engineer: Charles S. Gresser, P.E.

VITA

Curriculum Vitae

George D.O. Okwadha

833 N 14th Street Apt# 203
Milwaukee, WI 53233, USA

ogingad@hotmail.com, gokwadha@uwm.edu
Phone: (414) 526-1865

Education:

PhD in Environmental Engineering (GPA 3.521) May 2010
Minor: Mathematics
(University of Wisconsin-Milwaukee, Milwaukee, Wisconsin, USA)

Dissertation Title: Biocontainment of PCBs on flat concrete surfaces.

M.sc. in Environmental Engineering (GPA 3.667) Dec. 2006
(University of Wisconsin-Milwaukee, Milwaukee, Wisconsin, USA)

Thesis Title: Thermal removal of mercury in spent powdered activated carbon from the TOXECON™ process.

Bsc. in Civil Engineering (GPA 3.092) Dec. 2003
(University of Wisconsin-Milwaukee, Milwaukee, Wisconsin, USA)

B.sc. Environmental Science-Physical Systems (GPA 3.75) Dec. 2003
(University of Wisconsin-Green Bay, Green Bay, Wisconsin, USA)

Higher Diploma in Construction Engineering (Soil Mechanics & Foundation Engineering Option) (Credit Pass & Best Student Award) Dec. 1991
(The Kenya Polytechnic-Nairobi, Kenya)

Ordinary Diploma in Civil Engineering (Credit Pass) Dec. 1986
(The Mombasa Polytechnic-Mombasa, Kenya)

Other Trainings:

Wokingham, England:

- Roads and Transport for developing countries July 1996
- Appropriate technology roadworks for developing countries June 1996

Nairobi, Kenya:

- Geotechnical site investigation and laboratory analysis Oct. 1990

Publications

Refereed Journal Publications

- Dombrowski, F.J., Ramme, B.W., Okwadha, G.D.O., and Kollakowsky, D. "Evaluation of Surface Water Runoff from Fly Ash-Stabilized and Non-Stabilized Soil Surfaces". *Journal of Environmental Engineering*, 2009, doi: 10.1061/(ASCE)EE.1943-7870.0000214. (Article in press).
- Okwadha, G.D.O., Li, J., Ramme, B., Michaud, D., and Kollakowsky, D. "Thermal removal of mercury in spent powdered activated carbon from the TOXECON™ process". *Journal of Environmental Engineering*, 2009, 135(10), 1033-1040.

Technical Guidelines

- Okwadha, G. "Smoke Detectors Management". *We Energies Environmental Procedures, EN 1507, 1-7, December 9, 2008.*
- Okwadha, G. "Operation, handling and maintenance of the Dynamic Cone Penetrometer". *Materials Testing and Research Department, Ministry of Public Works-Kenya, August 1997.*

Reports

- Okwadha, G., and Li, J. "Thermal removal of mercury in spent powdered activated carbon (PAC) from the TOXECON™ process" *Report to We Energies, Milwaukee, Wisconsin, January 2007.*

Unpublished Research

- Okwadha, G. and Mutunga, G, "Calibration of the 5th Wheel Towed Bump Integrator with a Profile Beam in flexible pavements in Kenya". *Materials Testing and Research Department, Ministry of Public Works-Kenya, April 1998.*
- Okwadha, G., "The use of Dynamic Cone Penetrometer (DCP) to estimate the shear strength parameters, Cohesion C , and the angle of shearing resistance ϕ , for the design of foundations for low-cost housing in developing countries" *Higher Diploma Research project, Kenya Polytechnic, Dec. 1991.*

Research proposals

- Hazardous waste immobilization using fly ash-powdered activated carbon mixture
- The use of Dynamic Cone Penetrometer (DCP) to estimate the Relative Compaction of flexible pavement embankments.

On-going Research

- Biocontainment of PCBs on flat concrete surfaces
- Coprecipitation of metals in boiler chemical cleaning wastewater by microbial carbonate precipitation

Technical Experience

University of Wisconsin, Milwaukee, Wisconsin, USA

- Teaching Assistant Fall 2005-Fall 2009
(Statics, Water Quality Assessment, Soil Mechanics, Strength of Materials, Principles of Water Resources Design, and Coaching Mathematics)
- Research Assistant Spring 2005 & Spring 2010

We Energies, Milwaukee, Wisconsin, USA

April 2007 to date

- **Student Engineer Intern**
 - Bioremediation of PCBs (Literature review)
 - Manufacture of lightweight aggregates using Elastizell bio-foaming agent and fly ash.
 - Evaluation of Petroflag as a reliable test method for field determination of mineral insulating oil concentration.
 - Radiochemical analysis of coal combustion products
 - Boiler chemical cleaning wastewater treatment research

Giles Engineering & Associates, Waukesha, Wisconsin, USA

June 2004-Dec. 2006

- **Engineering Technician:** Construction materials testing
 - Quality control of asphalt road surfaces
 - Quality control of road embankments

Waukesha County Dept. of Public Works, Waukesha, Wisconsin, USA

May-Aug., 2003

- **Technical Intern:** Survey, paving operations supervision and inspection, culvert/bridge inspection, signs inventory, Automatic traffic counters installation and manual traffic counts.

Proctor & Gamble, Green Bay, Wisconsin, USA

January –May 2002

Company production activities' evaluation with regards to environmental pollution control and prevention. (Industrial Project)

Ministry of Roads and Public Works-Kenya

Jan. 1987- Dec. 1999

- **Senior Laboratory Technologist (Team leader):** Flexible pavement evaluation activities (Flexible pavement deflection measurements, in-situ California Bearing Ratio (CBR) measurements, moisture content and density measurements using nuclear techniques), and general laboratory testing of concrete, bitumen and soils for engineering purposes.

Kenya Institute of Highways and Building Technology (KIHBT)-Nairobi, Kenya

Jan. 1996- Dec. 1999

- **Lecturer:** Soil Mechanics; teaching and evaluation of students
- **Instructor:** International course on In-situ CBR measurements at KIHBT's School of Appropriate Technology.
- **Curriculum Developer:**
 - Preparation of training modules for the determination of CBR using Dynamic Cone Penetrometer (DCP), in collaboration with International Labor Organization (ILO/ASIST Program, Nairobi, Kenya).
 - Preparation of a technical syllabus for technical colleges in Kenya for the Kenya Institute of Education (KIE), Nairobi, Kenya.

Honors and activities

- Graduate Poster Competition (Honorary Mention) Spring 2010
- Graduate Poster Competition (Honorary Mention) Spring 2009
- Chancellor's Graduate School Award (UW-Milwaukee) Spring 2005-Fall 2009
- Dean's Honor List (UW-Milwaukee) Spring 2003
- Vice Chancellor's honors (UW-Green Bay) Spring 2000-2002
- Engineering Scholarship Award (UW-Green Bay) Spring 2001 & 2002
- Federation of Environmental Technologies (FET) Scholarship (UW-Green Bay) Spring 2001
- Member of the honor society of Phi Kappa Phi Spring 2000 to date
- Member of the National Scholars Honor Society Dec 2006 to date
- Member of the Air and Waste Management Association 2005 to date
- Member of American Society of Civil Engineers 2002
- Member of the National Society of Black Engineers 2002
- Member of the Institute of Transportation Engineers 2003
- Registered Graduate Technician Engineer (Engineers Registration Board-Kenya) 1993
- Best Student Award (Foundation Engineering-Kenya Polytechnic-Kenya) 1991

Community involvement

- Assisting individuals with mental disabilities carryout their activities of daily living (Dungarvin Wisconsin inc, Milwaukee)
- Coaching a senior citizen in the Lifetime Learner's Program (geared towards reducing the early onset of Alzheimer's disease) at Cardinal Stritch University carryout simple maths and economics.

References:

- (1) Dr. Jin Li (E-mail: li@uwm.edu, 414-229-6891)
- (2) Dr. Erik Christensen (E-mail: erc@uwm.edu, 414-229-4968)
- (3) Dr. Bruce Ramme (E-mail: bruce.ramme@we-energies.com, 414-221-2440)
- (4) Dr. Maurice Ogutu (E-mail: ogutu@illinois.edu, 708-352-0109)
- (5) Dr. Hani Titi (E-mail: hanititi@uwm.edu, 414-229-6893)
- (6) Dr. Rev. Fr Joseph Oduor Afulo, S.J.(E-mail: Oduorafulo@gmail.com, 254-20-3870436/254-20-3878015/254-20-3870489.



Major Professor

5/4/10

Date