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Accelerated biodegradation of cured cement paste by *Thiobacillus* species under simulation condition

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ABSTRACT

Biodegradation is one of the most important types of cement deterioration. Complex microbial populations take part in the biodegradation process of cement-based materials. Studies in this field show that the sulfur-oxidizing bacteria, including *Acidithiobacillus thiooxidans*, due to sulfuric acid formation, play a key role in this process. In this study, with the accelerated leaching process of calcium hydroxide of cement paste, cured under running tap water and exposed to sterile biogenic sulfuric acid for 6 days, the surface pH of the cement was reduced to a more favorable level for bacterial growth. In this case, the growth of *Thiobacillus* proceeded in the presence of cured cement paste specimens. After 90 days of exposure to a semi-continuous culture of *A. thiooxidans* with its pH less than 2 and continuous removal of damaged layers the compressive strength, length and mass of the samples dropped by 96%, 11% and 43%, in the order given. The mechanism of degradation and the structure of degraded specimens were analyzed by test laboratory techniques such as, XRD, SEM and EDAX analyses.

1. Introduction

Some microorganisms can grow on cement surfaces though their thin biofilms are invisible. They are not responsible for colored spots usually observed on the surface of concrete structures, but they have the ability to destroy them (Escadeillas et al., 2007). Some types of autotrophic and heterotrophic microorganisms, notably genus *Thiobacillus*, as a group of bacteria with serious destruction ability, were isolated and identified from the degraded concrete structures (Nica et al., 2000; Okabe et al., 2007). Most research reports have indicated that *Acidithiobacillus thiooxidans* plays a key role in the biodegradation process of cement (Milde et al., 1983; Sand and Bock, 1984; Diercks et al., 1991; Haile and Nakhla, 2009; Wei et al., 2010). These bacteria are available in water, air and soil (Waksman, 1922; Vidyalakshmi et al., 2009) and still the best and most suitable growth environment is found to be in sewage pipelines (Roberts et al., 2002), so any cement structure located nearby undergoes biodeterioration. These bacteria have the ability to oxidize organic and inorganic sulfur compounds in the presence of oxygen, carbon dioxide and moisture (Waksman, 1922; Wei et al., 2010). The final product of sulfur-oxidizing bacterial activity is sulfuric acid (Diercks et al., 1991; Roberts et al., 2002). A white gypsum layer is found by reaction of sulfuric acid and cement portlandite [$Ca(OH)_2$]. The reaction of gypsum and aluminate phase of the cement produces Ettringite ($3CaO{\cdot}Al_2O_3{\cdot}3CaSO_4{\cdot}32\ H_2O$), with expandability and low adhesion properties (Saricimen et al., 2003; Connell et al., 2010). With sulfuric acid formation by sulfur-oxidizing bacteria, the strong structure of cement is converted to a loose structure of gypsum and Ettringite at medium temperature (>15 °C) and Thaumasite ($CaSiO_3{\cdot}CaCO_3{\cdot}CaSO_4{\cdot}15H_2O$) at low temperature (<15 °C) (Cwalina, 2008).

First, *Thiobacillus* does not exhibit any sign of growth on concrete structures at initial pH of around 12–13 (Roberts et al., 2002), though from the beginning, some algae and fungi grow on cement surfaces as a suitable substrate (Diercks et al., 1991; Jayakumar and Saravanane, 2010; Wiktor et al., 2010). At longer time, the carbonation and leaching processes of cement portlandite occur by climatic changes such as snow, rain and other running water and the pH of cement surface are reduced to about 9 (Shook and Bell, 1998). *Thiobacillus thioparus* is the first acting species of *Thiobacillus* bacteria that has the ability to grow on the cement surface at pH 10. By production of polythionic acid, elemental sulfur and sulfuric acid, the pH of cement surface is dropped again and therefore other *Thiobacillus* strains start to grow. At pH 4.5, the various strains of *A. thiooxidans* find the ability to grow on the surface of cement (Roberts et al., 2002). By activity of these bacteria

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under suitable conditions, pH of the environment is dropped to less than 1. These bacteria have been found abundant in the 1–5 mm layer of concrete structures, but in deeper layers, their numbers are reduced logarithmically due to lower diffusion of oxygen and carbon dioxide gases of the air from the surface cement. So, sulfuric acid is produced on the concrete surface and penetrates into the lower layers and reacts with cement inner compounds (Nica et al., 2000; Yamanaka et al., 2002; Wiktor et al., 2010).

There are good review articles (Diercks et al., 1991; Monteny et al., 2000; Gaylarde et al., 2003; Cwalina, 2008; Connell et al., 2010) in relation to cement biodegradation. Considerable research (Magniont et al., 2011) works have been conducted on the biodegradation of concrete-based structures by synthetic organic acids (acetic and lactic acids) and comparisons are made with biogenic acid produced by *Escherichia coli* and silage effluent (acetic, propionic, butyric and isobutyric acids) (Bertron et al., 2005a,b, 2007). Biodegradation mechanism of cement structure is different in domestic and industrial sewage systems from that of agricultural silos, etc.; because of different microorganisms producing different acids. In agricultural and agro-food effluents, it is shown that some ionic salts and metals of cement are consumed by bacteria. Also it is particularly observed that bacteria cause more intense deterioration of cement compared to a medium without bacteria or in a synthetic acid solution (Magniont et al., 2011). In other studies chemical sulfuric acid (Chandra and Berntsson, 1983; Knight et al., 2002; Saricimen et al., 2003; Hewayde et al., 2007; Herisson et al., 2013) or sodium and magnesium sulfates (Monteny et al., 2000) are found to simulate the biodegradation process of cements, and it is shown that cement degradation with chemical sulfuric acid and sulfate salts take different course from biogenic sulfuric acid bacterially produced, even though the sodium and magnesium cations play important roles in degradation of cement. Some research works have focused their studies on presenting a model for biodegradation (Kaempfer and Berndt, 1999; Vollertsen et al., 2008; De Windt and Devillers, 2010) process by in situ tests (Monteny et al., 2000; Okabe et al., 2007; Alum et al., 2008; Herisson et al., 2013) and by a mixture of several bacteria (Sand and Bock, 1984; Wei et al., 2010; Herisson et al., 2013), or just in presence of one bacteria such as *A. thiooxidans* (Hormann et al., 1997; Vincke et al., 1999; Knight et al., 2002; Haile et al., 2008) and finally others have dealt with biodegradation of concrete structures using algae (Ismail et al., 1993; Bertron et al., 2007; Escadeillas et al., 2007, 2009; Alum et al., 2008; Jayakumar and Saravanane, 2010; Wiktor et al., 2010) and fungi (Gaylarde et al., 2003; De Windt and Devillers, 2010). In these studies, a variety of media are used including sulfur powder and thiosulfate with calcium, iron, aluminum and magnesium ions with the concrete, mortar and cement paste specimens in different shapes and sizes under variable temperature and humidity conditions. Scattered research data with various microorganisms and methods have produced complications to reach a unified conclusion for comparing the observations and data by various experiments on cement biodegradation. So, in-depth understanding of various interactive processes in structural biodegradation of cements and their constant changes are necessary.

In the present work, the growth of two species of *Thiobacillus* is studied under normal laboratory conditions in liquid media, free from ions in common with cements such as calcium, iron and sulfates, in which bacteria become fully colonized. In retrospect, the behavior of *Thiobacillus* has been assessed in the presence of a cement paste. The reduction in the pH of liquid medium to less than 2 leads to degradation in cement chemical structures. The study was further continued to evaluate the biodegradation of the cement in curing and degradation stages by *A. thiooxidans* in simulated laboratory conditions.

2. Materials and methods

2.1. Materials

2.1.1. Microorganisms and cultivation media

Two oxidizing-sulfur bacteria *T. thioparus* PTCC 1668 and *A. thiooxidans* PTCC 1717 were purchased from Persian Type Culture Collection, Iranian Research Organization for Science and Technology (IROST). The optimum pH for growth of the former was 7, while it was 4.5 for the latter microorganism at 30 °C. These microorganisms were kept in refrigerator at 4 °C in a liquid medium containing thiosulfate and they were re-cultivated once every 2–3 weeks. Liquid culture media for *T. thioparus* PTCC 1668 and *A. thiooxidans* PTCC 1717 (designated as M1 and M2 in Table 1) contained mineral salts (Merck Co., Germany) free of calcium, iron, aluminum, and sulfate ions. The optimum culture media for bacterial growth and their corresponding data are not published yet. The culture media were sterilized at 121 °C under 1.2 bar pressure for 20 min. The optimized volume of the inoculum in their culture media was 1 v/v(%) towards the end of the logarithmic phase of bacterial growth.

Symbols for various tests are presented in Table 2.

2.1.2. Cement paste specimen preparation

Portland cement was of Type II with chemical composition as presented in Table 3 and physical properties of Blaine Fineness of 302 m^2/kg and density of 3120 kg/m^3.

Cement paste was prepared by tap water of water/cement ratio of 0.35 and molded in 2 × 2 × 2 cm^3 cube blocks. The specimens were stored in an environment with high humidity 95% and room temperature for 1 day and molded and treated under curing condition.

2.2. Methods

2.2.1. Simulation experiments

After removing the specimens from the molds, in order to simulate ion leaching and pH reduction processes, the specimens were exposed to running tap water for 27 days for hydration of cement phases and leaching out the portlandite from their surface at room temperature. Then the specimens were exposed to sterile biogenic sulfuric acid with pH less than 2 and at room temperature for 6 days. This acid was obtained from the growth of *A. thiooxidans* PTCC 1717 in M2 medium after two days at shaker incubator conditions (30 °C, 150 rpm). The culture growth resulted in a pH that was in agreement with a report given for a 20-year old sewer condition (Kaempfer and Berndt, 1999). In all the tests, the volume ratio of biogenic sulfuric acid to cement paste specimens surfaces was equal to 5.

After simulation process and reduction of pH of the cement paste to its required level, the biodegradation of specimens was studied through different pathways of slow (1) and accelerated (2)

Table 1
Components of liquid culture media for *T. thioparus* PTCC 1668 (M1) and *A. thiooxidans* 1717 PTCC (M2).

Mineral salt (g)/1 l distilled water	M1	M2
NH_4Cl	0.40	2.43
$MgCl_2 \cdot 6H_2O$	0.20	0.41
Na_2CO_3	0.40	0.00
K_2HPO_4	2.00	0.00
KH_2PO_4	2.00	3.00
$Na_2S_2O_3 \cdot 5H_2O$	5.00	5.00