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Studies on Biodegradation of normal concrete surfaces by fungus *Fusarium* sp.

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ABSTRACT

The main objective of this study was to identify the potential of different concrete deteriorating acid producing microbes for removing surface layers of contaminated concrete structures. Studies were made using an acid producing fungus, *Fusarium* sp in a humidity chamber for one year and compared the degradation with the *Thiobacillus* sp. The growth of fungus as a black biofilm on the normal concrete surface in the humidity chamber was confirmed by culture techniques even after one year. Results of degradation parameters showed higher pH reduction, weight loss and thickness loss under *Fusarium* sp. biofilms. Epifluorescence micrographs clearly confirmed dense growth of filamentous *Fusarium* sp. Laser Raman spectroscopic and XRD analysis showed weakening of calcium silicate bands and presence of calcium oxalate bands confirming biodegradation. Thus by thinning of the concrete specimen by 2.27 mm in one year the potential of this species for cleaning contaminated concrete surfaces is demonstrated.

1. Introduction

Parker [1] isolated for the first time a highly acidophilic sulfur oxidizing bacteria (SOB) *Thiobacillus* sp. and reported the microbial involvement in the corrosion deterioration. These chemoautotrophs oxidize several sulfur compounds to produce sulfuric acid which reacts with free lime producing a corroding layer on concrete surface [2]. The potential of these concrete deteriorating acid producing microbes were studied for removing contaminated radioactive layers of concrete surfaces [3,4]. The porous nature of concrete makes cleanup of radioactive surfaces difficult. Physical operations such as sandblasting or pneumatic chisel generates lot of dust creating an air borne contamination, and working with wet surface reduce dust problem, but water cause soluble forms of contamination like uranyl nitrate to soak into the concrete. Techniques such as acid etching create a voluminous waste stream or further penetrate contaminants into concrete [5]. Microbial etching of concrete is known to occur in bridges and especially sewer systems where H_2S is present. Bacteria on the concrete, exposed to both air and H_2S, oxidize the sulfur compound to H_2SO_4 which attacks and dissolves the concrete. This process is being developed at the INEEL [5] as an intentional way of loosening the upper contaminated layers of concrete surfaces by using *Thiobacillus thiooxidans* bacteria. However various studies explain the difficulties in growing *Thiobacillus* sp. on concrete surface under humid environments alone as it needs special enrichment medium during isolation and maintenance [6,7]. Studies by Ji-Dong Gu et al. [8], Fomina et al. [9] and Wazny [10] indicated that fungi also play an important role in the deterioration of concrete. They observed both weight loss and release of calcium when concrete was exposed to *Fusarium* isolate. According to these workers degradation by fungi can proceed more rapidly than degradation by *Thiobacillus*. Fungal species are not so fastidious as *Thiobacillus* sp. and can easily grow in humid environments. Hence this study looks into the possibility of using an acid producing fungus for biodegradation purpose.

Under humid conditions, the fungi biofilm was encouraged to grow on the surface of the normal concrete specimens and allowed to slowly degrade the concrete because of its interaction with the products of microbial metabolism. The growth of fungus on the surface was followed by epifluorescence microscopy and culturing techniques. The degradation kinetics was followed by the weight loss, decrease in thickness and diameter of specimens and by pH decrease studies. Detailed Surface Enhanced Raman scattering experiments were carried out on the pure and fungi-developed concrete surfaces to identify the growth of *Fusarium* sp. on the concrete surface and the effect of these biofilms on concrete chemistry. XRD studies were also done to analyze the phase changes in concrete composition due to fungal growth. Comparative studies of biodegradation potential of *Fusarium* sp. were done with sulfur oxidizing *Thiobacillus* sp. and results are presented with possible explanation.

2. Experimental

2.1. Specimen preparation

The mix details of the normal concrete is given in Table 1. This concrete mix was referred to as normal concrete (N) and was crushed

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Table 1
Mix details of the normal concrete N/20/30/60 with a slump 60 mm.

Mix proportions	Free water	Cement	Fine aggregate	Coarse aggregate
Ratio	0.45	1	1.71	2.92
Kg m^{-3} of concrete	180	400	684	1168

with hard blue granite rock and river sand. These concrete mixes are basically on 20 mm maximum size aggregate (well graded aggregate), river sand (Palar River), O.P. Cement and water. Concrete specimens of sizes 35 mm diameter x 13 mm thickness (circular discs) were cast and cured in the concrete laboratory. The above specimens were cast from the mortar fraction of the above concrete mixes, as the thickness is 13 mm only, which cannot accommodate 20 mm size aggregate fraction.

2.2. Exposure studies in Humidity Chamber

The specimens were kept in a humidity chamber made of perspex. The perspex tank is filled with water up to half the height and specimens were kept in boats floating on the water. The tank was covered with a lid to prevent excessive evaporation of water. The lid has several small holes to permit aeration. Room temperature of 28 °C is maintained inside the chamber too. Below the lid, water condensations leads to dripping of water inside the chamber maintaining good humidity.

2.3. Development of Fungal biofilms on Concrete Specimens

Pure cultures of *Fusarium oxysporum* (MTCC No. 284) was brought from Microbial Type Collection Center (MTCC) of Institute of Microbial Technology, Chandigarh. The culture was maintained in Potato Sucrose Agar (Diced potato 200 g L^{-1}, Sucrose 20 g L^{-1}, agar 20 g L^{-1}, pH 6.5) as recommended by MTCC. Initially for a week the specimens were sprinkled once daily with *Fusarium* species cultured in Czapek Dox (CDOX) broth medium (Sucrose 30 g L^{-1}, Sodium nitrate 3 g L^{-1}, dipotassium phosphate 1 g L^{-1}, magnesium sulfate 0.5 g L^{-1}, potassium chloride 0.5 g L^{-1}, ferric sulfate 0.01 g L^{-1}, pH 7.3) to start the growth of this species on concrete surface. Subsequently CDOX broth alone was sprinkled on daily basis. This medium favored the fungal growth and the fungal film appeared as black patches on the specimens. The fungal film was isolated [11] on fungal agar plates and its morphology closely monitored to identify the growth of *Fusarium* sp. The specimens were withdrawn after 6 months and 1 year exposure for post exposure analysis.

2.4. Development of Thiobacillus sp. Biofilms on Concrete Specimens

Pure cultures of *Thiobacillus thiooxidans* (MTCC No. 468) was brought from Microbial Type Collection Center (MTCC) of Institute of Microbial Technology, Chandigarh. The culture was maintained in Starkey medium ($(NH)_2SO_4$ 0.2 g L^{-1}, $MgSO_4$ $7H_2O$ 0.5 g L^{-1}, $CaCl_2$ 0.25 g L^{-1}, KH_2PO_4 3.0 g L^{-1}, $FeSO_4$ 0.005 g L^{-1} with elemental S 1 g L^{-1}) as recommended by MTCC. To one set of specimens in humidity chamber *Thiobacillus* sp. culture was sprinkled for a week once daily. Then Starkey broth without S was sprinkled on daily basis. The specimens were withdrawn at known intervals to monitor growth of *Thiobacillus* sp. and other degradation parameters.

2.5. Post Exposure Studies

The normal concrete specimens with *Thiobacillus* sp. biofilm and *Fusarium* sp. biofilm were withdrawn and analysed for various parameters. Using DSLR Camera (Make Nikon D3) the photographs of exposed and unexposed samples were taken to get a clear picture of fungal growth on the surface. Fungal biofilm was scraped into fungal medium and the medium was observed under the binocular microscope to confirm the growth of *Fusarium* sp. After removing the biofilm, the weight loss, decrease in diameter and thickness of the concrete specimens was measured to follow the degradation kinetics. Reduction in pH on the surface was monitored using potable pH meter (pH3110) with a flat surface electrode of WTW GmbH, Germany make. Laser Raman studies were done on unexposed and exposed concrete specimens after removing the biofilms. Surface enhanced Raman scattering was also done on the fungi biofilms on the concrete surface. XRD studies were done to characterize the chemical changes in the concrete under fungal biofilm.

2.5.1. Epifluorescence microscope studies

Concrete specimens were gently washed with sterile water and air-dried in a sterile chamber and the surface was flooded using 0.1% acridine orange in distilled water [12]. After 2 min, the excess stain was drained off and washed in sterile water, dried and observed under a Nikon Eclipse E600 epifluorescence microscope (excitation filter BP 490; barrier filter O 515).

2.5.2. Raman protocol

All the experiments were carried out with an HR 800 (Jobin Yvon) Raman spectrometer equipped with 1800 grooves/mm holographic grating, He–Ne laser of 633 nm was used as an excitation source. The laser spot size focused on the surface was approximately 3 μm and laser power was 20 mW at the sample. Raman spectra were recorded using super cooled (<−110 °C) 1024 x 256 pixels CCD detector. The system consists of an Olympus optical microscope mounted at the entrance of the Raman spectrograph. Both Raman excitation and scattering were performed using a 10x long distance objective (180° back scattering). The holographic grating in the spectrograph covers the spectral regions from 150 to 4000 wave numbers, spectral resolution 4 cm^{-1}, with each exposure of the CCD detector. Data acquisition was controlled by a software package. The laser beam was focused tightly on the area of interests. Different regions of the biofilms were analyzed using Raman chemical Imaging. Raman spectra were obtained from unexposed normal concrete and exposed normal concrete with fungus biofilm. After cleaning the biofilm from the concrete surface mapping experiments were also done.

2.5.3. XRD studies

The powder method of X-ray diffraction was adopted in the present study by PANalytical Xpert with a X-ray source of Cu Kα radiation ($\lambda = 1.5418$ Å) was used. The scan step size was 0.02°, the collection time was 1 s, and in the scan range of 2θ from 5° to 65°. The X-ray tube voltage and current were fixed at 40 kV and 30 mA respectively. The standard database (JCPDS database) for X-ray powder diffraction pattern was used for phase identification for a large variety of crystalline phases in a sample. Unexposed and one year exposed concrete specimens were powdered and XRD analysis were done.

3. Results

Figs. 1–3 show the photographs of unexposed concrete, one year exposed concrete with *Fusarium* sp. biofilm and the exposed normal concrete specimens cleaned of all the biofilm, respectively. The growth of fungus as black film was visible with the naked eye (Fig. 2). Once the biofilm is removed by ultrasonication, the surface of exposed specimens showed removal of small aggregates and corresponding empty holes on the surface (Fig. 3). The biofilm of one year exposed concrete surface was ultrasonically removed into sterile buffer and was inoculated in Czapek Dox broth medium for culturing *Fusarium* sp. Optical microscopic observation (Fig. 4a, b) confirmed growth of *Fusarium* sp. in the one year biofilm.