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Biofilm Formation and Thermographic evaluation of Fly Ash concrete in sea water

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Abstract

Nuclear industry is opting for fly ash modified concrete structures to increase their resistance to seawater and microbial induced deterioration. This study focuses on comparative studies on biodeterioration of three types of concrete; control (unmodified concrete), fly ash and superplasticizer modified concrete exposed for 10 months in seawater environments. Biodeterioration of concrete specimens was evaluated by characterization of biofilm parameters like total bacteria density, density of anaerobic sulfate reducing bacteria (SRB) and aerobic sulfur oxidizing bacteria (SOB). Epifluorescence microscopy was used to visualize the biofilms on these concrete specimens. pH reduction on the exposed concrete specimen surface and the total concrete specimen was evaluated. Using Lock-in thermography (LT) phase angles and amplitude images were compared between unexposed and exposed concrete specimens to characterize deterioration under biofilms. Fly ash modified concrete showed least pH reduction and least density of total heterotrophic bacteria, SRB and SOB in the biofilms. Epifluorescence micrographs confirmed the delay in the onset of biofilm formation on fly ash modified concrete. LT study supported and confirmed that there is very little change in the phase angle and amplitude between one year seawater exposed and unexposed fly ash modified concrete indicating least deterioration even after 10 month exposure in seawater.

Keywords: Fly ash concrete; biofilms; biodeterioration; seawater; thermography

1. Introduction

Concrete is the mixture of calcium oxides, hydroxides and carbonates and sand with a typical pH range of 11-13 [1]. The surface pH could be as low as 9.5 in open environments due to atmospheric CO_2 [2] and then microorganism in the environment can attach and form biofilm [3]

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which can lead to biodeterioration of concrete [4]. Hydrogen sulphide formed either by decomposition of organic material or by sulphate reduction in the environment, when oxidized into sulphuric acid by microorganism leading to biodeterioration of concrete [5]. Thus deterioration of concrete by biogenic sulfuric acid is known as microbially induced concrete corrosion (MICC). Thiobacillus species, the main sulphur oxidizing bacteria produce H_2SO_4, which reacts with the free lime, and produces a corroding layer of gypsum ($CaSO_4.2H_2O$) [6]. A far more destructive reaction occurs between the newly formed gypsum crystals and calcium aluminate in the concrete. This reaction leads to the production of ettringite ($3Ca.Al_2O_3.3CaSO_4.32H_2O$) [7], which further contributes to the degradation of concrete by increasing the internal pressure, leading to the formation of cracks. The cracks in turn provide a larger area for corrosion processes and provide additional sites for acid penetration [8]. Some workers isolated Fusarium sp., a fungus from concrete surface and suggested that fungi also play an important role in concrete deterioration [9]. Their study suggested that fungus degradation proceeded more rapidly than Thiobacillus-mediated degradation.

Normally, nuclear power plants are designed for a life span of 30-40 years, but with the experience gained in the operation of nuclear reactors, future power plants will be designed for 60-100 years, to make nuclear power more economical. Therefore, maintenance of the integrity of the concrete structures of nuclear installations has assumed significance. In the cooling water system of nuclear reactors, there are many concrete structures in the form of tanks, pillars and reservoirs that come in contact with seawater. In nuclear industry fly ash modified concretes are being used that can give more durability, strength, resist faster pH reduction and thus delays deterioration.

This study involved comparing the biofilm formations and biodeterioration on three different types of concrete structures like control (unmodified concrete) (CC), concrete modified with fly ash (FA) and concrete modified with only superplasticizer (SP) exposed to seawater for more than 10 months. The traditional method of evaluation of biofilm formation was adopted in this study to enumerate microbes by culturing in nutrient media and microscopic observation of biofilm morphology by epifluorescence microscopy [10-11]. In addition this study explored an innovative application of an advanced technique like lock-in-thermography for identifying deterioration of concrete structures under the biofilms. There has been significant progress in the application of thermography for characterization of concrete degradation. Kim Roddis (1987) [12] used an infrared scanner and a video camera to sense temperature differences between delaminated and non-delaminated areas of bridge decks. Weirtz et al., 2003 [13] studied voids of different sizes and delaminations in a concrete specimen using Pulsed Phased Thermography. Arao Kamoi et al., 2004 [14] optimized test parameters and obtained reasonable estimates of defect parameters for air-filled voids and inclusions in concrete by using thermal NDT package developed at the Tomsk Institute of Introscopy. Tanaka et al., 2006 [15] and Lan Chung et al., 2006 [16] detected spalling of concrete by IR thermography. Ch. Maierhofer et al., 2006 [17] cracks in concrete by impulse thermography. In this work we have used lock-in-thermography based on thermal waves generated inside the specimen in the stationary regime. Using this method, phase angles and amplitude images were compared between unexposed and exposed concrete specimens to characterize deterioration under biofilms.

2. MATERIALS AND METHODS

2.1 Sample preparation

Mortar of three types of concrete mixes control (unmodified concrete) (M30) (CC), concrete with fly ash (M35) and SP (FA) and concrete with only SP (M30) (SP) were cast and cured for 28 days in the concrete laboratory of Indira Gandhi Centre for Atomic Research, Kalpakkam. Two different sizes of the samples viz.(i) bigger sample of 90 mm diameter x 15 mm thickness, circular discs (circular area projection – 5026 mm2) for biofilm characterization and thermography study and (ii) small sample of the size 35mm diameter x 10mm thickness for microscopic study were prepared.

Concrete-Mix N/30/20/60: Control Concrete

Ingredients	**Cement**	**Water**	**Fine agg.**	**Coarse agg.**
In Kg/m^3	(C)	(W)	(FA)	(CA)
Concrete	400	180	684	1168
Mix Preparation	C÷C :	W÷C :	FA÷C :	CA÷C
By Weight	1	0.45	1.71	2.92

Concrete-Mix N/35/20: Concrete with Fly Ash and Superplasticizer (SP)

Ingredients	**Cement**	**Water**	**Fly Ash**	**Binder**	**Sand**	**Coarse agg.**	**SP**
In Kg/m^3	(C)	(W)	(FA)	(B)	(FA)	(CA)	
	225	143	150	375	549	1130	6.75
Mix Preparation	C÷C :	W÷C :	FA	Total B	FA÷C :	CA÷C	B÷SP
By Weight	0.6	0.38	0.4	1	1.46	3.01	1.8%

Concrete-Mix N/30/20: Concrete with SP

Ingredients	**Cement**	**Water**	**Sand**	**Coarse agg.**	**SP**
In Kg/m^3	(C)	(W)	(FA)	(CA)	
Concrete	350	154	845	1101	4.2
Mix Preparation	C÷C :	W÷C :	FA÷C :	CA÷C	C÷SP
By Weight	1	0.44	2.41	3.15	0.9%

Ingredients:

Cement (C) : O.P.C./Penna/43 grade
Water (W) : Potable
Sand (FA) : River bed (Palar)
Coarse Agg. (CA) : Hard Blue Granite Rock Aggregate (Machine Crushed)
Fly Ash : Siliceous type IS 3812(Part–I)–2003
SP : Super Plasticizer SP-430

2.2 Exposure studies in seawater

Three types of concrete specimens, control concrete, concrete modified by fly ash and concrete modified by superplasticizer were exposed to Biofouling Test Loop (BFTL) seawater sump, near MAPS and triplicate specimens of each type of concrete were withdrawn after 30 days, 60 days, 100 days, 130 days, 160 days, 190 days, 250 days and 310 days for post exposure analysis.

2.3. Post exposure analysis of exposed specimens

2.3.1. Evaluation of pH of specimens

A set of specimens was used to measure the pH of the concrete surface using short-range pH papers. Another set of specimens were crushed and pH was measured using pH meter.

2.3.2. Evaluation of Biofilm Parameters

From one set of specimens, the biofilm were removed by sterile brush into sterile dilution buffer and various biofilm characterization studies were done. Parameters of interest were total bacterial density (TVC) using Sea Water Agar (SWA), total density of Sulfate Reducing Bacteria (SRB) using modified Postgate medium [18] and density of sulphur oxidizing bacteria (SOB) Thiobacillus sp. with starkey broth pH reduction.

The plates were incubated for 24–48 h at 32°C and colony forming units were counted and presented as cfu/cm^2 (TVC) [19]. Statistical analysis of the data was carried out using MYSTAT Software. Three replicates were analyzed for each experimental condition. A Student T-test was performed to assess significance in the difference between bacterial counts on control concrete and fly ash and between control concrete and superplasticizer modified concrete surfaces.

Another set of specimens of each condition exposed in seawater medium was used for direct microscopic observation using epifluorescence microscopy [11]. The specimens were gently washed with sterile water and air-dried in a sterile chamber and the surface was flooded using 0.1% acridine orange (AO) in distilled water. After 2 minutes, the excess stain was drained off and the specimens were washed in sterile water, dried and observed under a Nikon Eclipse E600 epifluorescence microscope (excitation filter BP 490; barrier filter O515). When AO intercalates with DNA it emits green fluorescence upon excitation at 480–490 nm and when AO complexes with RNA orange-red fluorescence is obtained. Thus active living cells and biofilm will fluoresce orange.

2.3.3. Thermographic studies

Different sets of three types of concrete specimens (Control, fly ash and SP modified) exposed to seawater environment were withdrawn for characterization using Lock-in thermography. The reference specimens and seawater exposed specimens of the three types of concrete after various exposure duration as described above were analyzed and compared using lock-in thermography. CEDIP Silver 420 camera was used for the study with thermal resolution of 25 mK. Two halogen lamps of 1000 W power each were used as optical light source. A function generator and an amplifier were used for generating sinusoidal stimulation. The samples were kept at a distance of 40 cm from the camera.

3. RESULTS

3.1 pH reduction on concrete specimens

pH reduced from 12 to less than 7 on the specimen surface of control concrete by 250 days exposure in seawater whereas the fly ash modified concrete showed least pH reduction (Table 1).

Table 1: pH of the concrete surface/crushed concrete specimens exposed in seawater

Specimen type/days of exposure	Not exposed	30 days	160 days	250 days
Control concrete	>12/12.3	10/11.8	8/8.2	7/7.3
Fly ash with SP	>12/12.4	12/12.1	10/10.6	9/9.2
Superplasticizer	>12/12.5	12/11.9	9/9.5	8/8.3

3.2 Biofilm parameters on concrete specimens

The results showed significant decrease in bacterial counts on concrete surface modified by fly ash compared to control concrete and concrete modified with superplasticizer. Biofilm of the fly ash concrete showed remarkable reduction in total aerobic bacterial density and density of anaerobic sulfate reducing bacteria. The error bar indicates the standard deviation (SD) of the mean and this clearly shows that the difference between the three experimental conditions, control concrete, FA concrete and SP concrete was significant (Figures 1 and 2). A Student T test performed using MYSTAT software gave a significant P value (Probability) of <0.01 between control concrete and superplasticizer modified concrete and a highly significant P of <0.001 between control and fly ash modified concrete surfaces. The density of sulfur oxidizing bacteria (SOB), the predominantly implicated bacteria in concrete biodeterioration was also least on the fly ash modified concrete surface as indicated by slower pH reduction in starkey broth culture (Table 2).

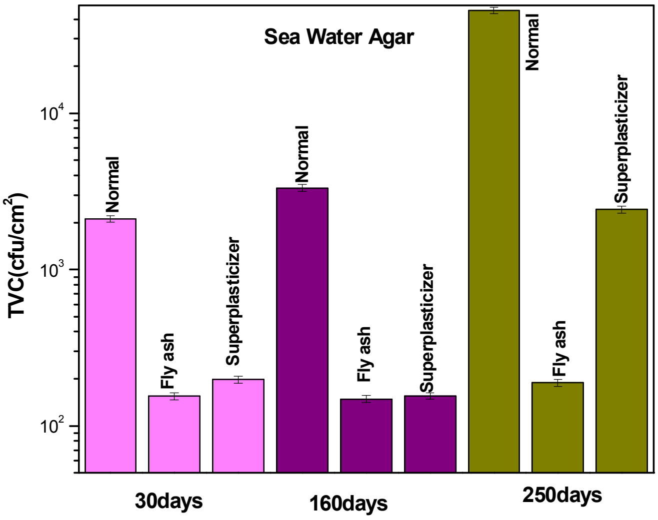

Figure 1: Density of aerobic bacteria in the biofilm on different concrete specimens exposed for 30days, 160days and 250 days in seawater

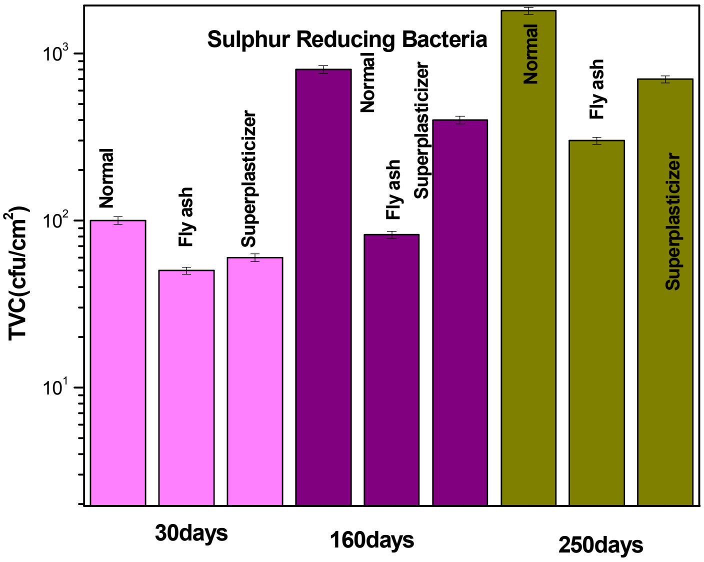

Figure 2: Density of anaerobic SRB in the biofilm on different concrete specimens exposed for 30days, 160days and 250 days in seawater

Table 2: pH reduction with time under the influence of growth of SOB in Starkey broth culture

Date	Control	Fly ash with SP	Superplasticizer
Initial pH of Starkey broth	7.5	7.5	7.5
30th day specimen	6.8 in 15 days	7.2 in 30 days	7.0 in 15 days
160th day	5.2 in 15 days	6.6 in 30 days	5.8 in 15 days
250th days	3.0 in 15 days	5.1 in 30 days	4.4 in 15 days

3.3. Epifluorescence Micrographs of concrete specimens

Epifluorescence micrographs of biofilms formed on three types of concrete specimens are shown in Figures 3 to 5. Orange fluorescing viable biofilms are there on both control and superplasticizer modified concrete surfaces. However, streaks of viable biofilms appeared on fly ash concrete surface only by 250 days exposure time.

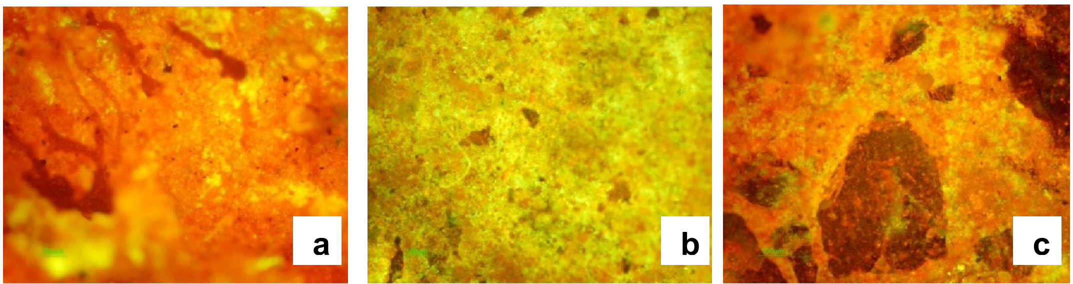

Figure 3: Epifluorescence micrographs of 30th day biofilm on (a) control (b) fly ash and (c) superplasticizer concrete specimens

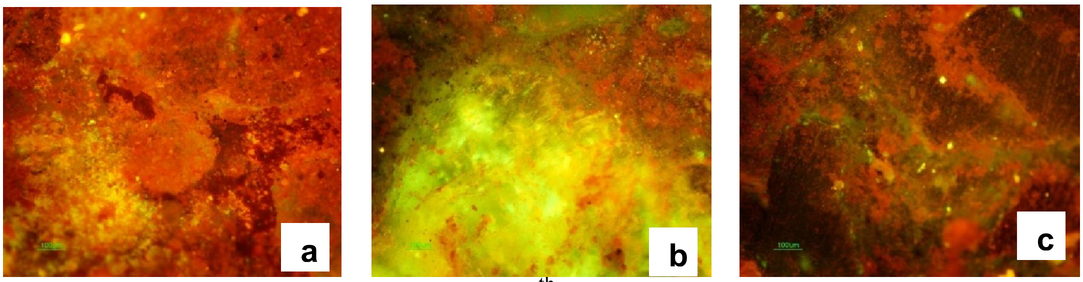

Figure 4: Epifluorescence micrographs of 160th day biofilm on (a) control (b) fly ash and (c) superplasticizer concrete specimens

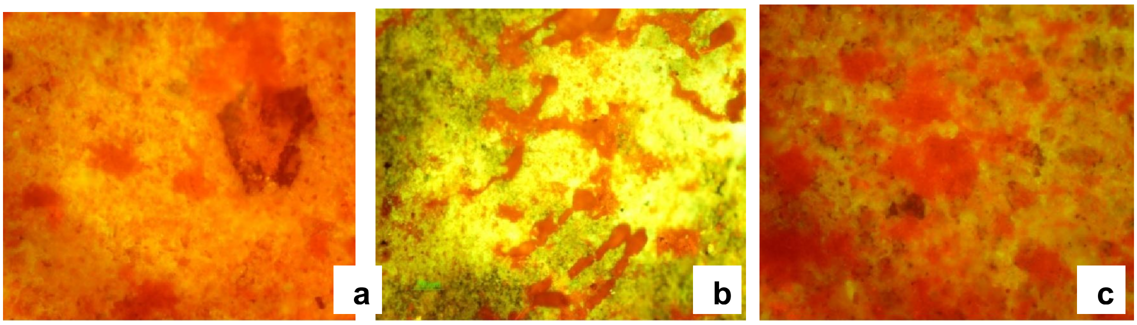

Figure 5: Epifluorescence micrographs of 250th day biofilm on (a) control (b) fly ash and (c) superplasticizer concrete specimens

3.4 Characterization of concrete deterioration under biofilms by thermography

Phase and amplitude images are analyzed for different sets of concrete samples of control, fly ash and superplasticizer. The experiments were carried out at various modulation frequencies ranging from 0.01 to 0.5 Hz. Amplitude and phase images were obtained for various numbers of exposure days and also for the unexposed specimens for comparison.

Results of amplitude difference and phase angle difference with different exposure time are presented. Figure 6 shows the amplitude differences between the unexposed and exposed specimens plotted with respect to number of exposure days for all the three types of concrete. Results showed that the amplitude difference is greatest between unexposed and exposed control concrete specimens. Fly ash concrete showed the least amplitude difference with the unexposed specimen. It can also be observed that all the three types of concrete specimens showed increase in amplitude difference with increase in exposure time.

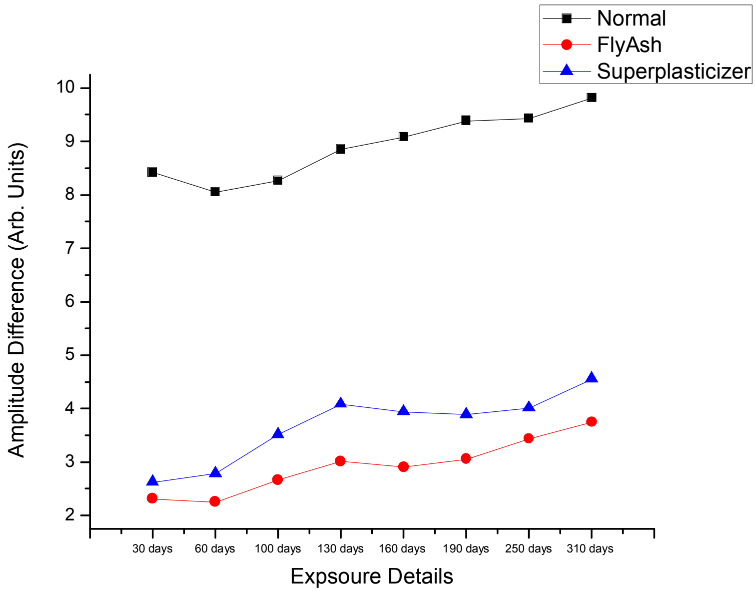

Figure 6: Amplitude difference on concrete surfaces with different exposure times

Phase angle difference between the exposed and unexposed concrete specimens was plotted against the exposure time in Figure 7 for three types of concrete. The results clearly revealed that control concrete specimens showed highest phase angle difference compared to other types. Fly ash concrete showed least phase angle difference. Another interesting observation was that phase angle difference increased with exposure time for control and superplasticizer concrete. However there was no significant raise in the phase angle difference with increasing exposure time for fly ash concrete. In the superplasticizer samples showed significant variation in phase angle after 130 days exposure to sea water.

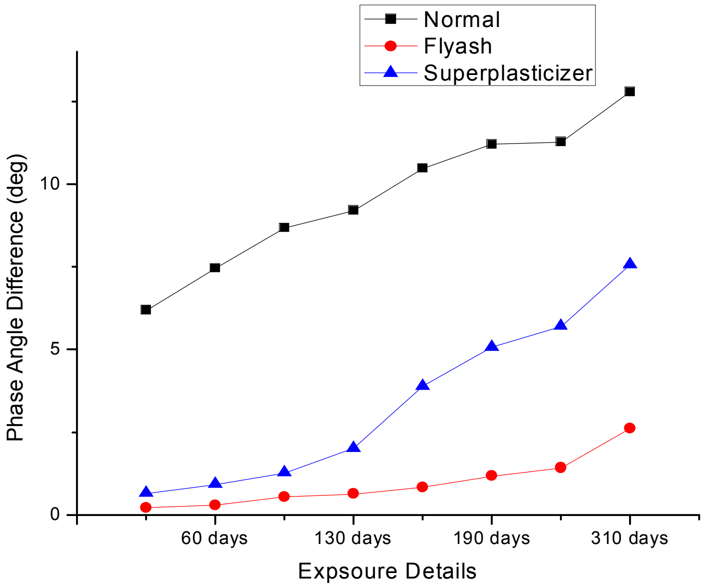

Figure 7: Phase angle variation with seawater exposure time for three types of concrete specimens

Figure 8 shows the amplitude images of the control, fly ash and superplasticizer concrete specimens which are unexposed and exposed to 30 and 250 days. The amplitude images of the unexposed and exposed specimens clearly showed variation in amplitude irrespective of control, fly ash and superplasticizer concrete. Comparison of the amplitude images of the specimens exposed to 30 days and 250 days showed that the variation in the amplitude is highest in the case of control when compared to superplasticizer and fly ash. The amplitude images of the fly ash concrete specimens exposed to 30 days and 250 days showed least change in amplitude as seen in the images revealing that the degradation of the concrete was least. It can be also observed from the images that 30 days and 250 days exposed superplasticizer concrete specimens showed higher amplitude variation at the edges of the specimens rather than the centre of the specimens.

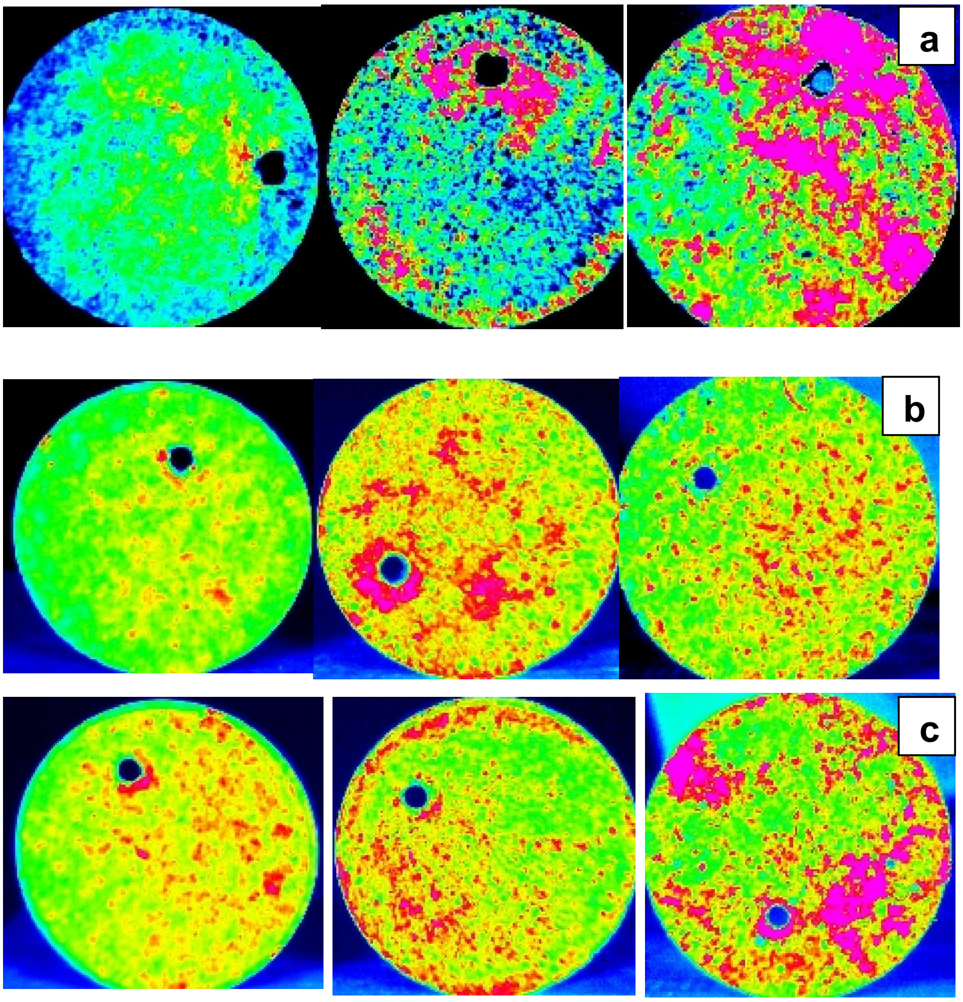

Figure 8: Amplitude images of three types of concrete (a) control (b) fly ash and (c) superplasticizer with different exposure times

4. DISCUSSION

Nuclear industry has planned to use fly ash concrete in a most of their future projects (Interim Report on HVFAC Project, Dr. P.C. Basu, AERB). The seawater environments have aggressive ions like chloride, sulfate and microorganisms which can be harmful to the structures during long-term exposures. Thus the main objective of this study was to do detailed microbiological investigations to compare the biofilm formation tendencies on fly ash modified concretes and correlate with deterioration kinetics. The comparison of the same with control and superplasticizer modified concrete was also planned. The important degradation parameters assessed were, pH reduction, biofilm parameters like total bacterial densities, density of two important bacteria implicated in sulfur cycle and biodeterioration of concrete, sulfate reducing bacteria and sulfur oxidizing bacteria. Then the results were correlated with amplitude images using Lock in thermography to characterize the corresponding deterioration.

For this, three different concrete mix proportions were prepared for the study (a) Control concrete (M 30), (b) Fly ash concrete (M 35) with SP (FA concrete) and (c) Superplasticizer admixture concrete (M 30) (SP concrete). These specimens were exposed for more than ten months in seawater.

Measurement of pH reduction was followed both on the surface of concrete specimens as well for total specimen by crushing and suspending in double distilled water for 24 hrs. Since concrete is a mixture of calcium oxides, hydroxides and carbonates with quartz minerals and water which is typically highly alkaline initially all the three types of concrete showed a pH in the range of > 12. However, it cannot remain basic throughout and exposure to atmospheric CO_2 or dissolved CO_2 in seawater will start reducing the pH [2]. The results also showed reduction of pH with time on all three types of concrete; however least reduction was again for the fly ash concrete. The main application for adding fly ash in concrete was to form stronger and durable concrete by reacting with calcium hydroxide and forming compounds. It reduced water requirement, increases workability, reduces bleeding, reduces segregation and reduces heat of hydration. It decrease drying shrinkage, improves water tightness in concrete, reduces Alkali-Silica reactivity in concrete, improves resistance of sulfate and acid attack, long term strength gain. Thus fly ash is providing an impermeable skin to concrete to withstand all weathering and hence the pH reduction is also decreased compared to control concrete.

Biofilm characterization parameters like total density of bacteria, density of anaerobic sulfate reducing bacteria and density of sulfur oxidizing bacteria was the least on FA concrete compared to others. Epifluorescence microscopic observation also showed that actively fluorescing biofilm started its presence only by 250 days. Literature [20-21] reports that depending upon the coal bed makeup the fly ash may contain some toxic elements like arsenic, beryllium, boron, cadmium, chromium, cobalt, lead, mercury etc. from trace amounts to several percent. However some earlier studies by directly inoculating fly ash in bacterial cultures could not confirm this antibacterial activity (BRNS report No:.2009/36/44-BRNS/1754). Earlier workers [1] have reported that some microbes could survive above the mentioned pH, as long as there is enough food for them. Hence in this study the result shows that the slower decrease in pH on FA concrete compared to other two types of concrete delayed the onset of attachment of biological life or organism on the FA concrete surface.

Lock in thermography was used to derive amplitude and phase images of the three types of concrete specimens exposed for more than 10 months in seawater environments to characterize concrete deterioration under biofilm forming conditions. As we are interested in surface mapping for biofilm study and degradation of concrete due to biofouling, both amplitude and phase angle were obtained. Experiments were carried out at various modulation frequencies and optimum frequency was identified to be 0.1 Hz where high phase contrast and amplitude difference were obtained between unexposed and exposed concrete specimens. Depth range of amplitude images is roughly given by thermal diffusion length μ when compared to phase image depth which is 1.8μ; hence the amplitude image gives more details about the surface of the specimen. The results of amplitude differences between the unexposed and exposed concrete specimens with exposure times showed greatest difference in control concrete and least difference in fly ash concrete. Amplitude values are clearly showing the surface variation due to the formation of biofilm on all the concrete surfaces. Thus the results of the amplitude difference are correlating well with the evaluation of biofilm parameters. It can be observed from both the techniques, control concrete samples are showing a very significant biofilm formation when compared to the other two concrete samples with the increase in number of exposure days. Similarly in the case of fly ash concrete samples, film formations were least.

Phase angle variation on the samples was also studied as prolonged exposure to sea water could have brought variation within the specimen. Results revealed that highest phase angle variation for control concrete is indicating deterioration and least variation for fly ash representing the minimum deterioration. With increasing exposure days, the phase angle variation increased for both control and superplasticizer concrete specimens indicating deterioration increases with time. When compared to the amplitude difference graph, slope of the phase angle difference graph is higher revealing the increase in the phase angle difference thus clearly shows that the degradation of specimens increase with the number of exposure days. Thermography results are correlating well with the biofilm characterization and pH reduction studies.

5. CONCLUSIONS

From this study, the following conclusions can be drawn:

1. pH reduced from 12 to less than 7 on the specimen surface of control concrete by 250 days exposure in seawater whereas the fly ash modified concrete showed least pH reduction.
2. The biofilm on the fly ash concrete showed remarkable reduction in total aerobic bacterial density, and density of anaerobic sulfate reducing bacteria. The density of sulfur oxidizing bacteria, the chiefly implicated bacteria in concrete biodeterioration was also least on the fly ash modified concrete surface as indicated by slow pH reduction in starkey broth culture.
3. Epifluorescence microscopy studies of biofilm on three types of concrete specimens showed that streaks of viable biofilms appeared on fly ash concrete surface only by 250 days exposure time.
4. Lock in thermography was used to derive amplitude and phase images of the three types of concrete specimens exposed for more than 10 months in seawater environments to characterize concrete deterioration under biofilm forming conditions. The results of amplitude differences between the unexposed and exposed concrete specimens with exposure times showed greatest difference in control concrete indicating more biofilm and least difference in fly ash concrete indicating least biofilm formation. Results revealed that highest phase angle variation is for control concrete indicating deterioration and least variation is for fly ash indicating minimum of deterioration.

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