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Microbial deterioration effect of cow dung ash modified concrete in freshwater environments

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Abstract

This paper explain the microbial deterioration of normal concrete (NC) and concrete modified with cow dung ash (CDA) in freshwater environments. Five different concrete mixes of M30 grade were prepared by replacing ordinary portland cement (OPC) with 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0% CDA by weight of cement and were compared with NC (0% CDA). First the antimicrobial properties of CDA powder were evaluated. All the specimens were cured for 28 days and then exposed in fresh water for 45 days to identify the best mix of concrete. pH degradation studies on the exposed samples were evaluated. Total viable bacterial counts (TVC) studies revealed that microbial growth was less in CDA modified concrete as compared to NC. Total dissolved solids (TDS) and Total suspended solids (TSS) were done to know the inorganic and organic content in the biofilm sample. Epifluorescence microscopic observation showed less number of fluorescing cells indicated the inhibition of biofilm formation on CDA modified concrete. XRD analysis was done to find the changes in the crystalline phases within the modified concrete microstructures and its antibacterial activity.

Keywords:Concrete; Cow dung ash; Microbial deterioration; Freshwater

1. Introduction

Concrete is the most widely used structural material in the construction industries worldwide. It is found in the form of tanks, pillars and reservoirs that come in contact with natural water/environment. Concrete structures damage when exposed to marine environments due to the action of number of physical and chemical deterioration processes [1]. Now days, materials such as fly ash slag, silica fumes, cow dung ash etc. are used as partial replacement of cement while production of concrete structures [2]. Cow dung ash (CDA) has pozzolanic qualities and thus can be classified as pozzolana and its replacement not exceeding 15% can be considered for the production of strong and quality concrete [3].

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Pavan Kumar et al. (2012) paid attention on significance and necessity of consumption of these waste materials for the manufacturing of sustainable concrete for construction of green buildings in future [4]. One of the major problems of concrete structure deterioration is microbial induced deterioration in aquatic environments. The microbes colonize on the concrete surface and its pores, capillaries and micro-cracks and cause damage through biodeterioration which is a serious problem in any environment [5].

In the recent years many researchers have tried to modify the concrete structure to control microbial corrosion. The antibacterial corrosion studies on concrete by different admixtures like fly ash and superplasticizer for prevention of biofouling was reported recently [6]. Cow dung acts as a natural inhibitor for microorganism when it is mixed in concrete structures acting as insect repellent and also prevents penetration of ultraviolet radiation. It is having natural antiseptic qualities and improves the resistance to disintegration. Houses coated with cow dung plaster are saved from nuclear radiation i.e. get protected from atomic emissions [7]. The use of green concrete structures can eliminate the negative impact of the cement industry. It minimizes the environmental effects therefore, we should try to reduce the quantity of cement used in construction and cement can be replaced with supplementary cementatious materials [8].

Literature says that the impact of construction products can be significantly reduced by substituting the use of finite natural resources for waste generated locally [9]. Asokan Pappu (2007) reported about the solid wastes generation and their recycling potentials and environmental implication in India [10]. Research has been reported on the CDA modified concrete associated with durability studies. But these types of underwater concrete structures need to identify the impact of microbial deterioration. Initially these microorganisms deteriorate the surface but after sometime it becomes the significant contributor to the deterioration of concrete that leads to the crack formation and reduces the life of the concrete. The contribution of microorganisms to the deterioration of materials as a whole may be in the range of 30% [11] relatively little attention has been given to biodeterioration of concrete [12]. X-Ray diffraction method is reliable, precise and very reproducible method to quantify the relative phase abundances of cementitious product [13]. The microstructural differences can be observed in the cement paste–aggregate interface as distinct from the bulk cement paste, formation of new compounds on account of exposure of concrete to different aggressive environments, characteristic patterns in the natural deterioration of concrete [14]. Ohira and Yamamoto (2012) found that small crystallite size showed greater antibacterial activity than those with a large crystallite size due to specific surface area [15].

This study is important to provide the information of microbial deterioration on normal concrete (NC) and concrete modified with CDA with different weight percentage of CDA and obtaining the better mix ratio.

2. Materials and methods

2.1. Preparation of specimens

Based on the requirements of the mix design, two types of M-30 grades of concrete mix namely NC and concrete modified with CDA were prepared as per IS 8112:1989. Ordinary Portland cement (43 Grade) conforming to IS 8112 -1989 were used in this study. The cement (Ordinary portland cement/Penna/43grade), water (Potable), fine aggregates (river bed, Palar), coarse aggregates (Hard Blue Granite Rock Aggregate - Machine Crushed) and superplasticizer (SP-430, Fosroc Chemicals Ltd., Bangalore, India) were used.

The cow dung has been collected, dried in the sunlight and burnt up to ignition temperature to obtain the ash and sieved through 0.425mm mesh. Physical properties and chemical compositions of the ordinary Portland cement (OPC) are shown Tables 1 and 2. Chemical compositions and physical properties of the CDA are shown Table 3. The chemical compositions and physical properties of the cattle manure ash was also reported by Shuguang Zhou et al (2012) [16].

The size of the specimens casted for the experiments was 200 x 100 mm cylindrical concrete. One set was NC whereas another set was concrete modified with CDA was prepared as 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 % of replacement of OPC by CDA. The water ratio was constant and superplasticizer (IS 9103:1999) percentage was adjusted based on the requirements. After 28 days of curing, all the specimens were sliced into equal size of 100 mm×9 mm before exposing in freshwater. They were exposed in freshwater for 45 days to identify the better mix for the long term study.

TABLE 1: PHYSICAL TEST ANALYSIS OF OPC/PENNA/43GRADE

Tested Parameters	Specific gravity	Fineness (cm^2/g) (Blaine's method)	Normal consistency	Setting Time (minutes)		Soundness Test (mm) (Le-Chatelier method)	Compressive strength (N/mm^2)		
				Initial	Final		3 days	7 days	28 days
Test results obtained	3.09	2602	30%	178	270	1.0	41.47	43.71	68.87
IS 8112/2013 limits	-	Min: 2250	-	Min:30 minutes	Max: 600 minutes	Max: 10 mm	Min: 23	Min: 33	Min: 43 Max : 58

TABLE 2: CHEMICAL TEST ANALYSIS OF OPC/PENNA/43GRADE

S.No.	Chemical Requirements	Result (%)
1	LOI	3.26
2	SiO2	19.28
3	Al2O3	6.32
4	Fe2O3	3.83
5	CaO	61.97
6	MgO	1.01
7	SO3	2.70
8	Na2O	0.60
9	K2O	0.50
10	Lime Saturation Factor (LSF)	0.94
11	Silica Modulus	1.90
12	Alumina Modulus	1.65
13	C3A	10.27

TABLE 3: CHEMICAL COMPOSITIONS AND PHYSICAL PROPERTIES OF CDA

	Test parameters	IS : 3812- (Pt-1)-2013		
S.No.	Chemical requirements (%)	Siliceous Fly ash	Calcareous Fly ash	Result (%)
1	LOI (Max)	5.0	5.0	4.56
2	SiO2 (Min)	35.0	25.0	68.08
3	Al2O3	NS	NS	9.73
4	Fe2O3	NS	NS	1.27
5	CaO	NS	NS	8.40
6	MgO (Max)	5.0	5.0	3.62
7	SO3 (Max)	3.0	3.0	1.18
8	Na2O	-	-	1.15
9	K2O	-	-	2.00
10	Available Alkalis (Max)	1.5	1.5	2.47
	Physical requirements			
1	Residue % (Retained on 45 Micron) Max	34.0		30.00
2	Fineness m2/ Kg Min	320		358.2
3	Sp.gravity (g/cm2)	NS		2.39
4	Lime reactivity test (N/mm2) Min	4.5		3.2

2.2. Antibacterial study of CDA powder

The cow dung is antiseptic, free from bacteria and also burning of cow dung as disinfectant and reduces the pathogenic effect of bacteria is mentioned during ancient times [17]. The *Bacillus subtilis* (gram positive) and Pseudomonas *aeruginosa* (gram negative) are the most common environmental bacteria. The antimicrobial activities of CDA were tested against isolated strains of these two bacteria using Total viable counts (TVC) techniques. Prepared 10 sets of 45ml nutrient broth and autoclaved. Then, added 5ml of *B. subtilis* in 5 sets and 5ml of P. *aeruginosa* pure culture in another 5 sets. After 24 hours, CDA of 0.5g, 1.0g, 1.5g and 2.0g was kept under UV light for 30 minutes and adding into the 4 sets of each culture respectively. One set for both the cultures were kept as control. All the sets of cultures were kept in the shaker for 24 hrs and estimated the TVC of bacteria with nutrient agar as per the APHA (1989) standards [18].

2.3. Exposure studies

After 28 days of curing of NC and concrete modified with CDA (2.5, 5.0, 7.5, 10.0, 12.5 and 15.0%) were immediately exposed to freshwater for 45 days and post exposure studies of the specimens were carried out.

2.4. Biofilm characterization studies

The biofilm characterization of NC and all the percentage of CDA modified concrete were carried out as after 45 days specimens were withdrawn. The significant part of biofilm characterization was evaluation of aerobic bacteria by culture techniques using nutrient agar (Hi Media-M001). The density of other microbes such as Pseudomonas sp., manganese-oxidizing bacteria, algae, fungi and anaerobic sulphate-reducing bacteria in the biofilm was estimated by culturing in Pseudomonas Agar (PSA) (Hi Media-MM119), Cyanophycean Agar (CA) (Hi Media-M699), Czapek Dox Agar (CDA) (Hi Media-M1170) (Vishwakarma et al., 2014) and modified Postgate medium [19] respectively.

As soon as the concrete specimens were collected from freshwater, it was slightly washed with tap water to remove extra cells. By using sterile brush, the biofilm on the NC and concrete modified with CDA of 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 % specimens were dispersed into 70 ml of sterile phosphate buffer (0.0425 g KH_2PO_4, 0.19 g $MgCl_2$ per liter). Then the serial dilutions of the bacterial cell suspension were prepared and 0.1 ml of each dilution was plated onto respective media. The plates were incubated for 24–48 h at 32◦C and the bacterial density was estimated as per standards (APHA, 1989). Three replicates of all the sets were analyzed by using MYSTAT Software. Student's t-test was performed to assess significance in the difference between bacterial counts on all the sets.

From the concrete biofilm, Total dissolved solids (TDS) and Total suspended solids (TSS) were estimated by the evaporation and filtration techniques respectively to know the inorganic and organic content in the biofilm sample. For TDS experiments, a known volume of biofilm sample in a preweighed beaker was completely evaporated taking care not to char the residue. The beaker was again weighed after cooling. The difference in weight would give the weight of the dissolved solids and was expressed as TDS of the specimens. For TSS analysis, a known volume of the biofilm sample (10ml) was filtered through a conditioned Millipore filter paper (0.45µm) using a vacuum pump. The filter paper was dried to constant weight at 100oC. The difference in weight would give the weight of the suspended solids and was expressed as TSS of the specimens.

2.5. pH degradation studies

The surface (WTW SenTix- 3110) and internal pH (Hanna, HI-2211) of NC and concrete modified with CDA of different percentage was measured before and after exposure of 45 days

in freshwater. The surface pH was measured once the specimens were taken out from water. The internal pH was checked by crushing the specimens of both NC as well as CDA modified concrete.

2.6. Epifluorescence micrograph

The epifluorescence microscopic study was performed to visualize the biofilm by nucleic acid stains such as acridine orange (AO). AO or 4, 6 diamino-2-phenylindole (DAPI), is a fluorescent dye used to differentiate between deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). The AO bonds with DNA and forms a complex that emits green fluorescence and when it bonds with RNA and forms a complex that emits orange fluorescence [20]. Thus, AO stains all the living active cells with lot of RNA in a biofilm and it will emit fluorescence orange and lesser active cells with more DNA with green fluorescence.

Absence or reduction in fluorescence indicates lesser biofilm formation. The NC and all the sets of 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 % of concrete modified with CDA biofilm was stained with 0.1% AO solution for 30 min and rinsed with deionized water to remove the excess stain. The stained specimens were observed under an epifluorescent microscope (Nikon Eclipse E600, excitation filter BP 490; barrier filter O515).

2.7. X-ray diffraction (XRD) study

Different environments cause the chemical changes in the concrete structures at the microstructure level. The X-ray diffraction studies were performed by powder X-ray diffractometer, Rigaku (9kW) smartLab and Copper (Kα) was used as a target material. The NC and concrete modified with CDA (10.0, and 15.0 %) exposed for 45 days in the freshwater were crushed to make the powder. It was analyzed and identified the unknown crystalline compounds by Brag Brentano method. The scan step size was 0.02o, the collection time 1s, and in the range 2θ Cu Kα from 10o to 90o.

The X-ray tube voltage and current were fixed at 30 kV and 100 mA respectively. The standard database (JCPDS) was used for phase identification for a large variety of crystalline phases in NC and concrete modified with CDA.

The crystallite size was calculated by using Scherrer's formula as

$$\text{Crystallite size } (T) = K\lambda/\beta.\cos\theta$$

Where, K is the Scherrer's constant

λ is the wavelength of X-ray

β is the Full width half maximum of the intensity observed

θ is the Bragg angle

The lattice strain analysis was calculated by using the formula, $\Delta d/d$

$\Delta d/d = d\text{-}d0/d0$, where d is the calculated d spacing

d0 is the JCPDS d spacing

3. Results

3.1. Results of antibacterial study of CDA powder

Different weight percentage of CDA such as 0.5g, 1.0g, 1.5g and 2.0g were tested and compared against *B. subtilis* (gram positive) and P. *aeruginosa* (gram negative) bacteria. Table 4 showed that on petriplates there was less growth of bacterial colony for *B. subtilis* added with CDA than B. *subtilis* without CDA. Also plating for pure P. *aeruginosa* and P. *aeruginosa* with added with CDA powder has been checked to know the effect of gram negative bacteria on the CDA powder. There is also reduction in growth of bacteria in P. *aeruginosa* with CDA powder (28.9×10^3 cfu/ml) than pure P. *aeruginosa* culture. The antibacterial activity against Cyanobacteria (C.bacteria), Staphylococcus aureus (S.aureus), Bacillus subtilis (B.subtilis) and Escherichia coli (E.coli) using different analytical techniques was studied by (Waziri and Suleiman, 2013) [21].

Generally growth of the bacterial culture depends on the pH condition [22].The optimum pH for the growth of *B. subtilis* is 7.0-7.5 and for P. *aeruginosa* is 7.0-8.0 maximum. The CDA powder is alkaline in nature (9.67) which is responsible for the antibacterial effects against these two bacteria. Though this is preliminary results but when compared both the cultures, it has been shown that *B. subtilis* cultures showed less active cell numbers than P. *aeruginosa* on petriplates.

TABLE 4: DENSITY OF MICROBIAL GROWTH IN CDA POWDER

Set of Samples	Counts (cfu/ml)
Bacillus without CDA	23.2×10^2
Bacillus with CDA (0.5g)	5.5×10^2
Bacillus with CDA (1.0g)	3.5×10^2
Bacillus with CDA (1.5g)	2.2×10^2
Bacillus with CDA (2.0g)	1.2×10^2
Pseudomonas without CDA	28.9×10^3
Pseudomonas with CDA (0.5g)	10.7×10^3
Pseudomonas with CDA(1.0g)	8.9×10^2
Pseudomonas with CDA(1.5g)	6.9×10^2
Pseudomonas with CDA(2.0g)	5.2×10^2

3.2. Biofilm characterization studies

Table 5 show the density of the NC and different percentage mix of concrete modified with CDA (2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 %) biofilm. Results of TVC were expressed as colony forming units per centimeter square (cfu/cm2). The density variations in the entire different microorganism revealed significantly. The lowest density of the biofilm was seen in CDA modified concrete compared to NC. Comparative studies of different percentage mix of CDA modified concrete showed that all the types of microbes was also least in the biofilm of 15%

replaced CDA modified concrete specimens. TDS and TSS analysis of all the modified concretes by CDA showed better results than NC concrete (Table 6.).

TABLE 5. DENSITY OF DIFFERENT TYPES OF MICROBIAL GROWTH IN THE BIOFILM OF NC AND CONCRETE MODIFIED WITH CDA IN FRESH WATER

Types of specimens	NA (cfu/cm^2)	PSA (cfu/cm^2)	CDA (cfu/cm^2)	CA (cfu/cm^2)	SRB (cfu/cm^2)
NC	10.0×10^3	5.6×10^2	2.1×10^2	5×10^1	No growth
2.5% CDA	9.3×10^2	4.9×10^2	9×10^1	3×10^1	No growth
5.0% CDA	7.7×10^2	4.0×10^2	7×10^1	1×10^1	No growth
7.5% CDA	4.5×10^2	1.7×10^2	6×10^1	1×10^1	No growth
10.0% CDA	3.7×10^2	1.4×10^2	3×10^1	1×10^1	No growth
12.5% CDA	2.4×10^1	4×10^1	1×10^1	No growth	No growth
15.0% CDA	1.8×10^1	3×10^1	1×10^1	No growth	No growth

TABLE 6. TDS AND TSS OF NC AND CONCRETE MODIFIED WITH CDA EXPOSED IN FRESHWATER

Types of specimens	TDS(mg/l)	TSS(mg/l)
NC	17.81	3.4
2.5% CDA	13.31	3.2
5.0% CDA	14.01	3.1
7.5% CDA	13.98	2.99
10.0% CDA	13.82	3.01
12.5% CDA	14.18	3.01
15.0% CDA	10.64	0.89

3.3 pH degradation studies

From the Table 7, it can be observed that pH reduction was less in CDA modified concrete. The comparative analysis of the entire percentage ratio stated that 15% replacement of CDA modified concrete showed small decline in both surface as well as internal pH.

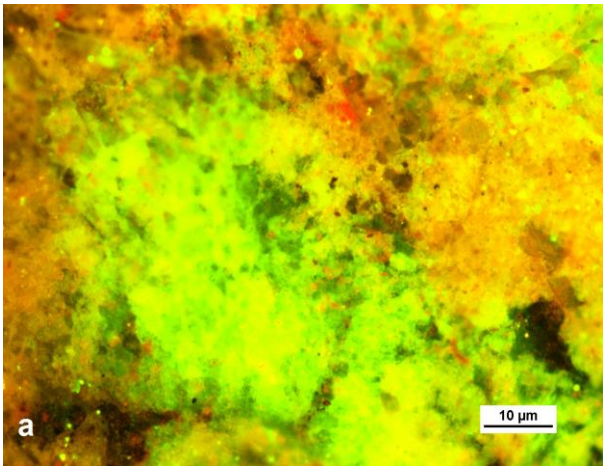
TABLE 7. SURFACE AND CRUSHED PH OF CONCRETE SPECIMENS EXPOSED IN FRESHWATER

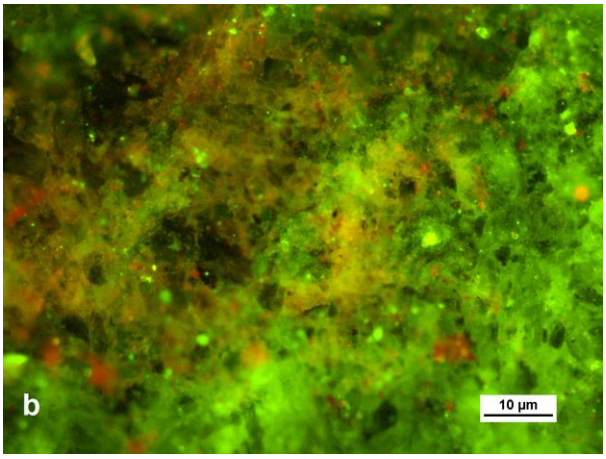
Types of Specimens	Surface pH	Crushed pH
NC	10.30	10.92
2.5% CDA	10.36	11.33
5.0% CDA	10.65	11.66
7.5% CDA	10.78	12.10
10.0% CDA	11.10	12.33
12.5% CDA	11.35	12.50
15.0% CDA	11.60	12.50

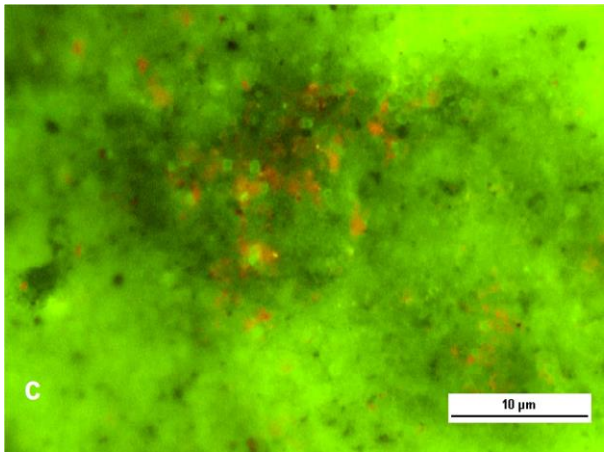
3.4 Epifluorescence microscopic study

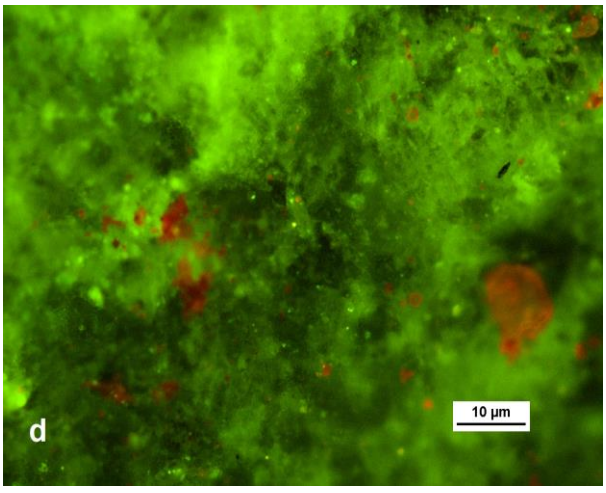
NC and concrete modified with CDA (2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 %) exposed in freshwater conditions stained with 0.1% AO solution for 30 min and rinsed with deionized water is shown

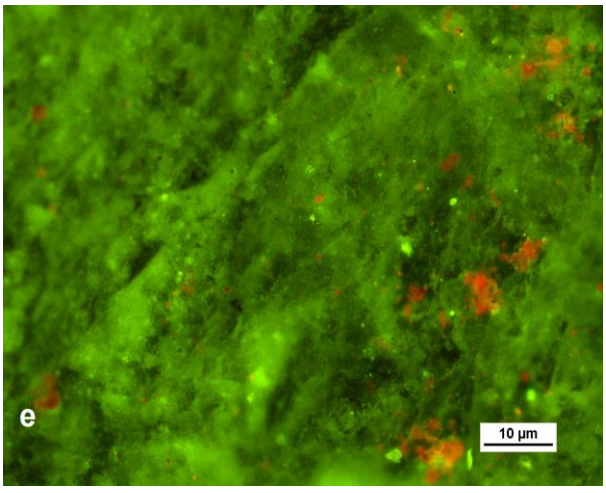
in Figure 1 The red fluorescence indicated good biofilm formation on NC (Figure 1a) whereas very less or no attached biofilm on concrete modified with CDA specimens confirmed with green fluorescence (Figure 1 b, c, d, e, f and g).











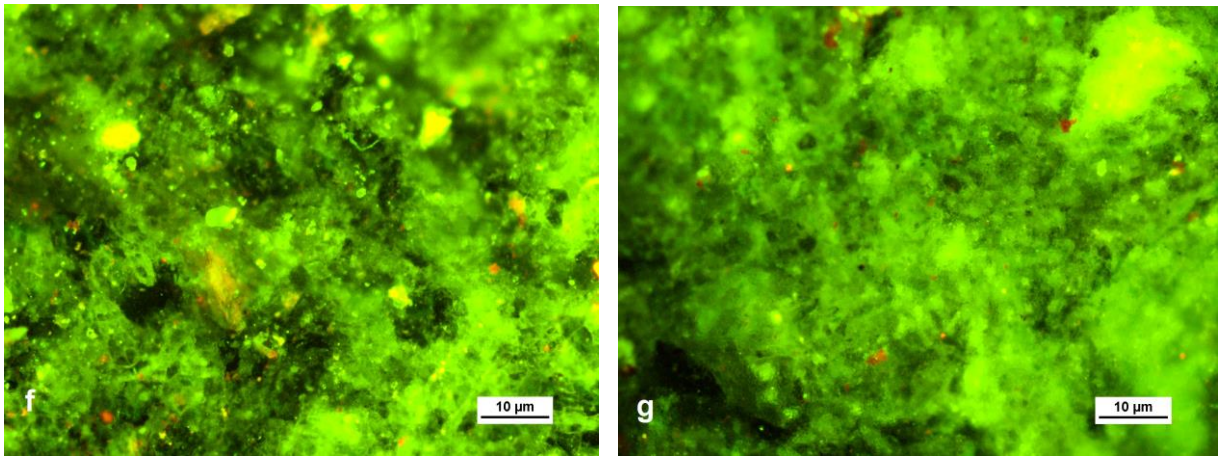


Figure 1. Epifluorescence micrographs of the biofilm on (a) NC and concrete modified with CDA (b) 2.5% (c) 5.0% (d) 7.5% (e) 10.0% (f) 12.5% and (g) 15.0% exposed in freshwater

3.5. Results of XRD studies

The XRD analysis showed the peak at 20.77, 26.68, 50.23, 68.16, 73.52, 81.39 and 83.78 corresponds to silicon dioxide peak (JCPDS - 461045). Calcium silicate hydrate peaks were observed at 28.41 and 59.97 (JCPDS – 330305). The peak at 29.47 and 59.97 shows the presence of calcium aluminum silicate hydrate (JCPDS – 391372). Calcium aluminum oxide sulfate peaks were observed at 23.65 and 42.86 (JCPDS – 330256). The peak observed at 33.98 and 54.87 showed the presence of calcium hydroxide (JCPDS – 040733) (Figure 2).

In Figure 3, calcium silicate hydrate peaks were observed at 28.41 corresponds to (022) plane and 59.97 corresponds to $(14\bar{1})$ plane. As the CDA percentage increases, the crystallite size of CSH peaks decreases. This type of nano structured CSH formation increases the hardening properties of the CDA modified concrete [23]. The strain was observed in the case of NC peaks which corresponds to (022) and (141) planes of CSH. The integral intensity of silica peak observed at 20.77 corresponds to (100) plane decreases (26.81, 9.75 and 6.00) with increasing CDA percent. This indicates that silica from NC also taking part in the formation of CSH in the CDA modified concrete.

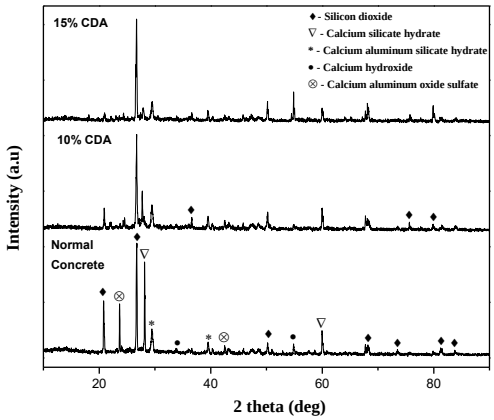


Figure 2. XRD analysis of NC and concrete modified with CDA with 10 and 15% exposed in fresh water

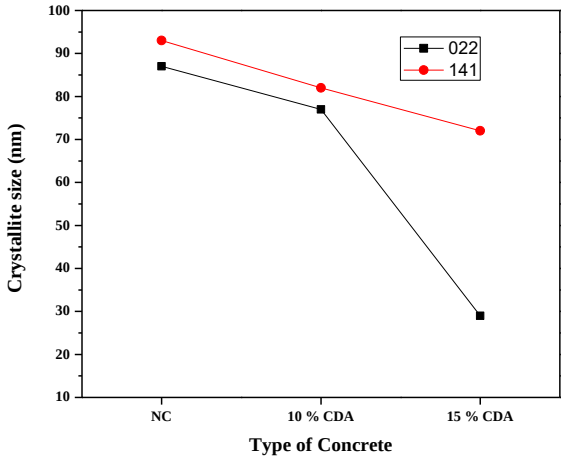


Figure 3. Crystallite size of calcium silicate hydrate by XRD

4. Discussion

The two different concrete mixes such as NC and concrete modified with CDA (2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 %) were prepared as cylindrical specimens of 200 x 100mm size. It was sliced into 100mm×9mm and exposed in the freshwater for 45 days to know the microbial deterioration on the CDA modified concrete and also to find out the better mix of these for the long term studies in the aquatic environments. Literature says that CDA contains cementatious materials enriched with silica which is a pozzolanic material [24].Also the partial replacement of cement with 5% to 10% silica fume improved resistance to microbiologically influenced corrosion (MIC) [25].

The antibacterial studies of cow dung ash powder were performed in both *Bacillus subtilis* (gram positive) and *Pseudomonas aeruginosa* (gram negative) bacterial pure culture. It has been found that increased the quantity of CDA powder from 0.5gm to 2.0gm showed less bacterial growths in agar plates in both types of bacterial culture. But when compared the both cultures with respect to presence of active cells gram negative showed more active cells than gram positive bacteria. The biofilm of 45 days exposed concrete specimens in freshwater was analyzed for pH degradation studies, TVC, TDS, TSS and epifluorescence microscopic studies. The biofilm characterization by TVC analysis on NC concrete was 10.0 ×103 cfu/cm2 and 1.8 ×101 on 15% CDA modified concrete with respect to aerobic bacteria. Same observation was found in Pseudomonas sp., fungal and algal species whereas no growth was observed in anaerobic bacteria (sulphate-reducing bacteria).

pH of the concrete one of the important factor for metabolic activities of bacteria. The pH degradation studies of both NC and concrete modified with CDA of all the percentage ratio maintained the basic nature from 10.30-11.60 for surface pH and 10.92-12.50 for crushed pH. pH was observed as alkaline in nature on the 15% modified concrete structures. Epifluorescence micrographs showed that the actively fluorescing was more on NC than CDA modified concrete and there was no fluorescence on 15% replaced CDA modified concrete. XRD analysis explained that the NC has excess of unreactive calcium hydroxide, which can also decrease the concrete strength. This calcium hydroxide undergoes secondary hydration reaction with silica available in the CDA modified concrete forming CSH. While increasing CDA percent in the concrete, the availability of silica increases and sufficient secondary hydration reaction occurs to form CSH. This could be also the possible mechanism to enhance the compressive strength of the concrete. The result showed that less crystallite size of CSH in CDA modified concrete which is the basis for reduced the pore size and hence decrease the microbial deterioration.

Thus this study showed the better results on 15% concrete modified with CDA under biofilm forming conditions, with respect to pH degradation and epifluorescence microscopic studies. However future studies will be taken for long term effects in the better mix of CDA modified concrete to know its biodeterioration properties in aquatic environments.

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