



Modified cement composites for protection against microbial induced concrete corrosion of marine structures

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ABSTRACT

Specific microorganisms involved in the deterioration of concrete result in a phenomenon called Microbial Induced Concrete Corrosion (MICC). The Reinforced cement concrete (RCC) structures constructed in and around coastal areas/backwaters are severely affected by various microbial communities in the form of MICC. Modification in concrete materials by the incorporation of mineral and chemical admixture have shown better resistance against MICC. The objective of this study is to evaluate the antimicrobial performance and corrosion protection of modified cement composites for coastal areas. Initially, four types of modified cement composite cube specimen were cast to evaluate its compressive strength. In order to find the microorganism responsible for concrete corrosion, microbe samples were collected from the seashore, isolated and sequenced followed by BLAST analysis for identification. The bacterium was identified to be *Serratia marcescens* and a phylogenetic tree was constructed showing the evolutionary relationship of the isolated bacterium. For the evaluation of antimicrobial performance, four types of semi-circular modified cement composite specimen were exposed in isolated microbial culture and total viable count (TVC) was calculated. The compressive strength results of inhibitor admixed cement composite showed an appreciable increase as compared with the other three cement composites. At the end of one week, the percentage increment of TVC in inhibitor admixed cement composite was least as compared to the other three cement composites. It can be concluded that inhibitor admixed cement composite possesses improved antimicrobial resistance as compared to cement composite without any admixture and polymer modified cement composites and recommended for corrosion protection of marine structures.

1. Introduction

Microbial induced concrete corrosion (MICC) is one of the major threats to concrete structures associated with the marine environment. Among different species of microorganisms identified in the marine environment, not all of them cause concrete deterioration. Concrete is highly alkaline in nature gets distressed mostly by acidophilic microorganisms (Islander et al., 1991). These microorganisms produce acids which will react with hydrates of cement in the hardened concrete causing a chemical change in the composition, reduction in pH and subsequent corrosion of steel rebar in the concrete structures. Reinforced cement concrete (RCC) structures which are exposed to the severe environment, such as sewer pipelines, water treatment plants, agricultural infrastructure, RCC cooling towers, RCC bridges and marine structures are getting corroded by the influence of microorganisms (Hu et al., 2011).

At this juncture, it is appropriate to define the meaning of MICC. In the Oxford advanced learners dictionary, published in 2005, says, "Microbe" is "an extremely small living thing that you can only see under the microscope and that may cause disease, the term "Induce" means "to cause something" and "Corrode" is to destroy something slowly". So collectively it can be said that "MICC" is the "slow destruction of concrete, caused by the influence of extremely small microorganisms". Rigdon and Beardsley (1958) has named these small microorganisms as "autotrophs" and stated that "Autotrophs belong to that class of ubiquitous one-celled organisms that are known as bacteria". He further states that mostly sulfur-oxidizing bacteria are involved in the deterioration of concrete (Rigdon and Beardsley, 1958, Sand and Bock, 1991).

Efforts to minimize or prevent MICC in concrete have been extensive. In general, there are three major preventative measures for the deterioration due to MICC: (1) coatings; (2) sewer treatment; and (3)

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modification of concrete materials (Hu et al., 2011). Many researchers have used mineral admixtures to enhance the property of concrete. Results show that the addition of mineral admixtures like fly ash and silica fume has significantly increased the resistance of concrete against MICC (Hu et al., 2011). Biofilm characterization studies and microscopic studies show excellent results of fly ash concrete. Laboratory exposure studies in pure cultures of *Thiobacillus thiooxidans* and *Fusarium oxysporum* were demonstrated for the inhibition of microbial growth on fly ash concrete. Epifluorescence and scanning electron microscopic studies supported the better performance of the fly ash concrete (Vishwakarma et al., 2014). In another similar study, in which fly ash modified concrete is tested using lock-in thermography showed a very little change in the phase angle and amplitude between one-year sea water exposures (Vishwakarma, 2012). It has been explored that, modification in concrete materials can be one of the effective methods in protecting the concrete from MICC. Moreover, incorporation of inhibitor for the protection of concrete against MICC is not extensively documented.

In this study, an attempt has been made to evaluate the antimicrobial performance and corrosion protection of modified cement composites for coastal areas. The modified cement composites were developed by the incorporation of mineral and chemical admixture in the cement composite. Initially, four types of modified cement composite cube and semi-circular specimens were cast. Cubes were used for the evaluation of compressive strength at various ages and semi-circular specimens were cast in order to find the antimicrobial performance of four types of modified cement composites. Simultaneously, Microbe samples from different places near sea shore were collected and isolated for the identification of microorganism responsible for concrete corrosion. In the end, the isolated microorganism was used to study the antimicrobial performance of modified cement composites.

2. Materials and methods

2.1. Materials

Portland pozzolana cement (PPC) conforming to IS 1489 (1): 1991 was used for the preparation of modified cement composite specimens. The specific gravity of PPC cement used was 2.76 whereas standard consistency was found to be 38% as per IS 4031 (11): 1988 and IS 4031 (4): 1988 respectively. Table 1 shows the test results of PPC cement. Manufactured sand (M-sand) with a maximum size of 4.75 mm and calculated specific gravity of 2.51 in accordance with IS 2386 (3): 1963, was used as fine aggregate. The bulk density of M-sand was determined in accordance with IS 2386 (3): 1963, having the value of 1670 kg/m³. The fineness modulus of M-sand was calculated as 3.2 (IS 2386 (1): 1963). The selection of fine aggregate was in agreement with IS 383: 1970. The physical properties of M-sand are given in Table 2. Fig. 1 shows the particle size distribution of M-sand which corresponds to grading Zone I. Three type of admixtures were used in this study namely styrene acrylate copolymer (SAR), acrylic polymer (AR) and sodium nitrite (SN) based mixed inhibitor (Haji Sheik Mohammed and Samuel Knight, 2008). Double distilled water was used both for casting and curing of four types of modified cement composite.

Table 1
Test results of PPC cement.

Sl. No.	Property	Value
1.	Fineness (%)	1.33
2.	Standard consistency (%)	38
3.	Specific gravity	2.76
4.	Initial setting time (min)	90
5.	Final setting time (min)	160

Table 2
Physical properties of fine aggregate.

Sl. No.	Property	Value
1.	Specific gravity	2.62
2.	Bulk density (kg/m ³)	1670
3.	Water absorption (%)	1.2
4.	Percentage voids (%)	36
5.	Fineness modulus	3.2
6.	Grading zone	1

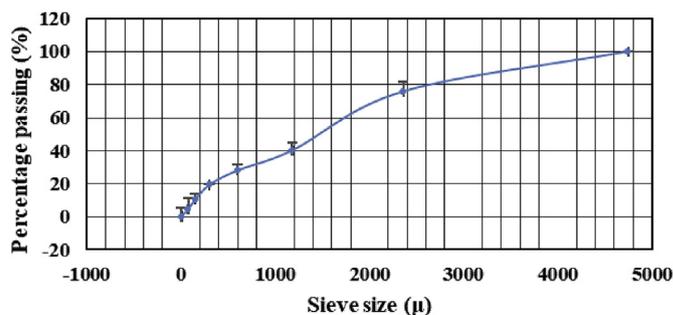


Fig. 1. Particle size distribution of fine aggregate.

2.2. Preparation of test samples

The mix design for the preparation of four types of modified cement composite was based on Hu et al. (2011). A mix ratio of 1:2.5 (1 part of cement: 2.5 parts of fine aggregate) was adopted. Four types of modified cement composite were prepared to have a designation as PPC, cement composite without any admixture; PPC-AR, cement composite prepared with 2% of AR polymer; PPC-SAR, cement composite prepared with 2% of SAR polymer; and PPC-SN, cement composite prepared with 2% SN based inhibitor. The w/c ratio for all the combinations was arrived by performing mini-slump test as per IS 4031 (7): 1988 with the fixed target flow 80–85%. Fig. 2 shows the mini-slump test in progress. Table 3 shows the details of the mix proportion of four types of modified cement composite.

Modified cement composites were prepared by thoroughly mixing cement, fine aggregate, and distilled water in a portable cement composite mixer machine. For the preparation of PPC-SN, PPC-AR, and PPC-SAR cement composite samples, initially 70% of total distilled water was added and the polymer or inhibitor was mixed along with the remaining 30% of distilled water. The properly mixed cement composite was placed inside the standard moulds and hand compacted. After casting, the samples were kept for 24 h at room temperature. The



Fig. 2. Mini-slump test in progress.

Table 3
Mix proportion of four types of modified cement composites.

Sl. No.	Modified cement composite	Admixture dosage (by weight of cement)	w/c ratio	Flow %	Target Flow %
1.	PPC	–	0.5	85.2	80–85%
2.	PPC-SN	2%	0.42	84.7	
3.	PPC-AR	2%	0.51	80.8	
4.	PPC-SAR	2%	0.49	83.5	

Table 4
Observation on compressive strength of four types of modified cement composites.

Type of sample	Compressive strength (MPa)		
	7 days	21 days	28 days
PPC	30.0	34.4	39.6
PPC-SAR	25.2	31.3	35.7
PPC-AR	21.1	28.3	28.8
PPC-SN	41.9	45.6	50.0

specimens were then cured in a distilled water for a specified period.

2.3. Compressive strength test

Compressive strength test was conducted in order to study the influence of polymer modification/inhibitor addition on the strength development of modified cement composites. Cube specimens of size 70 mm were cast and subjected to compressive strength test as per [IS 516: 1959](#) using a 500 kN capacity digital compressive testing machine. The compressive strength of the modified cement composite specimens at the age of 7, 21 and 28 days of curing were found and reported. Three specimens were cast for each combination and a total of 36 specimens were subjected to a compressive strength test.

2.4. Microbial samples collection

The microbial samples for the present study were collected from various locations such as walls, rock boulders near the seashore, and rust from an infrastructure facility, which is 10 km south-east of

Chennai and located within 100 m from the seashore. Visible corrosion could be noticed in the buildings, tanks and machinery present there. A total of 17 samples from specific locations such as walls, rocks, rust, concrete, mud, water samples etc., were collected and kept in 15 mL sterile falcon tubes. These were brought back to the lab and stored at 4 °C.

2.5. Isolation of microbes

Pour plate method was used for the isolation of microbe from the collected samples. 1 mL of serially diluted sample was placed in a sterile petri dish using a sterile pipette. The Luria-Bertani (LB) media was prepared and poured into the petri dish. Inversion of the plate was done after the solidification of the LB media and was left for overnight incubation at 37 °C. The pour plate method was followed by the streak plate method to isolate a pure microbial strain from a mixed microbial population ([Rasool et al., 2018](#)). After overnight incubation of the pour plate, the observation was made. A colony was picked from the pour plate with the help of inoculation loop and streaked over the petri dish. It was then incubated overnight at 37 °C ([Sah et al., 2019a, 2019b](#)).

2.6. Identification of microbes by 16s RNA sequencing

DNA was extracted from the microbial cultures using the Phenol-Chloroform method ([Kramvis et al., 1996](#)), which is based on liquid-liquid extraction technique. It separates molecular mixtures based on the solubility of the molecules individually, in two different immiscible liquids. 2 mL of microbial culture was pipetted from the test tube containing microbial culture and poured in a sterile 2 mL centrifuge vial. The sample was centrifuged using a tabletop centrifuge machine for 10 min at 10,000 rpm. The supernatant was discarded and the pellet was transferred to a 15 mL falcon tube. 2 mL of NaCl buffer was added to the pellet followed by 250 µL of 10% SDS. It was mixed well and was incubated in a hot water bath for at 60 °C for 15 min. Furthermore, 1000 µL of the 1:1 phenol-chloroform mixture was added followed by 500 µL of chloroform. The contents were centrifuged using a refrigerated centrifuge machine at 10,000 rpm for 5 min, maintained at 4 °C which led to the formation of three phases. The first phase was transferred to a sterile 15 mL falcon tube. An equal amount of 95% ice-cold ethanol was added to the falcon tube. The tube was then slowly inverted and mixed gently until the precipitation of DNA was seen. Next, the tube was incubated in –20 °C for 30 min. After which it was

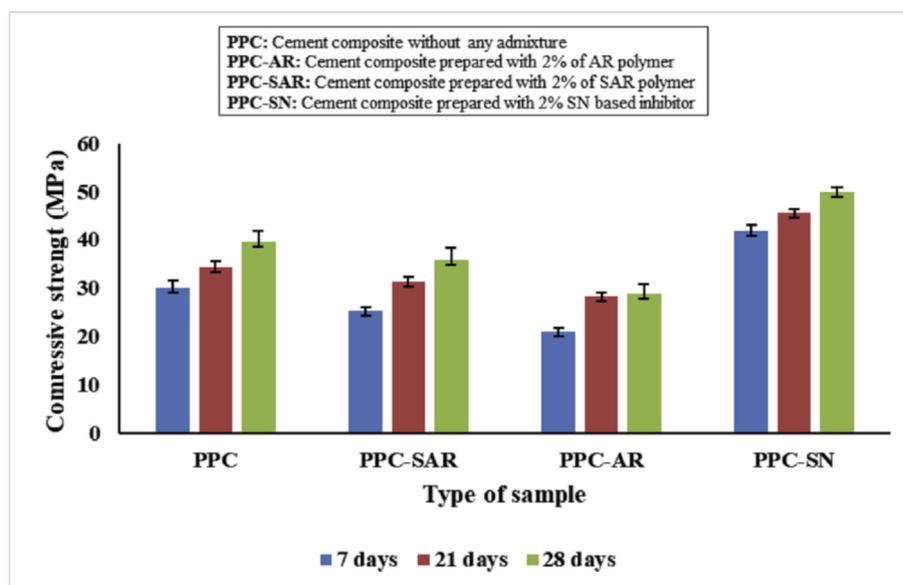


Fig. 3. Comparison of compressive strength of four types of modified cement composite.

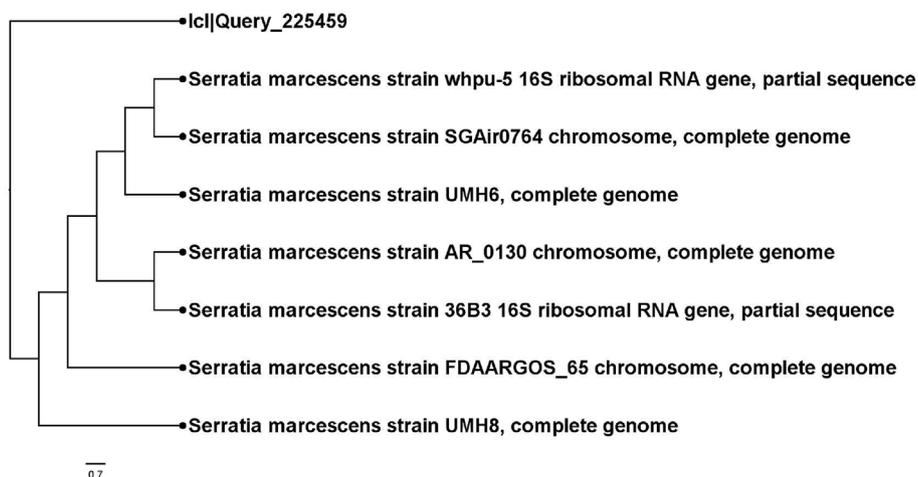


Fig. 4. Phylogenetic tree displaying the evolutionary relationship of the isolated bacterium.

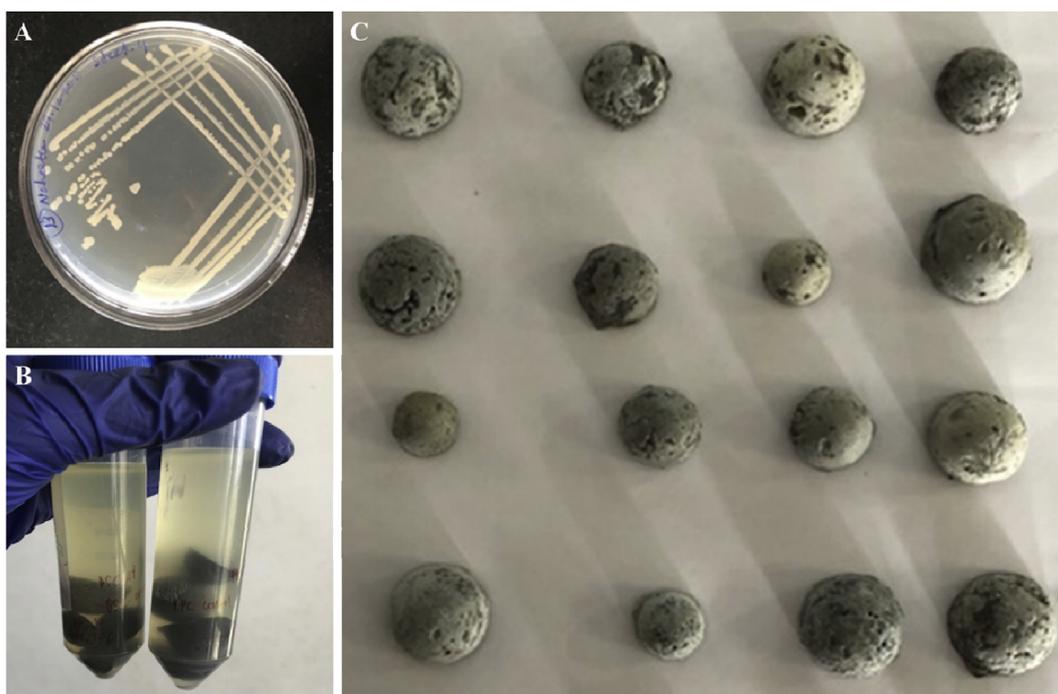


Fig. 5. A. Streak plate of the microbe *Serratia marcescens*; B. Four types of modified cement composite were inoculated in microbial broth; C. Four types of the modified cement composite after inoculation.

again centrifuged for 5 min at 10,000 rpm. At the end of ethanol wash, the pellet was dried in laminar air flow for 15 min. The pellet was then mixed with 50 μ L TE buffer. The extracted DNA was mixed with distilled water and stored at -80°C .

PCR amplification of 16s rRNA was carried out, using the isolated genomic DNA as a template and forward primers (5'AGAGTTTGATCC TGGCTCAG 3') and reverse primers (5' GGTTACCTTGTTACGACTT 3') were used. The amplification reaction was performed in a Master Cycler that was programmed as follows: initial denaturation at 95°C for 5 min; 30 cycles of denaturation of 94°C for 30 s, annealing at 56°C for 30 s, extension at 72°C for 2 min and the final extension at 72°C for 7 min. The PCR products were stored at -80°C for further use. The amplified products were quantified in 1% agarose gel electrophoresis. The gel was run at 100 V for 1 h 16s rRNA sequencing of the genomic DNA was done by Agrigenome, Smart City Kochi, Info park Road, Kakkanad, Kerala. The obtained sequence was run in the BLAST tool for microbial identification followed by phylogenetic tree construction (Akther et al., 2018).

2.7. Antimicrobial activity of modified cement composites

Four types of modified cement composite viz. PPC, PPC-AR and PPC-SAR, PPC-SN were used to evaluate their antimicrobial activity. LB Broth was prepared by dissolving 2.5 g of LB Broth powder in 100 mL distilled water and was autoclaved at 121°C for 15 min. Each of the five sterile 50 mL falcon tubes was filled with 30 mL of cooled LB broth. The microbial colony from the streak plate was inoculated into all the five tubes using an inoculation loop under sterile conditions. The falcon tubes were left for overnight shaking at 37°C . After overnight shaking, the falcon tubes exhibited a turbid appearance, depicting the growth of the microbe. The four types of modified cement composite samples were inoculated in each of these five falcon tubes and were again left for shaking at 37°C for 6 days. During the shaking period, the samples were taken after every 36 h to determine the Total Viable Count (TVC) (Vishwakarma et al., 2016; A.P.H. Association, 1915).

LB Agar was prepared by dissolving 2 g LB Agar powder in 50 mL distilled water, autoclaved at 121°C for 15 min 1 g of the cement

Table 5
TVC Value of modified cement composites for every 36 h.

Number of hours	Modified cement composite	Number of colony forming units (CFU)	Average microbe per mL of 1×10^6 dilution	Percentage increment (%)
36	PPC	48	480×10^6	0
	PPC-AR	55	550×10^6	0
	PPC-SAR	32	320×10^6	0
	PPC-SN	60	600×10^6	0
72	PPC	70	700×10^6	46
	PPC-AR	79	790×10^6	43
	PPC-SAR	58	580×10^6	81
	PPC-SN	87	870×10^6	45
108	PPC	95	950×10^6	35
	PPC-AR	99	990×10^6	25
	PPC-SAR	86	860×10^6	48
	PPC-SN	130	1300×10^6	49
144	PPC	200	2000×10^6	110
	PPC-AR	180	1800×10^6	82
	PPC-SAR	125	1250×10^6	45
	PPC-SN	167	1670×10^6	28

sample was taken and dissolved in 10 mL distilled. This was followed by serial dilution to 10^{-6} . 0.1 mL of the serially diluted sample was pipetted using a sterile pipette and placed in a sterile petri dish. Cooled agar was then poured over it and mixed well. The petri dish was left for 48-h incubation at 37 °C. At the end of the incubation period, the petri dish was observed using a colony counter for determining the number of microbial colonies or Colony forming units (CFU). From this, an average microbial per mL of serially diluted microbe was determined (Petersen and McLaughlin, 2016).

3. Results and discussion

3.1. Compressive strength test

Table 4 shows the observation on the compressive strength of four types of modified cement composite viz. PPC, PPC-SAR, PPC-AR, and PPC-SN at the age of 7, 21, and 28 days. It can be seen that PPC-SN cement composite offered significantly improved compressive strength as compared to PPC cement composite in all the tested ages. There is an increase in 28 days compressive strength of the order of 26% as compared to PPC cement composite. PPC-SAR cement composite exhibited marginally reduced compressive strength as compared to PPC cement

composite. There is a reduction in compressive strength of the order of 10% was observed when tested at the age of 28 days whereas PPC-AR cement composite was observed with a significant reduction in compressive strength of the order of 15–30% as compared to PPC cement composite. Also, the compressive strength of PPC-SAR is 24% higher when compared with PPC-AR. It can be said that PPC-SAR performing better when compared with PPC-AR whereas the compressive strength of PPC-SN cement composite is higher than other types of modified cement composite. The reason behind the higher strength of PPC-SN cement composite is lower w/c ratio when compared with the PPC cement composite for similar workability. Fig. 3 shows the comparison of the compressive strength of four types of modified cement composite.

3.2. Isolation and molecular characterization

A total of 17 samples from specific locations near seashore were collected for isolation. After running the sequencing data through BLAST and constructing a phylogenetic tree, it was observed that the organism was *Serratia marcescens* (Fig. 4). *S. marcescens* is a rod-shaped gram-negative bacterium, belonging to the family of *Enterobacteriaceae*. It is commonly present in the environment and due to its preference for damp conditions, it found abundantly in bathrooms and marine environment (Hejazi and Falkiner, 1997). A facultative anaerobic species *S. marcescens* ACE2 was identified in the degradation of diesel and diesel transporting pipeline (Rajasekar et al., 2007a; 2007b). Also, *S. marcescens* utilises the corrosion inhibitor used for the protection of petroleum product pipelines as a sole carbon source and causes microbial degradation (Rajasekar et al., 2007a). It was also found that new strains of *Pseudomonas eruginosa* and *S. marcescens* species showed relatively high capacity and wide spectrum to degrade the hydrocarbons in gasoline, kerosene, diesel, and lubricating oil. From the above discussion, it can be said that *S. marcescens* which abundantly found in the marine environment and has the potential to biodegrade diesel and steel pipeline can also be responsible for the deterioration of RCC structures near the seashore. Fig. 5A shows the growth of *S. marcescens* on a sterile agar plate.

3.3. Antimicrobial activity

The corrosion studies were carried out to evaluate the antimicrobial performance (Saroj et al., 2019b) of four types of modified cement composites. These specimens were inoculated in the microbial culture of *S. marcescens*. Fig. 5B and C shows four types of modified cement

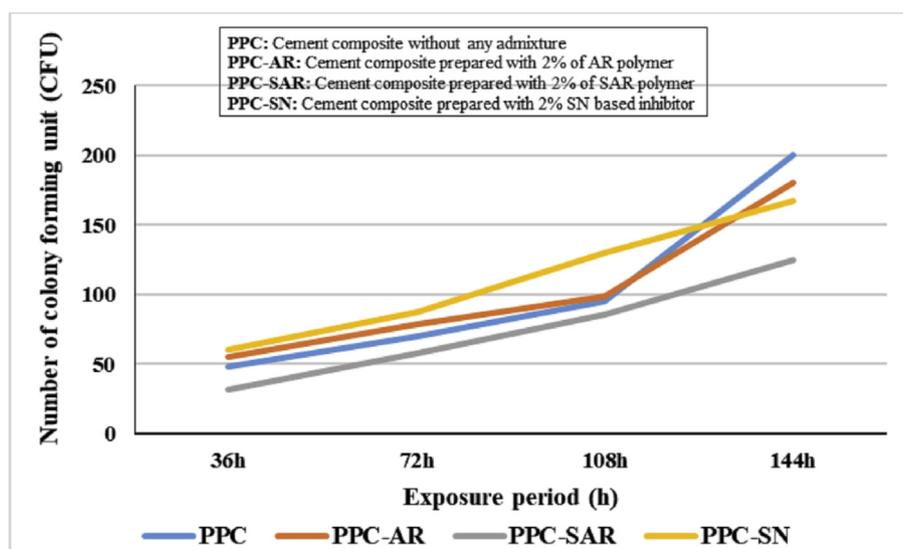


Fig. 6. Observation on CFU of four types of modified cement composite.

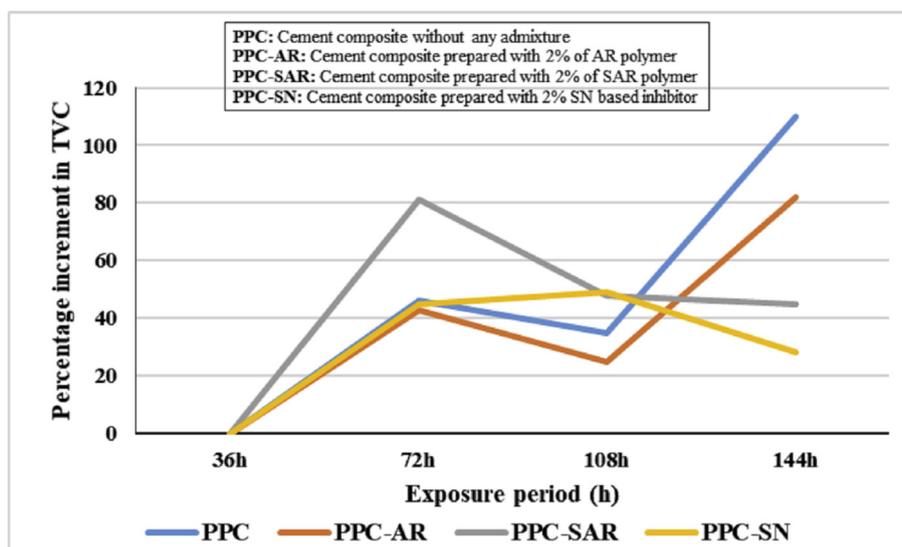


Fig. 7. Observation on percentage increment in TVC of four types of modified cement composite.

composite inoculated in microbial culture. The inoculation process was carried out for 144 h in which the modified cement composite samples were tested after every 36 h for TVC. According to the TVC results, at the end of 144 h, the microbial count (Rasool and Hemalatha, 2017) per mL in PPC and PPC-AR were the highest with very little difference whereas the microbial count per mL of PPC-SAR was the least. Also, the microbial count per mL of PPC-SN has shown a gradual increment with 16.5% less than the PPC and 33.6% higher than PPC-SAR at the end of 144 h. Table 5 shows the TVC value of the four types of modified cement composite.

Fig. 6 shows observation on percentage increment in TVC for four types of modified cement composite. It can be observed that initially, all the modified cement composites have shown similar increment except PPC-SAR. This may be due to insignificant resistance exhibited by styrene acrylate copolymer in the initial period against the growth of microbes. After 108 h, all the combinations of modified cement composite have shown a steep decrease in the percentage increment of TVC except in PPC-SN, which has a slight increment of 9% from the previous value. This can be attributed to improved resistance offered by all the modified cement composites against the growth of microbes. At the end of 144 h, only PPC-SN and PPC-SAR have shown a steep decrease in the percentage increment of TVC (Fig. 7). The percentage increment of TVC after 144 h for PPC-SN was only 28% which is 38% less than the increment shown by PPC-SAR. A similar reduction of TVC was observed when the same inhibitor was added in the concrete (Harilal et al., 2019).

Four types of modified cement composite were evaluated for the antimicrobial performance against *S. marcescens*. Results show that inhibitor admixed cement composites performed better as compared to other cement composites in antimicrobial performance with least percentage increment at the end of 144 h. It can be concluded that inhibitor admixed cement composite exhibited significantly improved capability to resist the growth of *S. marcescens* over RCC structures constructed in the marine environment (Sah and Hemalatha, 2015).

4. Conclusion

The present study manifest the incorporation of AR, SAR based polymer and SN based inhibitor in cement composites to evaluate their antimicrobial resistance. The 28th-day compressive strength results of PPC-SN shows 26.3, 40 and 73.6% higher values from PPC, PPC-SAR and PPC-AR respectively. It has also shown significant improvement in the antimicrobial resistance with least percentage increment in the TVC

count at the end of 144 h. It can be concluded that sodium nitrite mixed inhibitor based cement composite (PPC-SN) possess improved antimicrobial resistance as compared to other modified cement composites.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bcab.2019.101192>.

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