

ABSTRACT

Title of Document: MONITORING OF CORROSION IN
SUBMERGED REINFORCED CONCRETE
STRUCTURES USING BIOLOGICAL
INDICATORS

Jithin Thampi, Master of Science, 2017

Thesis directed by: Dr. Peter C. Chang, Department of Civil and
Environmental Engineering

Corrosion of steel has severe economic impacts on industry and infrastructure. Reinforcement corrosion and subsequent loss of strength is the reason for failures in numerous reinforced concrete structures that leads to billions of dollars in losses. Thus corrosion detection is of great importance. Currently, corrosion detection in existing structures is performed using non-destructive methods, which include ultrasonics, radiography, thermography, and enhanced photographic imaging. Such methods are quick but lack details and can be unreliable. An alternative approach may be measuring conditions like pH that affect corrosion. Lowering of pH can indicate that corrosion can occur. It has also been identified that certain biological factors like bacteria play a major role in corrosion through a process called Microbial Induced Corrosion (MIC). Bacteria accumulate over metal surfaces over time forming biofilms, creating environments favorable for corrosion. This thesis attempts to study the correlation between corrosion of reinforcement steel in concrete and bacterial activities measured at the concrete surface and thereby observe the effectiveness of using bacterial activities to indirectly detect corrosion in the reinforcement steel.

MONITORING OF CORROSION IN SUBMERGED REINFORCED CONCRETE
STRUCTURES USING BIOLOGICAL INDICATORS

by

Jithin Thampi

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master of Science
2017

Advisory Committee:
Associate Professor Peter C. Chang, Chair
Professor Amde M. Amde
Professor Yunfeng Zhang

© Copyright by
Jithin Thampi
2017

Dedication

To my beloved parents.

Acknowledgements

First of all, I would like to express my sincere gratitude towards my advisor Dr. Peter Chang for his support and motivation over the past two years. His help and guidance was crucial for the completion of this work.

I would like to thank Dr. Daniel Stein for allowing me to use the facilities and equipment in his lab for conducting this study and also for his valuable suggestions.

I learned a lot from my discussions with Dr. Birthe Kjellerup and I thank her for that.

I would also like to thank Dr. Amde M. Amde and Dr. Yunfeng Zhang for serving as the thesis committee members and also for all the knowledge I gained from their classes over the last two years.

And finally I would like to thank my family and friends for all the care and support.

Table of Contents

Dedication	ii
Acknowledgements	iii
Table of Contents	iv
List of Tables	vii
List of Figures	ix
Chapter 1: Introduction	1
Chapter 2: Literature Review and Background	5
2.1. Corrosion of Steel in Concrete	5
2.2. Methods of Corrosion Detection	6
2.3. Role of Bacteria in Corrosion	8
2.4. Bacteria and Corrosion Control	11
2.5. Colorimetric Determination of Carbohydrates	12
Chapter 3: Materials and Methodology	15
3.1. Preparation of Specimens	15
3.2. Details of Specimens	18
3.3. Measurement of Biological Activity	20
3.4. Measurement of pH	22
3.5. Measurement of Weight Loss due to Corrosion	23

3.6. Effect of Corrosion of Steel on Bacterial Activity on Reinforced Concrete Surface	25
3.6.1. Objective	25
3.6.2. Proposed theory.....	25
3.6.3. Medium.....	26
3.6.4. Parameters Measured	26
3.6.5. Procedure followed	27
3.7. Effect of Corrosion of Reinforcement Steel on Biological Activity in Surrounding Medium	28
3.7.1. Objective	28
3.7.2. Proposed theory.....	29
3.7.3. Medium.....	30
3.7.4. Parameters Measured	30
3.7.5. Procedure followed	30
3.8. Effect of Biological Factors in Increasing Steel Corrosion and Concrete Degradation.....	32
3.8.1. Objective	32
3.8.2. Proposed Theory	32
3.8.3. Medium.....	33
3.8.4. Parameters measured.....	33

3.8.5. Procedure followed	34
Chapter 4: Results and Discussion.....	37
4.1. Effect of Corrosion of Steel on Biological Activity on Concrete Surface	37
4.1.1. Expected Results and Experimental Findings.....	37
4.2. Effect of Exposure and Degradation of Steel on Surrounding Medium	45
4.2.1. Expected results and experimental findings.....	45
4.3. Effect of Biological Factors on Steel Corrosion and Concrete Degradation ..	48
4.3.1. Expected results and experimental findings.....	49
4.4. Experimental Assumptions and limitations.....	53
Chapter 5: Conclusion and Future Development	57
5.1. Summary	57
5.2. Recommendations for Future Development	59
Appendix: Experimental Data.....	62
References	67

List of Tables

Table 4.3-1. Parameters for concrete sample with 1 mm crack	48
Table 4.3-2. Parameters for Steel sample	49
Table A.1. Percentage Loss of weight for specimen with no crack	62
Table A.2. Percentage loss of weight for specimen with hairline crack	62
Table A.3. Percentage loss of weight for specimen with 1 mm crack	62
Table A.4. Percentage loss of weight for steel specimens	62
Table A.5. Surface Residue weight in g in concrete samples with no crack	62
Table A.6. Surface Residue weight in g in concrete samples with hairline crack	63
Table A.7. Surface Residue weight in g in concrete samples with 1 mm crack	63
Table A.8. Surface Residue OD in concrete samples with no crack	63
Table A.9. Surface Residue OD in concrete samples with hairline crack	63
Table A.10. Surface Residue OD in concrete samples with 1 mm crack	63
Table A.11. pH at surface for concrete samples with no crack	64
Table A.12. pH at surface in concrete samples with hairline crack	64
Table A.13. pH at surface in concrete samples with 1 mm crack	64
Table A.14. pH at level of steel in concrete samples with no crack	64
Table A.15. pH at level of steel in concrete samples with hairline crack	64
Table A.16. pH at level of steel in concrete samples with 1 mm crack	65

Table A.17. Medium pH in concrete samples with no crack	65
Table A.18. Medium pH in concrete samples with hairline crack	65
Table A.19. Medium pH in concrete samples with 1 mm crack	65
Table A.20. Medium OD for concrete samples with no crack	65
Table A.21. Medium OD for concrete samples with hairline crack	66
Table A.22. Medium OD for concrete samples with 1 mm crack	66

List of Figures

Figure 2.3-1. Mechanism of EMIC and CMIC	11
Figure 3.1-1. Preparation of specimens with hairline crack	16
Figure 3.1-2. Steps involved in the preparation of concrete specimens	16
Figure 3.1-3. Preparation of specimens with 1 mm wide crack using shims	17
Figure 3.1-4. Concrete samples with different crack sizes	18
Figure 3.2-1. Steel coupons before immersion	19
Figure 3.3-1. Surface residue in DI water after centrifuging	20
Figure 3.3-2. Samples used for testing carbohydrate concentration after addition of the anthrone reagent.....	22
Figure 3.5-1. Steel bars, before and after removing corrosion products	24
Figure 3.5-2. Steel coupons and bars after removal of corrosion products	24
Figure 4.1.1-1. Variation of Surface residue weight with loss of weight in steel.....	38
Figure 4.1.1-2. Variation of surface residue OD with loss of weight in steel.....	39
Figure 4.1.1-3. Variation of surface residue OD with surface residue weight	40
Figure 4.1.1-4. Variation of surface pH with surface residue weight	41
Figure 4.1.1-5. Variation of surface pH with loss of weight in steel	43
Figure 4.1.1-6. Variation of sub-surface pH with loss of weight in steel	43
Figure 4.2.1-1. Variation of medium pH with loss of weight in steel	46

Figure 4.2.1-2. Variation of medium OD with loss of weight in steel.....	47
Figure 4.3.1-1. Comparison of percentage loss of weight and surface residue weight in concrete specimens in sterile and non-sterile media	50
Figure 4.3.1-2. Comparison of medium OD and surface residue OD in concrete specimens in sterile and non-sterile media	50
Figure 4.3.1-3. Comparison of medium pH, surface pH and sub-surface pH in concrete specimens in sterile and non-sterile media	51
Figure 4.3.1-4. Comparison of medium OD and percentage loss of weight for steel specimens in sterile and non-sterile media	51

Chapter 1: Introduction

Reinforcing steel corrosion in reinforced concrete structures is one of the most common problems that require repair and rehabilitation of structures. Average losses due to corrosion are estimated to be billions of dollars. Because reinforcing steel is embedded in the concrete, detection of the corrosion problem is nontrivial. While the only direct method of determining the amount of corrosion is by weight change of the reinforcing steel, it is a destructive method that is costly and time consuming. As a result of this difficulty, a host of indirect nondestructive methods have been used. They include eddy current, ultrasonic, and radiography. These methods are labor intensive and they are commonly used to detect only local damage. Wide area damage detection methods employing imaging techniques include thermography, and enhanced photographic imaging. Such methods are quick but lack details. All the methods so far employed are costly, with the exception of photographic imaging, which so far has not shown an acceptable degree of accuracy (Dabous et al. 2017). Recent studies of corrosion problems in power plants show that bacterial biofilms may induce corrosion. These bacteria are found in the water circulation system despite attempts to make the environment as sterile as possible indicating that they can survive in a wide range of environments (Kjellerup et al. 2009).

Reinforced concrete and steel structures may be designed to be submerged or be submerged as a result of seawater rise. While those structures designed to be submerged are usually in contact with water continuously, a significant number of structures or part of these structure are in water intermittently; e.g. bridge piers in the

intertidal zone. Where moisture is present, either continuously or intermittently, bacteria have been observed to cause corrosion of the steel in a process known as microbiologically-influenced corrosion (MIC), or biological corrosion (bio-corrosion). MIC accounts for a significant amount of corrosion losses and is detrimental to the integrity of structures (Flemming 1996).

The biological and the subsequent chemical reaction processes may serve as an indirect measure of the corrosion. Studies conducted in heating systems have shown that the presence of bacteria greatly increased pitting corrosion. (Kjellerup et al 2005). Since bacterial colonies are not limited exclusively to the surface of steel components, it is reasonable to assume that they are present in the vicinity of the steel components undergoing MIC if conditions for those bacteria are favorable. For example, if the reinforcing steel under a layer of cracked concrete cover is corroding, then the iron oxidizing bacteria (IOB), sulfate reducing bacteria (SRB), and sulfate oxidizing bacteria (SOB) may form colonies on the exposed steel and the surface of concrete where cracks are formed (Videla and Herrera 2009). If a correlation between the steel corrosion and the bacteria influenced processes that take place at the surface of concrete can be established, then by measuring changes in these bacterial induced processes at the surface, it may be possible to determine the amount of steel corrosion indirectly. This work verifies the effectiveness of using bacteria as an indirect method for monitoring the corrosion in reinforcement steel in a structure without the need for using destructive testing.

Bacterial activities also affect several other parameters on the surface of concrete structures and in the surrounding medium. These include temperature, pH and

concentration of other chemical byproducts like sulfides. This study aims at establishing a relation between the bacterial activity at the concrete surface and the corrosion of steel leading to loss of weight. Other parameters like pH surrounding media are also studied as they may also be used to estimate the extent of corrosion taking place in reinforcement steel embedded in the concrete quickly and effectively.

In the current study, concrete samples were tested and the changes in the medium's pH, surface pH of concrete and bacterial activity in the medium and the concrete surface were monitored at varying degrees of steel corrosion. Bacterial activities produce enzymes and acidic compounds that lower pH and make conditions favorable for corrosion. Hence the measurement of pH may provide important information on corrosion. Metabolic activities in bacteria also lead to the formation of compounds like polysaccharides. Measuring concentration of these compounds can be used as indirect measurement of bacterial activity. Direct method for enumeration of specific bacterial strains was not performed in the current study. The corrosion of reinforcement steel in concrete was studied by keeping concrete specimens in water samples taken from the Chesapeake Bay. No additional nutrients or specific bacterial strains were added to the water sample. It is expected that bacteria present in the bay water sample will form biofilms on the concrete surface and the extent of biofilm formation may be related to the corrosion in steel. This study is an initial step in determining the relation between bacterial activity at the surface of concrete and corrosion of reinforcement. In the experiments conducted, the following parameters were observed:

1. Effect of corrosion of steel on activity of biological agents on concrete surface.
This was done by measuring the extent of biological activity at the surface of concrete and comparing it with the weight loss of reinforcing steel.
2. Effect of exposure and corrosion of steel on the surrounding medium. This was studied by measuring biological activity in the medium and relating it to the loss of steel weight.
3. Effect of biological factors on steel corrosion and concrete degradation. This was done by comparing the biological activities, pH of concrete and the medium, and the steel corrosion of specimens placed in a natural, non-sterile medium and an autoclaved sterile medium.

Chapter 2 reviews relevant studies on types and mechanisms of corrosion in concrete, techniques used for detection of corrosion and bio-corrosion. Chapter 3 includes details regarding the specimens used and protocols followed for testing. The results observed and conclusions drawn are presented in Chapters 4 and 5.

Chapter 2: Literature Review and Background

Reinforcement corrosion and subsequent loss of strength is the reason for a significant number of failures in concrete structures. Concrete is weak in tension and the embedded steel provides additional tensile strength. The loss of strength in steel thus has severe consequences. It is estimated by the Federal Highway Administration that the average losses per year on account of reinforcement corrosion are about 286 billion dollars (Arndt and Jalinoos 2009).

2.1. Corrosion of Steel in Concrete

Cracking of concrete cover can take place due to stresses produced in concrete by the corrosion of steel. The electrolytes present in the pores of concrete near the location of reinforcement steel undergo a reduction of pH after which the passive layer surrounding the reinforcement eroded (Tofutti 1982, Schiessl 1988). The pH reduction occurs due to the exposure to chlorides and sulfates from the surrounding environment. The reduction in pH and loss of passive layer initiates corrosion of steel. The consequent increase in volume of steel due to formation of corrosion products leads to the formation of micro-cracks that affects the bonding between concrete and steel. This in turn affects the strength of concrete. Over time the accumulation of rust and corrosion products further increase crack sizes and this increase of crack size lead to the degradation of the structure. Studies have been conducted to model the cracking in concrete as a result of corrosion-induced stresses (Bazant 1979) and to study the effect of corrosion on the integrity of the structure (Shodja et al.2010). It was also observed

that corrosion of steel is more prominent on the surface that is more exposed to the environment. This observation indicates that the corrosion process and the accumulation of corrosion products do not take place uniformly (Yuan and Ji 2009).

2.2. Methods of Corrosion Detection

There are several methods used for detection of reinforcement corrosion in concrete, most being non-destructive, as destructive tests cannot usually be performed on structures in service. Some methods attempt to quantify corrosion based on the electrochemical processes causing the corrosion. These include surface potential and concrete resistivity methods. But these methods require direct contact with the reinforcement bars, and are dependent on the environment within the concrete (Song and Saraswathy 2007). These shortcomings make them difficult to perform on existing structures.

Eddy current techniques are used for detecting defects in reinforcement in concrete structures. The results produced, however, depend on the electrical and magnetic permeability of the specimen, and this method is not very effective for detecting defects located deep under the surface. Also, if the distance between the test material and sensor cannot be maintained, the results may vary because of the testing procedure rather than the actual amount of corrosion. These are called lift-off effects (Shaikh et al. 2006) and this can affect the accuracy of the measurement.

Radiographic methods of inspection provide accurate details regarding corroding reinforcement bars in concrete. However there is the constant risk of overexposure which could be a severe safety hazard and the equipment used is not

suitable for in-situ measurements. This method also often requires access to both sides of the part being inspected, and this access may not always be possible (Song and Saraswathy 2007, Arndt and Jalinoos 2009).

Ultrasonic testing is yet another convenient method used for the detection of defects and cracks in concrete structure. This method relies on techniques like pulse transmission, pulse echo and resonance to identify defects within a structure. Ultrasonic testing does not give sufficient accuracy when it comes to detecting defects located deeper from the surface due to attenuation of the signal as it passes through the material. Also when used in a material like concrete which is not homogenous and has large amount of irregularities, the results produced are not always accurate (Tucker 1997, Zou et al. 2015).

A relatively low cost method of inspection is Infrared (IR) Thermography. An induction heater or even solar energy can be used to heat the reinforcement bars in concrete and the heat patterns are observed using thermographic images. Defects or cracks show up in the form of thermal variations as the defects present in the element affect the heating and cooling of the element. The emission of thermal radiation from the surface can be affected by several factors like surface coating, presence of dust and moisture among other environmental factors like temperature, wind speed and humidity. This in turn affects the resulting thermographic images (Vaghefi et al. 2011, Dabous et al. 2017). IR Thermography is commonly used in combination with other techniques like ultrasonic or Ground Penetrating Radar (GPR) to improve its accuracy.

GPR equipment transmits electromagnetic waves and records the reflection of the waves from the different layers within the material being tested. Reflection profile

is made using the amplitude of reflected waves from different points on the surface of the material being tested. The variation in reflection profiles is then analyzed by visual observation of the reflection profiles and the presence of defects can be identified. Signal attenuation and non-homogeneity of the material can affect the accuracy of the measurement (Tarussov et al. 2013, Dabous et al. 2017). At present GPR is used to determine delamination and the location of the reinforcing steel rather than the corrosion loss.

The above-mentioned methods of inspection all have their advantages and disadvantages. Most of them are highly sensitive to the surrounding environment and are effective for only local detection of defects.

2.3. Role of Bacteria in Corrosion

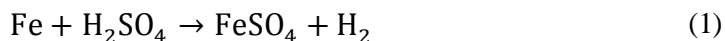
It has been estimated that millions of dollars are spent every year on repair and maintenance of pipelines and other equipment that were damaged as a result of microbially induced corrosion (MIC) (Flemming 1996)

Several types of bacteria have been identified as major causes of bio-corrosion. These include sulfate reducing bacteria (SRB), iron oxidizing bacteria (IOB), sulfate oxidizing bacteria (SOB), iron reducers and manganese reducers. These bacteria are known to promote corrosion by producing acidic compounds and other cellular secretions that create an environment that promotes corrosion (Hamilton 2003, Zuo 2007).

The bacterial biofilm on the surface of materials is formed by extracellular polymeric substance. Initially the biofilm is formed by aerobic bacteria. As the biofilm

As the biofilm matures more types of bacteria are included in the biofilm and the thickness of the biofilm increases. Therefore, most biofilms found on corroding surfaces are not made up of one type of bacteria but a consortium of bacteria with different characteristics. These biofilms, which house bacterial consortia, are known to cause severe corrosion (Videla and Herrera 2009, Zuo 2007). As the biofilm matures the aerobic bacteria carry out respiration process, depleting the oxygen and creating an anaerobic condition on the inner layers of the biofilm. This promotes the growth of anaerobic bacteria like SRB in the biofilm. The SRB then use various mechanisms of sulfide production that result in corrosion (Hamilton 1985, Nardy and van Veen 2015).

Initially corrosion occurs due to the activities of SOB and iron oxidizing bacteria (IOB) that are capable of forming biofilms. These biofilms mature and grow in thickness such that they create regions within the mature biofilm wherein there is lack of availability of oxygen. The presence of this anoxic condition gradually promotes the growth of SRB, and corrosion pitting occurs (Enning et al. 2012). The SRB may not be limited to anaerobic conditions. Studies conducted show that some SRB like *Desulfovibrio desulfuricans* are facultative anaerobes and are capable of surviving in aerobic environments also and may be involved in the initial biofilm formation (Lobo et.al 2007). Sulfur oxidizing bacteria oxidize sulfur species like sulfides, sulfites and thiosulfates to form sulfuric acid. This in turn reacts with the iron to form iron sulfate (Okabe et al. 2007).



Iron oxidizing bacteria like *Gallionella* are known to cause corrosion of iron in aerobic environments. They form biofilm that promote corrosion by the formation of differential aeration. These bacteria gain energy by converting ferrous iron into ferric iron (Erbs and Spain 2002).



Sulfate reducing bacteria produce hydrogen sulfide (H_2S) by various metabolic processes. This hydrogen sulfide reacts rapidly with iron to form iron sulfide (Enning et al. 2012).



SRB can cause corrosion directly by the uptake of electron from an electron donor like iron. This process is known as electrical microbially induced corrosion (EMIC). In another form of attack SRB reduce available sulfur containing compound and produce hydrogen sulfide that is highly corrosive and results in the precipitation of iron sulfide. This process is called chemical microbially induced corrosion (CMIC). In the case of EMIC, direct contact between microbes and metal surface is not required for transfer of electrons to take place and corrosion to occur (Enning et.al. 2012). These processes are shown in figure 2.3-1. The bacteria also promote the corrosion of iron in anaerobic conditions by the oxidation of the adsorbed hydrogen layer through the production hydrogenase enzymes and also through the reduction of insoluble ferric iron through processes that lower the pH of the medium (Hamilton 1985).

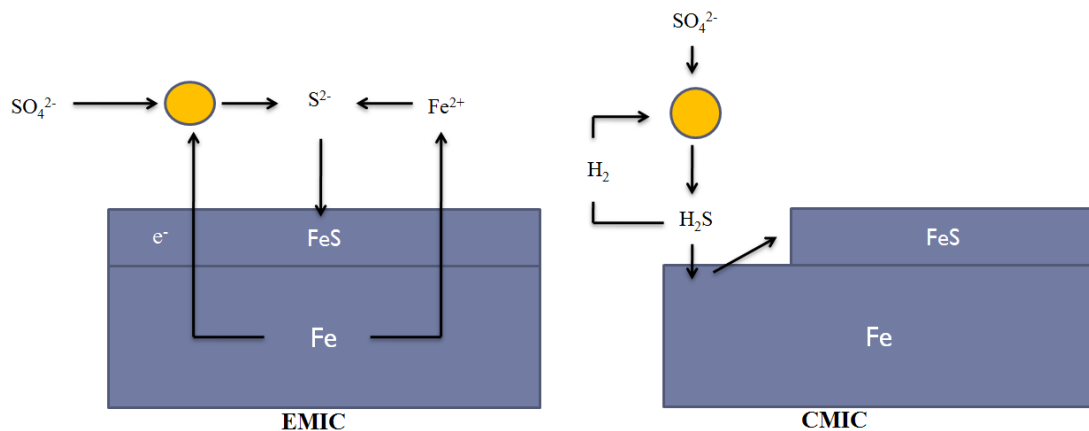


Figure 2.3-1. Mechanism of EMIC and CMIC

Bacteria are also involved in the degradation of concrete and stone structures. Bacterial biofilms are seen as slimy layers or dry powdery crust on the surface. Acidic secretions and enzymes produced by these bacterial colonies also contribute to the degradation of the surface. The degraded surface promotes the ingress of aggressive ions like chlorides and sulfates that may cause corrosion in the reinforcement steel (Warscheid and Braams 2000, Nardy and van Veen 2015).

2.4. Bacteria and Corrosion Control

While it is known that bacteria play a major role in causing metal corrosion, it has also been observed that certain bacteria have the property of inhibiting the corrosion process. They do so either by producing enzymes that inhibit the growth of bacteria that promote corrosion or by producing biofilms that are capable of acting as a passive, protective layer between the metal and the surrounding environment. These bacteria inhibit corrosion through two mechanisms, which may be the removal of oxygen from the immediate environment through aerobic respiration (Chongdar et.al 2005) and also by creating an unfavorable environment within the biofilm for other

sulfate reducing bacteria. (Videla and Herrera 2015, Alabbas et al. 2013). Several bacteria including *Bacillus subtilis*, *Bacillus brevis*, *Escherichia coli* and *Pseudomonas fragi* have been known to produce beneficial biofilms (Morikawa 2006). *E.coli* and *P.fragi* have been identified to produce a beneficial biofilm that is capable of reducing mass loss due to corrosion (Jayaraman et al. 1997). *B.brevis* is another type of bacteria capable of inhibiting microbial induced corrosion. It produces the antibiotic, gramicidin-S that inhibits the growth of SRBs, thereby controlling bio-corrosion (Zuo et al. 2007).

2.5. Colorimetric Determination of Carbohydrates

Activities of biological agents like bacteria in water lead to the formation of soluble microbial products and extracellular polymeric substances (Lapidou and Rittmann 2002, Le and Stuckey 2016). Studies have shown that these organic compounds are predominantly made of polysaccharides or carbohydrates. Bacteria have also been known to produce polysaccharides under various types of physiological conditions. Thus the presence and the amount of polysaccharides can be used as an indicator for biological activity (Flemming and Leis 2001, Sutherland 2001, Aquino 2004). It was also identified that both aerobic and anaerobic bacteria are capable of producing biofilm formations containing polysaccharides and these biofilms have been linked with microbially influenced corrosion (Beech and Gaylarde 1999). These findings suggest that measurement of carbohydrate content can be used to quantify biological activity in both aerobic and anaerobic biofilms. The anthrone reagent, which is composed of anthrone in concentrated sulfuric acid, reacts with the polysaccharides

to form a bluish green color (Dreywood 1946, Le and Stuckey 2016). The strongly acidic environment provided by the anthrone reagent and heating causes the glycosidic bonds in the polysaccharides to break resulting in the formation of monosaccharides. The dehydration of these monosaccharides leads to the formation of furfural derivatives. These compounds have a bluish green color. The intensity of this color depends on the concentration of saccharides and can be measured using a spectrophotometer (Dreywood 1946). This means the anthrone reagent can be used to quantify the biological activity in the medium.

The anthrone method is used to determine the total quantity of carbohydrates. Bacterial biofilms are made up polysaccharides and hence this method can be effective in quantifying biofilm growth (Le and Stuckey 2016). But since this method detects the total carbohydrates, biofilms with both dead and live cells will respond to the anthrone reagent. Hence, this method may not be able to identify active biofilm growth.

Corrosion is a major problem affecting the integrity of structures and biological agents play an important role in it. The existing inspection methods of non-destructive testing on reinforced concrete structures have several disadvantages. They are suitable only for local corrosion detection and they are affected by various environmental factors. It is now understood that microbes play an important role in corrosion (Enning et al. 2012). The process of corrosion detection in concrete would be greatly improved if there were parameters that can be easily measured at the concrete surface and can be related to the corrosion of the reinforcing steel inside the concrete. One such method could be the monitoring of bacterial activity or the pH at the concrete surface. These measurements may serve as an indirect measure of corrosion. The experiments

conducted in this study are a preliminary step in identifying the role of biological components in corrosion of steel. Certain bacteria like the anaerobic sulfate reducers have an important role in bio-corrosion. Studying specific strains of bacteria involved in corrosion and their impact on the biofilm formation and pH level at the concrete surface can provide further information on how bacterial activity can be used to effectively detect and monitor corrosion. While it is known that different types of bacteria are involved in different stages of corrosion, initial biofilm formation and corrosion may be contributed by aerobic iron oxidizers. Further corrosion in anaerobic conditions could be mediated by sulfate reducers. Identifying the type of bacteria associated with biofilms can provide information relating to the extent of corrosion. However at this point, such analysis is beyond the scope of this study. Here an attempt is made to relate corrosion of reinforcement steel with bacterial activity observed at the surface of concrete.

Chapter 3: Materials and Methodology

In the experiments conducted, steel and concrete specimens were used and were tested after immersion in water samples collected from the Chesapeake Bay. The detailed explanation regarding preparation of specimens and the experiments conducted are described in sections 3.1 to 3.8 of this chapter.

3.1. Preparation of Specimens

In this study, cracked reinforced concrete plates were used. A cover of 1 cm was used for all the specimens. Samples were to be 7 cm x 7 cm x 3 cm, with a no. 4 steel bar at mid depth. Steel bars were placed in the specimens with a cover of 1cm at the ends of the steel bar. The RC specimens were cast in high performance concrete with rapid early strength properties to accelerate the testing process. Specimens were made with two crack sizes, a hairline crack and a 1 mm crack to verify the influence of corrosion on pH and the bacterial activities.

The specimens with the hairline crack were prepared by first casting the top part which forms the 1 cm cover at the top. This was allowed to harden for 24 hours. The specimens after hardening are shown in figure 3.3.1 (a). After the top portions had hardened, they were taken out of the mold and a crack was induced by a three point bending test apparatus. Figure 3.3-1 (b) shows the top part after the hairline crack is made. The crack appears to be wider in figure 3.3-1 (b) because it was removed from the mold. The cracked portion was then placed in the mold. The ½ inch diameter (No.4) reinforcement bar was placed parallel to the crack and the bottom part was

poured. The specimens were left to harden for 24 hours and were removed from the mold. The steps involved in the preparation of the specimens are shown in figure 3.1-2.

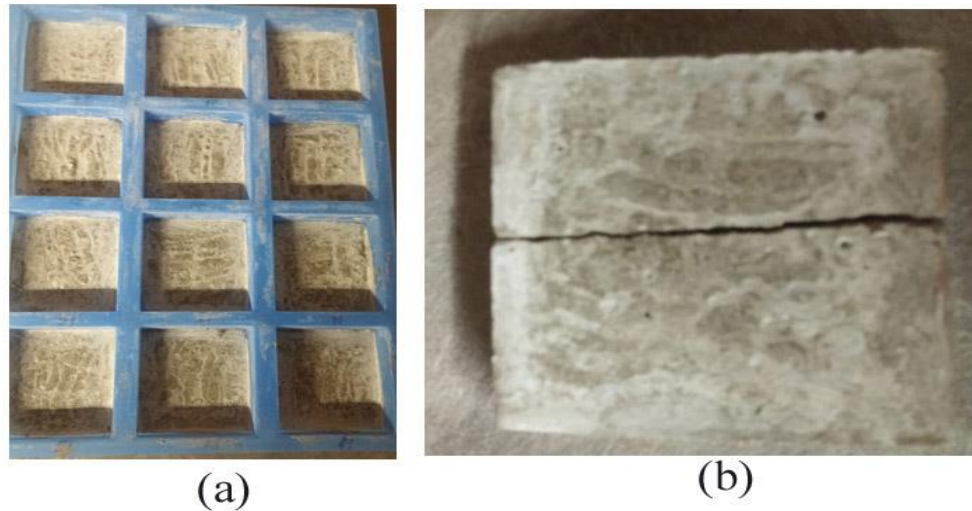


Figure 3.1-1. Preparation of specimens with hairline crack

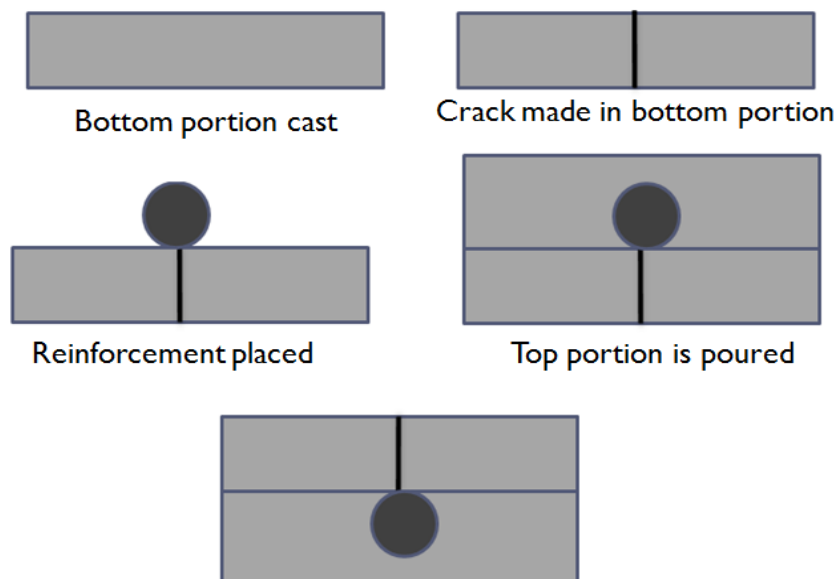


Figure 3.1-2. Steps involved in the preparation of concrete specimens

The specimens with a 1-mm crack were produced by using a shim during the casting of top portion which forms the 1 cm cover at the top. Figure 3.1-3 shows the casting of 1-mm crack samples using the shims. Once this part had hardened, the shim was removed and the two halves of this part were placed back in the mold. The reinforcement was placed parallel to the 1 mm gap and the bottom part was poured. The specimens were then allowed to harden for 24 hours and were later removed from the mold. Reinforced concrete specimens with no crack were added as control specimens for corrosion and bacterial activities. These specimens were also cast in two parts. Here, once one part had hardened, the reinforcement was kept in place and the other part was poured. The specimens with no crack would simulate the least steel exposure to the exterior environment. The steps involved in preparing the 1 mm crack specimens are similar to that described in figure 3.1-2. The difference here was that the crack was produced using a shim and not by three point loading. The specimens were then allowed a curing period of 1 week.



Figure 3.1-3. Preparation of specimens with 1 mm wide crack using shims

Figure 3.1-4 shows the finished specimen. The specimens with no crack are shown in the top row, the specimens with 1-mm crack are in the middle row and the specimens with a hairline crack are shown in the bottom row.



Figure 3.1-4. Concrete samples with different crack sizes

3.2. Details of Specimens

No. 4 bars steel bars were used in the reinforced concrete specimen. The bars used were ½ inch in diameter, 2 inches in length, and the weight per unit length was 0.376 pounds per foot. Chemical composition was: C- 0.22%, Si-0.15%, Mn-1%, P- 0.04%, S- 0.05%, Cr-0.3%, Ni-0.3%, Cu-0.3%, N-0.012%. All bars were cleaned using 150-grit sandpaper to remove any rust layer on the surface and degreased with acetone. After cleaning, the steel bars were numbered and weighed in a precision balance accurate up to one tenth of a milligram before being place in the concrete specimens.

Reinforced concrete (RC) specimens were prepared using the No. 4 steel bar. The specimens were 7 cm long, 7 wide and 3 cm thick. RC specimens were prepared using Type 3 cement (Density: 3.14g/cc, Magnesium Oxide: 1.55%, Sulfur trioxide: 3.12%, total alkali: 0.42%, Chloride ion: 0.006%). Hairline cracks were induced in RC specimen using 3 point loading and the 1 mm crack was produced using a shim, as described in section 3.1. The mix had cement and sand in the ratio 1:1.5 and a water/cement ratio of 0.4. Initial weight of each No: 4 steel bars were noted prior to immersion.

Hot rolled steel flats with dimensions 1/8 inch x 1 inch x 1 inch were used. The Chemical composition was: C – 0.10%, Mn – 0.60 %, P – 0.02%, S – 0.035%, Cu – 0.20%, Ni – 0.20%, Cr – 0.15%, Mo – 0.06%, V – 0.008%, Cb – 0.008%, Ti – 0.025%. The flats were degreased and sanded to remove rust layers after which they were weighed to record initial weights. The steel coupons after sanding and degreasing are shown in figure 3.2-1.



Figure 3.2-1. Steel coupons before immersion

3.3. Measurement of Biological Activity

The biological activity in the medium and on the surface of concrete was measured in terms of carbohydrate concentration. As bacteria grow, they produce biofilms. These biofilms are made up of polysaccharides. Measuring the carbohydrate content is therefore, an indirect measure of biofilm growth. The anthrone method for carbohydrate concentration determination (Dreywood 1946) was used. In this method, biofilms and residue formed on the surface of the samples were scraped off using a cell scraper into a 1.5 ml centrifuge tube and the volume was made up to 1 ml using De-Ionized (DI) water. This was then mixed thoroughly in a centrifuge at 3000 rpm for 10 minutes. This process separates the solid mass from the liquid. Figure 3.3-1 shows the centrifuge tubes with the solid mass collected at the bottom. The weight of solids was determined by drying these tubes in an oven at 105 °C for 1 hour. The supernatant liquid was used to measure the carbohydrate concentration. The reagent used for this test is the anthrone reagent, which was prepared by mixing 0.2 g of anthrone with 100 ml of concentrated Sulfuric acid. The reagent was prepared freshly immediately before use.



Figure 3.3-1. Surface residue in DI water after centrifuging

A 0.5 ml sample from the centrifuge tubes was transferred into a test tube using a pipette and the volume was made up to 1 ml using DI water. To this 4 ml of anthrone reagent was added. A blue green color formation was observed. The samples were then heated in a water bath at 100° C for 10 minutes. After that, the samples were allowed to cool to room temperature. A portion of each sample was transferred to an appropriate cuvette. Initially a blank measurement was observed using a spectrophotometer at a wavelength 630 nm using DI water. Thereafter, the absorbance of each sample was measured. The concentration of carbohydrates in the sample can be calculated from the expression.

$$C_{zs} = F(A - A_b)$$

Where, C_{zs} is the concentration. A is the absorbance of the sample, and A_b is the blank absorbance. The factor F is evaluated by standardization with known concentrations of glucose. This calculation shows a direct relation between carbohydrate concentration in the solution and its optical density (OD). In the experiments conducted, OD values were directly used as the measure of bacterial activity and conversion to concentration units was not performed. Figure 3.3-1 shows the surface residues samples collected in water. The supernatant from these samples were used for measuring the OD value. The supernatant liquid samples after treatment with anthrone reagent and heating in water bath are shown in figure 3.3-2

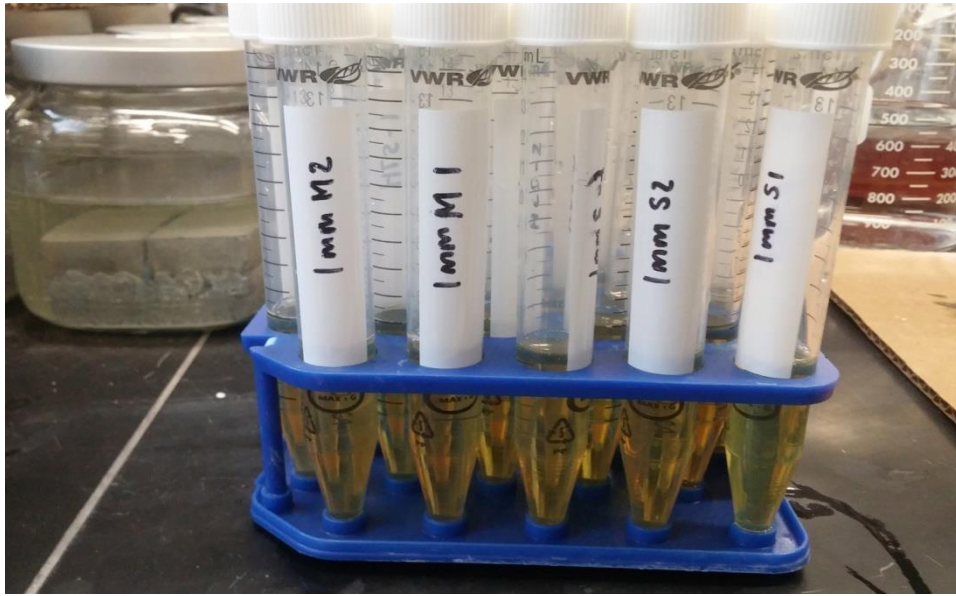


Figure 3.3-2. Samples used for testing carbohydrate concentration after addition of the anthrone reagent

3.4. Measurement of pH

pH at the surface of the concrete sample was measured according ASTM F 710 specifications. The surface was cleaned by sanding with 80-grit sandpaper. Once a layer of material, about 0.1 mm was removed, the surface was cleaned. A few drops of DI water were placed on the surface of concrete. After about 1 minute, the pH of the liquid was measured using pH strips and compared to a standard color chart as shown in figure 3.4-1. The same procedure was followed for the pH measurement for concrete at the level of steel after the samples were carefully cracked open. The pH of surrounding medium was measured using a properly calibrated pH meter. Using a pipette 10 ml of media samples were collected from the jars containing each specimen. The pH probe was then used to measure the pH of the collected liquid sample at 25°C.

3.5. Measurement of Weight Loss due to Corrosion

The steel bars in the concrete specimens were initially, polished with 150-grit sandpaper, degreased using acetone and dried before placing in concrete. After the experiments were completed, the steel bars were extracted from the concrete specimen. The corrosion products were removed according to ASTM G1 procedures. The cleaning solution was prepared with 3.5 g of Hexamethylenetetramine in 500 ml of 37% Hydrochloric acid and the volume was made up to 1000 ml using DI water. The samples were placed in this solution for 10 minutes. After the corrosion products were removed from the samples, they were wiped dry using a paper towel and then dried further in an oven at 80° C for 10 minutes. The samples were then allowed to cool to room temperature and the weights were measured in milligrams.

Figure 3.5-1 shows a corroded steel bar before cleaning on the top, and a steel bar after cleaning using HCl and hexamethylenetetramine below. The steel bars have a protective coating on the surface which was removed due to corrosion. The white patches are areas on the bars where the protective coating was removed. Figure 3.5-2 shows the steel coupons and bars after all the corrosion were removed.



Figure 3.5-1. Steel bars, before and after removing corrosion products

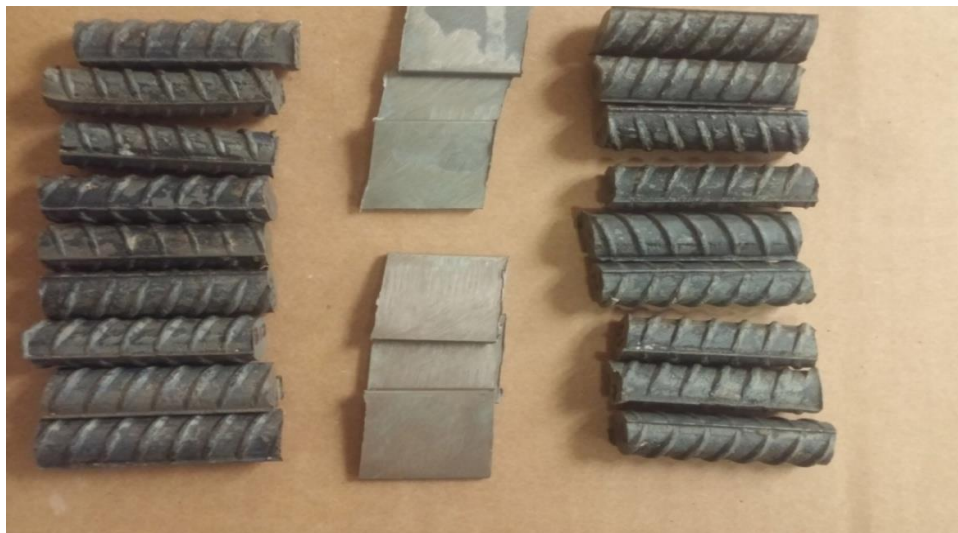


Figure 3.5-2. Steel coupons and bars after removal of corrosion products

3.6. Effect of Corrosion of Steel on Bacterial Activity on Reinforced Concrete Surface

3.6.1. Objective

This experiment tries to ascertain the influence of bacterial activity at the surface of the concrete, on the corrosion of reinforcement steel. Bacterial activity is measured in terms of carbohydrate concentration of the surface residue and weight of the surface residue. Corrosion in steel is measured in terms of loss of weight.

3.6.2. Proposed theory

The first step was to see if a biofilm was produced at the surface of the concrete samples and that the biofilm produced was an indicator of corrosion in reinforced concrete structures. Here the concrete specimens were placed in the collected water samples. Three types of concrete specimens were used, one with no crack, and another one with a hairline crack and a third one with a 1- mm wide crack. This experiment tried to ascertain if the occurrence of steel corrosion promotes bacterial growth and biofilm formation on the surface of the concrete. If the concrete specimens with no cracks showed no biofilm formation or highly reduced bacterial numbers, then it can be concluded that the presence of a biofilm is an indication of corrosion. This experiment aims to establish a relation between biofilm formation and occurrence of corrosion. The pH at the surface of concrete is normally in the range of 12-13. This experiment will also help to understand whether the bacteria are able to form colonies in such an environment and also to understand the effect of corrosive secretions like enzymes and acidic compounds from biofilms that may lower the pH of the concrete surface. In this

experiment the temperature, pH of the medium and nutrient content are kept constant for all the specimens at the start of the experiment.

3.6.3. Medium

Water sample collected from the Chesapeake Bay was used as the medium of immersion for this experiment. Each concrete specimen was immersed in 250 ml of the bay water sample with an initial pH of around 7.5.

3.6.4. Parameters Measured

Five parameters including, weight of residue obtained from concrete surface, carbohydrate content in the biofilm residue obtained from the surface of the sample, pH at the surface of concrete sample, pH of concrete at the level of steel in the specimen, and loss of weight of the reinforcement steel were measured in triplicate. The weight of reinforcement after immersion was measured after all the biofilms were removed from the surface of the sample. The steel reinforcement was initially weighed before being placed in concrete. The loss of steel weights were then correlated to the weight of surface residue, carbohydrate content of the residue and pH of concrete at the surface and at the level of steel. The experiment can be conducted on different media with varying salt content to study the effect of salinity on bacterial growth and loss of weight. However, at this point we are interested only in studying the effect of bacterial growth in corrosion of reinforced concrete in brackish water.

Step-by-step procedures of the experiment are described in the following section:

3.6.5. Procedure followed

1. Water samples to be used as medium were previously collected and stored.
2. Concrete specimens with two crack sizes; one with a hairline crack and one with a 1 mm crack were placed in glass jars.
3. Concrete specimen with no cracks was used as a control.
4. Prior to preparation of the concrete sample the reinforcement steel placed in them were cleaned as described in section 3.2 and weighed to record initial weight.
5. To each concrete specimen, 250 ml of water sample was added.
6. The jars were then kept undisturbed and stored at 25° C.
7. The samples were tested at the end of 12, 16 and 20 days.
8. On the day of the test, the specimens were removed from the water. A cell scraper was used to transfer biofilm and surface residue to a 1.5 ml centrifuge tube.
9. The volume of each sample in the centrifuge tube was made up to 1 ml using DI water and was centrifuged at 3000 rpm for 10 minutes. The separated solid mass and supernatant are shown in figure 3.3-1.
10. The supernatant was extracted and anthrone reagent was used to determine the amount carbohydrates in the liquid as described in section 3.3.
11. The settled solids in the centrifuge tubes were placed in an oven to dry and weighed. The weight of solids was obtained by subtracting the weight of the empty tubes.
12. The concrete surface was sanded after the specimens were dried. pH measurements at the concrete surface were done using pH strips as described in section 3.4.
13. The concrete specimens were then cracked open to extract the reinforcement steel.

14. Once the specimen was cracked open, pH was again measured at the level of steel using pH strips and DI water.
15. The extracted steel was sanded and cleaned to remove any concrete sticking to the surface.
16. After the preliminary cleaning, the steel was placed in the mixture of HCl and Hexamethylenetetramine for 10 minutes to remove corrosion products as described in section 3.5.
17. The steel was removed from the reagent and dried and cleaned again to remove any excess particles sticking to the surface.
18. Once dried, the steel reinforcement bars were weighed using a precision scale to measure the weight.
19. The previously recorded initial weight was used to calculate the percentage loss of weight.
20. The values measured were then recorded and are as shown in Tables A.1 to A.16.

3.7. Effect of Corrosion of Reinforcement Steel on Biological Activity in Surrounding Medium

3.7.1. Objective

In this experiment the effect of bacterial activity in the medium due to corrosion of reinforcement steel in the concrete was studied. Bacterial activity in the medium was measured in terms of carbohydrate concentration and pH of the surrounding medium. Corrosion of reinforcement steel was measured in terms of loss of weight.

3.7.2. Proposed theory

The first step was to measure the carbohydrate content of the medium and observe its impact on the corrosion. From the discussion in section 2.5, biofilm growth is expected to increase as the bacterial activities described in section 2.3 takes place. The bacteria present in the biofilm may secrete acids and enzymes. This may cause the pH of the medium to decrease, thereby creating favorable conditions for corrosion of steel. Three types of concrete samples: one with no crack, one with a hairline crack and one with a 1- mm wide crack were tested. In this experiment, an attempt is made see whether the corrosion of steel is related to bacterial growth in the medium and its pH. If the carbohydrate concentration and pH of the medium varies depending on the amount of corrosion of reinforcement steel in concrete, it can be concluded that the composition of surrounding media in a closed environment can be used as an indication of corrosion in the exposed reinforcing steel in concrete. This experiment aims to establish a relation between pH of the medium, the bacterial activity in the medium and the weight loss due to corrosion. The pH at the surface of concrete was initially measured to be in the range of 12-13. This experiment will also help understand whether the surrounding media affects degradation of concrete over time, which in turn leads to increased risk of corrosion. In this experiment also the temperature, pH of the medium and nutrient content of the medium are kept constant for all the specimens at the start of the experiment.

3.7.3. Medium

Water sample collected from the Chesapeake Bay was used as the medium of immersion for this experiment. Each concrete specimen was immersed in 250 ml of the bay water sample.

3.7.4. Parameters Measured

In this experiment, the parameters measured include pH of the medium, carbohydrate content in terms optical density (OD) value of the medium, surface pH of concrete, and loss of weight of the embedded steel. All the measurements were made in triplicate. The weight after immersion was measured after all the biofilms and surface residue were removed from the surface of the sample. The initial weight was measured prior to placing the steel bars in the concrete. The loss of steel weight of each specimen was then correlated with the carbohydrate content and pH of the surrounding medium. The experiment can be conducted on different media with varying salt content to study the effect of salinity on bacterial growth and loss of weight. However, at this point we are interested only in studying the effect of bacterial growth in corrosion in brackish water conditions.

Step-by-step procedures of the experiment are described in the following section:

3.7.5. Procedure followed

1. Water samples to be used as medium were previously collected and stored.
2. Concrete specimens with two crack sizes; one with a hairline crack and one with a 1 mm crack were placed in glass jars.

3. Concrete specimens with no cracks were used as a control.
4. Prior to preparation of the concrete specimens, the reinforcement steel placed in them were cleaned as described in section 3.2 and weighed to record initial weight.
5. To each concrete specimen, 250 ml of water sample was added.
6. The jars were then kept undisturbed and stored at 25° C.
7. The samples were tested at the end of 12, 16 and 20 days.
8. On the day of the test, 10 ml of media sample was collected from each specimen in the test tubes as described in section 3.4. The pH of each sample was measured using a pH probe.
9. 1 ml of media sample was collected from each specimen.
10. The anthrone reagent was used to determine the amount carbohydrates in the liquid samples as described in section 3.3.
11. The concrete surface was sanded after drying. The surface pH measurements were carried out using pH strips as explained in section 3.4.
12. The concrete specimens were then cracked open to extract the reinforcement steel.
13. The extracted steel was sanded and cleaned to remove any concrete sticking to the surface.
14. After the preliminary cleaning, the steel was placed in the mixture of HCl and Hexamethylenetetramine for 10 minutes to remove corrosion products as described in section 3.5.
15. The steel was removed from the reagent and dried and cleaned again to remove any excess particles sticking to the surface.

16. Once dried the steel reinforcement bars were weighed using a precision scale to measure the weight.

17. The values measured were then recorded and are as shown in Tables A.17 to A.22.

3.8. Effect of Biological Factors in Increasing Steel Corrosion and Concrete Degradation

3.8.1. Objective

Corrosion of steel in submerged conditions is due to biotic and abiotic factors. This experiment was performed to ascertain the extent of reinforcement corrosion caused by the presence of biological factors. This was done using concrete specimens with 1 mm crack immersed in the bay water. Another set of similar specimens were placed in sterilized bay water as a control. Various parameters were measured for both sets of samples and the results were compared. Measurements were also taken for the steel coupons placed in sterile and non-sterile bay water samples.

3.8.2. Proposed Theory

The effect of biofilm formation and the influence of biological agents on corrosion of reinforcing steel were studied in this experiment. Here the steel coupons and concrete specimens were placed in autoclaved bay water that was used as control. It is assumed that the autoclaving would completely sterilize the water and remove any microorganisms. This water sample would have the same chemical composition as that of the non-sterile water sample. Only the biological components are removed by the sterilization process. Concrete specimens with 1 mm crack, which simulates the maximum exposure condition, were used. This experiment attempts to understand

whether the presence of bacteria in water sample promotes corrosion. If the samples in water with the bacteria (non-sterile) showed a higher loss of weight for the reinforcement steel, then it can be concluded that the presence of a biofilm aggravates the corrosion process in reinforced concrete structures. This experiment aims to understand the influence of biological agents in the rate of corrosion. This experiment would also help understand the effect of corrosive biofilms in lowering the pH of the concrete surface. Parameters including, pH of the concrete at the surface and at the level of steel, pH of the medium, and the carbohydrate concentration in the surface residue and the medium were monitored for comparison. In this experiment the temperature, pH of the medium, and the nutrient content were kept constant at the start of the experiment for all the specimens.

3.8.3. Medium

Water sample collected from the Chesapeake Bay was used as the medium of immersion for this experiment. Each concrete specimen was immersed in 250 ml of the bay water sample. A part of the medium was sterilized by autoclaving. The control specimens used in this experiment were immersed in the sterilized medium.

3.8.4. Parameters measured

In this experiment, all the parameters including weight of residue obtained from concrete surface, carbohydrate content in the biofilm residue obtained from the surface of the specimen, pH at the surface of concrete specimen, pH of concrete at the level of steel in the sample, medium pH and medium OD, and loss of weight of the reinforcement steel were measured. All the measurements were made in triplicate. The

weight after immersion was measured after all the biofilms were removed from the surface of the sample. The measured parameters for the specimens placed in sterile and non-sterile medium were then compared.

Step-by-step procedures of the experiment are described in the following section:

3.8.5. Procedure followed

1. Water samples to be used as medium were previously collected and stored.
2. A portion of the water sample was autoclaved at 121° C and 15 psi pressure for 30 minutes. This was done to eliminate any bacteria and sterilize the water sample.
3. Concrete specimens with 1 mm crack and steel coupons were placed in glass jars. 3 specimens were used in this experiment.
4. Concrete specimens with 1-mm crack and steel coupons placed in sterile medium in an airtight jar were used as a control.
5. Prior to preparation of the concrete specimens the reinforcement steel placed in them were cleaned as described in section 3.2 and weighed to record initial weight.
6. To each steel coupon and concrete specimen, 250 ml of water sample was added.
7. The jars were then kept undisturbed and stored at 25° C.
8. The samples were tested at the end of 12, 16 and 20 days.
9. On the day of the test, the specimens were removed from the water. A cell scraper was used to transfer the biofilm and surface residue to a centrifuge tube.

10. The volume of each sample in the centrifuge tube was made up to 1 ml using DI and was centrifuged at 3000 rpm for 10 minutes. The separated solid mass and supernatant are shown in figure 3.3-1.
11. The supernatant was extracted and anthrone reagent was used to determine the amount carbohydrates in the liquid as described in section 3.3.
12. 1 ml of medium sample was also collected using pipette and subjected to analysis using anthrone reagent.
13. The settled solids in the centrifuge tubes were placed in an oven to dry and weighed. The weight of solids was obtained by subtracting the weight of the empty tubes.
14. The concrete surface was sanded after drying for surface pH measurements using pH strips as described in section 3.4.
15. The concrete specimens were then cracked open to extract the reinforcement steel.
16. Once the sample was cracked open, pH was again measured at the level of steel using pH strips and DI water.
17. The extracted steel was sanded and cleaned to remove any concrete sticking to the surface.
18. After the preliminary cleaning, the steel bars and coupons were placed in the mixture of HCl and Hexamethylenetetramine for 10 minutes to remove corrosion products as described in section 3.5.
19. The steel was removed from the reagent and dried and cleaned again to remove any excess particles sticking to the surface.
20. Once dried the steel reinforcement bars and coupons were weighed using a sensitive scale to measure the weight.

21. The previously recorded initial weight was used to calculate the percentage loss of weight.
22. The values measured were then recorded and are as shown in Tables 4.3-1 and 4.3-2 in Chapter 4.

Chapter 4: Results and Discussion

4.1. Effect of Corrosion of Steel on Biological Activity on Concrete Surface

Residue was collected from the surface of three types of concrete specimens at regular intervals. The percentage weight loss is recorded in Tables A.1 to A.4. The weights of the residue are recorded in Tables A.5 to A.7. The optical density (OD) values of the residue obtained from the spectrophotometer for the different types of specimens are recorded in Tables A.8 to A.10. The surface pH values of concrete are recorded in Tables A.11 to A.13 and pH values of concrete at the steel level are shown in Tables A.14 to A.16.

4.1.1. Expected Results and Experimental Findings

The samples with higher exposure of steel would provide a better condition for corrosion and from the discussion in section 2.3, it was understood that this would provide a somewhat better environment for the activity of biological agents. The exposed steel would be in contact with the medium containing the bacteria, and the presence of the electron donor, iron, in the medium would promote bacteria activities. The environment will be conducive to the growth of bacteria present in the medium when there was more exposure of steel. As a result of this increasing bacterial activity, the biofilm formation on the concrete surface was also expected to increase with the increase in corrosion. The increased growth of biofilm on the surface should lead to a decrease in pH at the surface of the concrete. This reduction of pH at the concrete surface would take place as a result of the release of enzymes and acidic cellular

secretion by the bacteria. The deterioration of the surface may also lead to the ingress of corrosive ions from the medium into the concrete leading to further deterioration. Due to the absence of immediate contact to steel and the lack of availability of electron donors, the biofilm growth was expected to be the least in the sample with no crack where the least amount of corrosion was expected to take place. The carbohydrate content measurement using spectrophotometer was used as an indirect method to quantify the biofilm growth, assuming that the biofilm is made up of carbohydrates. Different parameters including pH values, surface residue weight and OD values of the surface residue and medium were plotted and the variations were observed.

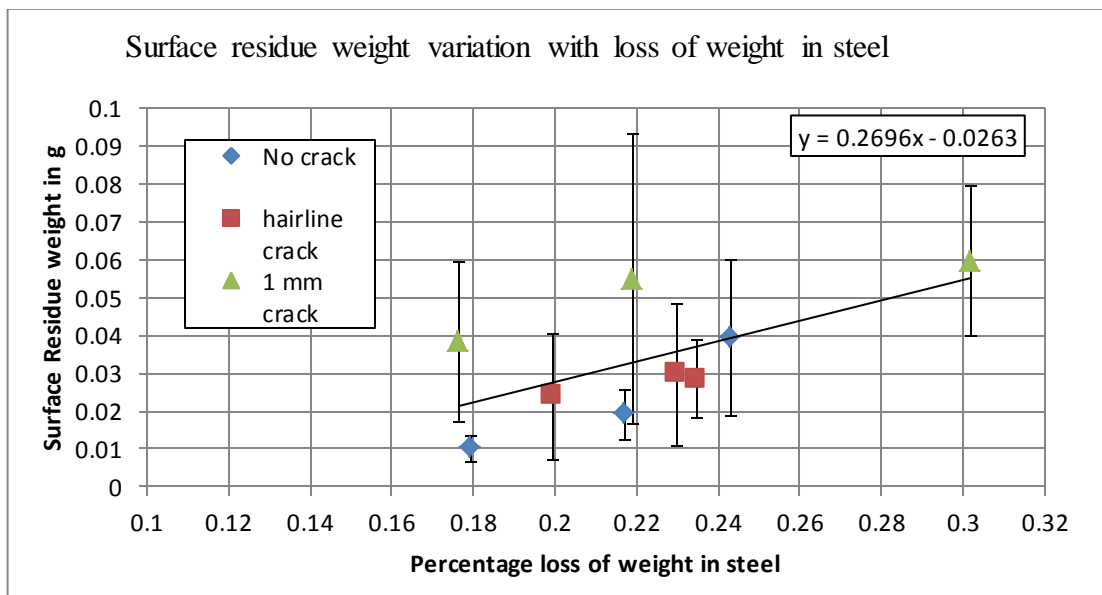


Figure 4.1.1-1. Variation of Surface residue weight with loss of weight in steel

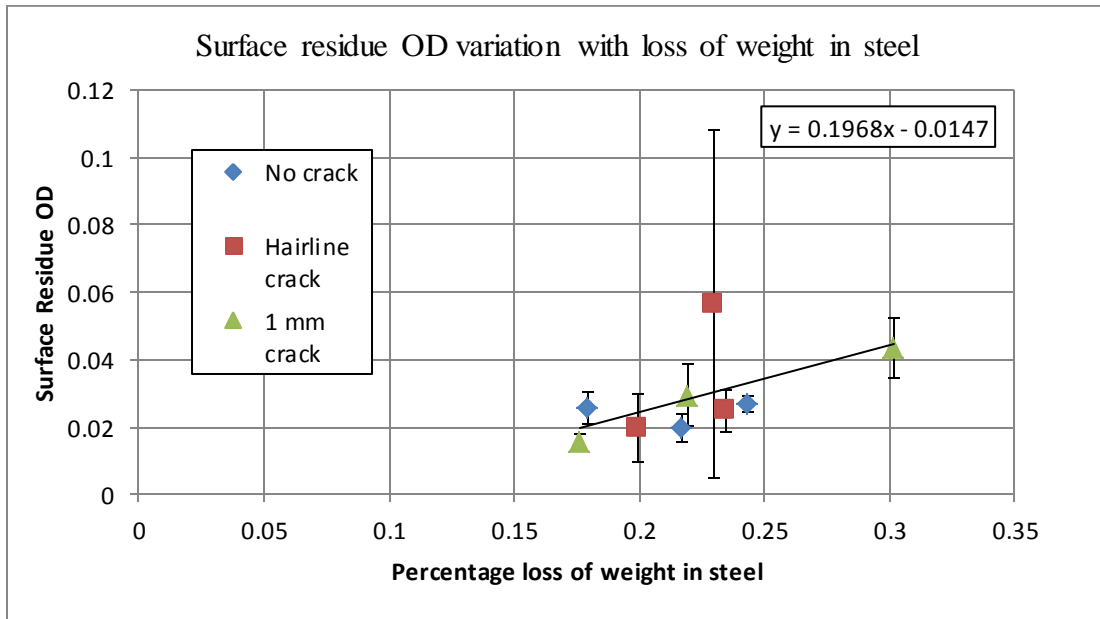


Figure 4.1.1-2. Variation of surface residue OD with loss of weight in steel

The weight of residue collected from the surface of concrete specimens was plotted with respect to the loss steel weight and a trend was observed as shown in figure 4.1.1-1. It can be seen that, higher weights of collected surface residue corresponds to higher loss of weight in steel, The sample with a 1- mm crack showed a much higher accumulation of residue on the surface. The exposed steel provides a condition favorable for growth of bacteria in the immediate regions surrounding the steel. This exposure of steel may have promoted the bacterial activity leading to an increase in biofilm growth at the surface close to the steel. This biofilm growth is measured as an increase in weight of residue collected from the surface of the concrete specimens. Figure 4.1.1-1 shows that the weight of biofilm on the concrete surface is correlated to the amount of corrosion of steel in the concrete. The correlation between

surface residue weight and corrosion suggests that biofilm growth at the surface of concrete is an indication of the amount of corrosion in the reinforcement steel.

In figure 4.1.1-2, the variation of the surface residue OD with respect to loss of steel weight is shown. The OD value indicates that the concentration of carbohydrates is a good measure of biological activity. As explained earlier in section 2.3, the bacterial growth increases with the corrosion of steel. As the conditions become favorable for bacterial activity, the biofilm growth also increases. These biofilms are made up of polysaccharides. Growth of biofilms would lead to an increase in the concentration of polysaccharides. Thus in a specimen where there is a higher bacterial activity, the concentration of carbohydrates collected would also be higher. This translates into higher OD values. From figure 4.1.1-2, the OD is seen to be increasing with increasing loss of weight. Therefore, this experiment suggests that measuring the biological activity in the biofilm formed at the surface in terms of carbohydrate content may also indicate the extent of corrosion in reinforcement steel inside concrete.

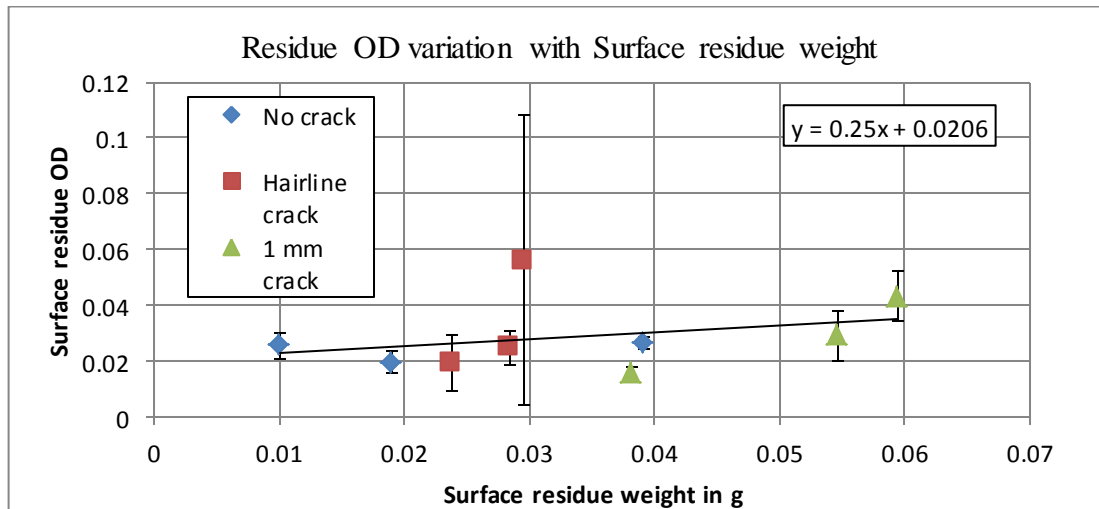


Figure 4.1.1-3. Variation of surface residue OD with surface residue weight

From figures 4.1.1-1 and 4.1.1-2, two parameters were used to quantify the growth of biofilms; weight of surface residue and OD value obtained from the mix of the surface residue and DI water. As the biofilm continues to grow, it can be expected that the amount of polysaccharides in them would also increase, as the biofilm matrix is made up of these compounds. Figure 4.1.1-3 shows that the increase in surface residue weight does correspond to a slight increase in the OD values. The OD value is a measurement of the carbohydrate content in the surface residue. The residue collected from the surface, however, may contain compounds other than carbohydrates, like degraded concrete that contribute to the weight of the collected residue. These compounds may not affect the OD measurement but would still increase the weight of the surface residue. Thus the increase in OD value with residue weight is not as high as expected.

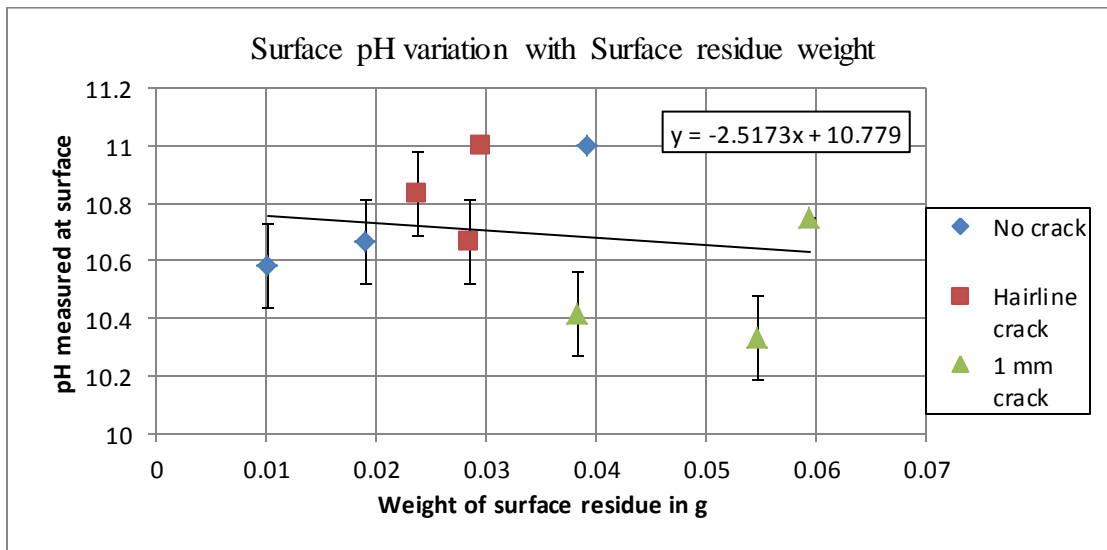


Figure 4.1.1-4. Variation of surface pH with surface residue weight

As biofilm growth continues, the weight of surface residue is seen to increase. It is possible that the bacterial activity in the biofilms is also increasing resulting in the deposition of more material at the concrete surface. As bacteria continued to grow, the acidic secretion from the cells would lower the pH of concrete at the surface where biofilm growth was taking place. This conditioning of surfaces by bacterial biofilms was explained in section 2.3. As the biofilm continue to grow, the bacterial activity at the surface would increase and this may reduce the pH of the surface. From figure 4.1.1-4, it was observed that, although there is a slight reduction in pH corresponding to higher weights of surface residue, the observed trend is not consistent. The reduction in pH at the surface creates a condition favorable for biofilm formation. This indicates that all the materials present in the biofilm and cellular secretion of the bacteria may not be acidic compounds that reduce surface pH. It can be expected that as the biofilm matures further, reduction in the surface pH may take place. In conditions where the biofilm matures, and sulfate reducing bacteria become active at the anaerobic zone within the biofilm, acidic compounds may be produced and a reduction in pH may be more pronounced. However at this point, the results shown in figure 4.1.1-4 do not give such an indication.

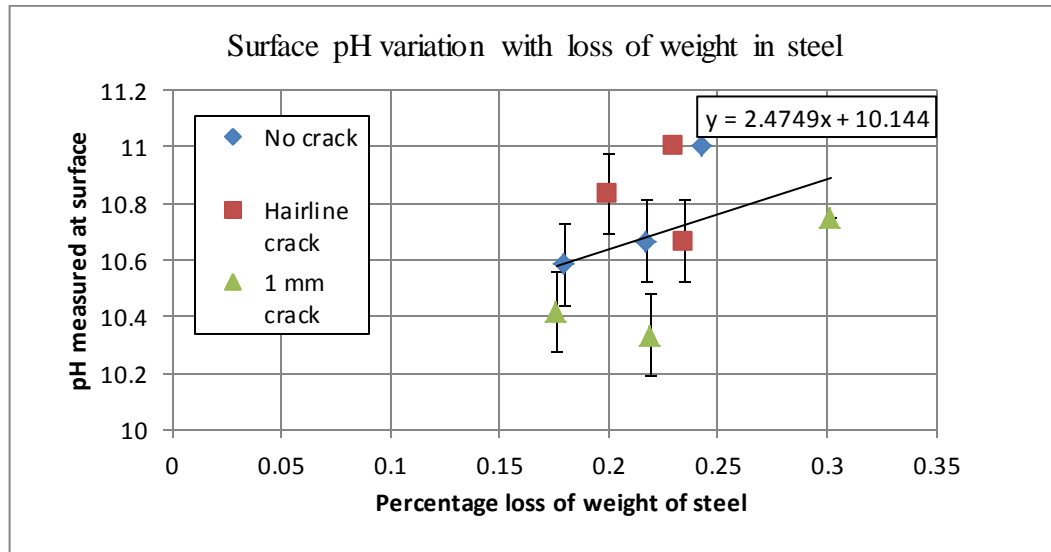


Figure 4.1.1-5. Variation of surface pH with loss of weight in steel

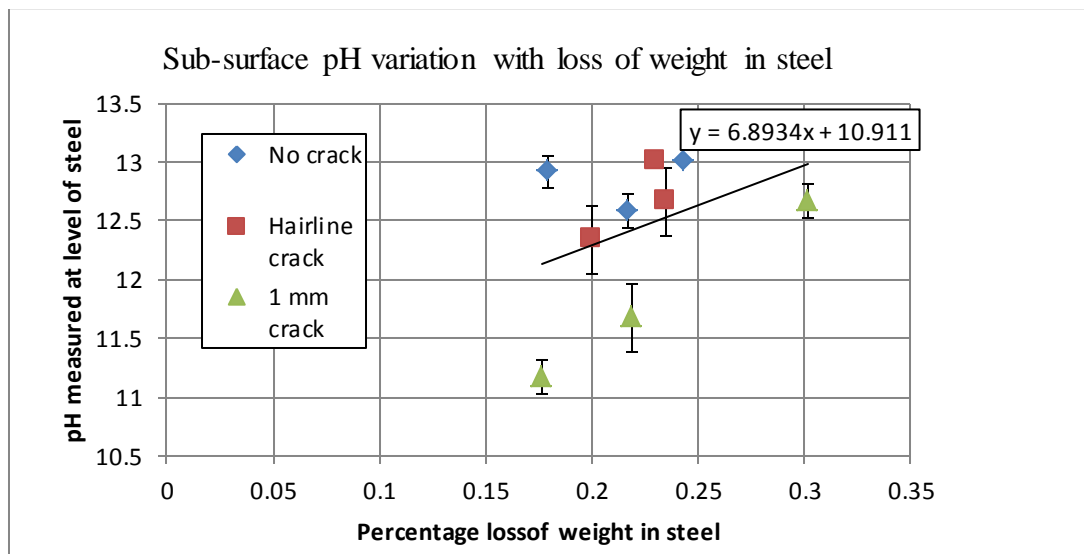


Figure 4.1.1-6. Variation of sub-surface pH with loss of weight in steel

From previous discussions and results, it can be seen that bacterial activities are related to the amount of corrosion in steel. It was expected that pH of concrete would decrease with increasing loss of weight. The corrosion of steel was expected to promote bacterial growth at the surface and subsequently cause a reduction in pH at the

surface. However, no clear correlation was observed from the experiment, between loss of weight in steel and pH at the surface of the concrete. This is shown in figure. 4.1.1-5. pH measurement at the level of steel in the concrete specimens and loss of steel weight also did not show a consistent trend as seen in figure 4.1.1-6. However, the surface pH was found to be much less than the pH at the level of the reinforcement steel that was located 1 cm from the surface. This may have been caused by the biological activities in the medium and at the surface of the concrete. Biological agents in the medium and in the biofilm at the surface are in contact with concrete surface, but not with the concrete layer at the level of steel. This could be the reason for the surface layer of concrete having a lower pH than the layer of concrete at the level of the reinforcing steel.

The results from these experiments showed that the pH measurement at the surface of concrete was not a reliable method to estimate corrosion in the reinforcement steel during the initial stages of corrosion. This may be because the bacterial secretions were not acidic enough to cause degradation of the surface. The biofilm may continue to grow but may not necessarily release highly acidic byproducts. At later stages of biofilm growth acid producing microorganisms may come into play. Acid production by bacteria at the surface may directly cause deterioration of concrete and leads to corrosion of steel as explained in section 2.3. In such conditions pH measurements may give indications regarding corrosion of steel. Another possible reason why concrete pH was not related to corrosion could be that, corrosion of steel starts with the degradation in concrete. This leads to the ingress of corrosive ions like

chloride and sulfates into the concrete causing corrosion. In these experiments, concrete specimens were pre-cracked. As a result, the ions in the medium had direct contact with steel from the beginning. Thus deterioration of the concrete was not necessary for the corrosion to take place. However, at this point, the measurement of bacterial activity in terms of OD value and weight of surface residue were found to be more indicative of the corrosion of steel and these measurements may be better at detecting and monitoring corrosion in reinforcement steel in concrete during initial stages of corrosion.

4.2. Effect of Exposure and Degradation of Steel on Surrounding Medium

The effect of bacterial activity in the medium in terms of pH and carbohydrate concentration on corrosion is observed in these experiments. Medium samples were collected at regular intervals from the containers in which the concrete specimens and steel specimens were kept immersed. The percentage losses of reinforcing steel weight were recorded in Tables A.1 to A.4. The medium pH values are shown in Tables A.17 to A.19 and the medium OD values are recorded in tables A.20 to A.22.

4.2.1. Expected results and experimental findings

The specimen in which the steel is more exposed and undergoes a higher rate of corrosion is expected to provide a somewhat better environment for biological activities due to the presence of the electron donor, iron in the medium. As the growth of the bacteria increases over time, the pH level was expected to decrease if the bacteria present in the medium started to follow anaerobic pathways for respiration resulting in the release of acidic compounds into the medium. With the increase in

biological activities and biofilm formation, the carbohydrate content was expected to increase. Due to the absence of an electron donor, the bacterial activity was expected to be highly reduced in the specimen where corrosion was significantly lower.

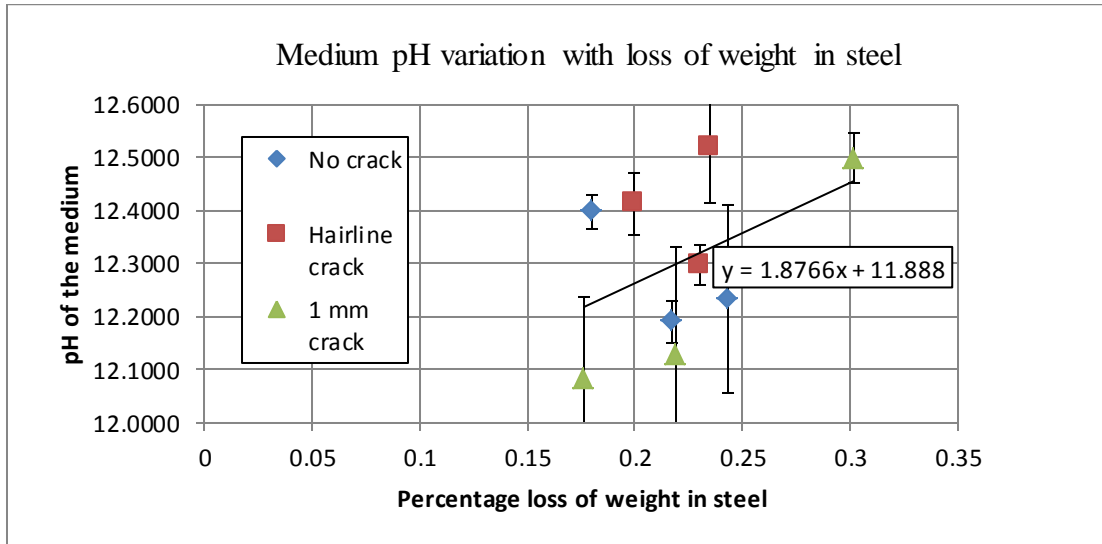


Figure 4.2.1-1. Variation of medium pH with loss of weight in steel

The pH of the medium was plotted with respect to the loss of weight in steel as shown in figure 4.2.1-1. With the increase in corrosion and bacterial activity, the pH was expected to decrease. However, the data shown in figure 4.2.1-1 shows an increase in pH instead. It is possible that bacteria present in the medium did not use pathways that result in the production of acid as byproducts of metabolic activities. In such a case the growth of bacteria may not lead to the reduction in pH in the medium. Also the medium is stagnant and constantly in contact with the concrete specimens. This may cause soluble products formed from the hydration of concrete to go into the medium. This may be another reason why the pH of the medium has shown an increase despite bacterial activities taking place in it. In a condition when pH of the medium is low, it

can be assumed that such a condition is favorable for corrosion and a lower pH would cause more severe corrosion. However in this case, it can be seen that pH value may not indicate that the bacterial activity is taking place in the medium that leads to corrosion of steel in reinforced concrete.

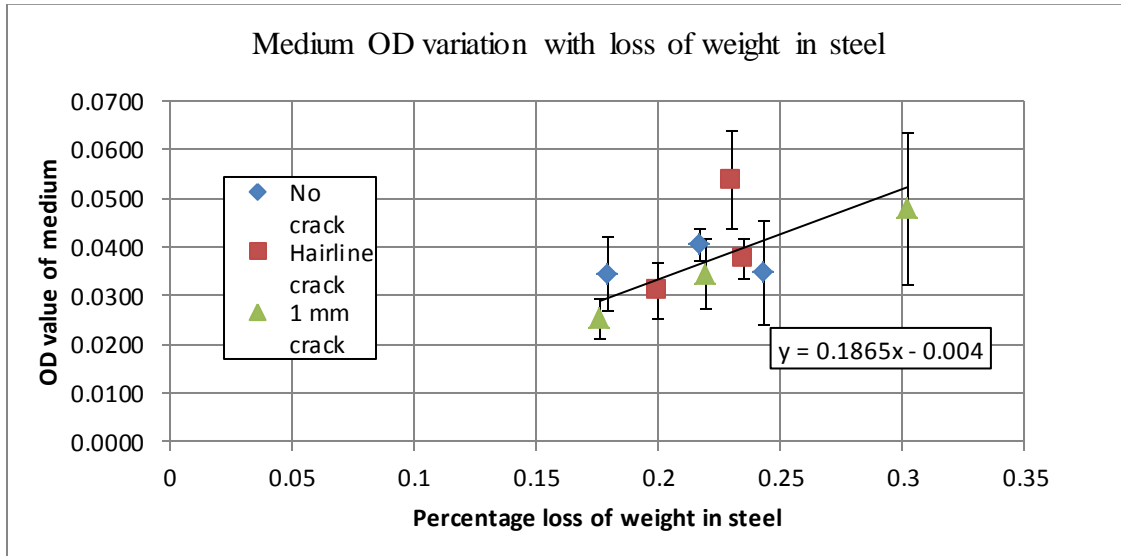


Figure 4.2.1-2. Variation of medium OD with loss of weight in steel

The medium OD (optical density) variation with loss of weight in steel is shown in figure 4.2.1-2. It is observed that the OD value of the medium increased with the increase in the weight loss in steel, with only a few outliers. This somewhat consistent trend confirms the initial assumption that corrosion of steel is influenced by bacterial activity in the medium. The bacterial activity in the medium was measured in terms of carbohydrate concentration. The results from the previous experiments in Chapter 3 show a relation between bacterial activity in the biofilm at the surface of concrete and the corrosion of steel. The results from this experiment further confirm the relation between bacterial activity and corrosion. Thus measuring the bacterial

activity in the surrounding medium may give a better indication regarding corrosion and loss of weight than measuring pH.

Figures 4.2.1-1 and 4.2.1-2 show that measuring bacterial activity in the medium surrounding a concrete structure is a more effective method to detect corrosion of reinforcement steel than measuring the pH of the medium. However in real-life condition, these measurements may not give reliable results as the medium is constantly changing and the pH and bacterial concentrations may change due to reasons other than corrosion. In this study the pH and bacterial activity in the medium are measured to corroborate the observation made in measurements of pH and bacterial activity at the concrete surface.

4.3. Effect of Biological Factors on Steel Corrosion and Concrete Degradation

The effect of presence of biological components in promoting corrosion is observed in this section. The parameters measured for concrete samples in sterile and non-sterile media are recorded in Tables 4.3-1. The observation made for the steel sample is shown in Table 4.3.1-2.

Table 4.3-1. Parameters for concrete sample with 1 mm crack

Parameter	Non- sterile	Sterile
Medium pH	12.08	12.65
Medium OD	0.025	0.018
Surface Residue OD	0.016	0.043
Surface pH	10.42	11.08
Sub-Surface pH	11.17	12.75
Surface Residue weight	0.18	0.14
Percentage weight loss	0.038	0.32

Table 4.3-2. Parameters for Steel sample

Parameter	Non- sterile	Sterile
Medium pH	7.09	7.29
Medium OD	0.043	0.061
Percentage weight loss	0.367	0.384

4.3.1. Expected results and experimental findings

Section 2.3 explained the reactions that take place during corrosion and how bacterial activities occur during this process. The samples with exposed steel placed in the non-sterile medium were expected to provide a somewhat better environment for biological activity and growth of biofilms due to the presence of bacteria and the exposed iron undergoing corrosion. As the growth of the bacteria increased over time, the measured OD value of the medium and the biofilm were expected to increase following a similar trend as the bacterial growth. In the autoclaved sample, the water medium was sterile and kept in a closed, air-tight jar limiting the activity of bacteria. Thus the corrosion tendency of steel was expected to be significantly less in the sterilized sample.

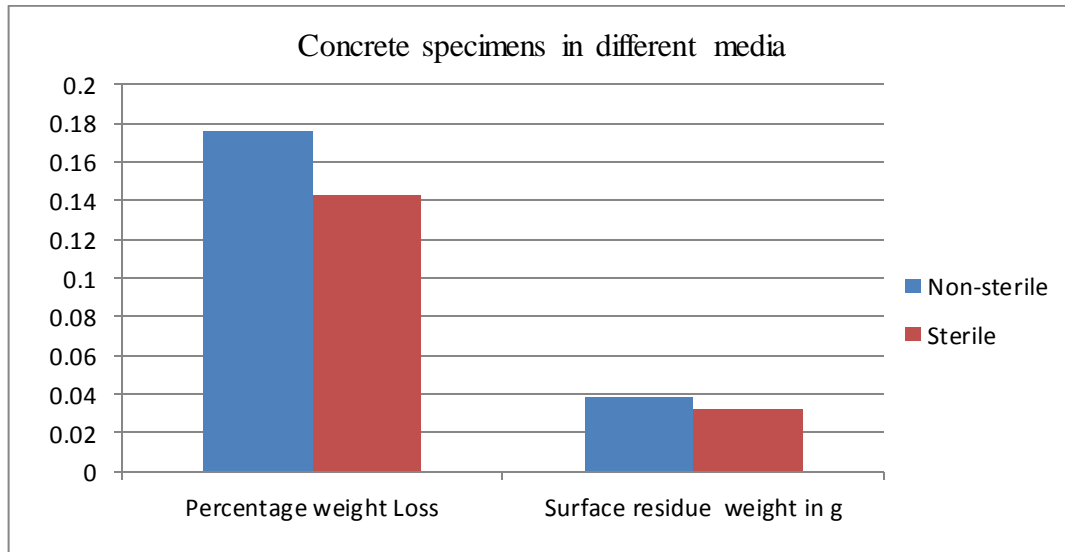


Figure 4.3.1-1. Comparison of percentage loss of weight and surface residue weight in concrete specimens in sterile and non-sterile media

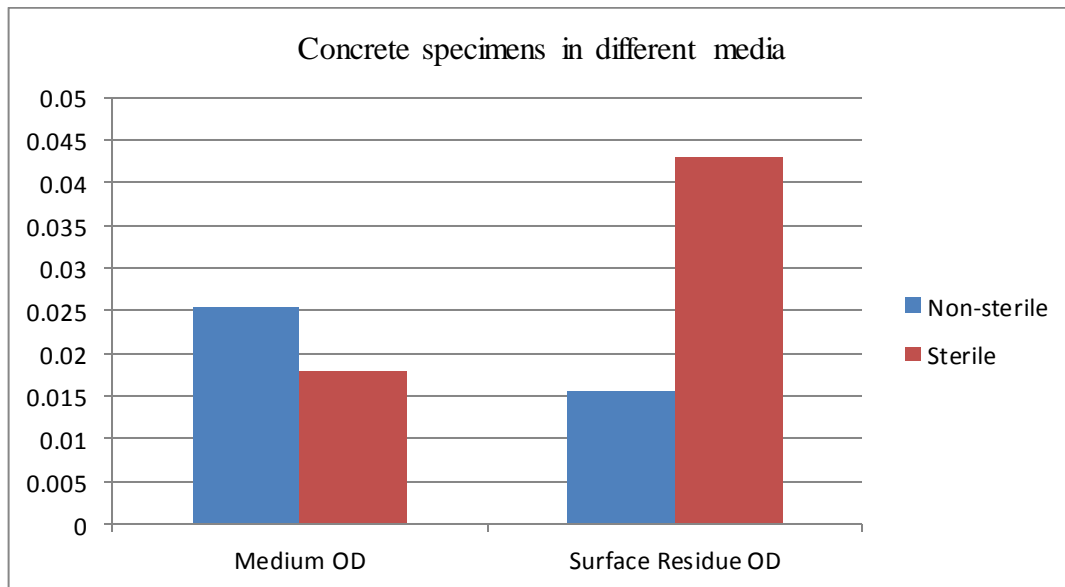


Figure 4.3.1-2. Comparison of medium OD and surface residue OD in concrete specimens in sterile and non-sterile media

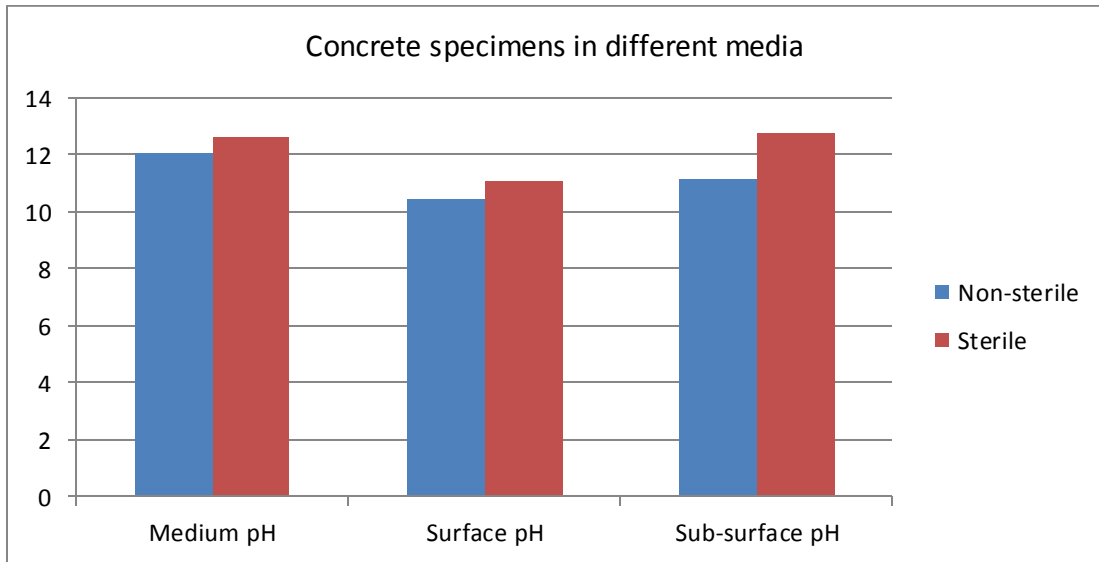


Figure 4.3.1-3. Comparison of medium pH, surface pH and sub-surface pH in concrete specimens in sterile and non-sterile media

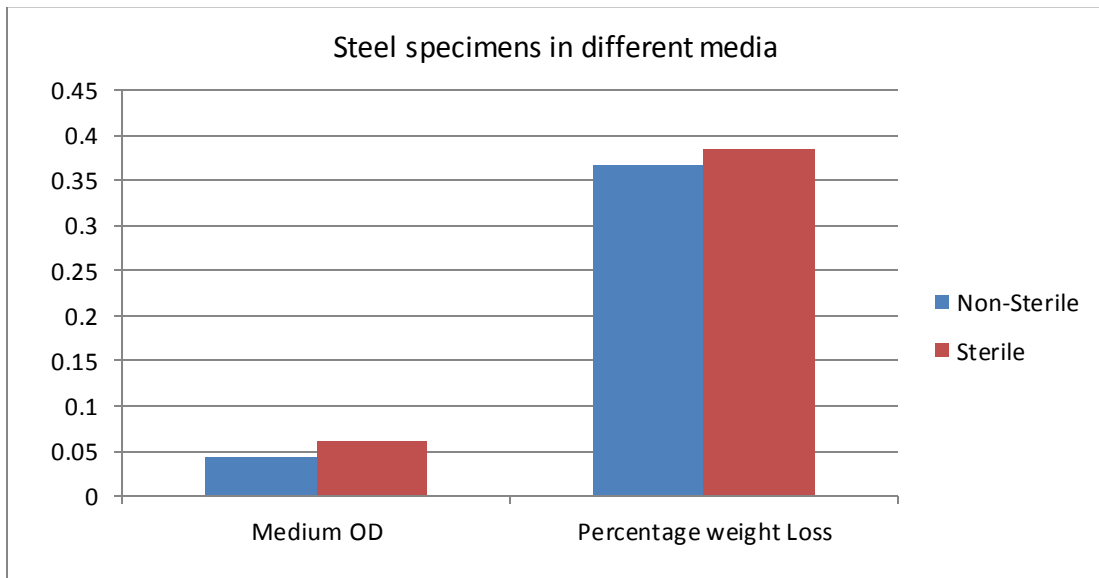


Figure 4.3.1-4. Comparison of medium OD and percentage loss of weight for steel specimens in sterile and non-sterile media

From figure 4.3.1-1 and 4.3.1-2, it can be seen that the loss of weight in steel, weight of surface residue and OD value of the medium are less in specimens placed in

the sterile medium, indicating reduced bacterial activity. Previous experiments have shown how the OD value relates to the biological activity and how the biological activity was related to the corrosion of steel. All the parameters related to bacterial activity were observed to be higher for the non-sterile sample which was as expected. It can be observed that some amount of biological activity was still observed in the specimens placed in sterile autoclaved water as shown in figures 4.3.1-1 to 4.3.1-3. However, the surface residue OD value of the sterile sample was found to be much higher than that of the non-sterile sample. This would mean the bacterial activity is higher on surface of the concrete specimens placed in the sterile medium. But the surface residue weight and OD value of the medium were observed to be lower for the specimens in sterile medium and this in turn indicates that bacterial activity is reduced in the medium and the concrete surface for the specimen in sterile water. Also, the surface OD value of the sterile medium was significantly higher than that of the non-sterile medium. The abnormally high value for surface residue OD measured for concrete samples placed in sterile water is contrary to all other results. The most likely cause for this inconsistency is an experimental error. Parameters like loss of weight, weight of surface residue, and medium OD measured for the concrete specimen show reduced values for specimens in the sterile medium as expected. All the measured pH values are also higher for the specimens in the sterile medium indicating a lower activity of biological components. All the parameters except for surface residue OD value indicate that the presence of biological agents increases corrosion of reinforced steel in concrete.

For the steel coupons the medium OD and loss of weight seem to be slightly higher in the sterile medium than in the non-sterile medium. Although the differences observed between the values measured in sterile and non-sterile medium were quite small, this was not expected. Figure 4.3.1-4 shows that both the medium OD value and loss of weight in steel were higher for the steel sample in sterile water. This indicates a higher amount of bacterial activity and corrosion in steel in the sterile medium. Although the water samples were sterilized, some bacteria may have been introduced during the immersion of specimens. Surface residue formation was also observed in the concrete specimens immersed in sterile medium, indicating biological activity. This may have happened due to the inefficiency of the autoclaving process or due to the lack of perfectly airtight storage of sterile medium.

4.4. Experimental Assumptions and limitations

All the above experiments were carried out in a stagnant medium. In real life conditions the surrounding medium is not likely to remain static, as in the case of a structure submerged in a flowing river or the sea. The surrounding medium in all the experiments conducted has the same composition at the beginning of the experiment. This can change over time due to the release of hydration products from the concrete and due to bacterial activities. This is evident from the experimental observation that the pH of the surrounding medium increased to the range of 11 to 12 over the period of immersion from an initial value of around 7.5. In real life conditions, constant dilution would result in the pH of the medium remaining more or less the same over time with slight changes that may have no correlation with the corrosion process. In actual

conditions where the composition of the medium is constantly changing, due to several other factors like temperature, light and presence of other chemicals, it is difficult to measure variation of associated parameters like pH and carbohydrate concentration of the medium and correlate these measurements to bacterial activity and corrosion. The measurement of pH and bacterial activity in the medium may not be useful in real life, but it was done in this study to corroborate other measurements of pH and bacterial activity at the concrete surface.

The collection of residue and biofilm from the surface of the concrete specimens was done using a cell scraper and was later transferred to a test tube by washing the surface of the cell scraper with water. Although extreme care was taken during the procedure to prevent loss of the collected surface residue, there was inevitably some amount of loss of material during this transfer. Also the collected surface residue may have also had some degraded concrete along with the biofilm material. These products could have been formed as a result of degradation of concrete. The presence of these compounds may affect the reaction of the surface residue sample with the anthrone reagent and also the weight of the surface residue. It was not possible to separate this material from the biofilm formation and its presence may affect the optical density reading and weights of surface residue samples collected from different concrete specimens.

The anthrone method is a color-based assay for quantifying the growth of biofilms by measuring the amount of carbohydrates. This method is used widely for estimating concentration of carbohydrates and gives reliable results. Biofilms are made of polysaccharides and thus concentration of carbohydrates can be used to quantify

biofilm growth. But this method is used to quantify the total carbohydrates content and therefore it does not make a distinction between dead cells and active cells. As a result this method cannot be used to identify active corrosion sites, where bacteria are actively multiplying and causing corrosion. Methods like crystal violet staining may be used to detect active bacterial growth and resultant corrosion.

Another limitation arises in the preparation of the concrete specimens. No two specimens are exactly identical. The distance to the crack from the surface of the sample, the crack width of each specimen and the orientation of the crack in these specimens are often not the same. The specimens also have many imperfections like voids and construction joints that are produced as part of the construction process. These construction joints may act similar to the hairline cracks, permitting ingress of surrounding media and resulting in exposure of the steel to corrosive environments.

The steel reinforcement used in the specimens had a surface coating that slowed the corrosion process. When the reinforcement bars were initially cleaned using sand paper, some of this coating may have been removed creating zones of accelerated corrosion within the steel. All these factors could influence the loss of weight, the rate of bacterial growth and the other dependent parameters. This would in turn contribute to a significant standard deviation in the measured results.

The pH at the surface of concrete was measured according to ASTM F 710 procedures, by sanding the surface to remove a certain quantity of material. The material must be sanded evenly from the surface so that the measurement is made only for a thin surface layer of concrete about 0.1 mm. pH measurements in concrete were carried out using pH strips and the pH values were accurate only up to 0.5 pH units. A

more accurate method was to sand the surface to get around 0.3 g of concrete, which would then be diluted in 1:2 ratio with DI water. The pH of this suspension would then be measured using a pH probe to give accurate results. Given the size of the concrete specimens (7cm x 7cm), this may not be possible without removing even more layers from the concrete surface and this could affect the accuracy of the surface pH measurements. However this method can be adopted in future studies that use larger specimens.

Chapter 5: Conclusion and Future Development

5.1. Summary

Previous studies have focused on corrosion of steel due to bacterial activities. In this study, experiments were conducted to understand the effect of corrosion of steel reinforcement in the concrete on the bacterial activity on the surface of reinforced concrete and in the medium surrounding the concrete. The effectiveness of measuring bacterial activity and pH at the surface of the concrete and the medium as an indirect method in determining corrosion of steel was observed. An attempt was also made to study the effect of biological agents in increasing the amount of steel corrosion.

- The effect of biological activity at the surface of concrete on the corrosion of steel reinforcement was studied. These experiments established that the bacterial activities in the biofilm can be linked to the extent of corrosion in the reinforcement steel. The results from the experiment show a direct correlation between weight of surface residue and loss of weight in steel. Based on the limited data from the experiment, the amount of corrosion is related to bacterial activity on the concrete surface as:

$$C = 3.717S_w + 0.097 \quad (4)$$

Where C is the corrosion in terms of percentage loss of steel weight and S_w is the bacterial activity measured in terms of weight of surface residue.

- Another somewhat consistent relation was observed between the optical density (OD) value of surface residue and its weights. The OD value indicates the concentration of carbohydrates, which is an indirect measurement of the bacterial

activity on the surface. From the limited data obtained from the experiment, the amount of corrosion is related to bacterial activity on the concrete surface as:

$$C = 5.102 S_{OD} + 0.071 \quad (5)$$

Where C is the corrosion in terms of percentage loss of steel weight and S_{OD} is the bacterial activity measured as OD value of the surface residue.

- The results from the experiment showed a somewhat consistent relation between the OD values measured for the surface residue and loss of weight in steel. This indicates that higher bacterial activity is associated with a higher loss of weight in steel. Thus it can be concluded that measuring the bacterial activity on the surface of concrete may give an indication regarding the corrosion of reinforcement steel.
- The effect of biological activity and pH of surrounding medium on corrosion of steel was also studied. Medium pH varied differently for different types of concrete specimens and did not show a consistent relation with steel corrosion.
- The bacterial activity in the medium measured in terms of the OD value of the medium showed a direct relationship with loss of weight due to corrosion. The OD value of medium sample indirectly determines the amount of carbohydrates in the medium. This is related to the biological activity in the medium. The experimental results indicate that a higher level of bacterial activity in the medium was associated with higher loss of weight in steel. Hence, it can be shown from the results that corrosion of steel can be estimated by observing the biological activity of the surrounding medium. This is done for a closed system where the medium is stagnant and is not to be used in real structures where the medium is constantly

changing. Based on the limited data available from the experiment, the amount of corrosion is related to bacterial activity in the surrounding medium as:

$$C = 5.375 M_{OD} + 0.022 \quad (6)$$

Where C is the corrosion in terms of percentage loss of steel weight and M_{OD} is the bacterial activity measured as OD value of the medium.

- A study was conducted to identify how the presence of biological components affects the corrosion rates in concrete by comparing a medium sterilized by autoclaving. The sterilized specimens were then stored in airtight containers. Various measurements for the concrete specimens showed a measurable difference in corrosion related parameters which include, surface residue weight, medium OD, surface pH of concrete and the resultant loss of weight in the specimens placed in sterile medium. These indicate how the biological activities accelerate corrosion.
- The comparison of parameters measured for specimens in sterile and non-sterile media show how biological agents contribute to increasing the effects of corrosion in steel coupons. This behavior was however not observed as expected in the case of the bare steel specimens. The values observed for different parameters were similar but slightly higher in the sterile sample. This may have been caused due to the introduction of bacteria into the sterile medium during the immersion of specimens.

5.2. Recommendations for Future Development

The current study uses a generalized method of quantifying carbohydrates in the medium and the surface residue to approximate biological activity. All the

components that contribute to carbohydrate production may not be directly related to the corrosion processes. More specific tests to identify bacterial species directly involved in the corrosion of steel in aerobic and anaerobic conditions can be used to quantify corrosion of embedded steel using surface measurements more accurately.

A group of bacteria commonly associated with corrosion are the sulfate reducing bacteria (SRB). The detection of sulfide concentration as an indicator of SRB can be an effective method to detect the extent of corrosion in metal and concrete surfaces. The experiments described above require the biofilm sample extracted from the field to be transported to the lab for accurate measurements. Another effective method to detect corrosion in submerged structures in-situ is by using other proxy parameters like intensity of light produced as a result of bioluminescence of the bacterial colonies on the concrete surface. Using further modified strains of bacteria involved with corrosion, which have bioluminescent qualities, might enable easier in-situ detection. The intensity of luminescence can be directly related to the extent of corrosion as it indicates the presence of a denser microbial colony. Sensitive sensors can capture the light emitted by the bacterial colonies and depending on the intensity of the light, the severity of corrosion can be classified. DNA editing techniques using CRISPR/CAS or recombinant DNA techniques may be utilized for introducing these attributes to the SRB, IOB or other microbes that grow in the presence of steel corrosion. These techniques require DNA sequencing and specific design of components based on genetic make-up of the bacterial strain being modified. But there are several regulations on the use of genetically modified bacteria in the environment and this could be a big challenge for the use of these techniques. These techniques

have recently been used in anaerobic bacteria like *Clostridium acetobutylicum* to introduce a fluorescent protein (Bruder et al. 2016). Fluorescence in alkaline media was observed in certain species of *Desulfovibrio* due to activity of an enzyme. However, the fluorescence produced is highly sensitive to temperature and light conditions in the medium (Barton and Carpenter 2013).

There exist bacteria that are capable of forming protective biofilm. These bacteria produce biocides and protective biofilms that can reduce and to a certain extent, prevent growth of anaerobic sulfate reducers (Zuo et al. 2004). The use of bacterial biocides and protective biofilms could replace the currently used chemical biocides that are damaging to the environment. This could be a more effective method to control corrosion in submerged structures. Usually metal surface are protected by using coatings. However these coating degrade with time and have to be reapplied on the metal surface. Protective bacterial biofilms also work like these coatings. Bacteria are known for their ability to adhere to surfaces and the coating of biofilm formed is also capable of maintaining itself (Jayaraman et al. 1996). This can be an efficient replacement for traditional corrosion protection systems

Appendix: Experimental Data

Table A.1. Percentage Loss of weight for specimen with no crack

	Sample 1	Sample 2	Sample 3	Average	Standard Deviation
Day 12	0.1987	0.2941	0.2372	0.2433	0.0479
Day 16	0.1883	0.1958	0.1548	0.1796	0.0218
Day 20	0.1684	0.2398	0.2433	0.2172	0.0422

Table A.2. Percentage loss of weight for specimen with hairline crack

	Sample 1	Sample 2	Sample 3	Average	Standard Deviation
Day 12	0.2710	0.2299	0.1888	0.2299	0.0410
Day 16	0.2236	0.2696	0.2118	0.2350	0.0305
Day 20	0.1877	0.2129	0.1985	0.1997	0.0126

Table A.3. Percentage loss of weight for specimen with 1mm crack

	Sample 1	Sample 2	Sample 3	Average	Standard Deviation
Day 12	0.3962	0.3069	0.2030	0.3021	0.0967
Day 16	0.2249	0.2221	0.2105	0.2192	0.0076
Day 20	-	0.1647	0.1879	0.1763	0.0178

Table A.4. Percentage loss of weight for steel specimens

	Sample 1	Sample 2	Sample 3	Average	Standard deviation
Day 12	0.2518	0.2595	0.3009	0.2707	0.0263
Day 16	0.3207	0.2878	0.2590	0.2892	0.0308
Day 20	0.3458	0.3668	0.3891	0.3672	0.0216

Table A.5. Surface Residue weight in g in concrete samples with no crack

Time	Sample 1	Sample 2	Sample 3	Average	Standard Deviation
Day 12	0.0297	0.0628	0.0251	0.0392	0.0206
Day 16	0.006	0.0123	0.12	0.0101	0.0035
Day 20	0.018	0.013	0.0262	0.0191	0.0067

Table A.6. Surface Residue weight in g in concrete samples with hairline crack

Time	Sample 1	Sample 2	Sample 3	Average	Standard Deviation
Day 12	0.0104	0.0308	0.0475	0.0296	0.0186
Day 16	0.0171	0.0371	0.0311	0.0284	0.0102
Day 20	0.0431	0.0146	0.0138	0.0238	0.0167

Table A.7. Surface Residue weight in g in concrete samples with 1 mm crack

Time	Sample 1	Sample 2	Sample 3	Average	Standard Deviation
Day 12	0.0578	0.0405	0.0802	0.0595	0.0199
Day 16	0.0846	0.0681	0.0115	0.0547	0.0383
Day 20	0.0298	0.0623	0.0228	0.0383	0.0211

Table A.8. Surface Residue OD in concrete samples with no crack

Time	Sample 1	Sample 2	Sample 3	Average	Standard Deviation
Day 12	0.028	0.028	0.024	0.0267	0.0267
Day 16	0.024	0.022	0.031	0.0257	0.0284
Day 20	0.024	0.016	0.019	0.0197	0.0238

Table A.9. Surface Residue OD in concrete samples with hairline crack

Time	Sample 1	Sample 2	Sample 3	Average	Standard Deviation
Day 12	0.0116	0.026	0.027	0.0563	0.0563
Day 16	0.02	0.023	0.032	0.025	0.025
Day 20	0.031	0.015	0.013	0.0197	0.0197

Table A.10. Surface Residue OD in concrete samples with 1 mm crack

Time	Sample 1	Sample 2	Sample 3	Average	Standard Deviation
Day 12	0.05	0.033	0.047	0.0433	0.0091
Day 16	0.021	0.028	0.039	0.0293	0.0091
Day 20	0.018	0.016	0.013	0.0157	0.0025

Table A.11. pH at surface for concrete samples with no crack

Time	Sample 1	Sample 2	Sample 3	Average	Standard Deviation
Day 12	11	11	11	11	0
Day 16	10.5	10.5	10.75	10.5833	0.1443
Day 20	10.75	10.5	10.75	10.6667	0.1443

Table A.12. pH at surface in concrete samples with hairline crack

Time	Sample 1	Sample 2	Sample 3	Average	Standard Deviation
Day 12	11	11	11	11	0
Day 16	10.75	10.75	10.5	10.6667	0.1443
Day 20	11	10.75	10.75	10.8333	0.1443

Table A.13. pH at surface in concrete samples with 1 mm crack

Time	Sample 1	Sample 2	Sample 3	Average	Standard Deviation
Day 12	10.75	10.75	10.75	10.75	0
Day 16	10.25	10.5	10.25	10.3333	0.1443
Day 20	10.5	10.25	10.5	10.4167	0.1443

Table A.14. pH at level of steel in concrete samples with no crack

Time	Sample 1	Sample 2	Sample 3	Average	Standard Deviation
Day 12	13	13	13	13	0
Day 16	12.75	13	13	12.9167	0.1443
Day 20	12.5	12.5	12.75	12.5833	0.1443

Table A.15. pH at level of steel in concrete samples with hairline crack

Time	Sample 1	Sample 2	Sample 3	Average	Standard Deviation
Day 12	13	13	13	13	0
Day 16	13	12.5	12.5	12.6667	0.2887
Day 20	12.5	12.5	12	12.3333	0.2887

Table A.16. pH at level of steel in concrete samples with 1 mm crack

Time	Sample 1	Sample 2	Sample 3	Average	Standard Deviation
Day 12	12.75	12.75	12.5	12.6667	0.1443
Day 16	11.5	12	11.5	11.6667	0.2887
Day 20	11	11.25	11.25	11.1667	0.2887

Table A.17. Medium pH in concrete samples with no crack

Time	Sample 1	Sample 2	Sample 3	Average	Standard Deviation
Day 12	12.03	12.36	12.31	12.3333	0.1779
Day 16	12.37	12.29	12.43	12.3967	0.0305
Day 20	12.15	12.19	12.23	12.1900	0.0400

Table A.18. Medium pH in concrete samples with hairline crack

Time	Sample 1	Sample 2	Sample 3	Average	Standard Deviation
Day 12	12.34	12.28	12.27	12.2967	0.0379
Day 16	12.63	12.42	12.51	12.5200	0.1054
Day 20	12.48	12.37	12.39	12.4133	0.0586

Table A.19. Medium pH in concrete samples with 1 mm crack

Time	Sample 1	Sample 2	Sample 3	Average	Standard Deviation
Day 12	12.55	12.48	12.46	12.4967	0.0473
Day 16	11.9	12.29	12.19	12.1267	0.2026
Day 20	12.18	11.9	12.16	12.0800	0.1562

Table A.20. Medium OD for concrete samples with no crack

Time	Sample 1	Sample 2	Sample 3	Average	Standard Deviation
Day 12	0.025	0.033	0.046	0.0347	0.0106
Day 16	0.031	0.043	0.029	0.0343	0.0076
Day 20	0.044	0.038	0.039	0.0403	0.0032

Table A.21. Medium OD for concrete samples with hairline crack

Time	Sample 1	Sample 2	Sample 3	Average	Standard Deviation
Day 12	0.055	0.043	0.063	0.0537	0.0100
Day 16	0.041	0.033	0.039	0.0377	0.0042
Day 20	0.032	0.036	0.025	0.0310	0.0056

Table A.22. Medium OD for concrete samples with 1 mm crack

Time	Sample 1	Sample 2	Sample 3	Average	Standard Deviation
Day 12	0.057	0.057	0.057	0.0480	0.0156
Day 16	0.038	0.039	0.026	0.0343	0.0072
Day 20	0.029	0.026	0.021	0.0253	0.0040

References

- Zuo, R., Ornek, D., Syrett, B.C., Green, R.M., Hsu C.H., Mansfeld, F.B., & Wood, T.K. (2004). Inhibiting mild steel corrosion from sulfate-reducing bacteria using antimicrobial-producing biofilms in Three-Mile-Island process water. *Applied Microbiology and Biotechnology*, 64(2), 275-283.
- Ornek, D., Wood, T.K., Hsu, C.H., & Mansfeld, F.B. (2002). Corrosion control using regenerative biofilms(CCURB) on brass in different media. *Corrosion Science*, 44, 2291–2302.
- Zuo, R., Kus, E., Mansfeld, F., & Wood, T.K., (2005). The importance of live biofilms in corrosion protection. *Corrosion Science*, 47, 279–287.
- Enning, D., & Garrelfs, J. (2014).Corrosion of iron by sulfate-reducing bacteria: new views of an old problem. *Applied Environmental Microbiology*, 80(4), 1226-1236.
- Lobo, S.A.L., Melo, A.M.P., Carita, J.N., Teixeira, M., & Saraiva, L.M. (2007). The anaerobe *Desulfovibrio desulfuricans* ATCC 27774 grows at nearly atmospheric oxygen levels. *FEBS Letters*, 581(3), 433-436.
- Jayaraman, A., Earthman, J.C., & Wood, T.K. (1997). Corrosion inhibition by aerobic biofilms on SAE 1018 steel. *Applied Microbiology and Biotechnology*, 47, 62-68.
- Alabbas, F.M., Bhola, S.M, Spear, J.R., Olson, D.L. & Mishra, B. (2013). The shielding effect of wild type iron reducing bacterial flora on the corrosion of linepipe steel Faisal. *Engineering Failure Analysis*, 33, 222–235.

Kjellerup, B.V., Gudmonsson, G., Sowers K., & Nielsen, P.H. (2006). Evaluation of analytical methods for determining the distribution of biofilm and active bacteria in a commercial heating system. *Biofouling*, 22(3), 133 – 139.

Kjellerup, B.V., Thomsen, T.R., Nielsen, J.L., Olesen B.H., Frolund, B., & Nielsen, P.H. (2005). Microbial diversity in biofilms from corroding heating systems. *Biofouling*, 21(1), 19 – 29.

Arndt, R., & Jalinoos, F. (2009). NDE for corrosion detection in reinforced concrete structures – a benchmark approach. *NDTCE*.

Tuutti, K. (1982). Corrosion of steel in concrete. *Swedish Cement and Concrete Research Institute*, 4, 468.

Schiessl, P.(1988) Corrosion of steel in concrete. *RILEM Report of Technical Committee*.

Balafas, I., & Burgoyne, J.C. (2011). Modeling the Structural Effects of Rust in Concrete Cover. *Journal of Engineering Mechanics*, 175-185.

Zou, X., Schmitt, T., Perloff, D., Wu, N., Yu, T., Wang, X. (2015) Nondestructive corrosion detection using fiber-optic photo-acoustic ultrasound generator. *Measurements*, 62, 74-80.

Bazant, Z.P., Physical model for steel corrosion in concrete sea structures-theory. (1979). *ASCE Journal of Structural Division*, 105, 1137-1153

Yuan, Y. S., & Ji, Y. S. (2009). Modeling corroded section configuration of steel bar in concrete structure. *Construction and Building Materials*, 23(6), 2461–2466.

Ning Xia, A.M., Ren, Q., Liang, R.Y., Payer, J., Patnaik, A.M. (2012). Non uniform Corrosion-Induced Stresses in Steel-Reinforced Concrete. *Journal of Engineering, mechanics*, 138(4).

Shaikh, H., Sivaibharasi, N., Sasi, B., Anita, T., Armirthalingam, R., Rao, B., Jayakumar, T., Khatak, H., & Raj, B. (2006). Use of eddy current testing method in detection and evaluation of sensitization and intergranular corrosion in austenitic stainless steels. *Corrosion Science*, 48, 1462–1482.

Hernandez-Valle, F., Clough, A.R., & Edwards, R.S. (2014). Stress corrosion cracking detection using non-contact ultrasonic techniques. *Corrosion Science*, 78, 335-342.

Song, H.W., & Saraswathy, V. (2007). Corrosion monitoring of reinforced concrete structures. *International Journal of Electrochemistry*, 2, 1-28.

Tucker, J.R. (1997). Ultrasonic spectroscopy for corrosion detection and multiple layer bond inspection. *Conference on Aging Aircraft*, 1537–1550.

Vaghefi, K., Silva, H., Harris, D., & Ahlborn, T. (2011). Application of Thermal IR imagery for concrete bridge inspection, *PCI National Bridge Conference*.

Dabous, S.A., Yaghi, S., Alkass, S., & Moselhi, O. (2017). Concrete bridge deck condition assessment using IR Thermography and Ground Penetrating Radar technologies. *Automation in Construction*, 81, 340-354.

- Tarussov, A., Vandry, M., & De La Haza, A. (2013). Condition assessment of concrete structures using a new analysis method: ground-penetrating radar computer-assisted visual interpretation. *Construction and Building Materials*, 38, 1246–1254
- Videla, H.A., & Herrera, L.K. (2005). Microbiologically influenced corrosion: looking to the future. *International Microbiology*, 8, 169-180
- Kip, N., & van Veen, J.A. (2015). The dual role of microbes in corrosion. *The ISME Journal* (2015) 9, 542–551
- Zuo, R. (2007). Biofilms: strategies for metal corrosion inhibition employing microorganisms. *Applied Microbiology and Biotechnology*, 76, 1245–1253.
- Hamilton, W.A. (2003). Microbially influenced corrosion as a model system for the study of metal microbe interactions: a unifying electron transfer hypothesis. *Biofouling*, 19, 65–76.
- Dawood, Z., & Brozel, V.S. (1998). Corrosion-enhancing potential of *Shewanella putrefaciens* isolated from industrial cooling waters. *Journal of Applied Microbiology*, 84, 929–936.
- Hamilton, W.A. (1985). Sulphate-reducing bacteria and anaerobic corrosion. *Annual Review of Microbiology*, 39, 195–217.
- Warscheid, T., & Braams, J. (2000). Biodeterioration of stone: a review. *International Biodeterioration and Biodegradation*, 46, 343–368.

Chongdar, S., Gunasekaran, G., & Kumar, P. (2005) Corrosion inhibition of mild steel by aerobic biofilm. *Electrochimica Acta*, 50, 4655–4665.

Le, C., & Stuckey, D.C. (2016). Colorimetric measurement of carbohydrates in biological wastewater treatment systems: A critical evaluation. *Water Research*, 94, 280-287.

Laspidou, C.D., & Rittmann, B.E. (2002). A unified theory for extracellular polymeric substances, soluble microbial products, and active and inert biomass. *Water Research*, 36 (11), 2711-2720.

Flemming, H.C., & Leis, A. (2001), Extracellular Polymeric Substances – the Construction Material of Biofilms. *Water Science and Technology*, 43, 1-8.

Dreywood, R. (1946). Qualitative test for carbohydrate material. *Industrial and Engineering Chemistry*, 18 (8), 499.

Sutherland, I.W., (2001). Bacterial exopolysaccharides: A strong and sticky framework. *Microbiology*, 147, 3-9.

Beech, I.B., & Garylarde, C.C., 1999. Recent advances in the study of biocorrosion- an overview. *Microbiology*, 30, 177-190.

Aquino, S.F., Hu, A.Y., Akram, A., & Stuckey, D.C. (2006). Characterization of dissolved compounds in submerged anaerobic membrane bioreactors (SAMBRs). *Journal of Chemical Technology and Biotechnology*, 81(12), 1894-1903.

- Okabe, O., Odagiri, M., Ito, T., Satoh, H. (2007). Succession of sulfur-oxidizing bacteria in the microbial community of corroding concrete in sewer systems. *Applied and Environmental Microbiology*, 73(3), 971-980.
- Barton, L.L. & Carpenter, C.M. (2013). Suitability of fluorescence measurements to quantify sulfate reducing bacteria. *Journal of Microbiological Methods*, 93, 192-197.
- Bruder, M.R., Pyne, M.E., Moo-Young, M., Chung, D.A., & Chou, P. (2016). Extending CRISPR-Cas9 technology from genome editing to transcriptional engineering in *Clostridium*. *Applied and Environmental Microbiology*, 82(20), 6109-6119.
- Shodja, H.M., Kiani, K., Hashemian, A. (2010). A model for the evolution of concrete deterioration due to reinforcement corrosion. *Mathematical and Computer Modelling*, 53, 1403-1422.
- Morikawa, M. (2006). Beneficial biofilm formation by industrial bacteria *Bacillus subtilis* and related species. *Journal of Bioscience and Bioengineering*, 101(1), 1-8.
- Erbs, M., & Spain, J. (2002). Microbial iron metabolism in natural environments. *Microbial Diversity*, 1-19.