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THE PREVENTION AND PREDICTION OF CORROSION
USING NOVEL METHODS

by

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in partial fulfillment of the requirements
for the degree of

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DEDICATION

To my parents, brothers, and sisters who offered me their full trust, unconditional love, and tireless support. They have been the source of encouragement and inspiration throughout my life.
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ABSTRACT

Corrosion causes huge economic losses worldwide with an annual direct cost estimated to be in hundreds of billions of dollars. More than 30% of this corrosion is microbiologically influenced corrosion (MIC). In the first part of this thesis, novel experiments were conducted to prevent MIC in several industrial applications.

The effect of adding specific natural oils to the oil-based coatings on their performance in sulfate reducing bacteria (SRB) and marine environments were evaluated. It was observed that the addition of optimum amounts of some natural oils to the oil-based coatings enhanced their performance and protection efficiency and increased their degradation resistance against the aggressive environments. These findings can lead to the development of new generations of environmentally-friendly oil-based coatings.

The degradability of the selected natural oils was studied in marine environments where they are mostly to be used. The difficult-to-degrade oils were selected for further tests on the remediation and control of MIC and on enhancing some of the coatings protection efficiency.

The antimicrobial effects of selected natural products against Shewanella putrefaciens bacteria and SRB which are known to be associated with MIC were evaluated. It was observed that both the black thorn (Acacia nilotica) and garlic (Allium sativum) possess bacteriostatic and bactericidal effects against these bacteria.

Natural hair-fibers were used to enhance the impermeability and reduce the shrinkage cracks of cement mortars to improve their performance when used as a lining material. The results showed that the hair-fibers were very effective in reducing shrinkage cracks and permeability of cement mortars by a remarkable amount of 92%.

The second part of this thesis was devoted to the modeling and simulation of MIC in different corrosive environments. Modeling of corrosion problems has the advantages of calculating and predicting the corrosion rates quickly, cheaply and accurately, mainly in situations where it is dangerous, very difficult or impossible to do that experimentally. To develop a novel and comprehensive MIC model, three modeling steps were adopted.

In the first modeling step a transient two-dimensional convective-diffusion model was developed to estimate the substrate concentration profiles on a biofilm surface formed on the inner surface of a given pipeline. In the second modeling step another model capable of calculating the biofilm growth rate and thickness in a pipeline system was developed. These two models were solved for different inlet substrate concentrations, operating temperatures and flow conditions (laminar and turbulent). The generated results were used as input for the pitting MIC model developed in the third modeling step.

The transient two-dimensional pitting MIC model was developed to study and estimate the pitting MIC of steel in SRB environments. The SRB cathodic depolarization theory was adopted as the SRB-influenced corrosion mechanism and was used in developing the model. The pitting MIC model was applied to marine, waste, and freshwater environments. The effects of substrate (sulfate) concentrations and SRB kinetic parameters on the shape, growth rate and depth of the corroded pits were also evaluated. The pitting MIC model was found to be very successful in estimating and predicting the pitting MIC in all the three studied environments. The model results were also found to be in a very good agreement with the corresponding experimental data found in the literature.
CHAPTER 1

INTRODUCTION

Corrosion is the deterioration of a metal by chemical and electrochemical reactions with its environment. Biocorrosion or microbiologically influenced corrosion (MIC) can be defined as the destruction or deterioration of materials by natural processes directly or indirectly related to the activity and/or growth of microorganisms. Sulfate reducing bacteria (SRB) are known to be the single most common causative organisms to MIC. Pitting MIC is usually a characteristic of the action of SRB on metals. MIC is a major hazard during the production of oil and gas causing considerable damage to both onshore and offshore production facilities and pipelines. Most of the other chemical industries also suffer from MIC. As a result of that, industries spend billions of dollars annually on coatings and biocidal chemicals to mitigate MIC.

For that, understanding MIC and developing new methods, strategies and techniques to combat it is a vital need. In this thesis, new materials, methods and techniques were developed and adopted to estimate and mitigate MIC. This thesis starts with the experimental investigations in which new materials were tested either for inhibiting the growth of the bacteria that cause MIC, or as effective additives to enhance the coatings protection efficiency. These experiments are introduced in Chapters 2 to 8. The thesis ends with introducing comprehensive mathematical models capable of calculating and estimating MIC rates and extents in different industrial applications. These modeling studies are presented in Chapters 9 to 11.

1.1 Experimental Investigations

In Chapter 2, the effects of SRB on the corrosion of both uncoated and coated mild steel coupons in the presence and absence of selected natural additives are discussed and evaluated. Alkyd oil-based coating was used with and without the addition of the selected natural additives to protect mild steel in a SRB environment. The effects of SRB and/or
their metabolites on the tested coatings and the adverse effect of those coatings and additives on the biofilm formation and the bacterial growth rate were also studied and introduced in Chapter 2.

In this study, bacteria populations, visual observations, scanning electron microscopy (SEM) analysis and computer image analyzer techniques were used. The two natural additives used in this study (olive and Manhaden fish oils) were found to be effective for MIC protection. The natural products were selected based on the environmental appeal of the products. The results also revealed that the addition of the natural additives improved the protection ability of commercial coatings with the best result with Manhaden fish oil.

The effects of the presence of selected natural oils (olive, mustard and fish oils) on the performance of the enamel oil-based coating exposed to simulated marine environments were also investigated and presented in Chapter 3. Laboratory tests were conducted according to the ASTM standards using the salt fog test corrosion chamber which is capable of simulating different marine environments.

The coatings performance and the nature and extent of degradation and corrosion on the coated coupon surfaces in the presence of one of the selected natural oils were monitored at different time intervals. Those samples and results were then compared with the corresponding control samples tested under the same conditions but without the addition of any natural additives. Various parameters such as surface roughness, weight loss and pit growth rate on the coated surfaces were measured using different techniques and various pieces of equipment such as the computer image analyzer, environmental scanning electron microscopy (ESEM) and the energy-dispersive X-ray analyzer (EDX).

From the generated results, it was concluded that the addition of specific amounts of some natural oils such as olive and fish oils to the enamel oil-based coating enhanced the coating protection efficiency under the simulated marine environments. These findings can lead to the development of new generations of environmentally-friendly oil-based coatings that can be used confidently in severe corrosive marine environments.
Since the successful natural oils in inhibiting corrosion are supposed to be used and applied in different corrosive environments like the marine environment, further studies on their stability and biodegradability were essential. Chapter 4 investigates qualitatively the biodegradability of various vegetable and fish oils under the influence of natural bacteria in seawater. In this investigation, the influence of nutrients and environment on changes in the bacterial numbers and the extent and rate of degradation for various test oils (olive, mustard, canola and cod liver oils) were studied over time. Time-series visual and microscopic observations were made to characterize the physical changes in the residual oils, formation of floating and precipitate particles, oil droplets and dispersion.

It was observed that the different oils responded in different rates and extents to biodegradation depending on their stability, viscosity and compositions. All the results clearly revealed a significant response of the oil-contaminated samples to both the seawater and wastewater environments. The results generated from this study revealed that the oils that are not easily biodegraded form excellent MIC prevention agents.

In Chapter 5, the degradability of the various vegetable and fish oils mentioned in Chapter 4 is quantitatively investigated. The tests were conducted in natural seawater, seawater inoculated with wastewater and seawater enriched with nutrients. The ESEM/EDX and the Iatroscan techniques were used to study and analyze the different oil-contaminated samples.

It was observed that the addition of the oils to each environment led to an increase in the population of marine and wastewater bacteria. The results also showed that both natural remediation (oxidation) and biodegradation took place in the oil-contaminated samples. Both the addition of nutrients to the seawater and the elevated level of broad microbial cultures in the wastewater enhanced the degradation processes. From the different generated results, it was clear that canola oil underwent the highest degradation, followed by mustard oil then cod oil and finally olive oil.
In Chapter 6, the antimicrobial effects of eight natural products (Neem, olive leaves, chamomile, *Salvia officinalis*, *Curcuma longa* (Turmeric), *Acacia nilotica* (black thorn), fresh and dry *Allium sativum* (garlic), and cactus) on *Shewanella putrefaciens* were studied. The bacteriostatic effects of all the above-mentioned natural products were investigated and only those which showed a pronounced bacteriostatic effect were later tested to study their bactericidal effects against the *Shewanella putrefaciens* bacteria.

The results showed that only black thorn and garlic possess bacteriostatic effects. For that, the minimum inhibitory concentrations, the apparent death rates and the decimal factors for both black thorn and garlic were calculated at different concentrations using the bactericidal test method.

In Chapter 7, both the influence of the surface topography of mild steel coupons and the addition of selected natural materials derived from garlic (*Allium sativum*) and black thorn (*Acacia nilotica*) on the SRB-biofilms growth rate and on the SRB-influenced corrosion rates were investigated on mild steel coupons immersed in a SRB medium.

For that, a set of experiments was conducted using mild steel coupons with different surface topographies immersed in SRB media for three months with and without the addition of the above mentioned natural materials. At the end of the experiment, the mild steel coupons were removed from the SRB media and their surfaces were investigated using different techniques. Both the SRB-biofilms growth rate and the SRB-influenced corrosion rates on the immersed mild steel coupon surfaces were studied using both visual observations and microscopic methods including the ESEM, EDX and the computer image analyzer techniques. The observations and results from the different studied samples were compared with each other and with the corresponding control samples.

It was observed that lower biofilm growth rates and lower MIC rates were detected on the smoother mild steel surfaces compared to the rougher ones under the same test conditions. It was also clear that the addition of the natural materials to the SRB media
inhibited both the biofilms growth rate and the associated MIC rates on the immersed mild steel coupon surfaces. As a result, it was concluded that both the surface topography and the addition of the selected natural materials were very influential in determining both the SRB-biofilm growth rates and the SRB-influenced corrosion rates and extents on the mild steel coupon surfaces immersed in the SRB media.

In many industrial processes, the pipeline systems are lined with a protective layer of cement mortar to protect them from the harsh flowing fluids. As an example, cement slurry is usually placed in a wellbore to harden into an impermeable mass that seals the annulus from fluid flow and protects the casing from corrosion for the life time of the well. Unfortunately, when uniform linings of neat cement fail in tension one or more small and/or large cracks may form. As a result, the pressurizing fluid or mud easily flows through the cracks causing extensive damage to the pipeline material. The necessity to check the damaging effect of plastic shrinkage in cement mortar and thus the formation of cracks has called for further new and advanced studies in this area.

For that, Chapter 8 summarizes the work that has been conducted regarding using human-hair as natural fibers to reinforce cement mortar and improve its impermeability. This investigation focuses on studying and understanding the effects of human-hair-fibers on the reduction of shrinkage cracks of cement mortars. The influence of the cement mortar mix proportions (cement/sand ratio (c/s), water/cement ratio (w/c) and hair-fiber content (f_h)) on the plastic shrinkage cracks of plain and hair-fibers reinforced cement mortars has also been studied. The results showed that human-hair-fibers proved to be very effective in reducing the plastic shrinkage cracks of cement mortars by a remarkable amount of 92%.

1.2 Modeling Studies

Biofouling is a major concern in all the industries in which microorganisms and their nutrients are available. In industrial systems the presence of biofilms can cause many problems like increase in the flow resistance of pipelines, energy losses in fluid transport
and heat exchangers, product contamination, materials deterioration and biocorrosion. As a result, biofilms contribute substantially to huge economic losses in industry.

There are many factors and parameters that affect the rates of biofilm growth on material surfaces. The substrate (nutrient) concentration near the biofilm surface is one of the important parameters that have a great effect on the biofilm growth rate. As a result, knowing and controlling the substrate concentrations in the system under investigation are very important in determining the extent and the type of the problems associated with the presence of biofilms like material degradation and biocorrosion.

The pipeline systems in many industries are the most susceptible structures to biofilm formation on their inner surfaces which in turn cause serious problems, involving degradation and corrosion. The operating conditions such as the substrate concentration, the bacteria type and growth rates, the flow velocity and the temperature are known to have a big impact on the rate and extent of the biofilms growth and the resulting biocorrosion.

One of the most dangerous and widely recognized classes of microorganisms in MIC is the SRB which is known to be responsible for the microbial fouling and the MIC in many industrial systems. The SRB are strict anaerobes with the capacity to reduce sulfate to sulfide which is a corrosive product to most of the metallic structures used in the industry. Therefore, understanding the biofilms and MIC and estimating their rates are very important in combating and preventing their presence and the associated effects.

The first step in developing the MIC comprehensive model is presented in Chapter 9. In this chapter, the substrate concentration profiles in the system under investigation were determined for later use as an input for the MIC model. In this study, a convective-diffusion model has been developed and solved under various flow conditions (laminar and turbulent) using the finite difference technique, employing the alternating difference implicit (ADI) method. The model assumes that a liquid containing substrate and bacteria is flowing in a pipeline with known concentrations at the inlet and then predicts the
variation of the transient substrate concentration along the pipeline and as a function of the pipe radius.

The model was then used to predict and estimate the substrate concentration profiles on the biofilm surface under different operating conditions. A parametric study was also conducted to study the effect of the different parameters influencing the substrate concentration profiles in the system and on the biofilm surface. The results showed that the model that has been presented in Chapter 9 is very successful in accurately estimating the substrate concentration profiles at any point in the pipeline system under different operating conditions.

In Chapter 10, the model that was presented in Chapter 9 was modified to account for the substrate consumption in the fluid bulk flow and to be capable of estimating the biofilm growth rate and thickness. This is step two in developing the comprehensive MIC model. In this step a convective-diffusion transient model that includes the substrate (sulfate) consumption rate term and predicts both the sulfate concentration profiles and the biofilm growth rate and thickness in the pipeline system has been developed and solved using the finite difference technique (FDT), employing the ADI method.

The above mentioned model was solved at different inlet sulfate concentrations, different operating temperatures and different flow conditions (laminar and turbulent). The effect of the turbulence eddies on the diffusion coefficient as well as on the biofilm growth rate was studied and thoroughly discussed. The instantaneous thicknesses of the biofilm along the pipe length were also calculated and presented under different operating conditions. A parametric study was also conducted to study the sensitivity of the model to each of the parameters influencing both the rates of substrate consumption and biofilm growth. From all the generated results, it was obvious that the biofilm model has succeeded in achieving its main goals which was mentioned in the previous paragraphs.

In Chapter 11, step three in the development of the MIC comprehensive model is presented. In this chapter a numerical model was developed to study the anaerobic pitting
MIC of steel in a SRB environment. A transient two-dimensional model in cylindrical coordinates was solved using the FDT, employing the ADI method. The SRB cathodic depolarization theory was adopted as the SRB-influenced corrosion mechanism. The pitting-MIC model was applied to marine, waste, and freshwater environments. The effects of the substrate (sulfate) concentration and the SRB kinetic parameters on the growth rate and magnitude of the pitting MIC were investigated.

The pitting-MIC model was successful in estimating and predicting the pitting-MIC growth rate, pit shape and depth in the three studied environments. The model results were also found to be in good agreement with the experimental data found in the literature for the same test conditions.

In conclusion and from all the results and discussions that have been presented in this thesis, it can be easily and confidently concluded that, the main objectives and goals of the research that has been conducted during the development of this thesis have been achieved. The main achieved goals were finding and developing new materials and methods to combat, prevent and estimate corrosion in different industrial applications.
CHAPTER 2

CONTROL OF THE MICROBIAL CORROSION USING COATINGS AND NATURAL ADDITIVES

2.1 Abstract

One of the major concerns in the oil and gas industry is corrosion. The microbial influenced corrosion (MIC) can be defined as the deterioration of metals by natural processes directly or indirectly related to the activity of microorganism. MIC affects many industries such as: petrochemical industries, ships and marine structures, power generation plants, aircraft fuel systems, wastewater facilities, cooling water systems, process industries, paper mills and water supply and distribution systems.

In this study, the influence of sulfate reducing bacteria (SRB) “grown in a lactate/sulfate culture medium” on the corrosion of both uncoated and coated mild steel coupons was evaluated in the presence and absence of selected natural additives. To achieve this, an oil-based coating (alkyd) was used with and without the addition of one of the selected natural additives to protect mild steel coupons immersed in a SRB environment. Another objective of this study was to investigate the effects of SRB and/or their metabolites on alkyd coating and the effect of this coating and additives on the SRB growth rate and biofilm formation.

In this paper, two natural additives were identified for effective MIC protection. The natural products were selected based on the environmental appeal of the products. Two additives derived from olive oil and Manhaden fish oil were found to be effective in reducing the MIC. In general 2-3\% of the natural additive was deemed adequate for effective MIC protection.
It was noticed that the number of SRB in the samples and their biofilms on the coated mild steel surfaces after being immersed for three months in the SRB media was greatly affected by the natural additives added to the alkyd coating.

A series of corrosion tests were performed to study the effectiveness of the various natural additives in inhibiting the MIC. The effects of the SRB on the coated and uncoated mild steel coupon surfaces were investigated using visual observations, scanning electron microscopy (SEM) and computer image analyzer techniques. It was observed that the presence of alkyd coating inhibited both the SRB-biofilm growth and the resulting MIC.

The results also revealed that the addition of one of the above mentioned natural additives improved the protection ability of the commercial alkyd coating, with the best result being with the Manhaden fish oil.

2.2 Introduction

Corrosion causes huge economic and ecological damage worldwide. MIC is extremely harmful to both industry and the environment. It is estimated that 20-30% of all corrosion is microbiologically influenced with a direct cost from $30-50 billions per year (Javaherdashti, 1999). One of the most important types of microbial corrosion is that due to the presence of sulfate reducing bacteria (SRB), which is most common in petroleum operations because of the prevailing anaerobic environment (Phelps et al., 1991).

Protection of structures against MIC has therefore become very critical in many industries including municipal pipelines, marine, storage vessels, sewage treatment facilities and so on (Geesey et al., 1994). The study of microbiologically influenced corrosion (MIC) has progressed from phenomenological case histories to a mature interdisciplinary science including electrochemical, metallurgical, surface analysis, microbiological, biotechnological and biophysical techniques (Little and Wagner, 1994).
Microorganisms such as bacteria, algae and fungi under certain conditions can thrive and accelerate the corrosion of many metals even in otherwise benign environments. Biological organisms can enhance the corrosion process by their physical presence, metabolic activities and direct involvement in the corrosion reaction (Hamilton, 1985). The occurrence of MIC is often characterized by unexpected severe metal attack, the presence of excessive deposits and in many cases the rotten-egg odor of hydrogen sulfide (Lee et al., 1995).

For a microorganism to grow, environmental conditions must be favorable. Essential nutrients required by most microbes include carbon, nitrogen, phosphorous, oxygen, sulfur and hydrogen. Other elements required in trace quantities include potassium, magnesium, calcium, iron, copper, zinc, cobalt and manganese. Carbon is required by all organisms for conversion into cell constituents (TANJI, 1999).

The main bacteria related to MIC are aerobic slime formers, acetate-producing bacteria, acetate-oxidizing bacteria, iron/manganese oxidizing bacteria, methane producers, organic acid producing bacteria, sulfur/sulfide-oxidizing bacteria (SOB), and sulfate-reducing bacteria (SRB).

The most important microbial corrosion is that due to the SRB. SRB thrive under anaerobic conditions, for example deep in soils and underneath deposits. The best-known examples of SRB are Desulfovibrio and Desulfotomaculum. In many cases SRB derive their carbon (for incorporation into cell material) from low molecular weight compounds such as lactate and fumarate. SRB possessing the enzyme hydrogenase and can obtain their energy from the oxidation of molecular hydrogen. SRB are capable of growing over a wide pH range (4-8) and at temperatures from 10-40 °C, although some thermophilic strains can grow in the temperature range 45-90 °C and a pressure up to 500 atm (Herbert and stott, 1983).

There is no universally acceptable mechanism to account for the corrosive action of SRB. It is believed that the iron sulfide formed on the metal surface is an efficient cathodic site for the reduction of hydrogen which has the effect of accelerating the corrosion process.
Another viewpoint suggests that oxygen made available from the sulfate reduction reaction shown in Equation (2-1) reacts with nascent hydrogen and therefore speed up the cathodic reaction (Pankhania et al., 1986).

\[ \text{SO}_4^{2-} \rightarrow \text{S}^2+2\text{O}_2 \quad (2-1) \]

The overall reaction of the anaerobic corrosion of iron induced by SRB can be described by (Pankhania et al., 1986):

\[ 4\text{Fe} + \text{SO}_4^{2-}+4\text{H}_2\text{O} \rightarrow \text{FeS} + 3\text{Fe(OH)}_2+2\text{OH}^- \quad (2-2) \]

Pankhania et al. (1986) proposed that the hydrogen sulfide (H₂S) acts as the cathodic reaction and showed that the sulfate reduction can occur with the cathodically formed hydrogen.

Chen et al. (1997) discussed many instrumental analysis of microbiologically influenced corrosion. They emphasized that detection and monitoring of microbiologically influenced corrosion is essential for understanding the mechanistic nature of the interactions and for obtaining control methods. The techniques include electrochemical noise (EN) measurements, concentric electrodes, scanning vibrating electrode probe (SVEP) mapping, electrochemical impedance spectroscopy, atomic fore microscopy, confocal laser microscopy, Fourier transform infrared spectroscopy, X-ray photoelectron spectroscopy and auger electron spectroscopy.

Rainha and Fonseca (1997) studied the influence of the sulfate reducing bacteria (SRB) *Desulfovibrio desulfuricans* ATCC 27774 grown in a lactate/sulfate medium on the anaerobic corrosion of mild steel. Higher corrosion rates, as well as the transpassive dissolution of Fe(0) or Fe(II) compounds to Fe(III) were observed in the presence of bacterial culture. Moreno et al. (1992) studied the pitting of stainless steel by the SRB and found that the biogenic sulfides enhanced the passivity breakdown in the presence of chloride anions.
Many workers studied the performance of different coatings exposed to biologically active environments (i.e.: Jones-Meehan et al., 1992; Jack et al., 1996). Jones-Meehan et al. (1992) studied coated steel exposed to mixed communities of marine microorganisms using the energy dispersive spectrometer (EDS). The EDS analysis detected breaching of epoxy, nylon and polyurethane coatings applied to steel coupons. SEM and ESEM studies have shown that all coated surfaces of steel were heavily colonized with a diverse assembling of bacteria (Jones-Meehan et al., 1992).

2.3 Experimental Work

The SRB *Desulfovibrio desulfuricans* culture medium was prepared with the composition: KH$_2$PO$_4$ (0.5 g/l), NH$_4$Cl (1.0 g/l), Na$_2$SO$_4$ (1.0 g/l), CaCl$_2$-2H$_2$O (0.1 g/l), MgSO$_4$.7H$_2$O (2.0 g/l), sodium lactate (3.5 g/l), yeast extract (1.0 g/l), cysteine solution (1.0 ml/l) and ferrous ammonium sulfate (5.0 ml/l). Preparation of this medium was performed by sequential addition of compounds to deionized water. The pH of the culture was adjusted to 7.6 and the medium was sterilized at 130 °C for 25 minutes. The medium was kept in sealed containers in an incubator at 33 °C under anaerobic conditions. After 30 hr of inoculation the population of the SRB was counted using the plate count method and was found to be 6×10$^6$ cells/ml.

The alkyd oil-based coating was used in this study to protect the mild steel coupon surfaces against the SRB and the corrosion effects. The oil based coatings include materials that are based entirely on oil such as linseed oil-based products, alkyds, alkyd enamels, oil based varnishes, and similar materials. One of the principal characteristics of this type of coatings is that they are generally applied in thin films. Another characteristic of these coatings is their wettability of most surfaces. On the other hand, one of the oil-based coating disadvantages is that they are not highly corrosion resistant. This is due to the relatively high moisture vapor transfer rate and the transfer of ions through the coating.
In this study, the alkyd coating was used because of its common usage. Another reason behind using the alkyd coating is that these coatings can be modified to improve their properties, mainly their degradation and corrosion resistance. The alkyd coating was applied (using a fine hair-brush) on 800-grit hand polished mild steel surfaces (using silicon carbide (SiC) abrasive papers). The average coating thickness was measured and found to be in the range of 4-5 mils.

The natural additives (olive and Manhaden fish oils) were added to the alkyd coating to study their effects on the protection efficiency of modified coatings. Nearly all the natural oils of either animal origin like Manhaden fish oil or plant origin like olive oil consist almost exclusively of the simple lipid class – triacylglycerols with the stereochemical configuration shown in Figure (2-1).

The different samples of mild steel coupons were immersed in the SRB medium and kept in sealed containers for three months. The studied mild steel coupons were: uncoated mild steel, mild steel coated with alkyd coating, mild steel coated with alkyd coating mixed with 2 vol.% Manhaden fish oil, and mild steel coated with alkyd coating mixed with 2 vol.% olive oil. After the three month incubation period, the samples were investigated using different microscopic and analytical techniques.

2.4 Results and Discussion

It was found that the growth rates of the SRB, as shown in Figure (2-2), were different from one sample to another depending on the environment and the coating system in that sample. This growth led to the formation of continuous and discrete biofilms and bacterial colonies attached to the coupon surfaces as can be seen from Figure (2-3). Within each biofilm, the local physical and chemical conditions created an environment that helped in accelerating the surface degradation. Those biofilms caused the degradation of the coating layer and MIC on the surfaces on which they grew. The scanning electron photomicrographs of the mild steel coupon surfaces underwent heavy microbial colonization after their exposure to the SRB environment for 3 months.
Figure (2-4) shows a scanning electron photomicrograph of the surface of one of the uncoated mild steel coupons. The bacterial colonies and biofilm matrices were observed to be attached to and between the layers of the heavy and dense corrosion products. Small holes (pits) were also observed under the corrosion product deposits. This can be attributed to both the SRB and the chloride attacks.

Most of MIC, however, manifests itself as localized corrosion attack because most of the microorganisms do not form completely continuous biofilms on the metal surfaces but rather they tend to settle as discrete colonies (Hamilton and Maxwell, 1986). This fact explains the localized pitting corrosion attack on some of the tested mild steel coupon surfaces as can be seen from Figure (2-5).

Figure (2-6) shows another scanning electron photomicrograph of a mild steel coupon coated with unmodified alkyd coating. From Figure (2-6), it can be seen that bacterial colonies and biofilm matrices are attached to the coated surface. Breaching of the coating was also detected on the surface as can be seen from Figure (2-7). This type of failure in the coating layer led to a severe localized corrosion attack on the mild steel surface underneath the coating. Biodegradation of the coating was also detected as small holes in the coating layer filled and surrounded by bacteria, as can be seen from Figure (2-8). Black ferrous sulfide deposits were detected wherever there was a crack or hole in the coating layer. It is believed that the presence of SRB reduced sulfate to sulfide, which reacted with iron and produced the black ferrous sulfide (Hamilton, 1985).

The scanning electron photomicrograph shown in Figure (2-9) is for the surface of a mild steel coupon coated with alkyd coating mixed with 2 vol.% olive oil. No biofilms were detected except a few small bacterial spots were scattered at different locations on the surface. Blistering with and without rupturing of the coating was observed on some areas of the coated surface, as can be seen from Figure (2-10). This was a clear indication of some local failure in the coating either as a result of coating disbondment or microbial processes occurring beneath the coating layer.
It is worth mentioning here that the SRB in the media and in the slim layers (biofilms) converted sulfates in the sample into sulfides, which in turn produced hydrogen sulfide (H₂S). Later, the H₂S and carbon dioxide (CO₂) reacted with water to produce mild acidic products that lower the pH of the substrate (metal) surface to levels favorable for the growth of bacteria, which at the end created a very acidic environment, thereby encouraged the rapid corrosion attack on those metal surfaces (Lee and Characklis, 1993; Lee et al., 1993).

Figure (2-11) shows the scanning electron photomicrograph of the surface of a mild steel coupon coated with alkyd coating mixed with 2 vol.% Manhaden fish oil. It was surprising to find only very few bacterial spots on this surface, which was shiny and almost clean. For that no breaches, blistering or deterioration were later detected on this surface when it was investigated under the microscope.

The above results were attributed to the marked inhibition of bacterial adhesion to the coated surface when one of the natural additives was added to that coating. Also it is believed that the natural additives increased the modified alkyd coatings protection efficiency by decreasing the ions and moisture vapor transfer rates through the coating layer.

As a result of those findings, it was concluded that the coated mild steel surfaces with alkyd coating mixed with 2 vol.% Manhaden fish oil were the most and very well protected surfaces followed by those coated with alkyd coating mixed with olive oil, while the least protected mild steel surfaces were those coated with the original alkyd coating.
2.5 Conclusions

From the results and discussions presented in the previous section, the following conclusions were withdrawn:

1. The black ferrous sulfide detected on some of the tested mild steel coupon surfaces and the rotten-egg smell confirmed the activity of the SRB on those surfaces and as a result the MIC attack.

2. SRB-biofilms were heavily attached to the uncoated mild steel coupon surfaces as discrete colonies rather than continuous biofilms and this fact led to the fast propagation rate of localized corrosion on those surfaces.

3. The mild steel coupon surfaces coated with the original alkyd coating were found to experience high degradation and failure of the coating and later pitting corrosion attack.

4. The coating degradation and the MIC attack appeared to proceed at much lower rates on the surfaces coated with alkyd coating mixed with one of the natural additives (olive or Manhaden fish oils), with the best results with the Manhaden fish oil.

5. In general 2-3% of the natural additive was deemed adequate for effective MIC protection.

From the above, it was confidently concluded that, mixing some natural additives with some oil-based coatings is promising towards improving the coatings protection efficiency and inhibiting both the biofilm formation and the microbial induced corrosion (MIC) effects on the metal surfaces immersed in corrosive microbial environments.
2.6 References


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Anaerobic Microbial Activities Including Hydrogen Mediated Acetogenesis Within


2.7 Appendices

2.7.1 Appendix A: Figures

![Chemical structure of triacylglycerols](image1)

Figure (2-1) Stereo-chemical structure and configuration of triacylglycerols

![SEM photomicrograph of SRB](image2)

Figure (2-2) SEM photomicrograph of the sulfate reducing bacteria (SRB)
Figure (2-3) SEM photomicrograph of the bacterial colonies and biofilms attached to the surface of one of the mild steel coupons.

Figure (2-4) SEM photomicrograph of uncoated mild steel coupon shows the corrosion products and the SRB attack on its surface.
Figure (2-5) SEM photomicrograph shows the localized corrosion attack (pitting) on the uncoated mild steel coupon surface.

Figure (2-6) SEM photomicrograph shows the heavy bacterial colonization and the biofilms attached to the surface of a mild steel coupon coated with alkyd coating.
Figure (2-7) SEM photomicrograph shows breaching and cracks and on a mild steel coupon surface coated with alkyd coating.

Figure (2-8) SEM photomicrograph of a coated mild steel surface with alkyd coating, shows the bacteria in and around the pits and cracks on the surface.
Figure (2-9) SEM photomicrograph shows some pinholes, spots, and localized attack on the surface of a coated mild steel coupon with alkyd mixed with olive oil

Figure (2-10) SEM photomicrograph shows blistering on the surface of a coated mild steel coupon with alkyd mixed with olive oil
Figure (2-11) SEM photomicrograph shows the surface of a well protected mild steel coupon coated with alkyd mixed with fish oil.
CHAPTER 3

USING NATURAL PRODUCTS TO ENHANCE THE PERFORMANCE OF OIL-BASED COATINGS IN SIMULATED MARINE ENVIRONMENTS

3.1 Abstract

Corrosion can be defined as the destruction or deterioration of a material because of its reaction with the environment. A recent study conducted by NACE, estimated the annual direct cost of corrosion in the United States to be $276 billion. It has also been estimated that 25-30% of the total annual direct corrosion cost could be saved by using state of the art corrosion management practices.

This work aims to study the impact of the presence of selected natural oils (olive, mustard, and salmon fish oils) on oil-based coated surfaces. To accomplish this, laboratory tests were conducted under simulated marine and acidic environments according to ASTM standards. The marine environment was simulated in a salt fog test corrosion chamber.

The form and extent of degradation and corrosion on the coated coupon surfaces in the presence of natural oils were monitored and compared with the corresponding control samples with no additives, under the same conditions after exposure periods of 1000, 2000, and 3000 hours in the salt fog test corrosion chamber. Various parameters such as surface roughness, weight loss, and pit growth rate on the coated surfaces were measured using different techniques and equipment like the image analyzer, the environmental scanning electron microscopy (ESEM) and the energy dispersive X-ray analyzer (EDX).

It was observed that, the addition of optimum amounts of some of the tested natural oils to the oil-based coating enhanced the coating protection efficiency and minimized its
degradability, under the simulated marine environment. It was clear that the addition of olive and fish oils to the oil-based coating enhanced its performance and protection efficiency against the aggressive marine environment. These findings can lead to the development of new generations of environmentally friendly oil-based coatings that can be used confidently in the corrosive marine environments.

3.2 Introduction

3.2.1 Background

Corrosion can be defined as the destruction or deterioration of a material because of its reaction with the environment (Fontana and Green, 1978). Corrosion causes huge economic and ecological damage worldwide. A two-year breakthrough study estimated the annual direct cost of corrosion in the United States to be $276 billion. This represents 3.1% of the U.S. Gross Domestic Product (GDP). It was also estimated in this study that, 25-30% of the total annual direct corrosion cost could be saved by using state of the art corrosion management practices (Koch et al., 2002). Environmental considerations are also part of the hidden costs of corrosion. For a safer and cleaner environment, and a safer and healthier public, effective corrosion measures should be implemented. Steps should be taken to make sure no irreparable damage is done to human life or the environment while taking anti-corrosion measures. Hence, it is very important to take engineering decisions in such a way that the risk involved is minimized.

The losses due to corrosion in marine environments are extensive because 70% of the earth surface consists of salt-water which is very aggressive on mainly metallic structures. Because of this, marine environments are the most vulnerable to corrosion problems (Muntasser, 2002). Marine corrosion of metallic structures is influenced by a number of factors (Fink and Boyd, 1970) like: water temperature, concentration of oxygen in the sea water, pH value of sea water, marine fouling on piling, salinity of sea water, velocity of the water flow over the structure, abrasive material in the water, and galvanic effect of unlike metals.
3.2.2 Corrosion Prevention Using Coatings

Protection of structures against corrosion has become very critical in many industrial systems including, pipelines, marine structures, storage vessels, sewage treatment plants, and oil and gas facilities (Geesey et al., 1994). There has also been a considerable increase in the construction of offshore structures in recent years, so coating methods and corrosion prevention techniques have developed significantly. As most of these structures are made of steel and are designed to operate in rough conditions for long periods of time, the coating system must perform well with minimum maintenance cost (Rangaswamy et al., 1995).

Protective coatings should possess many properties in order to provide high corrosion protection efficiency (Appleman, 1992; Muntasser, 2002). Some of those properties are: high resistance to abrasion, water, chemicals, and MIC; good adhesion to the metal surfaces; good expansion and contraction abilities; high temperature resistance, radiation resistance, resistance to cathodic disbonding; and acceptable ease of application.

Coating components could vary depending upon the proposed use. General coating components are: (a) binder, which is designed to hold the film together and provide the necessary adhesive strength (Munger, 1999), (b) pigment, which is a powdered material that provides the color as well as the inner characteristics and weathering resistance of the film, (c) solvent-thinner, which influences the development and formation of the coating. (Nowadays, stress on reduction of VOC in the environment has resulted in the rapid development of water-based latex paints that have little or no volatile solvent) (d) additives, which are used in low quantities as wetting, flattening agents, driers, plasticizers, emulsifiers, stabilizers, and cross linking agents (Gesser, 2002).

There are different types of coatings: oil, lacquer and water or emulsion-type based coatings, co-reactive, and inorganic coatings (ASM, 1987). Oil-based coatings include materials based completely on oil, such as linseed, alkyds, alkyd enamels, oil-based varnishes, and similar materials. This type of coating is applied in thin films and several
coats for best results. They react with the oxygen from air in order to provide a strong and resistant film. These materials have good workability due to the lubricating character, which makes application easy. Water-based, or emulsion-type coatings, are typically based on vinyl, acrylics, epoxies, and materials dispersed in water in the form of emulsions (Munger, 1999). They are easily workable and less viscous. The water content of the coating has to evaporate in a uniformly regulated fashion to produce a uniform dry coating layer. Moderate humidity and temperature is required for best results.

All coatings will fail eventually, but people are concerned about the failures that occur before time and are not anticipated. Various causes of coating failure are documented through experience in the industry. According to an estimate, 70% of all coating failures have resulted due to bad surface preparation (Bellassai, 1972). It is said that the cost of repair of failed coatings due to hidden deficiencies is much higher than the original application cost.

Coating researchers (Munger, 1999; Muntasser, 2002) reported different types of coating failures such as: cracking, wrinkling, discoloration, blistering, peeling, flaking, and delaminating. Contamination on the surface or incompatible systems prevents bonding of the paint to the surface (Appleman, 1992). Tator (1977) have discussed a procedure that should be followed by companies to investigate coating failures. The failure mechanism was illustrated by studying three different coating systems, which included alkyd, epoxy, and inorganic zinc, top coated with a vinyl system. Pearl and Kogler (1994) tested 47 different corrosion resistant coatings of various generic types in a marine environment, 38 of which had a volatile organic components (VOC) content of 2.8 lbs/gal or less. Low VOC test systems of various generic types performed well after 4 years of natural exposure in marine environments.

Extensive research has been conducted on the performance of coatings in marine environments. Indoor laboratory techniques have been developed by researchers to reduce the exposure time. Other researchers have carried out long outdoor exposure tests, which take several years to produce reliable data (Appleman, 1992). Several laboratory
accelerating tests of coatings were compared with those carried out in exterior marine environment by Appleman (1992). He concluded that a reliable accelerated laboratory test method for the prediction of field performance and durability of the coating systems is not available. A comprehensive method for coating selection in offshore environments was developed by Bone III (1989). In his study, he drew a correlation between accelerated laboratory tests and the actual field performance. Several other researchers investigated and discussed the characteristics, fabrication, construction technique, and quality control requirements needed to use coatings systems in marine environments (Munger, 1992; Szokolik, 1992; Chengde, 1995).

Even though there has been considerable development in the field of corrosion resistant coatings, very few systems have been developed taking their resistance to microbial influenced corrosion (MIC) into consideration (Pope and Morris III, 1995; Ray et al., 1997; Smart, 1997). Also the presence of hydrochloric and sulfuric acids in the surrounding atmosphere has a very strong impact on metal corrosion and coating deterioration, and for that, attention should be paid to their serious effects. Volatile acids are more corrosive than the non-volatile acids. Hydrochloric acid is volatile and hence considered to be highly corrosive. It moves through the atmosphere as a gas and reacts rapidly with iron and steel in the presence of moisture. Laboratory based measurements were conducted by Askey et al. (Askey et al., 1993), and the effect of atmospheric HCl on the corrosion of zinc and iron was studied. They concluded that mild steel samples exposed to HCl, corroded at a rate 18 times higher than in an unpolluted urban atmosphere. Ahmed et al. (Al-Sulaiman and Ahmed, 1995) proposed that the use of zinc primer coating could possibly prevent corrosion in such corrosive environments.

Many researchers have used different natural materials for corrosion inhibition and control purposes (El-Etre, 1998; El-Etre and Abdallah, 2000). In a study conducted by Mansour et al. (2003), green algae were tested as a natural additive for a paint formulation based on vinyl chloride copolymer (VYHH), to evaluate its efficiency for protection of steel against corrosion in seawater. Both suspended and extracted forms of algae were used to achieve optimum performance of the algae-contained coatings.
Poorest performance (protection of steel against corrosion is seawater) was obtained when algae was added in its suspended form, whereas the extracted form exhibited better performance based on impedance measurements.

Saeed et al. (2003) investigated the antimicrobial effects of garlic and black thorn against *Shewanella putrefaciens*, which is a bacterium implicated in pipeline corrosion. They concluded that both garlic and black thorn possess bacteriostatic effects against *Shewanella putrefaciens* and for that they can be used as bactericides to inhibit and prevent biocorrosion in environments containing *Shewanella putrefaciens*. Al-Darbi et al. (2002) mentioned that mixing natural products, mainly fish oils, with oil-based coatings like alkyd, showed a positive result towards inhibiting both the biofilm formation and the microbial induced corrosion (MIC) effects on mild steel surfaces.

As compared to normal paints, high performance coatings have been in use for a relatively short time, and have proved to be a very important tool used in corrosion engineering to mitigate corrosion. The high performance coatings have the ability to protect more surfaces and substrates from environmental damages (Munger, 1999). Harris and Lorenz (1993) discussed corrosion prevention in severely aggressive environment using cold applied tape based products. Murphy (2003) discussed the recent technical developments in Organic Coatings. Manning (1996) investigated the improvements that have been made to increase the corrosion protection performance of epoxy-coated reinforcing steel in North America

### 3.3 Experimental Work

The objectives of this study were to study the performance of oil-based coatings with and without the presence of selected natural oils and to develop a new coating system resistant to harsh environments.
3.3.1 Equipment

3.3.1.1 Salt fog test corrosion chamber

This equipment creates an environment that simulates the corrosive marine environmental conditions. A top opening cabinet with a volume of 150 cubic feet was used in this study (Model SF-850). The cabinet consists of: plastic-lined steel with no exposed metal or corrodible material in the interior testing area, an air saturation tower with auto level control, a salt solution reservoir with auto level control, plastic atomizing nozzles that are fixed in a central fog generation tower with internal baffling, a specimen support system, and provision for heating the cabinet. The temperature of the cabinet can be controlled up to 67°C and the humidity up to 100%. Salt solution is pumped into a nozzle where it meets a jet of humidified and compressed air, forming a fine droplet spray under controlled temperature and humidity.

3.3.1.2 Computer image analyzer

A computer image analyzer system (Model KS300) was used in this study. The setup consists of: an optical microscope which has a high magnification of 1000×, Axio-Cam which is a professional digital camera scanner used in light microscopy that has a digitization of 14 bits per color with a programmable spatial resolution of up to 3900×3090 pixels in each color channel to ensure excellent image quality, a high-speed desktop computer consisting of the two software, Axio-Vision and KS300.

3.3.1.3 Acid immersion chamber

An acid immersion chamber made of glass with the dimensions of 60×20×10 cm and a glass wall thickness of 0.5 cm was used in this study. A constant air supply was maintained in the solution. The pH was monitored using a pH meter. A pump was also used to supply the acid whenever needed. The supports used for the mild steel plates were also made of glass so that they do not react with the solution to prevent contamination. It
was made sure that the samples did not come in contact with each other to prevent any galvanic effect.

3.3.1.4 Electronic coating thickness meter

PosiTector 6000 was used as the electronic thickness meter to measure the coating thickness. It is designed for use with all metallic surfaces. It works on magnetic and eddy current principles and measures the thickness of coating on both ferrous and non-ferrous metals. It can measure coating thicknesses in the range of 0-60 mils, which is equivalent to 0-1500 μm, with an accuracy = (0.1 mils) + (the actual meter reading × 1%).

3.3.1.5 Abrasion chamber

The abrasion chamber (sandblasting chamber) was used to prepare the mild steel surfaces prior to the application of coatings. It works by shooting sand particles at the metallic surface using air compressor that propels the abrasive particles. The particles strike the surface of the metal and remove any unwanted substance present. The air pressure can be controlled using a valve. The sand-shooting gun that was used in this study has an internal diameter of 0.7 cm.

3.3.1.6 Surface roughness meter

DIAVITE DH-5 surface roughness meter was used to measure the surface roughness of the coated mild steel samples. This instrument can measure many parameters such as roughness average (Ra), maximum roughness depth (Rm), and mean roughness depth (Rz). The roughness meter range is between 0 and 10 μm with an increment of 0.01 μm. The accuracy of measurements meets the ANSI-B46.1, ISO and DIN standards, and MIL specifications.
3.3.1.7 ESEM/EDX

The ESEM uses electron beams to form a magnified image of the specimen. Detection of
the specific signals generated produces an image of the elemental composition of the
sample. Secondary electrons, backscattered electrons, and X-rays are the three major
signals providing information. The small diameter of the primary electron beam gives a
high-resolution image. Primary electron beams interact with the atoms in the sample,
causing shell transition, which results in the emission of X-rays.

The EDX is the detection and measurement of the energy permitting elemental analysis.
It provides qualitative and, with adequate standards, quantitative analysis of surfaces
elemental composition. The ESEM used in this study was an Electro Scan E3 ESEM with
an attached NORAN Voyager III EDX, using a NORAN Pioneer Detector.

3.3.2 Experimental Procedure

3.3.2.1 Materials

In this study, mild steel was used as the metallic substrate for the coated panels. The two
different panel sizes used were 75×75×3.5 mm for the acid test, and 75×50×3.5 mm for
the salt fog test. The chemical composition of the mild steel coupons is given in
Table (3-1).

Enamel oil-based coating was used as the coating material in this study. It was mixed
with three different types of natural oils. The coating thickness for all the systems was
recorded to be in the range of 4-5 mils. The details of the used coating systems are given
in Table (3-2).
3.3.2.2 Procedure

3.3.2.2.1 Application of coatings

Before the application of the coatings, all the mild steel coupon surfaces were polished and cleaned from any surface impurities, such as, dirt and rust using the abrasion chamber (Santagata et al., 1998). The mild steel coupon surfaces were painted using fine hair brush. Humbrol thinner was used with the enamel oil-based coating. The coated mild steel surfaces were left to dry for six hours (at a laboratory temperature = 25 °C) before being recoated. Three layers of the coating were applied on each sample. The coatings thicknesses were measured using the electronic coating thickness meter, to ensure the surface uniformity.

3.3.2.2.2 Salt fog test

The salt spray fog test corrosion chamber was used in this study following the ASTM B117 standard (ASTM, 2002). The temperature inside the cabinet was maintained at 35 °C and the relative humidity was kept at 100%. The salt solution used had a pH of 6.8. The panel edges were sealed with silicon rubber before exposure to avoid the edges effect and to prevent any leakage underneath the coating layer. For statistical reasons two coupons (duplicates) of each coating system were exposed in the corrosion chamber. All the results reported are the average of the two samples. All the samples were exposed to the simulated marine environment inside the corrosion chamber for 3000 hours in total. The specimens were examined after 1000, 2000, and 3000 hours of exposure, to study the ongoing degradation and corrosion on the coated surfaces.

The surface roughness of the samples surfaces was measured using the surface roughness meter after every 1000 hours of exposure. The Ra values were recorded at 20 random points on the surface of the sample and their average value was reported. The weights of the samples were measured before and after exposure in the corrosion chamber and the weight loss factor for each coating systems was reported. The KS-300 image analyzer
was used to monitor the degradation and the growth of pits on the surfaces of the coated samples. Both qualitative and quantitative results were reported. The ESEM/EDX system was used to investigate and analyze the surfaces of the coated samples, and the resulting degradation and corrosion products.

3.3.2.2.3 Acid test

This test was performed by immersing the coated samples in a hydrochloric acid solution of pH = 4 for 1000 hours. The test conditions were controlled to ensure adequate reproducibility of the results. The controlled test conditions included composition of solution, aeration, temperature of solution, and method of specimen support in the solution. The temperature of the solution was maintained at 25°C and air was supplied continuously. A pH meter was used to monitor and maintain a constant pH of the solution. A digital camera (1280×960 pixels) was used to take pictures of the coated surfaces and their deterioration as a result of the acidic corrosive environment. The blisters observed on those surfaces were compared with the ASTM D714-87 standards (ASTM, 2002).

3.4 Results and Discussion

3.4.1 Acidic Environments

The aim of this test was to observe the degrading and blistering effects of the acidic environment on the coated surfaces, and later on the corrosion forms and rates on the metallic substrates. Two samples of each coating system were tested in the same environment to be confident of the repeatability of the results.

Figure (3-1) shows the blistering effects of the acidic environment on both the control samples (system A) and the samples coated with the enamel oil-based coating mixed with one of the natural oils (systems B, C and D). The degree of blistering on each of the
samples was evaluated using the ASTM-D714-87 (ASTM, 2002) photographic reference standard. The results and findings are tabulated in Table (3-3).

The samples coated with the enamel oil-based coating (system A) showed very little or no sign of surface damage, while the samples coated with enamel coating mixed with one of the selected natural oils experienced either low or high degree of blistering. The highest degree of blistering was observed on the samples coated with the enamel coating mixed with 3 vol.% fish oil (system D). This was followed by the samples coated with the enamel coating mixed with 3 vol.% mustard oil (system B).

The samples coated with the enamel coating mixed with 3 vol.% olive oil showed anomalous behavior in terms of blister size. Initial surface contamination and difference in surface preparation could be the reason for the difference in the adhesive strength of the two samples coated with enamel coating mixed with olive oil.

From the above observations, it was concluded that the control samples coated with the enamel coating showed better resistance to blistering effects in the acidic environment studied. This also indicates that the presence of natural oils (such as mustard, olive, and fish oils) changes the adhesive properties of the oil-based coatings at low pH environments. These blisters can grow in size and frequency and hence degrade the coating quality and its protection efficiency.

3.4.2 Marine Environment (Salt Fog Tests)

3.4.2.1 Surface roughness

The deterioration and corrosion of a given surface are known to lead to an increase in its roughness. The average surface roughness value (Ra) for each sample was measured at 0 and 3000 hours of exposure in the salt fog test corrosion chamber. The Ra values are tabulated in Table (3-4) and the % differences in Ra values are plotted in Figure (3-2).
The maximum change in Ra values was observed in the samples coated with the enamel coating only (coating system A), followed by those coated with the enamel coating mixed with mustard oil (coating system B). The samples coated with the enamel coating mixed with either olive oil (coating system C), or salmon fish oil (coating system D) showed a much lower change in their Ra values (Figure 3-2). From this observation, it was concluded that the presence of olive or salmon fish oils in the enamel oil-based coating decreases the deterioration rate of the coated surface. Al-Darbi et al. (2002) observed higher coating protection efficiency when selected natural oils were added to the coating.

3.4.2.2 Weight loss

The weight loss is one of the most common methods used to quantify corrosion mainly when dealing with small panels and coupons (Fontana and Green, 1978). In this study, the rate and extent of corrosion on the surfaces of the different samples were estimated using this method. The weight of each sample was measured before and after exposure in the salt fog test corrosion chamber. The overall period of exposure for each sample was 3000 hours. The reported values are the average of duplicate samples of each coating system. The weight of the coated samples before and after the test, are summarized in Table (3-5) and plotted in Figure (3-3).

From Figure (3-3), it can be seen that the weight loss factor (WLF) was maximum for the samples coated with enamel coating only, closely followed by the samples coated with enamel coating mixed with mustard oil. From that, it was concluded that these two samples experienced high corrosion and erosion rates in the salt fog test corrosion chamber. On the other hand, the samples coated with enamel coating mixed with fish oil showed the lowest weight loss followed by the samples coated with enamel coating mixed with olive oil. It was obvious that the addition of fish and/or olive oils to the enamel oil-based coating decreased the rate of the coated surface deterioration, and as a result, decreased the associated substrate metal corrosion.
3.4.2.3 Image analyzer

The image analyzer system KS300 was used to monitor and investigate the coated surfaces deterioration and the growth of the localized corrosion reflected in the form of holes and pits on and beneath the coated surfaces. It was observed that, all the tested coating systems suffered from surface erosion, degradation, and metal corrosion, but with different rates, forms and extents. The holes and pits on the coated surfaces were photographed using a light microscope using a magnification of 10×. The pictures were then analyzed using the image analyzer technique. These pictures gave an idea about the severity and rates of the coating deterioration and the resulting corrosion. This method gave qualitative as well as quantitative results concerning the extent of corrosion in and around a given pit on the surface of a given coated sample (Muntasser et al., 2001). Photographs of some selected pits were taken after 1000, 2000 and 3000 hours of exposure in the salt fog corrosion chamber. The areas of those pits were also measured using the above mentioned image analyzer techniques.

The average values of the pits areas for each coating system at different time intervals are summarized in Table (3-6) and graphically represented in Figures (3-4 and 3-5). Figure (3-4) shows the average pits area for the different coating systems at different exposure times. Figure (3-5) shows the growth of pits with time for each coating system. The best fits of the experimental results shown in Figure (3-5), with $R^2 = 1$, were represented by Equations (3-1 to 3-4).

System A: $A_{pit}(t) = A_{pit}(0) + 6 \times 10^{-5} t^3 - 0.03714 t^2 + 694.91t$  \hspace{1cm} (3-1)

System B: $A_{pit}(t) = A_{pit}(0) + 2 \times 10^{-5} t^3 - 0.1228 t^2 + 229.92t$  \hspace{1cm} (3-2)

System C: $A_{pit}(t) = A_{pit}(0) + 8 \times 10^{-6} t^3 - 0.0469 t^2 + 87.184t$  \hspace{1cm} (3-3)

System D: $A_{pit}(t) = A_{pit}(0) + 2 \times 10^{-5} t^3 - 0.1318 t^2 + 244.37t$  \hspace{1cm} (3-4)
Figure (3-6) shows a comparison between the shapes and sizes of the pits on the surfaces of the different coating systems after an exposure time of 3000 hrs inside the salt fog test corrosion chamber. In Figure (3-6), the brownish and reddish colors in and around the pits are the different corrosion products, mainly comprising of ferric and ferrous ions. It is worth mentioning here that several limitations existed regarding the coating application method and the curing process. The size and growth of each pit is influenced by the initial surface contamination or breaks in the coating film.

The surface areas of the pits and the surrounding corrosion products were used to evaluate the performance of each coating system. From Figure (3-6), it was observed that the samples coated with the enamel coating only and enamel coating mixed with mustard oil, both showed the highest degree of localized corrosion. The lowest degree of surface degradation and localized corrosion was observed in the samples coated with enamel coating mixed with olive oil, where the overall surface damage and rusting on those samples were relatively low. The samples coated with enamel coating mixed with fish oil also suffered from coating degradation and localized corrosion attack, as can be seen from Figure (3-6). The amount of surface damage on these samples was higher compared to those on the surfaces of the samples coated with enamel coating mixed with olive oil.

From the previous findings, it was concluded that the presence of fish and/or olive oils in the coating system had a pronounced positive effect on increasing the coating resistance to the surrounding corrosive environment. The increase in the coating resistance (to the surrounding corrosive environment) when it is mixed with an optimum amount of a specific natural additive, might be attributed to the enhancement of the coating binding strength and to the increase in the moisture resistance of the coating.

3.4.2.4 ESEM / EDX analysis

Both the ESEM and the EDX were used to study and analyze the surfaces of the above mentioned coating systems. The EDX analysis technique is a well known method used for investigating the surfaces of metals and coatings. Meehan and Walch (Jones-Meehan
and Walch, 1992) studied coated steel exposed to mixed communities of marine microorganisms using EDX. There ESEM/EDX analysis detected the breaching of epoxy, nylon and polyurethane coatings applied to steel coupons.

Figures (3-7) shows the ESEM photomicrograph and the EDX spectra of the surface of a sample coated with enamel coating mixed with mustard oil, after an exposure time of 3000 hours in the salt fog test corrosion chamber. Cracks and pits were observed all over the surface of this sample. The EDX analysis of a particular spot on the surface shown in Figure (3-7-a) was conducted and the spectrum is shown in Figure (3-7-b). This spectrum revealed a high percentage of Si and Ti as they form a major part of the enamel oil-based coating. Iron (Fe) was detected on two different peaks on the spectra and that implies that iron was present in two valence forms (ferrous and ferric). From this observation, it was concluded that, the mild steel substrate had corroded and produced both ferric and ferrous oxides as part of the corrosion products. The EDX spectra also showed zinc (Zn) at the energy level of 1.03 KeV. The lower counts of zinc may be justified by the fact that, both Zn and the corrosion products in the form of ZnCl₂ were leached out and washed away from the surface. This fact makes the coating system much less protective towards any aggressive environment.

Figure (3-8) shows the ESEM photomicrograph and the EDX spectrum of the surface of the sample coated with enamel coating mixed with olive oil after 3000 hrs of exposure in the salt fog test corrosion chamber. Figure (3-8-a) shows that the surface was much less degraded with fewer pits, holes, and cracks compared to other coated surfaces. The EDX analysis of a spot on this surface is shown in Figure (3-8-b). From the spectra, it can be observed that iron was detected only on one peak. Zinc (Zn) on the other hand, was detected with a very high count as compared to that for coating systems B and D. This can be explained by the fact that, the amount of zinc leached out from the coating was quite low. This means that the addition of olive oil formed a homogeneous thin film on the metal surface which helped in making it much more protective.
Figures (3-9) shows the ESEM photomicrograph and the EDX spectrum of the surface of the sample coated with enamel coating mixed with fish oil (coating system D), after 3000 hrs of exposure inside the salt fog test corrosion chamber. Very few localized corrosion attacks were observed on the sample surface in the form of pits and cracks of almost the same shape and size. The amount of damage on the surface of coating system D was observed to be much lower than that for coating systems A and B. The EDX spectrum of a spot on the surface shown in Figure (3-9-a) is shown in (Figure 3-9-b). From Figure (3-9-b), it was observed that, Si and Ti had the highest peaks. Iron (Fe) was detected on two peaks implying that it was present in ferrous as well as ferric forms. The amount of chloride and zinc were detected to be very low. Zinc reacts with chlorides to form ZnCl₂ and that could have been washed out as it is a loose product (Munger, 1990).

From the above results, it was inferred that coating system C showed the best performance under the simulated marine environment, followed by coating system D, while coating systems A and B experienced the highest surface damage and poorest performance. The leaching of zinc from the coating surface indicates the degradation of the coating system as zinc starts behaving as a sacrificial anode. This phenomenon was observed in coating system B and a little bit in coating system D.

3.5 Conclusions

1. The presence of any of the natural oils: mustard, olive and fish oils in the enamel oil-based coating increased its susceptibility to deterioration in the acidic environments.

2. The addition of olive and/or fish oils to the enamel oil-based coating improved its performance in the simulated marine environments.

3. The change in the surface roughness values of the surfaces of the enamel oil-based coating systems (exposed to the simulated marine environment) were lowered by 40-45% by the addition of olive and/or fish oils to the original enamel coating.
4. The weight loss factor values of the mild steel coupons coated with the enamel oil-based coating was lowered by 40-50% by the addition of olive and/or fish oils to the original enamel coating.

5. The rate of surface degradation and growth of pits and cracks on the coated surfaces decreased in the presence of olive and/or fish oils in the enamel oil-based coating.

6. The presence of olive oil in the oil-based coating highly decreased the degradation and loss of the coating from the coated surfaces, thereby increased the coating system efficiency.

7. The use of olive, fish and other selected natural oils can lead to the development of new environmental friendly coating systems resistant to harsh corrosive environments.

3.6 References


### 3.7 Appendices

#### 3.7.1 Appendix A: Tables

**Table (3-1) Chemical composition of the mild steel coupons used in this study**

<table>
<thead>
<tr>
<th>Element</th>
<th>C</th>
<th>Mn</th>
<th>P</th>
<th>S</th>
<th>Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (%)</td>
<td>0.17-0.23</td>
<td>0.30-0.60</td>
<td>0.04</td>
<td>0.05</td>
<td>Balance</td>
</tr>
</tbody>
</table>

**Table (3-2) Details of the tested coating systems**

<table>
<thead>
<tr>
<th>Coating system</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>enamel oil-based coating</td>
</tr>
<tr>
<td>B</td>
<td>enamel oil-based coating + 3 vol.% mustard oil</td>
</tr>
<tr>
<td>C</td>
<td>enamel oil-based coating + 3 vol.% olive oil</td>
</tr>
<tr>
<td>D</td>
<td>enamel oil-based coating + 3 vol.% salmon fish oil</td>
</tr>
</tbody>
</table>
Table (3-3) Degree of blistering on the coated surfaces, evaluated according to the ASTM- D714-87 standards

<table>
<thead>
<tr>
<th>Coating system</th>
<th>Blister size</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>No. 6</td>
<td>Few</td>
</tr>
<tr>
<td>B</td>
<td>No. 4</td>
<td>Medium dense</td>
</tr>
<tr>
<td></td>
<td>No. 4</td>
<td>Medium</td>
</tr>
<tr>
<td>C</td>
<td>No. 6</td>
<td>Medium</td>
</tr>
<tr>
<td></td>
<td>No. 2</td>
<td>Medium dense</td>
</tr>
<tr>
<td>D</td>
<td>No. 2</td>
<td>Medium dense</td>
</tr>
<tr>
<td></td>
<td>No. 2</td>
<td>Medium dense</td>
</tr>
</tbody>
</table>

Table (3-4) The average surface roughness values “Ra” (μm) of the coated surfaces

<table>
<thead>
<tr>
<th>Coating System</th>
<th>t = 0 hrs</th>
<th>t = 3000 hrs</th>
<th>% Difference in Ra values</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.38</td>
<td>2.80</td>
<td>17.70</td>
</tr>
<tr>
<td>B</td>
<td>2.42</td>
<td>2.82</td>
<td>16.76</td>
</tr>
<tr>
<td>C</td>
<td>2.59</td>
<td>2.81</td>
<td>8.82</td>
</tr>
<tr>
<td>D</td>
<td>2.34</td>
<td>2.59</td>
<td>10.91</td>
</tr>
</tbody>
</table>
Table (3-5) Weight of the coated mild steel coupons before and after the test (g)

<table>
<thead>
<tr>
<th>Coating system</th>
<th>t = 0 hrs</th>
<th>t = 3000 hrs</th>
<th>Weight Loss</th>
<th>WLF (g/m²day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>45.08</td>
<td>44.85</td>
<td>0.23</td>
<td>1.563</td>
</tr>
<tr>
<td>B</td>
<td>43.26</td>
<td>43.07</td>
<td>0.19</td>
<td>1.352</td>
</tr>
<tr>
<td>C</td>
<td>46.75</td>
<td>46.61</td>
<td>0.14</td>
<td>0.932</td>
</tr>
<tr>
<td>D</td>
<td>46.00</td>
<td>45.88</td>
<td>0.12</td>
<td>0.785</td>
</tr>
</tbody>
</table>

Table (3-6) Average pit area for each coating system (micron squared)

<table>
<thead>
<tr>
<th>Coating System</th>
<th>Hours of exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1000</td>
</tr>
<tr>
<td>A</td>
<td>385496</td>
</tr>
<tr>
<td>B</td>
<td>127568</td>
</tr>
<tr>
<td>C</td>
<td>48187</td>
</tr>
<tr>
<td>D</td>
<td>134923</td>
</tr>
</tbody>
</table>
Figure (3-1) Digital photographs show 20×30 mm of the surfaces of the coated samples
Figure (3-2) Changes in the average surface roughness values (Ra) of the surfaces of the mild steel coupons coated with different enamel oil-based coating systems after 3000 hrs of exposure to the simulated marine environment inside the salt fog test corrosion chamber.
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CHAPTER 4

BIODEGRADATION OF NATURAL OILS IN SEAWATER

4.1 Abstract

Spills of non-petroleum hydrocarbons including vegetable oils and fish oils are of environmental concern because of their potential to cause serious effects on marine life and coastal environments. Biodegradation by indigenous microorganisms is an important and potentially ubiquitous process affecting both the chemical composition and physical properties of contaminant oils. Data on the environmental persistence of non-petroleum oils is now required for risk assessments and decision making by spill responders. This paper investigates the biodegradability of various vegetable and fish oils under the influence of natural bacteria in seawater.

The influence of nutrients and the microbial environment on changes in bacterial numbers and the extent and rate of degradation for various test oils (olive, mustard, canola and cod liver oils) were studied over time. Time-series visual and microscopic observations were made to characterize the physical changes in the residual oils and the other formed polymeric materials.

The biodegradation process was significantly influenced by environmental conditions, with a higher rate and extent of biodegradation observed in seawater amended with nutrients and wastewater that contained elevated level of broad microbial cultures and nutrients. It was observed that different oils responded in different rates and extents to biodegradation depending on their stability, viscosity and compositions. All results clearly revealed a significant response of the oil-contaminated samples to both the seawater and wastewater environments. Observations of changes in the physical properties of the residual oil may be important in the context of oil spill response strategies. For example, simple physical recovery methods may be used to recover polymeric lumps at the sea surface.
4.2 Introduction

Biodiesel (methyl esters of vegetable oil fatty acids) is handled in relatively small quantities and a worst-case scenario spill would be from road tanker haulage. More frequently, spills occurring from handling during refueling and are often flushed down the nearest sewer grating, irrespective of any hydrocarbon admixture. This brief study is intended to follow the degradation of the vegetable oil fatty acid methyl esters in seawater where bacterial action is likely to be important (Dunn and Knothe 2001).

A novel shoreline cleanup process has also been developed to aid in the removal of crude or fuel oil from shorelines using a highly effective biosolvent formulation based on vegetable oil methyl esters in combination with bioremediation enhancers. Mudge and Pereira (1999) conducted experiments using biodiesel derived from vegetable oils to study its effects on the biodegradation of crude oil. They found that the biodiesel demonstrated a considerable potential for removing crude oil from contaminated beaches. On the other hand, data on the environmental persistence of non-petroleum oils are now required for risk assessments and decision making by spill responders.

The term oil describes a broad range of hydrocarbon-based substances. Hydrocarbons are chemical compounds composed of the elements hydrogen and carbon. The basic unit of fats and oils, known as a triglyceride, consists of one molecule of glycerol combined with three molecules of fatty acid. If the material is liquid at ambient temperatures it is traditionally referred to as oil, and as a fat if solid. When all fatty acids of a triglyceride are of the same kind it is a simple triglyceride; if more than one kind is present, it is mixed. The kinds of fatty acid present in a triglyceride have marked effects upon its physical and chemical behavior. When the carbon atoms in the hydrocarbon chain of a fatty acid hold their full complement of hydrogen they are described as saturated. Where two adjoining carbon atoms in the hydrocarbon chain of a fatty acid each lack a hydrogen atom, a double bond forms between them. The fatty acid is then said to be unsaturated (Patterson, 1989).
Environmental Protection Agencies in Canada, USA and many other countries have found that petroleum oils, vegetable oils, and animal fats produce similar environmental effects following accidental spills in the environment as they share common physical properties. Impacts have been shown to result from a number of means including smothering, coating animals and plants with oil, oxygen depletion from the degradation of the oil, and toxicity of the oil and its byproducts. Studies on the environmental persistence of non-petroleum hydrocarbons are now required to determine the level of risk these compounds pose to our environment in the event of accidental spills (McKelvey et al., 1980).

In-situ biodegradation is the elimination of an organic compound from an ecosystem by the metabolic activity of the biocenosis actually present in this system. In theory, if the process were 100% efficient the final products of biodegradation would be biomass, carbon dioxide, and water. Biodegradability and toxicity can be considered as two basic criteria determining the behavior of chemical compounds in the environment. Biodegradation can be primary, environmentally acceptable and ultimate (Pitter and Chudoba, 1990). The intensity of biodegradation is influenced by several factors, such as the concentration of nutrients and oxygen, pH, composition, concentration and bioavailability of the contaminants, chemical and physical characteristics and the pollution history of the contaminated environment. Bioremediation attempts to accelerate the naturally occurring biodegradation of contaminants through the optimization of limiting conditions (Allard and Neilson, 1997).

Many hydrocarbon-contaminated environments are characterized by low or elevated temperatures, acidic or alkaline pH, high salt concentrations, or high pressure. Nevertheless, hydrocarbon-degrading microorganisms have adapted to grow and thrive in these environments and play an important role in recovery of polluted extreme habitats (Margesin and Schinner, 2001). Seawater contains a range of microorganisms that can partially or completely degrade oil to water-soluble compounds and eventually to carbon dioxide and water (Tango and Islam, 2002; Dean-Raymond and Bartha, 1975).
In contrast to non-petroleum oils, much work has been done to study the environmental factors influencing the biodegradation of petroleum hydrocarbons in marine waters (Atlas and Bartha, 1972; Atlas and Bartha, 1973; Sharma and Pant, 2000; Siron et al., 1995; Tango and Islam, 2002). The processes of spreading, evaporation, dispersion, emulsification and dissolution are most important during the early stages of a spill whilst oxidation, sedimentation and biodegradation are more important later on and determine the ultimate fate of the oil (Blumer et al., 1973).

In the United States, vegetable oil spills in freshwater are among the most common organic materials spilled. As a result, research is being carried out to characterize toxicity and the potential for degradation under both aerobic and anaerobic conditions, with a view to developing new oil spill countermeasure techniques (Sturman, 1973; Winicelle, 2001). In the marine environment, Pereira et al. (1998) studied the bacterial degradation of linseed and sunflower oils in sediments of a salt marsh in North Wales, UK. They determined the biodegradation of the oils by measuring changes in fatty acid composition via GC-MS. Three days after the addition of linseed oil, they observed that the numbers of aerobic heterotrophic bacteria increased 5 fold to around $1.7 \times 10^5 \text{ cfu/g wet sediment}$. This was followed by increases in anaerobic heterotrophs and sulfate reducing bacteria at 7 and 21 days respectively. Changes in the linseed oil fatty acid composition were noted after 14 days indicating a degree of degradation had occurred. They also noticed that the addition of sunflower oil to sediments did not affect the numbers of heterotrophic bacteria for over a month. This suggests that the sunflower oil polymerizes in sediments and makes it resistant to microbial breakdown. Therefore, the degradation of sunflower oil was reduced compared to that of linseed oil.

Canola oil spills are a major concern in Canada. Spills of this product, which now accounts for more value to the country than wheat, have occurred at a transshipment facility in Vancouver Harbor. Vegetable oil that escaped while being transferred through a pumping station to a tanker at Neptune Bulk Terminals reached Vancouver Stanley Park and contaminated birds in the harbor (ENS, 2000). Canola and other vegetable oil spills have resulted in massive losses in marine birds (McKelvey et al., 1980; Anon,
1994). The impact and persistence of the product on pelagic and benthic marine organisms are not known.

4.3 Experimental Set-up

One of the main objectives of this study is to investigate and understand the potential biodegradability of representative vegetable and fish oils in the marine environment. This information is needed for risk assessments and decision making in regards to the type and extent of spill response operations needed to protect environmental quality and human health. To accomplish this, the biodegradability of olive, mustard and canola vegetable oils and cod liver oil was studied in controlled laboratory shaker flask experiments. Two ml of each oil was added to 200 ml (1% volume/volume oil) of seawater and wastewater environments with and without the addition of nutrients. Seawater was taken from the Biology Department Laboratories of Dalhousie University, where it was being pumped into the labs from the North West Arm of the Atlantic Ocean in Halifax after passing through a sand filter. The wastewater was taken a few meters away from the effluent stream near the Halifax harbor.

Marine broth was prepared using seawater and was used as the nutrients enriched seawater samples. The ingredients of the marine broth were peptone (5 g), yeast extract (1 g), ferric citrate (0.1 g), sodium chloride (19.45 g), magnesium chloride (5.9 g), sodium sulfate (3.24 g), calcium chloride (1.8 g), potassium chloride (0.55 g), sodium bicarbonate (0.16 g), potassium bromide (0.08 g), strontium chloride (34.0 mg), boric acid (22.0 mg), sodium silicate (4.0 mg), sodium fluoride (2.4 mg), ammonium nitrate (1.6 mg), and disodium phosphate (8.0 mg) per liter, with a final pH of 7.6. The ingredients for the marine agar were the same with the addition of 15 g of agar. The marine agar and broth used for the bacteria counting was autoclaved for 20 minutes at 121 °C at 15 psi.

The oil-contaminated samples were put in Erlenmeyer flasks on a shaker (rpm = 170) in an incubator at 27 °C as shown in Figure (4-1). Another set of different oil-contaminated
samples was kept under static conditions to study the contact area and shaking effects on the biodegradation process. All the samples were studied in duplicates.

Regular surveys of the total number of bacteria in the oil-contaminated samples were performed during the studied period using the plate count method on marine agar Petri dishes. The bacteria were grown in an incubator at 27 °C for 36 hours. The effects of oil type as well as the nutrients and microbial environment (microorganisms present) on the extent and rate of oil degradation were studied. To accomplish this, visual observations, spectrophotometric measurements, bacteria counting, and light microscopic analyses were used.

4.4 Results and Discussion

The oil-contaminated samples in the flasks were visually observed at different time intervals to monitor the microbial growth and the changes in oil droplets shape, behavior, size, and presence in the sample. Figure (4-2-a) shows a canola oil-contaminated seawater sample, in which a white spherical particle can be seen floating on the surface and no oil droplets can be seen in the flask. For the canola oil-contaminated wastewater samples many small white flakes (2-5 mm long) were observed on the surface, while in the seawater containing nutrients samples, the oil droplets disappeared from the surfaces of those samples and no lumps were formed. The white flakes in the wastewater samples were formed much faster than in the seawater only samples.

Following the release of approximately 1500 tons of sunflower oil into the marine environments, Mudge et al. (1995) found that the oil formed a polymer in seawater, and produced relatively hard, intractable lumps. Figure (4-2-b) shows a light microscope picture of one of the white particles formed in canola oil samples. The particles dissolved in benzene and chloroform thus providing an indication that they are mostly soaps formed by the saponification process, in which triglycerides react with calcium, sodium or potassium hydroxide to produce glycerol and a fatty acid salt (soap).
For the mustard oil-contaminated samples, similar observations were noticed with the exception that many white flakes and lumps were formed and dispersed on the surface and bottom of the flasks. Figure (4-3-a) shows the floating particles formed in one of the mustard oil-contaminated seawater samples. Those flakes were observed growing faster in the wastewater samples, while in the seawater containing nutrients those flakes were not formed. Mudge et al. (1993) reported that the oil lumps formed after an oil spill was not available for bacterial degradation. Figure (4-3-b) shows a picture of one of those fine particles that were formed in the seawater contaminated with mustard oil. Solubility in benzene with heat (100 °C), confirmed that these particles were fatty acid salts (soaps).

The olive oil-contaminated samples were observed to have some oil spots and droplets still floating on the water surfaces even after 30 days. In the seawater samples, big oil droplets (2-4 mm in diameter) and white spots were observed on the surface. In the seawater containing nutrients samples, the size and concentration of the oil droplets were much smaller compared to seawater only samples (less than 1 mm in diameter). This might be attributed to the fact that the nutrients enhanced the biodegradation process. In the wastewater samples the oil droplets were very fine and few flakes and white spots were formed and dispersed on the surface as can be seen in Figure (4-4). No precipitates or floating particles were observed as in the case of canola and mustard oils. Pereira et al. (1998) mentioned that the fates of various types of oils in the same environment are different depending on their viscosity and composition.

In the case of cod liver oil-contaminated samples, it was observed that in the seawater samples there were yellow viscous spots attached to the flask surfaces near the water surface as waxy materials, and few precipitated particles (2-4 mm in diameter). In the seawater containing nutrients samples, much smaller yellowish particles (1 mm in diameter) and spots were observed either precipitated or floating on the water surface. In the wastewater samples, yellowish spherical particles were observed precipitated on the flask bottoms as can be seen in Figure (4-5-a). Figure (4-5-b) shows a magnified picture using a light microscope for one of those cod oil lumps. These particles were easily dissolved in chloroform. Thus as reported for canola oil, they are fatty acid
salts and not polymerized oil. Many researchers (Parker, 1967; Kemp et al., 1975; Perry, et al., 1979) reported that polyunsaturated fatty acids undergo preferential degradation relative to saturated fatty acids under the same environmental conditions.

To study the effect of shaking and contact area between the phases on the biodegradation process, a few oil-contaminated samples were kept under static conditions. These samples were compared with the corresponding samples that have been kept on the shaker during the study period. The visual observations as well as bacteria numbers monitoring showed an increase in oil degradation and bacteria numbers with shaking. In a wastewater sample contaminated with a high concentration of canola oil (a layer that covers the whole water surface), a polymeric thin white to gray film was observed at the interface between the oil and water phases. This film increased in thickness, size and density, with time until it collapsed and precipitated on the flask bottom surface (Figure 4-6). This film is believed to be a mix of fatty acid salts and oil polymeric materials.

The total bacteria populations for the different samples and at different time intervals were monitored using the plate count method using marine agar Petri dishes. The spectrophotometer was used at the beginning of the experiments to monitor the bacterial growth in the canola oil-contaminated samples. The first readings indicated a clear and measurable bacterial growth in the canola oil-contaminated samples comparing to the control samples (seawater alone). Due to the oil viscosity and the presence of dispersed fine oil droplets either on the liquid surface or suspended in the solution as white particles, the accuracy and the reproducibility of the spectrophotometer results were poor. For that the plate counts were used for the bacterial enumeration.

In general, it was observed that the numbers of marine oil degrading bacteria increased with time in the oil-contaminated samples. The increase in bacteria populations was more pronounced in the seawater containing nutrients and wastewater samples. Figure (4-7) shows the increase in bacterial population for the three different canola oil-contaminated samples (seawater, wastewater, and seawater containing nutrient). Mudge et al. (1995)
studied the biodegradation of sunflower and linseed oils and reported an increase in bacteria numbers due to the presence of oils.

Figures (4-8 to 4-11) show the bacteria populations in the different oil-contaminated samples during the period of study. It is obvious that both the availability of nutrients and the elevated level of broad microbial cultures enhanced the degradation process. By comparing the bacterial numbers for each type of oil with the corresponding numbers of other oils under the same conditions and time interval, and by taking advantage of the visual and microscopic observations it can be concluded that canola oil degraded with the least difficulty followed by mustard, cod and finally olive oils. Figures (4-8 to 4-11) show irregular trend in the data depending on the oil type and environment. Those findings can be explained by understanding the effect of oil structure and composition on the degradation processes.

The structure of a fatty acid molecule which is characterized by the length of the carbon chain (number of carbon atoms), the number of double bonds and also the exact position of these double bonds, define and determine the biological reactivity of the fatty acid molecule and even of the lipid containing those fatty acids. Olive oil is the richest in monoenes fatty acids. They have a unique double bond and the commonest are of the n-9 series, as oleic acid, which is probably the most common fatty acid (olive oil has a high content of this acid: about 60-70%). This is why it is the most stable compared to other types of oils studied, and that means it is expected to be the hardest to degrade. Linoleic acid is the most common polyunsaturated fatty acid, in plants and animal tissues. Canola oil contains around 33% linoleic acid, which is polyunsaturated fatty acid (Table 4-1). Olive oil contains less than 10% of this fatty acid and this is why canola oil is more susceptible to degradation than olive oil. Mustard oil on the other hand, contains up to 50% monounsaturated erucic acid with 22 carbon atoms. This is why it is much easier to degrade comparing to olive oil.

Table (4-1) shows that the percentages of fatty acids in mustard seed oil are 7% saturated, 63% monounsaturated and 30% polyunsaturated fatty acids. Oils from fish and marine
origins are characterized by a large range of fatty acids from 12 to 26 carbon atoms and 0 to 6 double bonds. The bulk of the fatty chains are contributed by saturated (15-25%), monoenes (35-60%) and polyenes (25-40%). Gadoleic acid with 20 carbon atoms was first noted in cod liver oil (Ratledge, 1994). Among all the oils studied in this investigation, cod liver oil is considered the least stable oil and as a result it is expected to be the easiest to be degraded (Ratledge, 1994). That was not the case in this study. This may be explained by the formation of antibacterial products during the oxidative degradation of cod liver oil (Erickson et. al., 1980).

Lipid peroxidation can be defined as the oxidative deterioration of lipids containing any number of carbon-carbon double bonds (Ratledge, 1994). The primary peroxidation products are hydroperoxides in which double bond(s) may have moved or/and changed configuration. These products may be structurally rearranged or converted into secondary peroxidation products, either smaller molecules by fission or bigger by dimerization. Some of the products of auto-oxidation have antibacterial properties that may kill the bacteria or cause bacterial stasis. The toxicity of the byproducts of oil metabolism processes is known as well, and has been studied by many researchers (Ratledge, 1994).

The observations, experimental results and bacteria numbers (Figures 4-12 to 4-14) in the different oil-contaminated samples are in agreement with the above discussion concerning oil stability, composition, and availability for biodegradation. As can be seen from Figures (4-12 to 4-14), bacteria numbers were, in general, the highest in the canola oil-contaminated samples, followed by mustard, cod, and olive oil samples.

As well as the biodegradation processes, auto-oxidation is taking part in oil deterioration with time. Examining the results can prove this observation. This fact may be explained also by the decrease in bacteria colonies with time depending on the oil type, as can be seen in Figures (4-8 to 4-11). The decrease in bacteria numbers may also be explained by running out of bioavailable substrate as the physical characteristics have changed. Nutrients limitation may also be a key role in that observation. From fatty acid composition perspective, one can rank the degradability of these oils as Canola, Cod liver,
mustard, and olive oil. Note that the rankings of Canola and Cod liver oils are close to each other. In terms of actual biodegradability, the ranking was Canola (most degradable), Mustard, Cod liver, and Olive oil. The discrepancy between observed and expected (from fatty acid considerations) biodegradabilities can be explained by noting that the generation of toxic products is different in each of the oils and does not depend on the fatty acid composition alone. In this respect, it is known that Cod liver oil produces the greatest amount of antibacterial products, followed by mustard, canola, and olive oils. This explains the sharp decrease in bacterial numbers in cod liver and mustard oil samples. The lack of nutrients after a long time is another cause of the sharp reduction in bacteria populations after reaching the peak (maximum) value (Figures 4-8 to 4-14), (Ratledge, 1994).

4.5 Conclusions

All results clearly revealed a significant response of the oil-contaminated samples to both the seawater and wastewater environments. The oil biodegradation activities in seawater were found to be significantly stimulated by nutrient enrichment and in the presence of mixed microbial consortia found in wastewater. It was also observed that different oils responded in different rates and extents to biodegradation depending on their viscosity, structure and compositions.

Visual observations showed that canola oil degraded with the greatest ease, followed by mustard, cod, and finally olive oil, which was the most stable oil used in this study. Bacteria numbers in the different samples showed a different trend for the different types of oils. Considering the effect of the degradation susceptibility of each oil type, and the antibacterial activity of metabolic byproducts for oil degradation, canola oil had the highest degradation, followed by mustard, cod and finally olive oil.

Both auto-oxidation and biodegradation took place in the oil weathering process. The oxidation process either accelerates the biodegradation rates by producing much smaller
and easier compounds to be biodegraded, or inhibits the microbial attacks by producing antibacterial products.

The formation of floating lumps may provide an operational advantage to spill responders, as the recovery of residual oil particles may be removed much more easily than surface oil slicks. This concludes that the bacteria populations of marine environments have the potential to degrade different types of oils with different rates and through different pathways.

4.6 References


4.7 Appendices

4.7.1 Appendix A: Tables

Table (4-1) Saturated and unsaturated fatty acids percentages of representative natural oils

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Olive</th>
<th>Canola</th>
<th>Mustard</th>
<th>Cod</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total saturated</td>
<td>15</td>
<td>6</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>Total monounsaturated</td>
<td>75</td>
<td>60</td>
<td>63</td>
<td>52</td>
</tr>
<tr>
<td>Total polyunsaturated</td>
<td>10</td>
<td>34</td>
<td>30</td>
<td>27</td>
</tr>
</tbody>
</table>

4.7.2 Appendix B: Figures

Figure (4-1) The oil-contaminated samples kept on a shaker in an incubation room at 27 °C
Figure (4-2) (a) Canola oil-contaminated seawater sample, 2x
(b) A light microscope picture of one of the floating particles in the sample shown in (a), 15x
Figure (4-3) (a) Mustard oil-contaminated seawater sample, 2x
(b) A light microscope picture of one of the floating particles in the mustard oil-contaminated seawater sample shown in (a), 15x
Figure (4-4) Olive oil-contaminated wastewater sample, 2x
Figure (4-5) (a) Cod liver oil-contaminated wastewater sample, 2x
(b) A light microscope picture of one of the precipitated particles in mustard oil-contaminated wastewater sample, 15x
Figure (4-6) Polymeric Film formation at the interface between the oil and water phases in a canola oil-contaminated wastewater sample, 2x

Figure (4-7) Bacteria colonies in the different canola oil-contaminated samples after two days of growth in the shaking flasks (a) not contaminated seawater control sample (b) seawater (c) seawater containing nutrients (d) wastewater
Figure (4-8) Bacteria numbers in the canola oil-contaminated samples

Figure (4-9) Bacteria numbers in the mustard oil-contaminated samples
Figure (4-10) Bacteria numbers in the olive oil-contaminated samples

Figure (4-11) Bacteria numbers in the cod liver oil-contaminated samples
Figure (4-12) Bacteria numbers in the oil-contaminated seawater samples

Figure (4-13) Bacteria numbers in the oil-contaminated seawater samples enriched with nutrients
Figure (4-14) Bacteria numbers in the oil-contaminated wastewater samples
CHAPTER 5

VEGETABLE AND ANIMAL OILS DEGRADATION IN MARINE ENVIRONMENTS

5.1 Abstract

Spills of animal and vegetable oils are a major concern because of their serious effects on marine life and their economic impact on coastal activities. Natural marine microorganisms have the potential to degrade different oil contaminants. This paper investigates the degradability of various vegetable and animal oils in natural seawater, seawater inoculated with wastewater, and seawater enriched with nutrients. The addition of any of the oils to each environment led to an increase in bacteria numbers.

The Environmental Scanning Electron Microscope and Energy Dispersive X-ray analyses and the Iatroscan techniques were used to study and analyze the different oil-contaminated samples. The results showed that both oxidation and biodegradation took place in the oil-contaminated samples. The addition of nutrients and the elevated level of broad microbial cultures in wastewater highly increased the degradation processes. Canola oil showed the highest degradation, followed by mustard and cod and then olive oil.

5.2 Introduction

The degradation of vegetable oils after spills is slow and complex (Jerald et al., 2000; Kemp et al., 1975; Mudge et al., 1995; Mudge, 1997; Pereira et al., 2002; Perry et al., 1979). The rate at which an oil spill spreads determines its effect on the environment and the chances of it being degraded. Factors that affect the spread ability of an oil spill include surface tension, specific gravity and viscosity. The lower the oil surface tension, the more likely a spill will spread, and since the water temperature reduces the oil surface tension more than the air temperature it is more likely to spread on warmer waters than on
very cold waters. Most triacylglycerol oils have a specific gravity less than water and initially float on the surface. The higher the viscosity of the oil, the slower it will spread, and the less available it will be for degradation processes.

Natural actions in the marine environments such as weathering, evaporation, oxidation, emulsification, and biodegradation reduce the severity of an oil spill and determine its fate (Dean-Raymond and Bartha, 1975). The processes of spreading, evaporation, dispersion, emulsification and dissolution are most important during the early stages of a spill whilst oxidation, sedimentation and biodegradation are more important later and determine the ultimate fate of the oil (Blumer et al., 1973). Vegetable oils and animal fats leave a thicker and more viscous residue compared to light refined petroleum products, and they are less likely to be affected by some of those natural actions. Bucas and Saliot (2002) investigated the non-petroleum oils spillages in marine environments. He mentioned that the drifting oils could mix with floating material to sink or form crust. He added that they could also be oxidized, dispersed, degraded by bacteria, and/or polymerize.

Danger from edible oil spills is a serious problem, and in some cases they produce more harmful effects than petroleum oil spills. Biodiesel (methyl esters of vegetable oil fatty acids) is a much less viscous material that for the present is handled in relatively small quantities and probably the worst-case scenario would be a spill from road tanker haulage. Spills from handling during refueling may become common and probably often would be flushed down the nearest sewer grating, irrespective of any hydrocarbon admixture (Dunn and Knothe, 2001). A spillage of rapeseed oil into Vancouver harbor killed 500 birds. This compares with 50 birds killed by many petroleum spills in the same place over a five-year period (McKelvey et al., 1980). Ingestion of large quantities of oil by birds, results in lipid aspiration pneumonia, furthermore, the oil can act as a laxative and lead to further dehydration and debilitation (Anon, 1994). Mudge (1995) reported that vegetable oils might have a direct toxicity towards animals or cause sub-lethal effects.

Each type of oil has distinct physical and chemical properties. These properties affect the way oil will spread and break down and the hazard it may pose to marine or freshwater life
forms. The variety of fatty acids in a vegetable oil offers the potential for different forms of attack by microorganisms. Natural edible oils are primarily composed of glycerol esters in the form of triacylglycerides of various saturated and unsaturated carboxylic acids. The major constituent carboxylic acids of four selected oils are shown in Table (5-1) (Hui, 1996; Patterson, 1989; Pryde, 1979). The properties and characteristics of oils are based on the individual carboxylic acids and their distribution. The oil can auto-oxidize and polymerize to form insoluble resinous compounds. Unsaturated fatty acids oxidize at the ethylenic bonds. The hydroperoxides tend to split producing short-chain water-soluble fragments that pass into the water for rapid degradation. Saturated fatty acids at least can often be hydrolyzed off the triacylglycerol molecule, and this gives a free carboxyl group as a point of attack.

Biodegradation is the elimination of an organic compound from an ecosystem by the metabolic activity of the biocenosis actually present in this system. Final products of biodegradation are biomass, carbon dioxide, and water. Biodegradation can be primary, environmentally acceptable and ultimate (Pitter and Chudoba, 1990). The intensity of biodegradation is influenced by several factors, such as nutrients, oxygen, pH value, composition, concentration and bioavailability of the contaminants, chemical and physical characteristics and the pollution history of the contaminated environment. Bioremediation, on the other hand, attempts to accelerate the naturally occurring biodegradation of contaminants through the optimization of limiting conditions, usually such as essential nutrients (Allard and Neilson, 1997). Seawater contains a range of microorganisms that can partially or completely degrade oil to water-soluble compounds and eventually to carbon dioxide and water (Tango and Islam, 2002). For that, the hydrocarbon degrading microorganisms, adapted to grow and thrive in these environments, play an important role in the biological treatment of polluted extreme habitats (Margesin and Schinner, 2001).

In a study of the biodegradability of several oils using the batch processing sturm test, it was observed that canola oil reached a final degradation level of 83% after 28 days in a mineral medium inoculated with an activated sludge (Sturm, 1973). This result indicates that canola oil is highly biodegradable in degradable bacteria environments. It was also
reported that the fungus *Acremonium alternata* exhibited a wide range of efficiencies in hydrolyzing various oils, with olive and groundnut oils having the maximum degradation efficiency (Kavitha *et al.*, 1997). Pereira *et al.* (1998) studied the bacterial degradation of vegetable oils in marine sediments in order to study the effects of oil spills on marine bacteria populations. They noticed that the addition of sunflower oil to sediments did not affect the numbers of heterotrophic bacteria for over a month. They suggested that the sunflower oil polymerizes in sediments making it resistant to microbial breakdown. Many other researchers studied the aerobic and anaerobic biodegradation of different natural oils in aquatic environments (Brian, 1999; Wincelle, 2001).

Our selection of oils is intended to include oils with different characteristics. Olive oil has only a low level of polyunsaturated fatty acids, and canola and mustard seed oils would be similar except the latter has a third of its mass as very long chain fatty acid with a high melting point temperature. The cod liver oil is a representative of fish oils.

### 5.3 Experimental Work

This brief study is intended to follow the degradation of selected vegetable oils in marine environments where bacterial action would probably be important. The selected oils in this study were: olive, mustard, canola and cod liver oils. The concentration of oil in each contaminated environmental regime was 0.5% (volume/volume). The environmental regimes studied were seawater with and without the addition of nutrients and seawater inoculated with wastewater. Wastewater was chosen as the inoculum because of its diverse microbial populations, high microbial activities and availability of nutrients. Seawater was taken from the North West Arm of the Atlantic Ocean in Halifax after passing through a sand filter. The wastewater was taken a few meters away from the effluent stream near Halifax harbor.

The oil-contaminated samples were put in 250-ml Erlenmeyer flasks closed with silver foil wrapped stoppers to minimize the oil loss and absorption on those stoppers during shaking. Those flasks were put on a shaker (rpm = 150) and kept in an incubator at 27 °C.
Bushnell-Haas medium (BH, DIFCO products) was used in this study as the nutrient source. The BH medium was prepared using seawater to produce the nutrient-enriched seawater samples. Marine broth and agar (DIFCO products) were used for the bacteria counting and samples dilution, after being autoclaved for 20 minutes at 121 °C and 15 psi (ZoBell, 1941).

Microbial growth and bacteria populations were monitored at different time intervals, using the plate count method. Duplicates from each oil-contaminated sample were plated on marine agar Petri dishes (ZoBell, 1941), and the colonies were counted after 24 hr of incubation at 27 °C. The flasks, marine agar media, broth, and equipment were sterilized using an autoclave at 121 °C and 15 psi for 20 minutes. Visual observations, light and environmental scanning electron microscopic analyses were also used in this study.

By the end of the experiment (15 days), the remaining oil and the associated water insoluble products were extracted from each sample. To accomplish that, each of the oil-contaminated samples was transferred to a 500 ml separation funnel. OmniSolv® (EM Science) dichloromethane (75 ml) was then added to each funnel, shaken vigorously for 30 seconds and then allowed to stand for 2 hours. The bottom phase, comprising the dichloromethane and the oil dissolved in it, was then removed and kept in a clean 250 ml Erlenmeyer flask. Ten grams of anhydrous sodium sulfate was then added to the Erlenmeyer flasks and left for 1 hour with occasional gentle agitation. After that the samples were filtered under vacuum into a 200 ml pre-weighed round bottom flasks. A rotary evaporator with aspirator vacuum was then used to evaporate the dichloromethane from the samples. The rotary evaporator was first cleaned with acetone, and nitrogen was then used to break the vacuum to reduce any oxidation. The oil residue in the round bottom flasks was treated under vacuum for 10 minutes in a sonicator bath to ensure the removal of any dichloromethane traces. The round bottom flasks were weighed and the weight difference was calculated as the amount of oil recovered from each sample. The oily residue in each flask was diluted with chloroform to a concentration of 10 mg/ml to prepare it for the Iatroscan analysis by application of 1 μl to silica gel chromarods SIII. The solvent system that was used in the Iatroscan lipid class analysis was 65% hexane, 35% ethyl
acetate, and 0.04% formic acid. The chromarods were developed for 20 minutes, and scanned as described by Ackman et al. (1990).

5.4 Results and Discussion

One of the main objectives of this study is to investigate and understand the biodegradability of selected edible natural oils in marine environments and the effect of nutrients and microbial availability on the rate and extent of degradation processes. This will help in treating spills of edible natural oils in marine environments in an efficient and effective way to protect the quality of the environment, marine life and human health.

5.4.1 Visual and Microscopic Observations

The oil-contaminated samples were visually observed and inspected with time to monitor the microbial growth, agglomeration, and any changes in oil layer, droplets shape, size, color, and presence in the sample. It was observed that in some of the oil-contaminated samples, aggregates and viscous lumps were formed. The time of insoluble particles formation, size and shape, were different from one sample to another depending on the oil type and environment. These results and observations were in agreement with the findings of Mudge et al. (1995) in their study of sunflower and linseed oils biodegradation on salt marshes. They found that sunflower oil polymerized and formed relatively hard and intractable lumps at the surface after 28 days while linseed oil did not form any polymers. The above observations were also in agreement with the findings of Pereira et al. (2002) and Li et al. (2001).

In canola oil, mustard oil, and cod liver oil-contaminated samples, lumps were observed either floating on the water surface or precipitated on the bottom surface of the flask. Figure (5-1) shows the precipitated particles and lumps formed in one of the mustard oil-contaminated seawater samples. Those lumps were smaller in size, irregular in shape, formed faster and were more numerous in the wastewater contaminated samples compared to the seawater samples (Mudge et al., 1995). On the other hand, almost no lumps or very
fine particles were observed in the seawater amended with nutrients. Mudge et al. (1992; 1993) reported that the bacteria cannot breakdown the polymer in short-term experiments, and the oil lumps formed after an oil spill are not available for bacterial degradation. In the case of olive oil-contaminated seawater samples, small dispersed oil droplets were observed floating on the water surface. Those droplets were smaller in size and fewer in numbers in both the contaminated wastewater and seawater amended with nutrients. Most of those droplets remained unchanged until the end of the experiment (day 15). Pereira et al. (1998) reported that the fates of different types of oils in the same environment are different depending on their viscosity and composition.

The environmental scanning electron microscopy (ESEM) and EDX techniques were used to study the particles and lumps formed in the oil-contaminated samples. Figure (5-2) shows the EDX spectrum of the surface of one of the particles formed in the mustard oil-contaminated seawater samples. The EDX spectrum (Figure 5-2) shows clearly the presence of sodium and calcium on the surface of the particle, which is an indication that part of those particles are in fact different fatty acid salts formed by the saponification process. In this process the triglycerides partially react with calcium or sodium hydroxide to produce glycerol and fatty acid salt, called 'soap'.

5.4.2 Bacterial Growth and Populations

The total viable bacteria populations in the oil-contaminated samples were counted at different time intervals using the plate count method. Marine agar Petri dishes and sterile seawater for the serial dilutions were used in the counting process. Duplicates from each sample were studied and the results were compared with the corresponding control samples for each environment. Figures (5-3 to 5-6) show the bacteria numbers in the different oil-contaminated samples at different time intervals. It can be observed that all samples exhibited an increase in the bacteria numbers after oil is introduced to the sample. This is expected since marine bacteria are often carbon limited and the addition of oil remedies this (Mudge et al., 1995; Mudge et al., 2001). From Figures (5-3 to 5-6) it can also be observed that the rate of increase in bacteria numbers was higher in samples amended with nutrients.
This leads to the conclusion that the availability of nutrients enhances microbial growth, which in turn, might increase the rate of biodegradation. The same observation holds true for the contaminated wastewater samples. The elevated level of broad microbial cultures and nutrient availability in the wastewater environments can possibly explain this observation. Cornish (1993) reported that the mixed microbial environments showed higher degradation rates compared to seawater only samples. It is worth mentioning here that the maximum bacteria numbers and their corresponding time were different depending on the oil type and on the environment. This might be dependent on the oil susceptibility to degradation, and on the chances of the formation of polymeric and undegradable particles (Pereira et al., 1998).

Comparing between the bacteria numbers for the different types of oil under the same environmental conditions and at the corresponding time intervals, it can be concluded that canola oil exhibited the highest degradation rate, followed by mustard oil, then cod liver oil, and finally and least, the olive oil. Many researchers (Kemp et al., 1975; Parker, 1967; Perry et al., 1979) reported that polyunsaturated fatty acids are subjected to preferential degradation relative to saturated acids under the same environmental conditions. This phenomenon explains the difference in the bacteria numbers in the different oil-contaminated samples.

The bacterial growth might be affected by the fact that, some of the oils produce antibacterial materials and products as a result of their auto-oxidation and biodegradation processes. This affects the bacterial growth rate either by killing or causing bacterial stasis. As a result of that and as can be seen from Figures (5-3 to 5-6), there were some irregular trends in the bacteria counts depending on the oil type and environment (as in the case of cod liver oil). In principle, cod liver oil should produce the highest level of antibacterial products followed by mustard oil, then canola oil, and finally olive oil (Erickson et al., 1980). This might explain the sharp decrease in the bacteria numbers in the cod liver oil-contaminated samples. The lack and unavailability of nutrients with time can be other causes that might explain the sharp reduction in the bacteria populations after reaching peak values as can be seen in Figures (5-3 to 5-6).
From the previous discussion and observations, it can be concluded that, due to the multiple factors possibly affecting the bacteria numbers, it is difficult and even misleading to reach conclusions on the rate and extent of degradation by depending only on bacteria monitoring. For all of that, it was very important to use a more precise technique to analyze the oil and its chemical evolution with time in the different contaminated environmental regimes. One of the best, cheapest and easiest methods to be used is the Iatroscan technique, which was adopted for this study to examine the different lipid classes. It has found favor among marine scientists with similar lipid problems (Parrish, 1987; Volkman, 1980).

5.4.3 Iatroscan Analysis

After the remaining oil was extracted from each contaminated sample, it was analyzed using the Iatroscan technique as described by Ackman et al. (1990). In this technology “essentially thin layer chromatography on silica gel”, the polar material is that part of the sample applied in solution that remains at the point of application on the silica gel Chromarod. It can be polymeric, or contain multiple groups of material with strong affinity for silica gel (e.g. hydroxyl groups). Since formic acid is in the developing solvent, soaps are not normally a problem. The response of the flame ionization detector (FID) is basically to the carbon atoms in the molecules, provided they are not already attached to oxygen. In this preliminary study there was no calibration or corrections for this or other factors, and the FID recorder area response is assumed to be proportional to the mass of the particular lipid class. Figures (5-7 to 5-10) show the Iatroscan lipid analysis results for each oil in the different contaminated environmental regimes. As can be seen from the figures (Figures 5-7 to 5-10), both the oil type and the environment greatly affected the final lipid class percentages in each sample.

To study the effect of environment on the oil degradation process, lipid class percentages for each oil that remained by the end of the experiment were compared for the three contaminated environments (Figures 5-7 to 5-10). From those figures, it can be concluded
that the percentages of remaining triglycerides (TG) were the highest in the oil-contaminated seawater samples, followed by the oil-contaminated wastewater samples and least in the oil-contaminated seawater enriched with nutrients samples. The percentages of free fatty acids (FFA), polar material (PM) and monoglycerides (MG) were the highest in the contaminated seawater amended with nutrients, followed by the contaminated wastewater, and least in the contaminated seawater.

To study the effect of oil type on the degradation process, lipid class percentages in each environment were compared for the four types of oil studied. From Figures (5-7 to 5-10), it was observed that the percentages of TG were the highest in the olive oil-contaminated samples, followed by mustard and cod liver and least in the canola oil-contaminated samples. The percentages of FFA and MG were the highest in the canola oil-contaminated samples, followed by cod, then mustard and least in the olive oil-contaminated samples. The percentages of PM were the highest in the cod liver oil-contaminated samples, followed by canola, then olive and least in the mustard oil-contaminated samples.

The above observations and findings can be explained by separating the effect of the environment from that of the oil constituents. In the case of seawater amended with nutrients, and wastewater that contains elevated level of broad microbial cultures and nutrients, the degradation was more pronounced and it was much easier and faster to break down the oils into simpler and smaller components. This explains the higher percentages of TG, and the lower percentages of FFA and MG in the contaminated seawater samples comparing to both the wastewater and seawater amended with nutrients contaminated samples.

The effect of oil composition can be understood by examining Table (5-1). In it, it can be seen that olive oil has the highest percentage of saturated fatty acids among the four vegetable oils and the lowest percentage of polyunsaturated fatty acids. Since the degradation of polyunsaturated fatty acids is much higher than that of any saturated fatty acid, it is to be expected that the olive oil will have the minimum degradation rate compared to the other three oils investigated in this study. On the other hand, canola oil has
the highest percentages of polyunsaturated and the lowest saturated fatty acids among all the other types of oil investigated in this study. For that, canola oil would be expected to have the highest rate of degradation. Mustard oil is very similar to canola oil, with lower percentages of the polyunsaturated fatty acids. Also, it has a third of longer chains of fatty acids, which makes it more difficult to be dissolved in water and be attacked by microorganisms. Cod liver oil has a high percentage of saturated and more highly unsaturated fatty acids, besides containing longer chains of the polyunsaturated fatty acids than canola and mustard oils. For that, cod liver oil comes next after canola and mustard oils in its susceptibility for degradation in seawater environments (Ratledge, 1994).

It is also expected that auto-oxidation took part in the oil deterioration process besides the biodegradation processes. Bucas and Saliot (2002) mentioned that vegetable and animal oils in marine environments could also be oxidized, dispersed, degraded by bacteria, or polymerized. This fact explains the results of the Iatroscan analysis, in which different concentrations of polar materials were found in many of the oil-contaminated samples. From the above results it may be concluded that in the wastewater and seawater amended with nutrients, the bacteria can break down the PM if they have adequate nutrients.

To fully understand the Iatroscan results, the lipid peroxidation process should be understood first. Lipid peroxidation can be defined as the oxidative deterioration of lipids containing any number of carbon-carbon double bonds (Ratledge, 1994). The primary peroxidation products are hydroperoxides. The initial products may be structurally rearranged or converted into secondary peroxidation products, either smaller molecules by fission or bigger by dimerization. These observations were further supported by all the Iatroscan lipid analysis results.

5.5 Conclusions

1. Marine microorganisms were able to use and degrade the four studied oils but in different rates and pathways.
2. From the bacteria counts and Iatroscan analysis results, it can be concluded that the rate of oil degradation is dependent on the oil type, and environmental regime.

3. Addition of nutrients and mixed microbial communities greatly enhanced the oil degradation process.

4. The lipid analysis at the end of the experiment indicated that both auto-oxidation and biodegradation took place in the oil-contaminated samples.

5. The rate and amount of polymeric materials and lumps formed in the oil-contaminated samples were different from one sample to another.

6. The formation of floating lumps was an advantage in gathering together the oil undegraded particles, so it can be either controlled or removed much more easily than the oil layer itself.

In general, and from all the results and observations in this study, it can be concluded that canola oil exhibited the highest rate of degradation, followed by mustard and cod liver oil, while olive oil had the lowest degradation rate.

5.6 References


Anon. 1994. Danger from Edible Oil Spills Debated Again, Lipid Technology, 6:40-44.


Table (5-1) The major constituent fatty acids (>1%) of selected natural oils, %

<table>
<thead>
<tr>
<th>Fatty acid (trivial name)</th>
<th>Olive</th>
<th>Canola</th>
<th>Mustard</th>
<th>Cod liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetradecanoic (Myristic) 14:0</td>
<td></td>
<td></td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Hexadecanoic (Palmitic) 16:0</td>
<td>12</td>
<td>4</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>Octadecanoic (Stearic) 18:0</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Total saturated</td>
<td>15</td>
<td>6</td>
<td>6</td>
<td>21</td>
</tr>
<tr>
<td>Hexadecenoic (Palmitoleic) 16:1</td>
<td>1</td>
<td></td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>Octadecenoic (Oleic) 18:1</td>
<td>74</td>
<td>58</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>Eicosenoic (Gadoleic) 20:1</td>
<td>2</td>
<td>18</td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>Docosenoic (Erucic) 22:1</td>
<td></td>
<td>33</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Total monounsaturated</td>
<td>75</td>
<td>60</td>
<td>66</td>
<td>52</td>
</tr>
<tr>
<td>Octadecadienoic (Linoleic) 18:2</td>
<td>10</td>
<td>22</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>Octadecatrienoic (Linolenic) 18:3</td>
<td>12</td>
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<td></td>
</tr>
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<td>Eicosapentaenoic 20:5</td>
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<td></td>
<td>9</td>
</tr>
<tr>
<td>Docosapentaenoic 22:5</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Docosahexaenoic 22:6</td>
<td></td>
<td></td>
<td></td>
<td>13</td>
</tr>
<tr>
<td>Total polyunsaturated</td>
<td>10</td>
<td>34</td>
<td>28</td>
<td>27</td>
</tr>
</tbody>
</table>
5.7.2 Appendix B: Figures

Figure (5-1) Precipitated particles and lumps in one of the mustard oil-contaminated seawater samples

Figure (5-2) EDX spectrum of the surface of one of the lumps formed in the mustard oil-contaminated seawater samples
Figure (5-3) Bacteria numbers in the canola oil-contaminated samples

Figure (5-4) Bacteria numbers in the mustard oil-contaminated samples
Figure (5-5) Bacteria numbers in the olive oil-contaminated samples

Figure (5-6) Bacteria numbers in the cod liver oil-contaminated samples
Figure (5-7) Lipid class percentages in the olive oil-contaminated samples

Figure (5-8) Lipid class percentages in the canola oil-contaminated samples
Figure (5-9) Lipid class percentages in the mustard oil-contaminated samples

Figure (5-10) Lipid class percentages in the cod liver oil-contaminated samples
CHAPTER 6

THE ANTIBACTERIAL EFFECTS OF SELECTED NATURAL MATERIALS AGAINST SHEWANELLA PUTREFACIENS BACTERIA

6.1 Abstract

*Shewanella putrefaciens* bacteria are facultative anaerobes implicated in the microbial influenced corrosion (MIC) in many industrial systems. These bacteria are also one of the very few isolated microorganisms able to use Fe (III) as an electron acceptor.

In this paper, the antimicrobial effects of eight natural materials (Neem, olive leaves, chamomile, *Salvia officinalis*, *Curcuma longa* (turmeric), *Acacia nilotica* (black thorn), fresh and dry *Allium sativum* (garlic), and cactus) on *S. putrefaciens* were studied. In this investigation the bacteriostatic effects of all the above-mentioned natural products were evaluated and those which showed a pronounced bacteriostatic effect were later tested to study their bactericidal effects on the *S. putrefaciens* bacteria.

From the experimental results generated in this study, it was observed that only black thorn and garlic possess bacteriostatic effects against *Shewanella putrefaciens* bacteria. The minimum inhibitory concentrations, the apparent death rate and the decimal factor values were calculated at different concentrations of black thorn and garlic using the bactericidal test method.

6.2 Introduction

*Shewanella putrefaciens* is a facultative anaerobe (Moser and Nealson, 1996) that is capable of surviving in aerobic and anaerobic environments. Also it has an outstanding ability to use a variety of organic and inorganic electron acceptors such as oxygen, ferric iron (III), manganese, nitrate, nitrite, sulfite and elemental sulfur. Due to this versatility of
electron sinks, *S. putrefaciens* may occur in many environments, including oil fields, marine water, freshwater lakes, and sediments (Ledyard and Butler, 1997).

The ability of *S. putrefaciens* to reduce Fe (III) and produce sulfides can be a cause of microbial corrosion of metals, and like many Gram-negative bacteria, it is capable of being attached to surfaces, and when grown in relatively nutrient-rich environments forms a thick biofilm. Biofilm investigation experiments on steel surfaces have shown that this bacteria, like many others, produce a fibrous net of exopolysaccharides in which the bacteria proliferate.

*S. putrefaciens* is a powerful agent of MIC, and more detrimental than the sulfate reducing bacteria (SRB). That was concluded from an interesting study in Canada conducted by the water resource center (Brozel et al., 1997) on the role-played by *Shewanella* and *Sulfide-producing* bacteria in metallic corrosion in industrial water systems (specifically cooling systems). They reported that *S. putrefaciens* reduced sulfite at dissolved oxygen concentration levels less than 1.5 mg/l. This may explain why hydrogen sulfide is often detected in highly aerated industrial cooling-water systems, even where biofilms growth is kept to a minimum and biofilms are consequently thin, with few anaerobic zones.

A simulated model of a cooling-water system inoculated with *S. putrefaciens*, *Desulfovibrio vulgaris* (SRB) and *Pseudomonas aeruginosa*, yielded biofilms in which the SRB did not survive well. It was found that *S. putrefaciens* dominated the biofilms together with the aerobic *P. Aeruginosa*, showing that *S. putrefaciens* grow better in biofilms and sulfidogenic/aerated environments than the SRB. It was also shown that *S. putrefaciens* possesses the metabolic capabilities required to induce MIC, accelerates the corrosion rate of mild steel and outperforms the SRB (Brozel et al., 1997).

Most of the natural products used in this study were chosen depending on their known medical uses. The antimicrobial effects of two of them (garlic and black thorn) are briefly mentioned in the next paragraphs.
Black thorn is the fruit of the *acacia nilotica* tree, which is widely distributed in tropical and subtropical Africa from Egypt and Mauritania to South Africa. In Africa and the Indian subcontinent, *acacia nilotica* is extensively used as a browse, timber and fire-wood species. The bark and seeds of *acacia nilotica* are used as a source of tannin. The species are also used for medical purposes. The bark of *acacia nilotica* has been used for treating hemorrhages, colds, diarrhea, tuberculosis and leprosy, while the roots have been used as an aphrodisiac and the flowers for treating syphilis lesions. The aqueous extract of the black thorn is rich in tannin (18-23%), which has the empirical formula $C_{78}H_{32}O_{16}$ (Carter, 1994).

Garlic has many properties and the most important among them is its antimicrobial effect. Garlic is an antibiotic, antifungal, and antiviral. It inhibits most of the common infection agents. It was believed that the main active component of garlic is allicin. However, other sulfurous chemicals, similar to allicin, are now credited with the beneficial effects. The crude juices and the powder form of garlic have antimicrobial effects against many Gram positive and Gram negative organisms, including *Escherichia coli*, *Pseudomonas pyocyaneus*, *Streptococcus viridans*, *S. haemolyticus*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Providencia* species, *Citrobacter* species, *Hafnia* species, and *Aeromonas* species. Many researches proved that allicin can block two groups of enzymes, cysteine proteinases and alcohol dehydrogenases, by reacting with one of their important components known as sulfhydryl (SH) groups, or thiols (Snyder, 1997; Yoshiok, 1920; Koch and Lawssor, 1996).

In this study, the antimicrobial effects of eight natural products (Neem, olive leaves, chamomile, *Salvia officinalis*, *Curcuma longa* (turmeric), *Acacia nilotica* (black thorn), fresh and older-less *Allium sativum* (garlic), and cactus) on *S. putrefaciens* were studied. In this investigation the bacteriostatic effect of all the above-mentioned natural products were conducted and those which showed a pronounced bacteriostatic effect were later tested to study their bactericidal effect (Videla, 1996).
The bacteriostatic test (Benson, 1998) for each natural product was conducted using the hot water and organic-solvent extracts and the dry mash of each product. Both the Ditch plate and the Kirby-Bauer techniques were used in this test. The Ditch plate technique was used for testing the products in their solid formulation, and the Kirby-Bauer technique for the products in their liquid formulation.

6.3 Materials and Methods

6.3.1 Test Organism and Preparation of Media

*Shewanella putrefaciens* obtained form ATCC (cat. # 49138) was cultured in tryptic soy broth. The medium consisted of 17.0 g peptone from casein, 3.0 g peptone from soy meal, 5.0 g sodium chloride, and 2.5 g di-potassium hydrogen phosphate. The medium was prepared by dissolving the previous mentioned 27.5 g in 1 L of demineralized water. The pH of the solution was adjusted to 7.3 and autoclaved for 15 min. After that the culture was incubated at 27 °C for 24 hrs. The tryptic soy agar which was used in the plating count method consisted of 15.0 g peptone from casein, 5.0 g of peptone from soymeal, 5.0 g sodium chloride, and 15.0 g agar. The agar medium was prepared by suspending 40 g of the agar in 1 L of demineralized water and then the mixture was autoclaved for 15 minutes at 121 °C.

6.3.2 Hot Water and Organic Solvent Extracts Preparation

For the hot-water extraction process, 40.0 g of each material was added to 100 ml sterilized distilled water. The mixture was first heated for 15 minutes in a water bath at 45 °C and then it was soaked for 30 minutes and finally filtered using a Hirsch funnel.

For the organic solvent extraction, a 50:50 percent ethanol-water mixture was prepared. After that, 40.0 g of each material was soaked for 15 minutes in 100 ml of this mixture. Using a Hirsch funnel the soaked material was filtered, then the filtrate was heated for 30
minutes at 80°C to evaporate the organic solvent (since it has a lower boiling point than the extract).

6.3.3 Evaluation of the Bacteriostatic Effects

The ditch plate technique was used to evaluate the bacteriostatic effects for all the natural materials used in this study. The bacteriostatic test is a method used to determine the ability of a given material to inhibit the growth of bacteria. From the S. putrefaciens culture medium, 50 µl was streaked using a sterilized glass rod onto the Petri dishes contain tryptic soy agar media. The plates were cross streaked in at least three different directions to cover most of the agar surface area. A 6 mm-wide ditch was cut in the agar plate and in it 0.3 g from each solid material (after being dried in an oven at 45 °C for 24 hr) were placed. The only exception was with Neem oil which was added directly to the ditch. The plates were then incubated at 27 °C for 24 hr. After that, the extent of inhibition was measured and recorded.

The Kirby-Bauer technique (which is also known as the agar diffusion test) was used to find the minimum inhibitory concentrations for the both black thorn and garlic, which are the natural materials that showed positive results in the bacteriostatic test. The minimum inhibitory concentration of a given material is the lowest concentration preventing visible bacterial growth. In this test four dilutions of black thorn and garlic (0.15, 0.015, 0.005, and 0.002 g/ml) were prepared from the extracts. Circular filter papers with a diameter of 6 mm were used in this test after being autoclaved for 15 minutes. Using forceps, the filter paper-disks were dipped into the prepared extracts. After that, each saturated paper-disk was placed onto the surface of the tryptic soy agar plates which were previously streaked with the S. Putrefaciens bacteria.

Particular care was taken to prevent any movement of the paper disk on the surface of the agar medium. The treated plates were kept inverted in a 27 °C incubator. After 24 hr of incubation, the diameter of the inhibited zone surrounding each paper-disk was measured with a metric ruler. Six millimeters (which is the diameter of the paper-disk) was
subtracted from the diameter of the inhibited zone to get the exact diameter of the real inhibited area. These measurements were then compared with the standard Kirby-Bauer table to determine the significance of the inhibited zone diameter. These comparisons helped in determining whether the particular strain of *S. putrefaciens* were resistant, intermediate, or sensitive to each natural material.

6.3.4 Evaluation of the Bactericidal Effects

The bactericidal test, which is also known as the time-kill test, is the technique that involves the contact of a microbial population with an antimicrobial agent for specified or varying periods of time after which the surviving organisms are recovered and counted.

In this test, three concentrations from the hot-water extract of both black thorn and garlic were prepared and used to evaluate their bactericidal effects against the *S. Putrefaciens* bacteria. After that, 0.1 ml from the *S. putrefaciens* culture medium, which approximately contains $2\times10^9$ cfu/ml, was added to each sample. After that, at each of the time intervals: 0, 3, 5, 10, 20, 30, 40, 60, 90 and 120 minutes, a 200 µl of the extract solution that contains the bacteria was withdrawn and then diluted with the tryptic soy broth. After that, 50µl from the diluted samples was plated on the soy agar and the plates were incubated at 27 °C for 24 hrs.

6.4 Results and Discussion

6.4.1 Evaluation of the Bacteriostatic Effects

Using the ditch plate technique for testing the natural products in their solid phase, and the Kirby-Bauer technique for the hot water and solvent extracts, only black thorn and garlic showed bacteriostatic and bactericidal effects against the *S. putrefaciens* bacteria. Figure (6-1) shows the clear zones in the Petri dishes around the black thorn and garlic respectively, and compare them with the control samples. It was observed that, the black thorn colored the agar with a dark color and that was related to the tannin compound, which
is one of the black thorn main components. It is believed that, tannin is the responsible compound for the bacteriostatic effects of black thorn.

Figure (6-2) shows the clear zones around the ditches that are filled with either fresh or dry (odorless) garlic. It was observed that, the clear zone area around the ditch was much higher in the case of fresh garlic compared to that for the dry (odorless) garlic. In the case of garlic, it is believed that allicin is the component that has the bacteriostatic effects against the *S. putrefaciens* bacteria. This fact is in agreement with the above findings since fresh garlic contains much higher amounts of allicin that dry (odorless) garlic.

The bacteriostatic effects of the hot water and solvent extracts of garlic against *S. putrefaciens* were evaluated using the Kirby-Bauer technique, as can be seen from Figure (6-3). The diameters of the inhibited zones around the soaked filter paper-disks with different concentrations of black thorn and garlic extracts (Figure 6-3) were measured, analyzed and compared with the standard diameters of zones of inhibition shown in Table (6-1). The degree of *S. putrefaciens* bacteria response to the different concentrations of both black thorn and garlic are shown in Table (6-2). The minimum inhibitory concentrations for both black thorn and garlic were estimated using the Kirby-Bauer technique. It is worth mentioning here that, the natural materials organic solvent extracts gave the same results as their hot water extracts.

6.4.2 Evaluation of the Bactericidal Effects

The bactericidal effects of different concentrations of black thorn and garlic against *S. putrefaciens* were studied and shown in Figures (6-4 and 6-5), respectively. In these figures the readings on the \(X/X_0\) ordinate (the survival ratio of bacteria) were the average of triplicate samples. Figures (6-4 and 6-5) show a reduction in the survival ratio of the bacteria with time for all studied concentrations of black thorn and garlic. Figures (6-4) and (6-5) also show that an increase in concentrations of black thorn and garlic enhances their bactericidal effects against the *S. putrefaciens* bacteria.
The bacterial death rate can be best described with first-order reaction kinetics (Pedilla et al., 1998; Sawi et al., 2000; Ruiz-Ordoz, 1998; Lambart et al., 1999).

The experimental data shown in Figures (6-4 and 6-5) were in good agreement with the first order decay assumption. It is known that, for the first order decay, the change in the viable bacterial population level \( (X) \) with time can be represented as shown in Equation (6-1):

\[
\frac{dX}{dt} = -K_d \cdot X
\]  

(6-1)

where:

\( X \) = number of viable cells  
\( K_d \) = death rate constant  
\( t \) = time

From Equation (6-1), it is clear that, \( K_d \) can be found by simply integrating Equation (6-1) to obtain:

\[
X(t) = X_0 \cdot e^{-K_d t}
\]  

(6-2)

where:

\( X_0 \) = the initial concentration of the bacterial cells at \( t = 0 \)

From Equation (6-2), it is clear that, the slopes of the semi-log plots shown in Figures (6-4 and 6-5) are in fact the apparent first-order decay death rate constant values \( (K_d) \). For that, the \( K_d \) values for the different concentrations of black thorn and garlic were calculated using Figures (6-4 and 6-5) and then tabulated in Tables (6-3 and 6-4), respectively. From
these tables it can be easily observed that, increasing the concentration of both black thorn and garlic increases the value of $K_d$.

On the other hand, tables (6-3 and 6-4) show that, the $K_d$ values for black thorn are higher than those for garlic at the same concentrations. This fact implies that, the black thorn has stronger bactericidal effects against the *S. putrefaciens* bacteria than garlic.

The decimal reduction factor ($D_r$) which is equal to $2.303/K_d$ and can be defined as the time needed to reduce the viable population of bacteria by a factor of 10, was also calculated for each black thorn and garlic concentration and the values were tabulated in tables (6-3 and 6-4). From these tables it can be observed that the decimal factor for both black thorn and garlic decreased with increasing their concentrations.

### 6.5 Conclusions

1. The results of both the ditch plate and Kirby-Bauer techniques showed that, only black thorn and garlic possess bacteriostatic effects against *S. Putrefaciens* bacteria and for that the time-kill test was used to study their bactericidal effects against *S. Putrefaciens*.

2. The Minimum inhibitory concentrations of black thorn and garlic were estimated using the Kirby-Bauer technique and it was found that the black thorn has a lower minimum inhibitory concentration compared to that for garlic.

3. The apparent death rate ($K_d$) and the decimal factor ($D_r$) values for different concentrations of black thorn and garlic were calculated using the bactericidal test method and it was found that the $K_d$ values increase with increasing both the black thorn and garlic concentrations.

4. It was observed that, at the same concentration of additives, the values of $K_d$ for black thorn were higher than the corresponding values for garlic, implying that the black thorn has a stronger bactericidal effect against *S. Putrefaciens* bacteria than garlic.
5. In general, it was concluded that both the black thorn and garlic are effective to be used as bactericides against the *S. Putrefaciens* bacteria.

### 6.6 References


### 6.7 Appendices

#### 6.7.1 Appendix A: Tables

**Table (6-1) Standard interpretation of the inhibited zones of specific test cultures (Madigan, 2000)**

<table>
<thead>
<tr>
<th>Diameter of the inhibited zone (mm)</th>
<th>Bacteria response</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 or less</td>
<td>Resistant</td>
</tr>
<tr>
<td>11-15</td>
<td>Intermediate</td>
</tr>
<tr>
<td>16 or more</td>
<td>Sensitive</td>
</tr>
</tbody>
</table>

**Table (6-2) The response of *S. putrefaciens* bacteria to the different concentrations of black thorn and garlic**

<table>
<thead>
<tr>
<th>Black thorn conc. (g/ml)</th>
<th>Garlic conc. (g/ml)</th>
<th>Bacteria response</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.002</td>
<td>0.002</td>
<td>Resistant</td>
</tr>
<tr>
<td>0.005</td>
<td>0.005</td>
<td>Intermediate</td>
</tr>
<tr>
<td>0.015</td>
<td>0.015</td>
<td>Intermediate</td>
</tr>
<tr>
<td>0.150</td>
<td>0.150</td>
<td>Sensitive</td>
</tr>
</tbody>
</table>
Table (6-3) The death rate constant \((K_d)\) and the decimal reduction factor \((D_r)\) for black thorn

<table>
<thead>
<tr>
<th>Concentration (g/ml)</th>
<th>(K_d) ((s^{-1}))</th>
<th>(D_r) ((s))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.005</td>
<td>0.006</td>
<td>397</td>
</tr>
<tr>
<td>0.015</td>
<td>0.021</td>
<td>110</td>
</tr>
<tr>
<td>0.150</td>
<td>0.159</td>
<td>14</td>
</tr>
</tbody>
</table>

Table (6-4) The death rate constant \((K_d)\) and the decimal reduction factor \((D_r)\) for garlic

<table>
<thead>
<tr>
<th>Concentration (g/ml)</th>
<th>(K_d) ((s^{-1}))</th>
<th>(D_r) ((s))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.005</td>
<td>0.004</td>
<td>562</td>
</tr>
<tr>
<td>0.015</td>
<td>0.008</td>
<td>277</td>
</tr>
<tr>
<td>0.64(0.15)</td>
<td>0.085</td>
<td>27</td>
</tr>
</tbody>
</table>
Figure (6-1) Digital photographs show the bacteriostatic effects of (a) black thorn (b) garlic, against *S. putrefaciens* bacteria, using the ditch plate technique.
Figure (6-2) Comparison between the bacteriostatic effects of (a) odorless (dry) garlic and (b) fresh garlic against *S. putrefaciens* bacteria.
Figure (6-3) The bacteriostatic effects of garlic against *S. putrefaciens* using Kirby-Bauer technique
Figure (6-4) The bactericidal effects of different concentrations of black thorn against the *S. putrefaciens* bacteria
Figure (6-5) The bactericidal effects of different concentrations of garlic against the *S. putrefaciens* bacteria
CHAPTER 7

EFFECTS OF SURFACE TOPOGRAPHY AND NATURAL BIOCIDES ON BIOFILMS GROWTH AND BIOCORROSION RATES ON MILD STEEL SURFACES

7.1 Abstract

Corrosion causes huge economic damage worldwide with an annual direct cost estimated to be in the hundreds of billions of dollars. Biocorrosion can be defined as the destruction or deterioration of materials by natural processes directly or indirectly related to the activity and growth of microorganisms. Biocorrosion is a major hazard during the production of oil and gas, causing considerable damage to both onshore and offshore production facilities and pipelines. Sulfate Reducing Bacteria (SRB) are the single most common causative organisms in biocorrosion. Pitting biocorrosion is usually a characteristic of the action of the SRB on metals. For that, industries spend billions of dollars annually on biocidal chemicals to mitigate biocorrosion.

In this study, both the influence of the surface topography (geometry) of mild steel coupons, and the addition of selected natural materials derived from garlic (*Allium sativum*) and black thorn (*Acacia nilotica*) on biofilms growth and biocorrosion rates, were investigated on mild steel coupons immersed in SRB media.

To accomplish this, a set of experiments was conducted on mild steel coupons with different surface topographies, immersed in the SRB media for three months, with and without the addition of natural materials. This set of experiments conducted was also used to study the effects of the addition of the selected natural materials to the SRB media, on both biofilms growth and biocorrosion rates of the immersed mild steel coupons. At the end of the experiment, the coupons were removed from the SRB media and their surfaces were investigated using different techniques.
Both the biofilms growth and the biocorrosion rates on the immersed mild steel coupon surfaces were studied using both visual observations and microscopic methods including the environmental scanning electron microscopy (ESEM), the energy dispersive X-ray analyzer (EDX) and the computer image analyzer techniques. The observations and results from the different samples were compared with each other, and with the corresponding control samples.

It was observed that, lower biofilms growth rate, and lower biocorrosion rates prevail on the smoother mild metal surfaces compared to the rougher ones under the same test conditions. It was also clear that the addition of the natural materials tested to the SRB media inhibited both the biofilms growth, and the associated biocorrosion rates on the surfaces of the immersed coupons. It can thus be concluded that both the surface topography and the addition of the selected natural materials are very important factors in determining both the biofilms growth and the biocorrosion rates of mild steel surfaces.

7.2 Introduction

Corrosion can be defined as the destruction or deterioration of a material because of its reaction with the environment, and it causes huge economic and ecological damage worldwide. A two-year breakthrough study estimated the annual direct cost of corrosion in the United States to be $276 billion. This represents 3.1% of the U.S. Gross Domestic Product (GDP). It was also estimated in this study that 25-30% of the total annual direct corrosion cost could be saved by using state of the art corrosion management practices (Koch et al., 2002).

Biocorrosion refers to corrosion that is influenced by the presence and/or activities of microorganisms, and it is extremely harmful to both the industry and the environment (Jawaherdashti, 1999). One of the most important and dangerous types of biocorrosion is that due to the presence of Sulfate Reducing Bacteria (SRB), which is most common in petroleum operations, because of the prevailing anaerobic environment (Phelps
et al., 1991). The sulfate reducers are the single most common causative organisms in biocorrosion, which may in turn constitute 50% of all instances of corrosion (Booth, 1964).

Protection of structures against biocorrosion has become very critical in many industrial systems including pipelines, marine structures, storage vessels, sewage treatment plants, and the oil and gas facilities (Geesey et al., 1994). The oil and gas industries estimate that 30-90% of their serious "pitting-type" corrosion is biocorrosion. In the United States, industries spend $1.2 billion annually on biocidal chemicals to mitigate biocorrosion. The extent of biocorrosion and the cost associated with it are only recently being recognized (Little and Wagner, 1994).

Biological organisms can enhance the corrosion process by their physical presence, metabolic activities and direct involvement in the corrosion reactions (Hamilton, 1985). The occurrence of biocorrosion is often characterized by unexpected severe metal attack, the presence of excessive deposits, and in many cases the rotten-egg odor of hydrogen sulfide (Lee et al., 1995). The most important and dangerous biocorrosion is that due to sulfate reducing bacteria (SRB), which thrive under anaerobic conditions. SRB possessing the enzyme hydrogenase and can obtain their energy from the oxidation of molecular hydrogen. They are also capable of growing over a wide range of pH (4-8) and at temperatures from 10-40°C, although some thermophilic strains can grow in the temperature range 45-90°C and a pressure up to 500 atm (Herbert and Stott, 1983).

It has been agreed that the overall reaction of the anaerobic corrosion of iron induced by SRB can be described by (Pankhania et al., 1986):

\[ 4\text{Fe} + \text{SO}_4^{2-} + 4\text{H}_2\text{O} \rightarrow \text{FeS} + 3\text{Fe(OH)}_2 + 2\text{OH}^- \]  

\[(7-1)\]

Pankhania et al. (1986) proposed that hydrogen sulfide (H₂S) acts as the cathodic reaction, and showed that the sulfate reduction can occur with the cathodically formed
hydrogen. On the other hand, Costello (1974) proposed that hydrogen sulfide (H₂S) rather than the hydrogen ion, would act as cathodic reactant, i.e.:

\[ 2\text{H}_2\text{S} + 2e^- \rightarrow 2\text{HS}^- + \text{H}_2 \]  

(7-2)

According to Costello (1974), the bacterial hydrogenase system may play a secondary role by removing molecular hydrogen (H₂), favoring the production of the hydrogen sulfide (HS⁻). Rainha and Fonseca (1997) studied the influence of the SRB *Desulfovibrio desulfuricans* ATCC 27774 grown in a lactate/sulfate medium, on the anaerobic corrosion of mild steel. They observed higher corrosion rates in the presence of bacterial culture. Moreno *et al.* (1992) studied the pitting of stainless steel by SRB and found that the biogenic sulfides enhanced the passivity breakdown in the presence of chloride anions.

The development of new and appropriate methods for mitigation and prevention of biocorrosion in the industry has been the ultimate goal of biocorrosion researchers since its inception (Jones-Meehan *et al.*, 1992; Jack *et al.*, 1996). The uncontrolled growth and activity of these microorganisms in an oil field can create environmental and operational problems. Mitigation of biocorrosion must be done only after careful monitoring and consideration of the situation, otherwise considerable time and money can be spent performing unnecessary treatment. Currently, the predominant way of biocorrosion mitigation consists of massive use of biocides, in amounts that are most of the time oversized. This includes significant over costs and may lead to severe pollution.

Many researchers have used different natural materials for corrosion inhibition purposes (El-Etre, 1998; El-Etre and Abdallah, 2000). In a study conducted by Mansour *et al.* (2003), green algae were tested as a natural additive for a paint formulation, based on vinyl chloride copolymer (VYHH), to evaluate its efficiency for protection of steel against corrosion in seawater. Both suspended and extracted forms of algae were used to achieve optimum performance of the algae-contained coatings. Poorest performance was obtained when algae were added in suspended form, whereas the extracted form
exhibited better performance based on impedance measurements. Saeed et al. (2003) investigated the antimicrobial effects of garlic and black thorn against *Shewanella putrefaciens*, which is a bacterium implicated in pipeline corrosion. They concluded that both garlic and black thorn possess bacteriostatic effects against *Shewanella putrefaciens* and that therefore they can be used as bactericides to inhibit and prevent biocorrosion in environments containing *Shewanella putrefaciens* bacteria.

Medilanski et al. (2002) studied the influence of surface topography of stainless steel on bacterial adhesion. In their investigation, five types of steel surface finish corresponding to roughness values between 0.03 and 0.89 μm were produced and studied. They found that, rougher surfaces exhibiting wider scratches allowed a higher fraction of bacteria to adhere in other orientations, whereas the orientation of cells adhered to the smoothest surface was completely random.

### 7.3 Materials, Methods, and Experimental Work

1. Mild steel coupons (4x3x0.2 cm) were polished to a surface finish of 120 and 600 grits using silicon carbide (SiC) abrasive papers. In this study, the coarse surfaces (120 grits) were referred to as rough surfaces, while the fine surfaces (600 grits) were referred to as smooth surfaces. The mild steel coupons went through a surface preparation process according to the ASTM standard G1-90 (ASTM, 1999) before being immersed in the SRB media.

2. A lactate/sulfate Postgate liquid medium (Postgate, 1984) was inoculated with the SRB *Desulfovibrio desulfuricans* culture, and was used as the corrosive environment in this study.

3. The selected natural materials, derived from garlic (*Allium sativum*) and black thorn (*Acacia nilotica*) were added to some of the SRB culture media, in the amount of 1 g/100 ml.
4. After that, the mild steel coupons were immersed in the containers containing the SRB culture media following the ASTM standard G 31-72 (ASTM, 1999). All sealed containers were then incubated at 30°C for three months under anaerobic conditions.

5. At the end of the experiment (after 3 months), the mild steel coupons were removed from the SRB media and then investigated using both visual observations, and microscopic analysis, including ESEM, EDX and the computer image analyzer techniques.

6. Both the biofilm and the biocorrosion types and products on the mild steel coupon surfaces were studied. The observations and results from the different coupon surfaces were then compared with each other, and with the corresponding control samples.

7.4 Results and Discussion

When the mild steel coupons were removed from the SRB media after being immersed for three months; biofilms and biocorrosion products, were observed attached to the coupon surfaces with a reddish brown color with many black spots (Figures 7-1 to 7-6). A black layer adherent to the metal surface was also observed underneath the outer loose reddish brown thick layer of the corrosion products. The biofilms density, thickness, and adherence to the mild steel coupon surfaces as well as the biocorrosion forms and products, were found to be different from one sample to another, depending on the environment, metal surface, and the interaction between them.

In the case of the SRB media containing natural materials, it was observed that the surfaces of the immersed mild steel coupons were covered with thin and loose biofilms and corrosion products, as can be seen in Figures (7-1 to 7-4). It was also observed that the thickness, density, and the attachment of those biofilms and corrosion products were bigger and higher on the surfaces of the corresponding coupons in the SRB media without the presence of any natural materials (Figures 7-5 and 7-6) (Sunny-Cheung and Beech, 1996). That was attributed to the antibacterial effects of both natural materials
added to the SRB media, which inhibited both the biofilms growth and the biocorrosion rates and extents (Saeed et al., 2003).

It is worth mentioning here that, under the same test conditions, and in the SRB media containing natural materials, the effects of black thorn were more pronounced than those of garlic, in inhibiting both the biofilms growth and the associated biocorrosion on the mild steel surfaces having the same surface finish (Figures 7-1 to 7-4). This was related to the fact that, tannin, which is the active antimicrobial component in the black thorn, was more effective against the SRB growth than allicin, which is the active antimicrobial component in garlic (Saeed et al., 2003).

Under the same test conditions, and by comparing the photographs shown in Figures (7-1 to 7-6, it was clear that both the biofilms growth, and the biocorrosion rates and extents were higher on the rough coupon surfaces compared to the smooth ones. Those findings are in agreement with the fact that rough surfaces usually attract the bacteria to be attached to them to form their biofilms, which in turn initiates and/or accelerates the biocorrosion attack (Medilanski et al., 2002). Also, it was proven that biofilms contain many hundred times the organisms present in the liquid phase, and the biocides are very much less effective against sessile organisms within biofilms than against planktonic populations (Costerton and Lashen, 1984; Little and Ray, 2002). Since, in this study, the adherence, thickness and density of the biofilms on the rough coupon surfaces were found to be higher than those on the smooth coupon surfaces, the added natural materials to the SRB media were less effective in inhibiting both the bacterial growth, and the biocorrosion effects on the rough surfaces of the mild steel coupons compared to their effects on the smooth ones.

Within each biofilm, the local physical and chemical conditions create an environment that helps the microorganisms to attack the substratum (Beech et al., 1994). SRB take the form of biofilms and allow for the creation of anaerobic microenvironments within a bulk aerobic environment (Lee et al., 1995). The SRB existing in the biofilms convert sulfates
in the sample into hydrogen sulfide (Videla and Characklis, 1992). These facts were proven in this study using the ESEM/EDX techniques.

The hydrogen sulfide ($\text{H}_2\text{S}$) and carbon dioxide ($\text{CO}_2$) react with water to produce a mild acidic condition that affects the metal surfaces. This process also lowers the pH of the substrate surfaces to levels favorable for the growth of bacteria that at the end creates a very acidic environment, thereby encouraging rapid corrosion. This explains the severity of the localized attack underneath the spotty areas, where heavy and dense colonization were detected as can be seen in Figure (7-7).

The reason behind the predominance of localized corrosion on the coupon surface can be explained by the fact that most of the organisms do not form a continuous and uniform film on the metal surface (Lee et al., 1995; Wagner et al., 1996). Microscopic organisms tend to settle on metal surfaces in the form of discrete colonies or at least spotty, rather than continuous films, and this explains the localized biocorrosion on the mild steel coupon surfaces. Pitting corrosion is a characteristic of the action of the sulfate reducers on steels (Angeles-Chavez et al., 2001), with the pits being open and filled with soft black corrosion products in the form of iron sulfides (Hamilton, 1985), as can be seen in Figures (7-8 and 7-9).

ESEM/EDX techniques were used to analyze the biofilms and corrosion products on the mild steel coupon surfaces. Figure (7-10) shows an ESEM photomicrograph that shows the biofilm and the corrosion products on the surface of one of the mild steel coupons after being immersed in the SRB media for 3 months, while Figure (7-9) shows the EDX elemental analysis of the biofilm and the corrosion products on the surface of the above mentioned coupon.

In conclusion, it was obvious that the biofilms, corrosion products, and the severity of the localized damage on the coupon surfaces were the highest on the rough mild steel surfaces, followed by smooth mild steel surfaces both immersed in the SRB media
without the presence of natural materials. After that, comes the rough then the smooth mild steel surfaces immersed in the SRB media containing natural materials.

7.5 Conclusions

1. Both the rotten-egg odor and the black ferrous sulfide detected on the mild steel coupon surfaces, confirmed the activity of the SRB and the associated biocorrosion attack.

2. Pitting biocorrosion was heavily detected on the mild steel coupon surfaces immersed in the SRB media, which is “usually” a characteristic of the action of the SRB on metals.

3. The presence of SRB has reduced sulfate to sulfide, which in turn reacted with either iron and produced the black ferrous sulfide, or hydrogen and produced hydrogen sulfide.

4. The SRB biofilms were found to be thicker and more adherent to the mild steel coupon surfaces in the SRB media containing no natural materials, compared to the ones containing natural materials.

5. The severity of corrosion on the surfaces of the coupons immersed in the natural materials containing SRB media were found to be much lower than the corresponding coupon surfaces in the SRB media with no natural materials.

6. The biofilms growth and attachment to the rough coupon surfaces were found to be higher than those at the smooth coupon surfaces, under the same test conditions.

7. The biocorrosion rates and extents on the rough coupon surfaces were found to be higher than those at the smooth coupon surfaces, under the same test conditions.

8. The efficiency of black thorn was more pronounced than that of garlic in inhibiting both the biofilms growth and the associated biocorrosion on mild steel surfaces having the same surface finish, under the same test conditions.

9. It was concluded that both the coupon surface topography and the presence of selected natural materials are very effective and important in determining the rates and the mechanisms of biofilms growth and biocorrosion on mild steel surfaces.
7.6 References


7.7 Appendices

7.7.1 Appendix A: Figures

Figure (7-1) Photograph of the surface of a rough mild steel coupon after being immersed for 3 months in the SRB media containing garlic
Figure (7-2) Photograph of the surface of a rough mild steel coupon after being immersed for 3 months in the SRB media containing black thorn.
Figure (7-3) Photograph of the surface of a smooth mild steel coupon after being immersed for 3 months in the SRB media containing garlic
Figure (7-4) Photograph of the surface of a smooth mild steel coupon after being immersed for 3 months in the SRB media containing black thorn
Figure (7-5) Photograph of the surface of a rough mild steel coupon after being immersed for 3 months in the SRB media without the addition of any natural materials.
Figure (7-6) Photograph of the surface of a smooth mild steel coupon after being immersed for 3 months in the SRB media without the addition of any natural materials
Figure (7-7) Photograph shows the severity of the localized corrosion attack underneath heavy and dense biofilms and corrosion products on the surface of mild steel coupon after being immersed for 3 months in the SRB media.
Figure (7-8) Photograph shows the severe pitting corrosion (open pits filled with soft black corrosion products) detected on the surface of a mild steel coupon after being immersed for 3 months in the SRB media.
Figure (7-9) EDX surface analysis (spectra) of the biofilm and the corrosion products that filled the pits detected on the surface of a rough mild steel coupon after being immersed for 3 months in the SRB media.
Figure (7-10) ESEM photomicrograph shows the layers of the biofilm and the corrosion products on the surface of a mild steel coupon after being immersed for 3 months in the SRB media.
CHAPTER 8

A NOVEL METHOD TO REDUCE SHRINKAGE CRACKS IN CEMENT MORTARS USING NATURAL HAIR-FIBERS

8.1 Abstract

In many industrial processes the pipeline systems are lined with a protective layer of cement mortar. Cement slurry is also placed in wellbores to harden into an impermeable mass that seals the annulus from fluid flow and protects the casing from corrosion for the life time of the well. When uniform linings of neat cement fail in tension, small and large cracks are formed, which causes the pressurizing fluid or mud to easily flow through them towards the metal substrate. The necessity to study and understand the parameters and conditions affecting the formation and intensity of plastic shrinkage cracks in cement mortar has called for further and advanced studies in this area.

In this investigation, human hair, which is a waste material, was used as a new natural fiber to reinforce cement mortar and improve its impermeability. The study reported herein focuses on the effects of human hair-fibers on the reduction of shrinkage cracks in cement mortars. The influence of the cement mortar mix proportions on the plastic shrinkage of the hair-fiber reinforced cement mortar has also been studied. The approach selected in this study was based on the factorial design of experiments, in which the considered parameters for investigation were: cement/sand ratio (c/s), water/cement ratio (w/s) and hair-fibers volume fraction percentage (f_h).

The results showed that, the human hair-fibers were very effective in reducing the plastic shrinkage cracks of cement mortar by a remarkable amount of 92%.
8.2 Introduction

Cement mortar lining is a process by which metal pipelines are coated internally to protect their structures. There are several advantages to the cement mortar lining of pipes compared to other pipeline protection methods. Some of these advantages are: preventing pipe leakage, protecting the inner surface of the pipe against corrosion by forming an alkaline environment in contact with the pipe material where corrosion of the steel is inhibited, decreasing the pumping energy consumption by providing a smooth interior surface with a high flow coefficient, and reducing pipe maintenance.

The objective of cementing the annulus, which is present between the casing and the formation, is to provide zonal isolation of the formations that have been penetrated by the wellbore. Moreover, cementing and lining the annulus protect the casing from corrosion for the life of the well. No fluid communication should develop during the life of the well among these various formations (whether they are saturated with water, oil, or gas) and the surface (Thiercelin et al., 1998; Nowak and Patout, 1997). When uniform linings of neat cement fail in tension, one or more large cracks are formed and the pressurizing fluid or mud flows easily through them. When the cement mortar containing fibers fails in tension, it usually forms many small cracks. The cement matrix fails first by forming microcracks, and then the fibers take over the loading. The fiber-laced cracks give a high resistance to fluid leak off. When fiber cement samples are subjected to high impact loads, the cement matrix shatters but the fibers hold the broken matrix together (Stewart et al., 1997).

In addition to that, fibers also reduce the plastic shrinkage of cement mortars. In the setting process, cement slurries shrink and that causes the cement hydrostatic pressure to drop. The hydrostatic pressure is important, as gas starts to flow into the cement when the pressure of the cement column falls below that of a gas bearing formation. After the gas has entered the pore system of the cement, the gas inside may overcome the tensile strength of the cement structure, break the cement matrix, and migrate through the microfractures. A low shrinkage rate is preferable because the decline in the resulting hydrostatic pressure will be slower than that for slurry with a higher shrinkage rate. Slow shrinkage has two advantages: (a) the pressure equilibrium between formation and slurry columns can be
reached and (b) the driving force behind the flow of pore fluid into the cement will be lower. Both factors should reduce the risk of early gas migration (Backe et al., 2001; Backe et al., 1999; Sabins and Wiggins, 1997).

The cement sheath integrity is important for safe and economical operation of gas storage, geothermal and producing wells. Loss of cement integrity can cause the following serious events: loss of gas reserves, unsafe operations, premature water of gas cap production, extra costs because of unplanned remedial operations, and well shutdown (Karen, 2002).

Plastic shrinkage cracks are random cracks that occur in the exposed surface of fresh mortar during the first few hours after the mortar is placed, while the mortar is still plastic and before attaining any significant strength (Shaeles and Hover, 1988; Samman et al. 1996). As drying starts, the mortar near the surface dries and shrinks faster than the inner mortar, causing tensile stress and possible cracks. Plastic shrinkage cracking is usually associated with hot-weather concreting; however it can occur under ambient conditions that produce rapid evaporation of moisture from the mortar surface (Kosmatka et al., 1995).

Cement mortar products are notable for their weakness in tension, and for their lack of toughness, which gives risk to frequent cracking under impact loads, thermal shocks or dimensional changes due to humidity variation. Fibers have been used for decades to overcome such deficiencies, and to improve impermeability and minimize shrinkage, which are essential requirement properties of concrete besides its strength. The three main types of fibers that may be used as reinforcement for concrete are steel, glass and organic (natural and synthetic) fibers. As far as natural fibers are concerned, animal and vegetable fibers (i.e.: wood-cellulose, sisal, bast, coconut and bagasse) are all being used in various sheet materials (Padron and Zollo 1990; Krenchel et al., 1980; Majumdar, 1978). The drawbacks of using these natural materials are the high water absorption which must be allowed for in the mixing process. Moreover, the biological deterioration of the fiber (if not treated) and the strength loss that may occur in alkaline environments are also serious concerns. Additional processing may be required as in the case of
bagasse (to remove the sugar from the fiber) (Soroushian and Ravanbakhsh, 1998; Cook, 1980).

In this research, human-hair-waste is introduced as a new cement mortar reinforcing material. It would be highly pertinent to talk about the hair morphology, mechanical, chemical, physical, and electrical characteristics, to elucidate and provide the reader with the unique characteristics of hair that encouraged using it as a fiber in cement matrices. Human hair consists of five definite morphological components: cuticle, cortex, medulla, melanin granules and cell membrane complex, each distinct in morphology and chemical composition. Human hair consists of approximately 80% protein, 15% water, and 5% lipids (Potsch 1995). The water content of hair varies directly with the ambient relative humidity (Potsch 1995).

Regarding the mechanical properties of human hair, it was reported that, the load required to cause breakage of a natural and healthy hair-fiber varies between 50 and 100 g (Katz and Chatt, 1988). The average healthy human head which contains approximately 120,000 hair-fibers, may handle 12 metric tons (Katz and Chatt, 1988). For an average hair-fiber, the distribution point corresponds to a load of 12 kg/mm², and this exceeds that of aluminum. The unusual strength of hair is related to keratin which is a type of protein found in the hair cortex. The long keratin molecules in the cortex are compressed to form a regular structure, which is not only strong but also flexible. Keratin is unique in that, its chains contain high concentrations of a particular amino acid called cystine. Every cystine unit contains two cystine amino acids in different chains that have come to lie near to each other and are linked together by two sulfur atoms, forming a very strong chemical bond known as a disulphide linkage. Many disulphide bonds form down the length of the keratin chains, joining them together like the rungs of a ladder (Gray, 2000).

Hair has a high frictional coefficient, higher than that of vegetable or synthetic fibers. The high frictional coefficient of hair is attributed to its special surface structure (presence of scales), as can be seen in Figure (8-1) (Katz and Chatt, 1988). Hair is permeable to water both in liquid and vapor forms. After sufficient contact, hair keratin can absorb water up to
35 or 40% of its weight. The absorbed water is partially linked to the keratin protein by hydrogen bonds, but it also can exist in the free form. When water is absorbed by keratin the hair diameter can increase by 15-20%, while its length can increase only by 0.5-2%. The water absorption and subsequent swelling depends mainly on the pH level. Swelling is limited if the pH is low, and greatly enhanced if the pH is high (Zviak 1986).

It was found recently that human hair is a good absorbent material. For instance, in 1998, the U.S. Environmental Protection Agency (E.P.A) Oil Spill Program Internal Journal published an astonishing report on hair as a good absorbent material for crude oil spill clean up. It was reported therein that the National Aeronautical and Space Administration (NASA) was testing an unusual absorbent material (human hair) for oil spill clean up after a series of experiments conducted by McCrory (1998). The use of human hair waste as a phenol biosorbent was also reported by Fawazi and Sameer (2001). This property was established as well for the removal of different metal ions from contaminated environments (Tan et al., 1985).

The objective of this research is to optimize the cement mortar mix composition (i.e.: cement-sand ratio (c/s), water-cement ratio (w/c) and hair-fibers volume fraction percentage (f_b)) in order to minimize its plastic shrinkage. To achieve this, the factorial design method was used. Some of the advantages of this method compared to traditional methods are: it is much faster and shorter, it reveals the variables with the most significant effect on the process, and it measures the interactions between them. In contrast, and in the traditional methods, everything used to be kept constant while changing only one variable at a time. Also using traditional methods for investigating scientific experiments with many variables is time consuming and fails to measure the interactions between the variables (Box et al., 1978).
8.3 Experimental Procedure

8.3.1 Materials and Methods

Portland cement type I (ASTM C-150-86, 1990) was selected for the entire program of this work. The chemical properties of this cement are listed in Table (8-1). Silica sand with grain sizes greater than 0.125 mm, human-hair pieces with a length approximately equal to 2 cm, and tap water were also used to prepare the cement mortar mixtures.

8.3.2 Preparation of the Hair-Fibers and the Cement Mortar Mix Proportions

The human-hair was first washed and dried at room temperature. Subsequently, the human samples were cut into small pieces before adding to the cement mortar mix. The ASTM standard method (C305) for the mechanical mixing of mortar was adopted for mixing the cement mortar components. After that, the hair-fibers were slowly added to the cement mortar mix to ensure a good and uniform distribution of the fibers throughout the mix.

8.3.3 Test Procedure to Study the Plastic Shrinkage Cracks of Cement Mortars

To investigate the effect of the addition of hair-fibers to cement mortar on its susceptibility to experience the formation of shrinkage cracks, the test procedure was divided into two steps. In the first step, a set of preliminary experiments was conducted to decide either to proceed with this research if the results were promising, or it is not worthy to do so. The experimental setup for the preliminary experiments is shown in Figure (8-2), while the mix proportions are shown in Table (8-2).

In the second step, the factorial design method was adopted to study the effects of the cement/sand ratio (c/s), the water/cement ratio (w/s) and the hair-fibers volume fraction percentage (fₕ) on the rate of formation and intensity of the plastic shrinkage cracks in cement mortar slabs.
The test procedure proposed by Kraii (1985) and refined by Shaeles and Hover (1988) was used to evaluate the effects of the hair-fibers on the plastic shrinkage cracking of cement mortar. In spite of the extensive use of synthetic fibers in many fields and the importance of controlling the shrinkage cracks in cement mortar linings, a standard test method is not available yet to evaluate the potential reduction of shrinkage cracks in cement mortars using natural fibers (Balaguru, 1994).

In the preliminary experiments, two 30x30 cm slabs of plain and fibrous cement mortar (cement mortar containing hair-fibers) with a thickness of 3 cm were cast side by side and exposed to identical environmental conditions (temperature = 20±1°C, humidity = 56±1%), as can be seen from Figure (8-2). A vertical partition was used between the two slabs (panels) to prevent any non-uniformity arising from the interference between the fans and the slabs. The formation of the plastic shrinkage cracks on the slabs surfaces was then monitored with time.

8.4 Results and Discussion

8.4.1 Preliminary Tests

From the results of the preliminary tests, in which two cement mortar slabs (plain and fibrous) of the same c/s and w/c, exposed to the same environmental conditions, it was observed that the rate and intensity of the surface shrinkage cracks were much higher in the plain cement mortar slabs. This can be seen from seen from Figure (8-3).

The shrinkage cracks started to appear on the slab surfaces 60 to 75 minutes after casting them and starting the fans. The fans were later stopped after 4 hr of operation. The crack width, length and total area were measured for each slab using optical lenses. Each crack width and length was taken to be the average of three measurements. Characterizing the cracks by their total area instead of their width or length, helped to account for the fact that some cracks were very thin and short, while others were much wider and longer. Figure (8-2) shows the experiment setup used for this study.
A tremendous reduction in the cracks area of 92% was noticed in the cement mortar containing hair-fibers compared to the plain cement mortar. This result can be seen clearly from Figure (8-3) and it is depicted graphically in Figure (8-4). The length of some cracks in the plain cement mortar slabs reached high values up to 27 cm. The crack area percentages were calculated by dividing the total area of cracks by the total area of the cement mortar slab surface.

This interesting reduction in the shrinkage cracks in the cement mortar samples containing hair-fibers is believed to be the result of the unique hair-fiber characteristics. Some of these characteristics are the hair-fiber dimensions (length and diameter) (Ali et al., 1978), and the bond type and strength between the cement mortar matrix and the hair-fibers. It can also be observed from Figure (8-3) that the sheen disappeared from the surface of the plain mortar slab, whereas, it remained in the fibrous mortar slab. The disappearance of the sheen from the surface of cement structures indicates that, the rate of evaporation has exceeded the rate at which the bleeding water rises to the cement structure surface (Shah, 1998).

The previous result indicates that the rate of water evaporation was greater than the rate of rise of the bleeding water in the plain cement mortar, while the bleeding rate was retarded by the hair-fibers in the fibrous cement mortar. It can be concluded that the quantity of surface water was significantly reduced by the addition of hair-fibers. The hair-fibers seem to cause a reduction in the consolidation process, thus eliminating the damaging capillary bleed channels and causing an increase in the intergranular strength in the cement mortar. The fast evaporation of water is believed to decrease the capillary pores pressure which in turn decreases the volume which causes the contractions (Soroushian et al. 1995).

The high reduction of shrinkage cracks in the fibrous cement mortar can also be a result of the effective diameter of the hair-fibers, which varies between 57 to 120 μm (Gray, 2002). This size range of the hair-fibers is similar to that for the cement particle sizes. This fact promotes a close and very effective packing and development of a dense bulk and interface microstructure in the matrix (Walton and Majumdar, 1978). The relatively high surface
area and the close spacing of hair-fibers make them quite effective in the stabilization and suppression of microcracks in the cement mortar samples.

The length of the hair-fibers and their distribution in the cement mortar mix are also believed to affect the reduction of cracks in two ways. If the fibers are relatively long and far apart they will have no ability to arrest the microcracks, but they can arrest the propagation of the macrocracks and substantially improve the toughness of the composite. This is probably due to the fact that cracking in the cement mortar matrix first occurs at the micro level. However, small fibers could bridge even the microcracks.

The high aspect ratio of the hair-fibers (length/diameter) may also be accountable for the high reduction of cracks in the fibrous cement mortar slabs. The hair-fibers used in this study have a small diameter range. The small diameter gives the fibers less surface area and consequently fewer flaws that might propagate during cracking. The aspect ratio for the hair-fibers used with a length of 2 cm ranges between 160 and 350. For the same length (2 cm) and a diameter range between 0.25 to 0.65 mm, the aspect ratio for steel fibers would range between 30 and 80, and for polypropylene with a diameter of 0.38 mm, the aspect ratio would be 50 (Lee, 1992).

Another explanation for the reduction of shrinkage cracks in cement mortars using hair-fibers is that, the hair-fibers increase the amount of large pores in the cement paste. These groups of pores are probably attributed to the interfacial zone between the fiber and the cement paste. The formation of large pores is believed to reduce the capillary pressure in the cement paste, thus reducing the plastic shrinkage cracking of the cement matrix.

The effect of the hair morphology on the reduction of shrinkage cracks in the cement mortar is believed to be attributed to the hair high friction coefficients, which are higher than those for the vegetable or synthetic fibers. The high hair frictional coefficient value is attributed to its special surface structure, which has scales arranged in a specific orientation, as can be seen from Figure (8-1). The high friction of the hair surface increases the shear and friction forces between the hair-fibers and the cement matrix. As the matrix shrinks, the
shear stress along the hair-fibers and cement matrix interface develops. The hair-fibers are subjected to tension while the cement matrix is subjected to compression. The shrinkage of the cement mortar matrix in any direction would then be restrained by the aligned hair-fibers of effective lengths parallel to the direction of the shrinkage strain (Zhang and Li, 2001).

Another fact that might explain the effectiveness of hair-fibers in reducing the shrinkage cracks of cement mortars is their behavior when they are in contact with water. Hair is known to be permeable to water in both the liquid and vapor forms. It was reported that, hair keratin can absorb water up to 35 or 40% of its weight (Katz, 1988). This fact apparently explains why hair could be a good sorbent material in the clean up of oil spills. For that, the hair-fibers are capable of retaining a substantial amount of the evaporated water from the cement mortar surface, and this reduces the shrinkage cracks. The water absorption and subsequent swelling depend mainly on the mix pH level. Swelling is limited if the pH is low (acidic) and greatly enhanced if the pH is high (alkaline). Since cement mortar has high pH values (11-12), its swelling is high and this results in a high shrinkage reduction.

8.4.2 Factorial Design Tests

After the encouraging results generated from the preliminary experiments, the same procedure was repeated for the factorial design study, from which the effects of c/s, w/s, and $f_h$ on the shrinkage cracks of cement mortars were estimated.

The main objective of this test was to find the optimum composition of the cement mortar matrix that produces the lowest shrinkage cracks. To obtain the optimum composition of the cement mortar matrix and the effective amount of hair-fibers to be added to it, the Box-Hunter statistical method was used (Box et al., 1978). In this investigation, the adopted experimental design for optimizing the cement mortar mix proportions included the three variables c/s, w/c and $f_h$. The upper and lower values for each variable are listed in Table (8-3).
In the factorial design method as many variables as needed can be considered to check which individual variables, or interactions between variables, appear to have considerable effects on the measurement of interest, which is in this investigation the shrinkage cracks area. The experimental design with the above-mentioned three variables requires eight experiments. The set of experiments is represented as the spheres at the corners of the Box-Hunter Cube, as can be seen from Figure (8-5).

Using the Box-Hunter statistical method, the main effect of each variable was determined, then the interaction effect between two variables was evaluated, and finally the effect of the three variables together was calculated. The main effect, which is the average response of a variable over all conditions of the other variables, was calculated from the data \( Y_i \) obtained at the eight corners \( (i = 1-8) \) as follows:

\[
\text{Main effect} = \bar{Y}_+ - \bar{Y}_- \quad (8-1)
\]

Where \( \bar{Y}_+ \) is the average response for the upper level of the variable, and \( \bar{Y}_- \) is the average response for the lower level of the variable.

\[
f_h \text{ effect} = \frac{Y_2 + Y_4 + Y_6 + Y_8} {4} - \frac{Y_1 + Y_3 + Y_5 + Y_7} {4} \quad (8-2)
\]

\[
c/s \text{ effect} = \frac{Y_3 + Y_4 + Y_7 + Y_8} {4} - \frac{Y_1 + Y_2 + Y_5 + Y_6} {4} \quad (8-3)
\]

\[
w/c \text{ effect} = \frac{Y_5 + Y_6 + Y_7 + Y_8} {4} - \frac{Y_1 + Y_2 + Y_3 + Y_4} {4} \quad (8-4)
\]

The two variables interaction is the difference between the average response for the two variables when they are both in their higher or lower levels and the average response
when one of them is in its higher level and the other is in its lower level. The interactions between each of the two variables were calculated according to the following equations:

\[
f_{h \times c/s \text{ effect}} = \frac{Y_1 + Y_4 + Y_5 + Y_8}{4} - \frac{Y_2 + Y_3 + Y_6 + Y_7}{4}
\]  \hspace{1cm} (8-5)

\[
f_{h \times w/s \text{ effect}} = \frac{Y_1 + Y_3 + Y_6 + Y_8}{4} - \frac{Y_2 + Y_4 + Y_5 + Y_7}{4}
\]  \hspace{1cm} (8-6)

\[
c/s \times w/c \text{ effect} = \frac{Y_1 + Y_2 + Y_7 + Y_8}{4} - \frac{Y_3 + Y_4 + Y_5 + Y_6}{4}
\]  \hspace{1cm} (8-7)

Finally, the interaction between all three variables was given by:

\[
f_{h \times c/s \times w/c \text{ effect}} = \frac{Y_5 + Y_6 + Y_7 + Y_8}{4} - \frac{Y_1 + Y_2 + Y_3 + Y_4}{4}
\]  \hspace{1cm} (8-8)

Figure (8-6) shows the shrinkage cracks area versus c/s and w/c for both the plain \( (f_h = 0) \) and the fibrous \( (f_h = 0.4) \) cement mortar slabs. It can be observed that, the highest shrinkage cracks area was in the mixture with c/s = 1, w/c = 0.5 and \( f_h = 0 \). This result was expected since the higher level of w/c was used with the higher level of c/s and no hair-fiber was added to the mix. This is in contrast to the mixture with c/s = 0.67, w/c = 0.38 and \( f_h = 0.4 \) which experienced the lowest shrinkage effects. This result also met the expectations since the lower level of w/c was used with the lowest level of c/s, and \( f_h = 0.4 \).

Table (8-4) contains the results of the factorial design test. The main effect of a variable should be individually interpreted only if there is no evidence that this variable interacts with other variables. When there is evidence that one or more of such interactions are there, the interacting variables should be considered jointly. The negative sign of the values means a reduction effect of that variable(s) on the shrinkage cracks area. For example, the
c/s variable had a reduction effect on the shrinkage cracks area by 11 units, and that was the highest main effect of a given variable in reducing the shrinkage cracks area.

That above-mentioned result, which is also tabulated in Table (8-4), was expected since sand is an inert material that greatly reduces the amount of cement used in a cement mortar mixture, thus decreasing the creep and shrinkage of the cement mortar structures (Popovics, 1982). This is in addition to the fact that, the quartz (silicon dioxide) sand used in the entire program of this study is a very low adsorption aggregate. It is known that the low adsorption aggregates have low shrinkage properties and vice versa (Kosmatka et al., 1995). All of that explains and supports the above result that the c/s variable has the highest impact on the shrinkage properties of cement mortar structures.

In addition to the c/s high effect of the shrinkage behavior of cement mortar slabs, the f_h was also found to have a similar effect like that for c/s as can be seen in Table (8-4). But since the c/s and f_h variables were highly interacting with each other (f_h×c/s effect in Table 4-4), their individual effects should not be taken into consideration alone.

On the other hand, the w/c was found to have a low effect on increasing the shrinkage of cement mortar slabs, as can be seen from Table (8-4). The other interesting observation was that, there was no evidence that the w/c is interacting with any of the c/s or f_h variables. The above results were not expected since it is believed that the most important controllable variable affecting shrinkage is the amount of water per unit volume of cement mortar mixture.

Many researchers have reported the importance of the w/c on the shrinkage susceptibility of cement mortar mixtures. Kosmatka et al. (1995) found that, for each 1% increase in the mixing water, the concrete shrinkage increased by 2% which contradicts the result reported in this study. This observation may be explained and justified by the fact that, the hair ability to absorb substantial amounts of water is dominating other effects. But since this study is the first of its kind to use the human hair as a binding fiber, further studies are needed and recommended in this area.
8.5 Conclusions

Based on the experiments conducted in this study, the following conclusions can be reached:

1. Human hair, a waste material that would have been a nuisance to the environment, can be converted into a useful material of economic value, replacing expensive polymeric fibers.

2. This study has shown a novel method of reducing the shrinkage cracks arising from the unequal dissipation of water from the cement mortar structures and the rising of bleeding water to the cement mortar surfaces.

3. The addition of hair-fibers to the cement mortar matrix was very effective in reducing its shrinkage cracks by a remarkable amount of 92%.

4. The effect of the hair-fibers volume fraction in reducing the cement mortar shrinkage cracks was close to the established effect of cement to sand ratio.

5. It was observed that the effect of water to cement ratio on the shrinkage of cement mortars was lower in the fibrous cement mortars compared to the plain ones.

6. The results of this study demonstrated that the commercial application of the hair-fibrous cement mortar in lining pipelines and cementing hydrocarbon wells is feasible.

8.6 References


Kraii, P. P. 1985. Proposed Test to Determine the Cracking Potential Due to Drying Shrinkage of Concrete. Concrete construction 30: 775-778.


8.7 Appendices

8.7.1 Appendix A: Tables

Table (8-1) Chemical composition of the ordinary Portland cement (Karii, 1985)

<table>
<thead>
<tr>
<th>Component</th>
<th>Weight %</th>
</tr>
</thead>
<tbody>
<tr>
<td>SiO₂</td>
<td>21.60</td>
</tr>
<tr>
<td>Fe₂O₃</td>
<td>1.84</td>
</tr>
<tr>
<td>TiO₂</td>
<td>0.31</td>
</tr>
<tr>
<td>P₂O₅</td>
<td>0.35</td>
</tr>
<tr>
<td>Mn₂O₃</td>
<td>0.15</td>
</tr>
<tr>
<td>Al₂O₃</td>
<td>4.31</td>
</tr>
<tr>
<td>CaO</td>
<td>65.28</td>
</tr>
<tr>
<td>MgO</td>
<td>1.18</td>
</tr>
<tr>
<td>Na₂O</td>
<td>0.15</td>
</tr>
<tr>
<td>K₂O</td>
<td>0.42</td>
</tr>
<tr>
<td>SO₃</td>
<td>3.06</td>
</tr>
<tr>
<td>Loss on ignition</td>
<td>1.04</td>
</tr>
<tr>
<td>Free lime</td>
<td>1.94</td>
</tr>
</tbody>
</table>

Table (8-2) Cement mortar mix proportions for the set of the preliminary experiments

<table>
<thead>
<tr>
<th>Sample</th>
<th>fₜ</th>
<th>c/s</th>
<th>w/c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plain</td>
<td>0</td>
<td>1</td>
<td>0.42</td>
</tr>
<tr>
<td>Fibrous</td>
<td>0.4</td>
<td>1</td>
<td>0.42</td>
</tr>
</tbody>
</table>
Table (8-3) The upper and lower values of \( c/s \), \( w/c \) and \( f_h \)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Lower value</th>
<th>Upper value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( f_h )</td>
<td>0</td>
<td>0.4</td>
</tr>
<tr>
<td>( c/s )</td>
<td>0.67</td>
<td>1</td>
</tr>
<tr>
<td>( w/c )</td>
<td>0.38</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Table (8-4) The estimated variable effects on the shrinkage cracks area of cement mortar using the \( 2^3 \) factorial design method

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Main effects and interactions ( (10^{-2}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Main effect</td>
</tr>
<tr>
<td>( f_h )</td>
<td>-9.21</td>
</tr>
<tr>
<td>( c/s )</td>
<td>-11.01</td>
</tr>
<tr>
<td>( w/c )</td>
<td>1.53</td>
</tr>
<tr>
<td></td>
<td>Two factors interactions</td>
</tr>
<tr>
<td>( f_h \times w/c )</td>
<td>-1.67</td>
</tr>
<tr>
<td>( f_h \times c/s )</td>
<td>8.66</td>
</tr>
<tr>
<td>( c/s \times w/c )</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>Three factors interactions</td>
</tr>
<tr>
<td>( f_h \times c/s \times w/c )</td>
<td>6.00</td>
</tr>
</tbody>
</table>
8.7.2 Appendix B: Figures

Figure (8-1) SEM photomicrograph of a human hair fiber

Figure (8-2) Schematic diagram of the designed experimental setup to study shrinkage cracks in cement mortar
Figure (8-3) Shrinkage cracks in (a) plain and (b) fibrous cement mortars
Figure (8-4) Comparison between the shrinkage cracks area in plain and fibrous cement mortar slabs
Figure (8-5) Schematic representation of the $2^3$ factorial design method
Figure (8-6) The percentage of shrinkage cracks area to the total surface area of the cement mortar slabs for different values of c/s, w/c and $f_h$.
CHAPTER 9

ESTIMATION OF THE SUBSTRATE CONCENTRATION PROFILES IN INDUSTRIAL SYSTEMS CONTAINING BIOFILMS

9.1 Abstract

In industrial systems, biofilm formation can cause many problems such as increase in the flow resistance of pipelines, energy losses in fluid transport and heat exchangers, product contamination, material deterioration and biocorrosion. As a result, biofilms contribute substantially to huge economical losses in the industry.

Substrate concentrations in the system and near the biofilm surface are some of the parameters that have a great impact on the extent of the problems associated with biofilms. In this study, a convective-diffusion model under various flow conditions (laminar and turbulent) has been solved using the finite difference technique, employing the alternating difference implicit (ADI) method. The model assumes that a liquid containing substrate and bacteria is flowing in a pipeline with known concentrations at the inlet and then predicts the variation of the transient substrate concentrations along the pipeline and as a function of the pipe radius.

The model was then used to predict and estimate the substrate concentration profiles on the biofilm surface under different operating conditions. A parametric study was also conducted to study the effect of different parameters that influence the substrate concentration profiles in the system and on the biofilm surface. All the results indicated that the model presented in this paper was very successful in estimating the substrate concentration at any point in the pipeline system under any particular specific operating conditions.
9.2 Introduction

Biofouling is a general term that refers to the undesirable accumulation of biotic deposits on a surface (Mattila, 2002). Microbial cells attach firmly to almost any surface submerged in an aquatic environment. The immobilized cells grow, reproduce, and produce extracellular polymeric substances (EPS) that frequently extend from the cell, forming a tangled mass of fibers lending structure to the entire assemblage which shall be termed a biofilm (Characklis and Cooksey, 1983).

Biofilms are traditionally represented as thin layers of microbial cells attached to flat impermeable surfaces and in contact with liquid phases of constant substrate concentrations. In addition, the biofilm is idealized as having a constant thickness and uniform cell density (Pizarro et al., 2001). Cloete (2003) reported that there are a number of factors that promote the adhesion of microorganisms to surfaces. Surface roughness and composition play a major role in the early stages of biofilm formation and may influence the rate of cell accumulation and cell distribution. Another key factor in microbial adhesion is hydrodynamic shear stress.

Biofilms play important roles, some beneficial and others detrimental, in many natural and technological processes (Characklis and Cooksey, 1983). Important examples where biofilms occur are: heat exchangers, trickling filters, marine and freshwater sediments, ship hulls, water and sewage pipelines, desalination, cooling towers, paper machines and bioreactors (Kreft et al., 2001). Biofilms are of great practical importance for beneficial technologies such as water and wastewater treatment and bioremediation of groundwater and soil. Biofilms are also implicated in a wide range of petroleum process problems, from the production field to gas station storage tanks.

Mass transport can have a significant influence on the rate of various biotransformation reactions occurring in biofilm systems since it is often the rate limiting process. Therefore, to fully understand and optimize processes occurring in natural and industrial biofilms, it is necessary to determine the rates of both diffusive and convective mass transport.
In a biofilm system, substrate transport involves convection from the bulk liquid to the near vicinity of the cell clusters, diffusion through the mass transfer boundary layer, and diffusion through the cell clusters to the cells. The concentration difference between the bulk liquid and the biofilm surface, the diffusion coefficient of the solute, and the thickness of the mass transfer boundary layer, determine the diffusive solute flux to the biofilm (De Beer et al., 1996). The thickness of the concentration layer was found to depend on surface structure which depends on the substrate loading and the hydrodynamic conditions during the growth phase of the biofilm (Wasche et al., 2000). Biofilm density and maximum substrate flux were also influenced by growth conditions.

The steady-state flux of substrate into a biofilm can be expressed as a function of the concentration of substrate in the bulk, the thickness of the stagnant liquid layer surrounding the biofilm, and the minimum concentration of substrate capable of sustaining growth (Suidan et al., 1989). Wasche et al. (2002) found that the hydrodynamic conditions and the substrate load during the growth phase of the biofilm in biofilm systems are two key parameters that influence the biofilm growth, particularly the structure, density and thickness. They used the measured substrate conversion rates, biofilm densities and the boundary layer thickness to formulate an equation for the mass transfer in biofilm tube reactors.

The surface averaged relative effective diffusivities ($D_\text{s}$) of the substrate were higher in biofilms grown at high substrate (glucose) concentrations and at low flow velocities, and it was influenced by the glucose concentration to a much larger extent than by the flow velocity (Beyenal and Lewandowski, 2000). The effect of turbulent diffusion on the substrate uptake rate by biofilms was studied by Nagaoka and Sugio (1994). It was concluded that the substrate uptake rate by biofilms was dependent on the turbulence near biofilms (Nagaoka and Sugio, 1994).

Both the biofilm and the suspended bacteria play critical roles in the bioprocesses occurring in a given system. Suspended and attached growth kinetics was studied by Ozer and
Kasirga (1995) to explore the significance of aeration in sewer trunk lines. Soluble substrate consumption of suspended growth microorganisms was taken into consideration by means of an experimental approach and they were estimated by using respiration rates. Ozer and Kasirga (1995) developed an empirical relationship which provides the utilization rate in correspondence with diminishing substrate concentrations along a sewer line based upon their experimental results.

Dibenzofuran uptake-associated kinetic parameters of suspended and attached *Sphingomonas sp.* strain HH19k cells were compared with each other in a study conducted by Harms and Zehnder (1994). The suspended cells were studied in a batch system, whereas glass beads in percolated columns were used as the solid support for attached cells. The maximum specific activities of cells in the two systems were the same. The apparent half-maximum uptake rate-associated concentrations of attached cells, however, were considerably greater than those of suspended cells and depended on cell density and on percolation velocity. A mathematical model was developed to explain the observed differences in terms of substrate transport to the cells.

Palumbo *et al.* (1983) studied the efficient utilization of dissolved free amino acids by suspended marine bacteria. Their data affirmed that suspended marine bacteria efficiently utilize dissolved free amino acids and that acidification should not be used to stop the incubation of samples to be filtered for measurement of substrate incorporation. Chen (2001) investigated the growth kinetics of *Pseudomonas aeruginosa* in continuous culture. His results showed that the half saturation coefficient of oxygen for colony biofilm was lower than the half saturation coefficient of oxygen for planktonic culture (Chen, 2001).

Yu *et al.* (2001) conducted a study in which they demonstrated that, during the two-step biodegradation of toluene in an aerobic circulating-bed biofilm reactor, biofilm and suspended bacteria played critical roles. Although the suspended bacteria were less than 1% of the total amount of biomass in the system, they transformed up to 30% of the toluene into its intermediate in the bulk liquid phase. On the other hand Yu *et al.* (2001) found that
most of the toluene intermediate was removed inside the biofilm, where diffusion resistance reduced the toluene concentration, thereby relieving inhibition to the degradation reaction of the intermediate. They concluded that suspended bacteria are most important for rapidly biodegraded substrates, for which diffusion limitation controls the kinetics in the biofilm. Suspended bacteria lose importance when the effects of an inhibiting substrate must be overcome (Yu et al., 2001).

Mathematical models allow the simulation of microorganism growth and substrate transport and utilization in biofilm systems. A simple dynamic model was presented by Rauch et al. (1999) for fast simulation of the removal of multiple substrates by different bacterial species growing in a biofilm reactor. Nagaoka (1997) developed a mathematical model to describe substrate profiles in biofilms under oscillatory flow conditions. In his model, he suggested that the substrate uptake rate by biofilms is proportional to the square root of Reynolds number (Nagaoka, 1997).

A steady-state mathematical model has been developed by Pivasant et al. (1997) to predict axial-concentration profiles of a pollutant in an extractive-membrane bioreactor (EMB). Other models describe the pollutant concentration profiles in the membrane-attached biofilms in a direction perpendicular to the membrane. In contrast, the model presented in Pivasant et al. (1997) describes not only the radial profiles, but also the axial profiles along the membrane length. A diffusion-reaction model was employed to describe the diffusion and reaction in the biofilm in the radial direction.

In this study, a general model will be developed and solved to enable estimating the key substrate concentration profiles at any point in the system of interest. In this model both sessile and suspended bacteria will be taken into consideration. It is also worth mentioning here that the model will be capable of solving transient industrial systems.
9.3 Modeling and Simulation

The aim of this work is to develop a model capable of estimating the substrate concentration profiles in industrial systems containing biofilms. The model derivation extended previous modeling work by including diffusional transport and a generalized bulk reaction mechanism, and by solving the resulting transport equation for unsteady flow conditions. The model is based on the principle of mass transport and it can simulate a species disappearance mechanism under different flow conditions. The model accounts for the axial convection and radial diffusion in a pipe under different flow regimes.

The transport of a given species in a liquid can be described quantitatively by the spatial and temporal evolution of its concentration; in other words, a differential equation for the concentration \( C(x, y, z, t) \). The differential equation must also account for the reactions (sources or sinks of the species).

The principle of conservation of mass requires that the chemical flux \( F = (F_x, F_y, F_z) \) and the concentration \( C \), satisfy the following mass balance equation (Perry and Green, 1997):

\[
-\left( \frac{\partial F_x}{\partial x} + \frac{\partial F_y}{\partial y} + \frac{\partial F_z}{\partial z} \right) + R - S = \frac{\partial C}{\partial t} \tag{9-1}
\]

The term inside the brackets on the left hand side of Equation (9-1) represents the chemical influx minus efflux. The right hand side of Equation (9-1) represents the temporal change of solute in the bulk fluid. \( R \) and \( S \) represent the source and sink terms, respectively.

In this investigation, a 2-D modeling was considered and due to the nature of the systems involved a cylindrical coordinate system was chosen. Therefore the above mass balance equation (Equation 9-1) can be re-written in the following form:

\[
-\left( \frac{\partial F_r}{\partial x} + \frac{1}{r} \frac{\partial (r F_r)}{\partial r} \right) + R - S = \frac{\partial C}{\partial t} \tag{9-2}
\]
For a 2-D cylindrical transport, the axial \((F_x)\) and radial \((F_r)\) solute flux can be derived solely from advection and dispersion. For that, \(F_x\) and \(F_r\) can be described as:

\[
F_x = u_x C - D_x \frac{\partial C}{\partial x} \tag{9-2-a}
\]

\[
F_r = u_r C - D_r \frac{\partial C}{\partial r} \tag{9-2-b}
\]

Where \(u_x\) and \(u_r\) are the velocity components in the \(x\) and \(r\) directions. In this investigation the radial velocity was neglected due to the assumption of symmetry. Therefore, Equation (9-2) can be re-written as (Bird et al., 2001):

\[
-\left( \frac{\partial}{\partial x} \left( u_x C - D_x \frac{\partial C}{\partial x} \right) - \frac{1}{r} \frac{\partial}{\partial r} \left( -r D_r \frac{\partial C}{\partial r} \right) \right) + R - S = \frac{\partial C}{\partial t} \tag{9-3}
\]

Equation (9-3) represents a very general differential equation that can be applied in different applications like a fluid flow in cylindrical devices such as pipes. In this investigation the above differential equation will be used to study the concentration profiles of a given substrate in a pipeline system.

The solution of the convective-diffusive mass balance equation given above (Equation 9-3) depends on the system under investigation. In other words, the solution of Equation (9-3) has to be subjected to prescribed boundary and initial conditions, which are characteristics of each system.

The system in our investigation is a pipeline containing biofilm at its inner surface, and a fluid containing substrate flowing through it. The substrate concentrations model in cylindrical coordinates can be derived using the principle of mass conservation in a pipe. A mass balance over a fluid element in a single pipe was developed, accounting for the
transport by convection and radial diffusion, and for the substrate consumption in the bulk fluid according to a first-order reaction term.

For unsteady transport of a substrate in a pipe of length \( L^* \) and a radius \( r_0^* \), the equation of material conservation accounting for the aforementioned mechanisms can be written as:

\[
\frac{\partial C^*}{\partial t^*} = -\frac{\partial (U^* f(r^*) C^*)}{\partial x^*} + \frac{\partial}{\partial x^*} \left( D \frac{\partial C^*}{\partial x^*} \right) + \frac{1}{r^*} \frac{\partial}{\partial r^*} \left( r^* D \frac{\partial C^*}{\partial r^*} \right) + R^* \tag{9-4}
\]

The term on the left-hand side of Equation (9-4) accounts for changes in the substrate concentration with time. The first term on the right-hand side of Equation (9-4) describes advective transport, whereas the second and the third terms represent diffusion in the axial and radial directions, respectively. The last term on the right hand-side of Equation (9-1), \( R^* \), can be either a general sink term or the substrate consumption rate by bacteria at any point in the system. The \( f(r^*) \) is a term that accounts for the flow profile present in the pipe. \( U^* \) is the average or hydraulic velocity of the liquid flowing in the pipe under consideration. \( D \) is the diffusion coefficient of the substrate in the bulk flow.

The system can be described using cylindrical coordinates and having the center of the pipe at the inlet as the origin, as can be seen in Figure (9-1). The boundary and initial conditions for the model shown in Figure (9-1) can be written as:

\[
x^* = 0 \quad ; \quad C^* = C_0^* \tag{9-5-a}
\]

\[
x^* = L \quad ; \quad \frac{\partial^2 C^*}{\partial x^* r_0^*} = 0 \tag{9-5-b}
\]

\[
r^* = 0 \quad ; \quad \frac{\partial C^*}{\partial r^*} = 0 \tag{9-5-c}
\]
\[ r^* = r_0 \quad ; \quad D \frac{\partial C^*}{\partial r^*} = -R_b^* \quad (9-5-d) \]

where \( R_b^* \) is the substrate consumption rate on the biofilm surface, and inside the biofilm.

\[ r^* = 0 \quad ; \quad C^* (x^*, r^*) = C_{\text{initial}} (x^*, r^*) = 0 \quad (9-5-e) \]

The function \( f(r^*) \) describes the radial velocity distribution in the pipe depending on the flow type. For a laminar flow (\( Re < 2100 \)) in a pipe, \( f(r^*) \) has a parabolic shape which is described by the Poiseuille formulation:

\[ f(r^*) = 2 \left( 1 - \left( \frac{r^*}{r_0} \right)^2 \right) \quad (9-6) \]

for turbulent flow, a uniform velocity profile can be assumed:

\[ f(r^*) = 1 \quad (9-7) \]

and for stagnant conditions:

\[ f(r^*) = 0 \quad (9-8) \]

As was mentioned before, \( D \) is the diffusion coefficient of the substrate in the liquid under investigation. In the laminar flow regimes, \( D \) is equal to the molecular diffusion coefficient \( D_m \). On the other hand and in the turbulent flow regimes, \( D \) represents the turbulent diffusivity which is the sum of the molecular diffusion coefficient \( (D_m) \) and the eddy diffusivity \( (\varepsilon) \), which is dependent on the distance from the wall. Based on Reichardt’s law for a pipe, \( \varepsilon \) can be calculated from:
\[ \varepsilon = 0.4U^{*}(r_0 - r^*) \cdot \frac{\left(1 + \frac{r^*}{r_0}\right) \cdot \left(1 + 2 \left(\frac{r^*}{r_0}\right)^2\right)}{\sqrt{8}} \cdot \frac{\alpha}{6} \]  

(9-9)

where \( \alpha \) is given by:

\[ \alpha = \frac{1.32547}{\left[ \ln\left(\frac{e}{3.7\{2r^*\}} + \frac{5.74}{(Re)^{0.9}}\right) \right]^2} \]  

(9-10)

Concerning the term \( R^* \) in Equation (9-4) which is the rate of substrate consumption in the pipe, it can be calculated using the Monod equation. That is because microbial growth is usually represented with Monod kinetics and the rate of substrate utilization is assumed proportional to the rate of microbial growth (Grady et al., 1999). From that, the substrate utilization rate can be calculated as follows:

\[ R^* = \frac{q \cdot X_c \cdot C^*}{K_m + C^*} \]  

(9-11)

where:

\( R^* \) = substrate consumption rate, \( \text{mol/m}^3\text{-h} \)

\( q \) = maximum substrate utilization rate, \( \text{mol/g-h} \)

\( X_c \) = bacteria concentration, \( \text{g/m}^3 \)

\( K_m \) = Monod half velocity coefficient, \( \text{mol/m}^3 \)

The Monod equation can also be written as: 
\[ R^* = \frac{k_1 C^*}{1 + k_2 C^*}; \text{ where } k_1 \text{ and } k_2 \text{ are constants} \]

with the units of \( \text{s}^{-1} \) and \( \text{l/mg} \), respectively.
The local average concentration (based on cross-sectional area) was calculated from the detailed substrate concentration profile \(C(X, r, t)\) by the following integral:

\[
C(X, t) = \frac{\int_0^r C(x, r, t) \cdot r \cdot dr}{\int_0^r r \cdot dr}
\]  \hspace{1cm} (9-12)

where \(X\) is the non-dimensional \(x\) position along the pipe.

### 9.4 Numerical Techniques

The resulting governing equation with its initial and boundary conditions that describe the substrate concentration in the pipe system was solved numerically under different conditions. In order to solve these model equations numerically for different combinations of parameters, the governing equation with its initial and boundary conditions were first transformed to the non-dimensional form then discretized by an Alternating Difference Implicit (ADI) scheme.

The ADI scheme is superior to many other numerical methods such as Barakat Clark and Crank-Nicholson methods, for several reasons (Carnahan et al., 1969; Ozdemir and Ger, 1999). First, the ADI scheme produces a stable solution regardless of the choice of the time step and the discretization grid size, and the model results will therefore always be reasonable. Second, the ADI method creates unsteady solution using the finite differences in the spatial directions \(x\) and \(r\) to find a solution at the next time level, while the Crank-Nicholson scheme uses finite differences in one spatial dimension to obtain the steady state solution throughout the grid. Furthermore the Crank-Nicholson scheme is not capable of accounting for the axial spreading of species by diffusion.

The ADI scheme which is being used in this study to find the solution of the general model of substrate transport and consumption in a pipe belongs to a class of numerical methods
that are unconditionally stable and are second-order accurate with a total error of the order \( O[(\Delta r)^2, (\Delta x)^2, (\Delta r)^2] \). Regardless of the choice of the step sizes in both the time and the spatial dimensions, the method produces stable results.

In applying the ADI scheme, finite difference techniques were used to advance the solution in time at each location in the grid. In the ADI scheme, each time step is split into two half steps. During the first time step, the solution at the time level \((n+1/2)\) was created by using finite differences at the new time level \((n+1/2)\) for all partial derivatives in \(r\), and finite differences at the old time level \(n\) for all partial differences in \(x\). After that and following the same procedure the solution at the end of the time step \((n+1)\) was considered with respect to \(x\).

It should be noted that Equation (9-12) was converted to a programmable form by employing the discrete values of \(C\) in a computational grid as follows:

\[
C(X,t) = \frac{\sum_{j=1}^{j_{\text{max}}} C(i, j, t) \cdot r_j \cdot \Delta r_j}{\sum_{j=1}^{j_{\text{max}}} r_j \cdot \Delta r_j} \quad (9-13)
\]

**9.5 Parameters and Constants used in solving the Model**

In this investigation, the model was solved for water containing glucose substrate and *Pseudomonas aeruginosa* bacteria flowing in a pipe containing biofilm. The inlet substrate concentration \((C_0)\) was taken to be 100 mg/l. The pipe length \((L)\) and radius \((r)\) were taken to be 10 m, and 1 cm, respectively. The diffusion coefficient of the substrate in water was taken to be \(6 \times 10^{-6} \text{ cm}^2/\text{s} \) (Baillod and Boyle, 1970).

The Monod equation was used to calculate the substrate consumption rate in the bulk flow. The kinetic parameters \(k_1\) and \(k_2\) in the Monod equation were taken to be 0.06 s\(^{-1}\) and 0.0083 l/mg, respectively (Kirkpatrick *et al.*, 1980). In the model solution, it was assumed
that all of the substrate that reaches the biofilm surface will be consumed. The model was then solved for different flow regimes with different Reynolds numbers.

9.6 Results and Discussion

As it was mentioned before, the main objective of solving this model is to estimate the substrate concentration profiles on the biofilm surface and at any other point in the system under investigation (pipeline).

Figure (9-2) shows the substrate concentration profiles development along the pipeline under laminar flow conditions. It is clear from Figure (9-2) that the model was successful in calculating the transient substrate concentration values at any point in the pipe (at each point of the numerical grid). The steady state conditions were achieved after 1 hour. As Figure (9-2) also shows, the concentration values were calculated at the biofilm surface, and as area average values. The importance of calculating the substrate concentration values at the biofilm surface is that these values determine the rate of consumption of the substrate on the biofilm surface, which in turn affects the associated processes resulting from the presence and growth of those biofilms, like degradation and corrosion.

It can also be observed from Figure (9-2) that the curvature of the area average concentration profile curve is less steep that the curvature of the concentration profile curve on the biofilm surface. This is due to the fact that as we move from the biofilm surface, the rate of substrate consumption decreases.

Figure (9-3) shows the steady state concentration profiles at the biofilm surface and along the center of the pipe under laminar flow conditions. The steady state area average concentration profile is also shown in Figure (9-3). It is clear that the area average substrate concentration values are closer to the substrate concentration values at the pipe center line more than the substrate concentration values on the biofilm surface. This is because the localized concentration profile values used in the calculation of the area average
concentrations (refer to Equation 9-12), were of a parabolic shape as will be explained in the next paragraphs.

It can also be observed from Figure (9-3) that the curvature of the concentration profile curves was the lowest at the center of the pipe and the highest on the biofilm surface. This is because the centerline represents the farthest point from the biofilm and therefore, it is the least affected by the biofilm consumption of the substrate.

Another observation from Figure (9-3) was that the rate of change in the substrate concentration was decreasing along the pipe length. This observation can be explained by the fact that the substrate concentration itself is decreasing along the pipe length.

Figure (9-4) shows the steady state concentration profiles at the biofilm surface and along the center of the pipe under turbulent flow conditions. The area average concentration profiles are also shown in Figure (9-4). As was the case for the laminar flow, it is clear that the area average substrate concentration values were closer to the substrate concentration values at the pipe center line more than those on the biofilm surface, for the same reason given above.

At $Re = 5000$, which is the case shown in Figure (9-4), the turbulent eddies were very high to the point that they contributed to more than 95% of the diffusion parameter at the centerline of the pipe. This fact along with the constant velocity profile (Equation 9-7) has made the concentration at the centerline the dominant factor in the cross-sectional area average concentration. As a result the relationship between the area average concentration and $X$ was almost a straight line as shown in Figure (9-4).

Figure (9-5) shows the dependence of the substrate concentration profiles on the biofilm surface on Reynolds number values within the laminar flow range. It can be seen from Figure (9-5) that the steady state concentration values on the biofilm surface increase by increasing Reynolds number. This is due to the fact that increasing the flow velocity
decreases the exposure time of substrate to the bacteria and the biofilm and, therefore, less substrate will be consumed.

The biofilm process rates may be controlled by mass transfer limitations in the bulk fluid phase. The substrate removal rate is dependent on fluid velocity past the biofilm. At low fluid velocities, a relatively thick mass transfer boundary layer can cause a fluid phase mass transfer resistance that decreases substrate concentration at the fluid-biofilm interface, thereby decreasing the substrate removal rate. Two factors may result in low mass transfer rates from the bulk fluid to the biofilm: low fluid velocities, and the transport of dilute liquid phase concentrations of the material (Characklis and Cooksey, 1983).

Figure (9-6) shows the dependence of the area average substrate concentration profiles on Reynolds number values within the laminar flow range. It can be seen from Figure (9-6) that the area average steady state substrate concentration values increase by increasing Reynolds number. Also by increasing Reynolds number values, the rate of decrease in the substrate concentration values along the pipe decreases. These observations can be explained in the same way as was mentioned in the case for the substrate concentration profiles on the biofilm surface.

Figure (9-7) shows the substrate concentration profiles in the radial direction under laminar flow conditions. From Figure (9-7) it can be observed that the concentration profiles in the radial direction have a parabolic shape (low at the surface of the pipe, max at the center). These results and observations are in agreement with our model and with the fact that the rate of substrate consumption at the biofilm surface is the highest compared to the consumption at any other point in the pipe.

Figure (9-8) shows the substrate concentration profiles in the radial direction under turbulent flow conditions. These concentration profiles are less steep than those under laminar flow conditions. This observation is the result of the velocity profiles that were used for the turbulent flow conditions. The effect of the eddy diffusivity is clear from the chaotic behavior of the concentration profiles in the radial direction. The turbulent flow
behavior has resulted in a better mixing phenomenon (as compared to laminar flow) and, therefore, higher concentration values were obtained at the biofilm. Kirkpatrick et al. (1980) reported that for laminar flow, utilization of substrate is diffusion limited in the fluid since radial mass transport is solely by molecular diffusion. They also found that in a turbulent flow system, radial velocity fluctuations yielded a much higher effective diffusivity which results in a well-mixed fluid phase.

Figure (9-9) presents the concentration profiles at the pipe outlet and gives further evidence about the effect of eddies on the substrate consumption rates in the pipe and mainly on the biofilm surface. For the particular case shown in Figure (9-9) \( (Re = 5000) \), it was predicted that the fluid will reach the end of the pipe in about 40 seconds, and this explains the big difference between the profiles at 40 and 80 Seconds.

The effect of Reynolds number on the outlet substrate concentration values under both laminar and turbulent flow conditions is shown in Figure (9-10). It is clear that increasing Reynolds number which in this investigation means increasing the velocity in the pipe increases the value of the outlet substrate concentration. As was discussed before, increasing the flow velocity decreases the residence time of the flowing fluid that contains the substrate, and that in turn reduces the consumed amounts of the substrate.

Figure (9-10) also shows a very interesting observation which is the sharp drop in the transition from turbulent to laminar flow. This sharp drop in the substrate concentration values is attributed to the eddy effects associated with turbulent flow. Those eddies increase the rate of mixing and lead to higher mass transfer in the pipe and towards the biofilm surface which in turn results in higher substrate removal rates and thus lower the outlet substrate concentrations.
9.7 Conclusions

A transient two-dimensional model incorporating substrate transport by advection as well as axial and radial diffusions along with a generalized sink term $R$ (to account for substrate consumption in bulk fluid) was established. The model was then solved for various flow scenarios and the results were presented and thoroughly discussed. The modeling results were found to be fully consistent with many experimental findings generated by other researchers from similar applications.

In light of the results presented above, the following conclusions can be drawn:

1. The model presented herein was very successful in estimating the substrate concentration profiles at any point in the pipeline system

2. Increasing the flow velocity decreases the residence time of the flowing fluid which contains the substrate, and that in turn reduces the consumed amounts of the substrate.

3. Turbulent flow produces eddies that increase the rate of mixing and lead to higher mass transfer in the pipe and towards the biofilm surface which in turn results in higher substrate consumption rates.

4. The sink term (substrate consumption in the bulk solution) was found to highly affect the resulting substrate concentration profiles

5. The model presented in this paper can be applied in a wide range of industrial applications either under static or flow conditions.

9.8 References


Mattila, K. 2002. Biofilms on Stainless Steels Exposed to Process Waters. Academic Dissertation in Microbiology, Department of Applied Chemistry and Microbiology, Division of Microbiology, University of Helsinki, Finland.


**9.9 Appendices**

9.9.1 Appendix A: Figures

![Figure (9-1) Schematic diagram of the pipeline system under investigation](image-url)
Figure (9-2) Development of the substrate concentration profiles along the pipeline under laminar flow conditions (Re = 1000)
Figure (9-3) Steady state substrate concentration profiles at the biofilm surface and along the center of the pipeline as well as the area average concentration profiles, under laminar flow conditions (Re = 1000)
Figure (9-4) Steady state substrate concentration profiles at the biofilm surface and along the center of the pipeline as well as the area average concentration profiles, under turbulent flow conditions (Re = 5000)
Figure (9-5) The effect of Reynolds number (in the laminar range) on the steady state substrate concentration profiles on the biofilm surface along the pipeline.
Figure (9-6) The effect of Reynolds number (in the laminar range) on the steady state area average substrate concentration profiles along the pipeline
Figure (9-7) The steady state substrate concentration profiles in the radial direction at the middle and at the end of the pipe, under laminar flow conditions (Re = 1000)
Figure (9-8) The development of the substrate concentration profiles in the radial direction at the middle of the pipe, under turbulent flow conditions (Re = 5000)
Figure (9-9) The development of the substrate concentration profiles in the radial direction at the end of the pipe, under turbulent flow conditions (Re = 5000)
Figure (9-10) The effect of Reynolds number on the outlet area average substrate concentration values under both laminar and turbulent flow conditions.
CHAPTER 10

MODELING SUBSTRATE CONCENTRATIONS AND BIOFILM GROWTH IN PIPELINE SYSTEMS

10.1 Abstract

Biofouling is a major concern in all the industries where microorganisms and their nutrients are available. Pipelines are susceptible to biofilm formation on their inner surfaces, which in turn causes serious problems, such as corrosion. Substrate concentrations, bacteria growth rates, velocity, and temperature are known to be important parameters affecting the rate and extent of biofilm growth and the resulting corrosion.

In this study, a convective-diffusion transient model “including the substrate consumption rate term”, that predicts both the substrate concentration profiles and the biofilm growth rates in a pipeline system has been developed and solved using the finite difference technique (FDT), employing the alternating direction implicit (ADI) method.

The model was solved at different inlet substrate concentrations, different operating temperatures and under both laminar and turbulent flow conditions to estimate the instantaneous thickness and growth rate of a biofilm formed along the inner surface of a pipeline. Finally, a parametric study was conducted to determine the sensitivity of the model to each of the parameters influencing the rates of substrate consumption and biofilms growth rate and thickness.
10.2 Nomenclature

$C_0^*$  inlet substrate concentration to the pipe, g.m$^{-3}$

$R_B^*$  substrate consumption rate in the biofilm, g.m$^{-3}$.s$^{-1}$

$\Delta r$  radial position step size used in the numerical solution

$\Delta t$  time step size used in the numerical solution

$\Delta x$  axial position step size used in the numerical solution

$C$  non-dimensional substrate concentration

$C^* (x, r, t)$  time dependent substrate concentration at any point in the pipe, g.m$^{-3}$

$D$  diffusion coefficient of the substrate in the bulk flow, m$^2$.s$^{-1}$

$D_m$  molecular diffusion, m$^2$.s$^{-1}$

$D_x$  diffusion coefficient in the x direction, m$^2$.s$^{-1}$

$D_y$  diffusion coefficient in the y direction, m$^2$.s$^{-1}$

$F$  substrate flux, g.m$^{-2}$.s$^{-1}$

$F_r$  substrate flux in the radial direction, g.m$^{-2}$.s$^{-1}$

$F_x$  substrate flux in the x direction, g.m$^{-2}$.s$^{-1}$

$F_y$  substrate flux in the y direction, g.m$^{-2}$.s$^{-1}$

$F_z$  substrate flux in the z direction, g.m$^{-2}$.s$^{-1}$

$k_1$  constant in Monod equation, s$^{-1}$

$k_2$  constant in Monod equation, L.mg$^{-1}$

$K_m$  Monod half velocity coefficient, mol.m$^{-3}$

$L^*$  length of the pipe, m

$n$  number of time steps in the numerical solution

$q$  maximum substrate utilization rate, mol.g$^{-1}$.h$^{-1}$

$Q_{10}$  a factor by which $R(C)$ increases for a 10 °C rise above $T_e$

$QD_{10}$  a factor by which $R(C)$ decreases for a 10 °C rise close to $T_D$

$r$  non-dimensional radial distance
\( r^* \)  radial distance, m
\( R^* \)  substrate consumption rate in the bulk solution, g.m\(^{-3}\).s\(^{-1}\)
\( r_0^* \)  radius of the pipe, m
\( r_B \)  distance from the center of the pipe to the surface of the biofilm, m
\( Re \)  Reynolds number
\( R_n \)  the reaction term in the mass balance equation, g.m\(^{-3}\).s\(^{-1}\)
\( S \)  sink term in the mass balance equation, g.m\(^{-3}\).s\(^{-1}\)
\( T \)  temperature, °C
\( t \)  time, s
\( T_D \)  temperature at which bacteria death occurs, °C
\( T_e \)  temperature at which the reaction is measured experimentally, °C
\( t_L \)  average residence time in the pipe, s
\( U^* \)  the average velocity of the liquid flowing in the pipe, m.s\(^{-1}\)
\( u_r \)  velocity component in the \( r \) (radial) direction, m.s\(^{-1}\)
\( u_x \)  velocity component in the \( x \) (axial) direction, m.s\(^{-1}\)
\( X \)  non-dimensional axial distance
\( x^* \)  axial distance (along the pipeline), m
\( X_c \)  bacteria concentration, g.m\(^{-3}\)
\( Y_{B/s} \)  biomass yield (g biomass.g substrate\(^{-1}\))

*Greek Symbols*

\( \varepsilon \)  eddy diffusivity, m\(^2\).s\(^{-1}\)
\( \mu \)  viscosity, g.cm\(^{-1}\).s\(^{-1}\)
\( \rho \)  biofilm density, g.cm\(^{-3}\)
\( \tau \)  non-dimensional time
10.3 Introduction

Biofouling is the term used to describe the attachment of biological materials to surfaces (Mattila, 2002). Biofouling is a persistent problem for many industries. When microorganisms attach to surfaces they develop biofilms (O'Toole et al., 2000). A biofilm can therefore be defined as a structured community of bacterial cells enveloped in a self-produced polymeric matrix adherent to an inert or living surface (Costerton et al., 1999).

There are a number of factors that promote the adhesion of microorganisms to surfaces. Surface roughness, composition and hydrodynamic shear stress play a major role in the early stages of a biofilm formation and may influence the rate of cell accumulation and cell distribution (Cloete, 2003).

Biofilms play important roles, both beneficial and detrimental, in many natural and technological processes (Characklis and Cooksey, 1983). Important examples where biofilms occur are: trickling filters, marine and freshwater sediments, ship hulls, water and sewage pipelines, desalination, cooling towers, paper machines, porous media and bioreactors (Chen and Li, 1999; Kreft et al., 2001).

Biofilm process rates are controlled by mass transfer limitations in the bulk fluid phase and the compactness of the biofilm. For that, the substrate removal rate is dependent on fluid velocity past the biofilm (Characklis and Cooksey, 1983; Flora et al., 1993; Cheng et al., 1997; Soini et al., 2002). For laminar flow, utilization of substrate is diffusion limited in the fluid since radial mass transport is solely by molecular diffusion. In turbulent flow system, radial velocity fluctuations yield a much higher effective diffusivity. This results in a well-mixed fluid phase with the principal resistance residing in the biofilm (Kirkpatrick et al., 1980). Picioreanu et al. (2000) reported that the more compact the biofilm, the higher the global conversion rate of substrate.
To fully understand and optimize processes occurring in natural and industrial biofilms, it is necessary to determine the rates of both diffusive and convective mass transport. The concentration difference between the bulk liquid and the biofilm surface, the diffusion coefficient of the solute, and the thickness of the mass transfer boundary layer, determine the diffusive solute flux to the biofilm (De Beer et al., 1996).

Camper (1995) investigated the conditions contributing to the growth of biofilm bacteria, particularly coliforms, in simulated drinking water distribution systems. He found that substrate loading, temperature, hydraulic residence time, presence of chlorine, pipe material, and the initial growth rate of the introduced coliforms all affected the bacterial numbers and the biofilm growth. Suidan et al. (1989) reported that the dimensionless steady-state flux of substrate into a biofilm can be expressed as a function of the concentration of substrate in the bulk, the thickness of the stagnant liquid layer surrounding the biofilm, and the minimum concentration of substrate capable of sustaining growth.

In a long-term study on heterotrophic biofilms in tube reactors, Wasche et al. (2002) concluded that the hydrodynamic conditions and the substrate load during the growth phase of the biofilm in biofilm systems are two key parameters that influence the biofilm growth, particularly the structure, density and thickness. Zhu and Chen (2001) investigated the relationship between total ammonia nitrogen (TAN) removal rate and the Reynolds number \((Re)\) in a steady-state nitrification fixed biofilm system. Their results indicated that the hydraulic condition is an important factor that limits TAN removal rate.

Modeling and simulation of biofilm growth and substrate utilization rates have been approached by many researchers (Barton and Zhang, 1998; Lardon et al., 2002). Mathematical models allow the simulation of microorganism and biofilm growth and substrate transport and consumption rates in biofilm systems (Rittmann and McCarty, 1980; Wasche et al., 2000). Nagaoka (1997) developed a mathematical model to describe substrate profiles in biofilms under oscillatory flow conditions. He found that the substrate uptake rate by biofilms is proportional to the square root of Reynolds number and that the substrate uptake rate decreased with the decrease of Reynolds number of the wave motion.
Nagaoka and Sugio (1994) studied the effect of turbulent diffusion on substrate uptake rate by biofilms and developed a new turbulent diffusion biofilm model. They concluded that the substrate uptake rate by biofilms was dependent on the turbulence near biofilms.

Many researchers have studied the effect of temperature on biofilm growth rate and the rate of substrate consumption. Pedersen (1982) found that increased flow velocity, temperature, and nutrient concentration increased the biofilm production rate. Fdz-Polanco et al. (1994) reported that the temperature is a key parameter in the nitrification process producing two opposite effects: bacteria activation and free ammonia inhibition.

In calculating the rate of substrate consumption in a system containing both biofilm and suspended bacteria, both should be taken into consideration (Chen, 2001). Ozer and Kasirga (1995) studied the suspended and attached growth kinetics to explore the significance of aeration in sewer trunks. In their model, soluble substrate consumption of suspended growth microorganisms were taken into consideration.

In another study conducted by Yu et al. (2001), it was demonstrated that, during the two-step biodegradation of toluene in an aerobic circulating-bed biofilm reactor, both biofilm and suspended bacteria played critical roles. They found that although the suspended bacteria were less than 1% of the total amount of biomass in the system, they transformed up to 30% of the toluene into its intermediate in the bulk liquid phase.

Microbial activity within the biofilm creates a substrate flux from the bulk liquid to the biofilm. Microbial growth is usually represented with Monod kinetics; the rate of substrate utilization is assumed proportional to the rate of microbial growth and the flux of substrate is described as due solely to diffusion (Grady et al., 1999).
10.4 Derivation of the Model

10.4.1 Background

The model presented in this study extended previous modeling work by including diffusional transport, a generalized bulk reaction, and a sink term, and by solving the resulting transport equation for unsteady flow patterns. The model is based on the principle of mass transport and it can simulate a species consumption rate under different flow conditions. The model accounts for the axial convection and radial diffusion in a pipe under different flow regimes.

The transport of a given species in a liquid can be described quantitatively by the spatial and temporal evolution of its concentration; in other words, a differential equation for the concentration $C(x, y, z, t)$. The differential equation must account for the reactions (sources or sinks of the species).

The principle of conservation of mass requires that the chemical flux $F = (F_x, F_y, F_z)$ and the concentration $(C)$, satisfy the following mass balance equation (Perry and Green, 1997):

$$-\left(\frac{\partial F_x}{\partial x} + \frac{\partial F_y}{\partial y} + \frac{\partial F_z}{\partial z}\right) + R - S = \frac{\partial C}{\partial t}$$

(10-1)

The term inside the brackets on the left hand side of Equation (10-1) represents the chemical influx minus efflux. The right hand side of Equation (10-1) represents the temporal change of solute in the bulk fluid. $R$ and $S$ represent the source and sink terms, respectively.

10.4.2 Modeling Fluid Flow in a Pipeline

In this investigation, two-dimensional modeling was considered. Due to the nature of the system involved in this study (pipeline), a cylindrical coordinate system was chosen
(Figure 10-1-a). Therefore the above mass balance equation (Equation 10-1) can be re-written in the following form:

\[- \left( \frac{\partial F_x}{\partial x} + \frac{1}{r} \frac{\partial (r F_r)}{\partial r} \right) + R - S = \frac{\partial C}{\partial t} \]  

\[ (10-2) \]

For a two-dimensional cylindrical transport, the axial \((F_x)\) and radial \((F_r)\) solute flux can be derived solely from advection and dispersion as can be seen in Figure (10-1-b). For that, \(F_x\) and \(F_r\) can be described as:

\[ F_x = u_x C - D_x \frac{\partial C}{\partial x} \]  

\[ (10-2-a) \]

\[ F_r = u_r C - D_r \frac{\partial C}{\partial r} \]  

\[ (10-2-b) \]

In this investigation the radial velocity was neglected due to the assumption of symmetry. Therefore, Equation (10-2) can be re-written as (Bird et al., 2001):

\[- \left( \frac{\partial}{\partial x} \left( u_x C - D_x \frac{\partial C}{\partial x} \right) \right) - \frac{1}{r} \frac{\partial}{\partial r} \left( -r D_r \frac{\partial C}{\partial r} \right) + R - S = \frac{\partial C}{\partial t} \]

\[ (10-3) \]

Equation (10-3) represents a very general differential equation that can be applied in different applications like a fluid flow in cylindrical devices such as pipes. In this investigation the above differential equation was used to study the concentration profiles of a given substrate in a pipeline system.

The solution of the convective–diffusive mass balance equation (Equation 10-3) depends on the system under investigation. In other words, the solution of Equation (10-3) has to be subjected to prescribed boundary and initial conditions, which are characteristic of each system.
For unsteady transport of a substrate in a pipe of length $L^*$ and a radius $r_0^*$, the equation of material conservation accounting for the aforementioned mechanisms can be written as:

$$\frac{\partial C^*}{\partial t^*} = - \frac{\partial (U^* f(r^*) C^*)}{\partial x^*} + \frac{\partial}{\partial x^*} \left( D \frac{\partial C^*}{\partial x^*} \right) + \frac{1}{r^*} \frac{\partial}{\partial r^*} \left( r^* D \frac{\partial C^*}{\partial r^*} \right) + R^* \tag{10-4}$$

The term on the left-hand side of the Equation (10-4) accounts for changes in the substrate concentration with time. The first term on the right-hand side of Equation (10-4) describes advective transport, whereas the second and the third terms represent diffusion in the axial and radial directions, respectively. The last term on the right hand-side of Equation (10-1), $R^*$, can be either a general sink term or the substrate consumption rate by bacteria at any point in the system (pipe is this study). The $f(r^*)$ is a term that accounts for the flow profile present in the pipe.

The boundary and initial conditions required for the model solution can be formulated as:

$$x^* = 0 \quad ; \quad C^* = C_{0}^* \quad \tag{10-5-a}$$

$$x^* = L \quad ; \quad \frac{\partial^2 C^*}{\partial x^*} = 0 \quad \tag{10-5-b}$$

$$r^* = 0 \quad ; \quad \frac{\partial C^*}{\partial r^*} = 0 \quad \tag{10-5-c}$$

$$r^* = r_0 \quad ; \quad D \frac{\partial C^*}{\partial r^*} = -R_B^* \quad \tag{10-5-d}$$

$$t^* = 0 \quad ; \quad C^*(x^*, r^*) = C_{initial}^*(x^*, r^*) = 0 \quad \tag{10-5-e}$$
The function \( f(r^*) \) describes the radial velocity distribution in the pipe depending on the flow regime. For a laminar flow \((Re < 2100)\) in a pipe, \( f(r^*) \) has a parabolic shape which is described by the Poiseuille formulation:

\[
f(r^*) = 2 \left( 1 - \left( \frac{r^*}{r_0} \right)^2 \right)
\] (10-6)

For turbulent flow \((Re > 2100)\), a uniform velocity profile can be assumed:

\[
f(r^*) = 1
\] (10-7)

For stagnant conditions:

\[
f(r^*) = 0
\] (10-8)

As was mentioned before, \( D \) is the diffusion coefficient of the substrate in the liquid under investigation. In laminar flow, \( D \) is equal to the molecular diffusion coefficient \( D_m \). In turbulent flow, \( D \) represents the turbulent diffusivity which is the sum of the molecular diffusion coefficient \( (D_m) \) and the eddy diffusivity \( (\epsilon) \), which is dependent on the distance from the wall. Based on Reichardt’s law for a pipe, \( \epsilon \) can be calculated from:

\[
\epsilon = 0.4U^* \cdot (r_0^* - r^*) \sqrt{\frac{\alpha}{8}} \cdot \frac{1 + r^*}{r_0^*} \cdot \frac{1 + 2 \left( \frac{r^*}{r_0^*} \right)^2}{6}
\] (10-9)

where \( \alpha \) is given by:

\[
\alpha = \frac{1.32547}{\left[ \ln \left( \frac{e}{3.7(2r^*) + \frac{5.74}{(Re)^{0.89}}} \right) \right]^2}
\] (10-10)
In order to reduce the modeling effort and to make the model applicable to a wider range of cases, Equation (10-4) was transformed into a dimensionless form. The non-dimensional form of the model was obtained by defining the following dimensionless quantities:

\[ C = \frac{C^*}{C_0^*} \quad (10-11-a) \]

\[ X = \frac{x^*}{L^*} \quad (10-11-b) \]

\[ r = \frac{r^*}{r_0^*} \quad (10-11-c) \]

\[ \tau = \frac{t^*}{t_L} \quad (10-11-d) \]

\( t_L \) can be calculated as follows:

\[ t_L = \frac{L^*}{U^*} \quad (10-12) \]

Using Equations (10-11-a to 10-11-d), Equation (10-4) can be rewritten as:

\[ \frac{\partial C}{\partial \tau} = -f(r) \frac{\partial C}{\partial X} + \frac{D}{U^*L^*} \frac{\partial^2 C}{\partial X^2} + \frac{1}{r} \frac{\partial}{\partial r} \left( \frac{L^*}{r_0^*} \frac{U^*}{r} \frac{\partial C}{\partial r} \right) + \frac{L^*}{U^*C_0^*} R^* \quad (10-13) \]

The boundary and initial conditions were also rewritten in the non-dimensional form as follows:

\[ X = 0, \quad C = 1 \quad (10-14-a) \]

\[ X = 1, \quad \frac{\partial^2 C}{\partial x^2} = 0 \quad (10-14-b) \]
\[ r = 0, \quad \frac{\partial C}{\partial r} = 0 \]  
\[ (10-14-c) \]

\[ r = 1, \quad D \frac{\partial C}{\partial r} = -\frac{r_0^*}{C_0} R_B^* \]  
\[ (10-14-d) \]

\[ t = 0, \quad C(\mathbf{x}, r) = C_{\text{initial}}(\mathbf{x}, r) = 0 \]  
\[ (10-14-e) \]

10.4.3 Substrate Consumption Rate

The rate of substrate consumption in the bulk flow (\( R^* \) in Equation 10-4) and in the biofilm (\( R_B^* \) in Equations 10-14 and 10-15) can be calculated using the Monod equation (Equation 10-15). That is because microbial growth is usually represented with Monod kinetics and the rate of substrate utilization is assumed proportional to the rate of microbial growth (Grady et al., 1999).

\[ R^* = \frac{q X_v C^*}{K_m + C^*} \]  
\[ (10-15) \]

In calculating the rate of the substrate consumption at any point in the bulk solution, the bulk concentration of the substrate at that point was taken into consideration. On the other hand, to calculate the rate of substrate consumption in the biofilm, the substrate concentration at the biofilm surface was taken into consideration.

It is worth mentioning here that Monod equation can also be written as:

\[ R^* = \frac{k_1 C^*}{1 + k_2 C^*} \]  
\[ (10-16) \]
10.4.4 Biofilm Growth Rate

Since the rate of substrate utilization is proportional to the rate of microbial growth (Grady et al., 1999), the increment in the biofilm thickness can be calculated from the mass balance on the biofilm surface (Equation 10-17). Part of the substrate entering the biofilm will be converted to biomass. The ratio between the biomass produced from the consumption of a known mass of the substrate is called the biomass yield ($Y_{B/s}$).

$$r_B^{(t+\Delta t)} = r_B' + \frac{D_m C_0 \left[ Y_{B/s} \right]}{\rho r_0^2} \left( \frac{\partial C}{\partial r} \right)_{r=r_s} \Delta t$$  (10-17)

It should be mentioned here that in this study the biofilm detachment was not taken into consideration.

10.4.5 Effects of the Operating Temperature

The substrate consumption rate is strongly temperature dependent. The temperature dependent consumption rate can be written as (Kirkpatrick et al., 1980):

$$\frac{R(T)}{R(T_c)} A \exp\left(-\frac{E}{T}\right) - A' \exp\left(-\frac{E'}{T^2}\right) \quad T < T_D$$  (10-18)

where:

$$E = \frac{T_c (T_c + 10) \ln Q_{10}}{10}$$  (10-18-a)

$$A = \exp\left(\frac{E}{T_c}\right)$$  (10-18-b)
\[ E' = \frac{(T_D - 10)^2T_D^2 \ln QD_{10}}{T_D^2 - (T_D - 10)^2} \]  
\hspace{1cm} (10-18-c)

\[ A' = A \exp\left(\frac{E}{T_D}\right) \exp\left(\frac{E}{T_D^2}\right) \]  
\hspace{1cm} (10-18-d)

### 10.5 Numerical Solution of the Model

In order to solve the model equations numerically for different combinations of parameters, the governing equation with its initial and boundary conditions were first transformed to the non-dimensional form then discretized using the FDT employing the ADI method.

The ADI scheme is superior comparing to many other numerical approaches such as the Barakat Clark and Crank-Nicholson methods, for several reasons (Carnahan et al., 1969; Ozdemir and Ger, 1999). First, the ADI scheme produces a stable solution regardless of the choice of the time step and the discretization grid size, and for that the model results will always be reasonable. Second, the ADI method creates a transient solution using the finite differences in the spatial directions \(x\) and \(r\) to find a solution at the next time level, while the Crank-Nicholson scheme uses finite differences in one spatial direction to obtain the steady state solution throughout the grid. Furthermore the Crank-Nicholson scheme is not capable of accounting for the axial spreading of species by diffusion. The ADI scheme also belongs to the class of numerical methods that are unconditionally stable and are second-order accurate with a total error of the order \(O((\Delta t)^2, (\Delta x)^2, (\Delta r)^2)\).

In applying the ADI scheme, the FDT were used to advance the solution in time at each location in the grid. In the ADI scheme, each time step is split into two half steps. During the first time step, the solution at the time level \((n+1/2)\) is created by using finite differences at the new time level \((n+1/2)\) for all partial derivatives in \(r\), and finite differences at the old time level \(n\) for all partial differences in \(x\). After that, and following
the same procedure, the solution at the end of the time step \((n + 1)\) will be considered with respect to \(x\).

It is worth mentioning here that the local area average substrate concentration, \(\overline{C}(X, t)\) (based on the pipe cross-sectional area), was calculated from the detailed substrate concentration profile \(C(x, r, t)\) by the following integral:

\[
\overline{C}(X, t) = \frac{\int_{0}^{r} C(x, r, t) \cdot r \cdot dr}{\int_{0}^{r} r \cdot dr}
\]  

(10-19)

Equation (10-19) was converted to a programmable form by employing the discrete values of \(C\) in a computational grid as follows:

\[
\overline{C}(X, t) = \frac{\sum_{j=1}^{j_{\text{max}}} C(i, j, t) \cdot r_{j} \cdot \Delta r_{j}}{\sum_{j=1}^{j_{\text{max}}} r_{j} \cdot \Delta r_{j}}
\]  

(10-20)

10.6 The Parameters and Constants used in solving the Model

The model was applied on a pipeline system that has a biofilm on its inner surface and water containing substrate (glucose) and bacteria (\textit{Pseudomonas aeruginosa}) is flowing through it. The inlet substrate concentration \((C_{0})\) was taken to be 100 mg.L\(^{-1}\). The pipe length \((L)\) and radius \((r)\) were taken to be 100 m, and 2.5 cm, respectively. The diffusion coefficient of the substrate in water was taken to be \(6 \times 10^{-6}\) cm\(^2\).s\(^{-1}\) (Baillard and Boyle, 1970).

The Monod equation was used to calculate the substrate consumption rates in both the biofilm and in the bulk flow. For the biofilm, the kinetic parameters in Monod equation
(Equation 10-16) $k_1$ and $k_2$ were taken to be 0.06 s$^{-1}$ and 0.0083 L.mg$^{-1}$ (Kirkpatrick et al., 1980), respectively. It was assumed that the amount of substrate being consumed in the bulk solution by the suspended bacteria is equal to 10% of that consumed in the biofilm (Yu et al., 2001). $Q_{10}$ and $QD_{10}$ in Equations (10-18-a and 10-18-c) were taken to be 2 and 5 respectively (Kirkpatrick et al., 1980).

The biomass yield ($Y_{B0}$) was taken to be 0.61 g biomass.g substrate$^{-1}$ (Bakke et al., 1990). The biofilm density was taken to be 1 g.cm$^{-3}$ which is the density of water. That is because approximately 87-99% of the content of a biofilm is water (Characklis et al., 1981). The temperature inside the pipe was taken to be 20, 30 and 45 °C. Different values of Re number both in the laminar and turbulent regimes were also taken into consideration.

10.7 Results and Discussion

The results presented in this study are of importance in the industrial system that often requires the knowledge of the substrate concentration values in a pipeline. Indeed, the substrate concentration values determine the rates and extents of the processes associated with the presence of bacteria and biofilm growth.

An example of that is the microbiologically influenced corrosion (MIC), where the sulfate concentration which is the substrate for the sulfate reducing bacteria (SRB), plays a very important role in determining the rate and extent of the resulting MIC. Another example worth mentioning here is the heat loss due to the formation of biofilms on the inner surfaces of the pipelines used in heating applications like in heat exchangers.

From the results of this investigation, and as a general conclusion, it was observed that the biofilm thickness values and the rate of increase in the biofilm thickness were decreasing along the pipe.
10.7.1 Effect of Reynolds Number (Flow Velocity)

10.7.1.1 Laminar flow regime

It was observed that increasing $Re$ number increases the substrate steady state concentration values on the biofilm surface at any point along the pipe, as can be seen from Figure (10-2). Examining Figure (10-2) also shows that the effect of changing $Re$ number from 500 to 1000 was more pronounced than the change from 1000 to 1500. This observation can be justified by the fact that, in the laminar flow regimes (low $Re$) the substrate transport and utilization are diffusion limited. As $Re$ increases, the diffusional limitations become less and less pronounced.

Also increasing $Re$ number, which in this investigation means increasing the flow velocity in the pipe, decreases the residence time of the flowing fluid which contains the substrate in the pipe, and that in turn reduces the consumed amounts of the substrate along the pipe. The reduction in the substrate consumption increases the concentration values of the substrate at any point in the pipe.

It was also noticed from the results shown in Figure (10-2) that increasing $Re$ number increases the difference between the steady state values of the substrate concentration on the biofilm surface at $x = 0.25$ and $0.5$. On the other hand, it was found that increasing $Re$ number has a very little or no effect on the time needed for the substrate concentration to reach steady state values on the biofilm surface along the pipe. Those findings are in agreement with the previous discussion.

Figure (10-3) shows the effect of $Re$ number on the biofilm growth rate and thickness. From Figure (10-3), it is obvious that as $Re$ number increases the biofilm thickness at a given time and location in the pipe increases. The effect of increasing $Re$ number from 500 to 1000 was bigger than that from 1000 to 1500. The rate of increase in the biofilm thickness at a given point in the pipe was also found to increase by increasing $Re$ number as can be seen in Figure (10-3).
For laminar flow, utilization of substrate is diffusion-limited in the fluid since radial mass transport is solely by molecular diffusion (Flora et al., 1993). Consequently, an increase in Re increases the rate of mass transport (Flora et al., 1993). Since the rate of substrate utilization is proportional to the rate of microbial growth (Grady et al., 1999), the increment in the biofilm thickness would be proportional to the substrate consumption rate which is dependent on the fluid velocity past the biofilm (Flora et al., 1993; Cheng et al., 1997; Soini et al., 2002). These phenomena are in agreement with the observations made in this numerical study.

10.7.1.2 Turbulent flow regime

It was observed that increasing Re number in the turbulent flow regime greatly decreased the substrate steady state concentration values on the biofilm surface at any point along the pipe. Figure (10-4) shows the above finding at $x = 0.25$ and 0.5. Figure (10-4) also shows that the effect of increasing Re number from 2500 to 5000 was much more pronounced than the change from 5000 to 7500. In turbulent flow regimes the eddies increase the rate of mixing and lead to higher mass transfer in the pipe and towards the biofilm surface, which in turn results in higher substrate removal rates (Soini et al., 2002). This characteristic of the turbulent flow explains the results discussed above.

It was also mentioned before, that increasing Re number decreases the residence time of the flowing fluid which contains the substrate in the pipe. This fact causes a reduction in the consumed amounts of the substrate. On the other hand, increasing Re increases the mass transfer in the flowing fluid and that causes more efficient mixing in the pipe. This mixing allows the high substrate concentrations to reach to any point in the pipe and on the biofilm surface. As was mentioned before, the higher the substrate concentration, the higher would be the rate of consumption of the substrate (Characklis, 1990). As a result of the high consumption rate of substrate, its concentration will decrease on the biofilm surface along the pipe.
It was also noticed from Figure (10-4), that increasing $Re$ number decreases the difference between the steady state values of the substrate concentration on the biofilm surface at $x = 0.25$ and 0.5. On the other hand, and different from the findings in the laminar flow regime, it was found that increasing $Re$ number in the turbulent regime decreases the time needed for the substrate concentrations to reach steady state values on the biofilm surface at $x = 0.25$ and 0.5. Those results were expected and they are in agreement with what was mentioned before, resulting from the turbulent flow regime, higher diffusion, transport, and mixing of the substrate in the flowing fluid in the pipe.

The effect of $Re$ number in the turbulent regime on the biofilm growth and thickness is demonstrated in Figure (10-5). Examining Figure (10-5) leads to an interesting finding which is that: as $Re$ number increases the biofilm thickness at a given time and location in the pipe decreases. The effect of increasing $Re$ number from 2500 to 5000 was much bigger than the increase from 5000 to 7500. It was also observed from Figure (10-5) that the rate of increase in the biofilm thickness at any point along the pipe is decreasing by increasing $Re$.

The above results can be explained as follows: increasing $Re$ number increased the substrate consumption rate in the bulk solution due to the high mass transfer and the efficient mixing of the substrate in the pipe. The amount of substrate that was consumed in the bulk solution did not contribute to the biofilm growth.

On the other hand and due to the high substrate consumption in the bulk flow, the amount of substrate that managed to reach the biofilm surface was low. This in turn decreased the substrate concentrations on the biofilm surface. The low substrate concentrations on the biofilm surface reduced the amount of substrate that was consumed by the biofilm, and that results in a low biofilm growth rate.
10.7.2 Effect of Operating Temperature

10.7.2.1 Laminar flow regime

The increase in the operating temperature decreased the steady state values of the substrate concentration along the pipe as can be seen from Figure (10-6). When the temperature was increased from 20 to 30 °C, the decrease in the steady state substrate concentration values was observed to be bigger than the decrease when the temperature was increased from 30 to 45°C. When the temperature was increased above 20 °C, the substrate consumption rates were increased. The increase in the substrate consumption decreased the values of the substrate concentrations in the pipe.

The results demonstrated in Figure (10-6) also show that increasing the temperature decreased the difference between the substrate concentration values at $x = 0.25$ and 0.5. Increasing the temperature also decreased the time needed for the substrate concentration to reach steady state values at the biofilm surface, see Figure (10-6). Also Figure (10-6) shows that at a given time the difference between the substrate concentration values at $x = 0.25$ and $x = 0.5$, decreased by increasing the temperature.

On the other hand, as can be seen in Figure (10-7), the biofilm thickness values were found to decrease by increasing the temperature. This result contradicted the expected result which was that: since increasing the temperature above 20 °C increased the rate of substrate consumption, the biofilm growth rate and thickness were supposed to increase accordingly.

The above results can be justified and explained by the fact that increasing the temperature increased the substrate consumption rate in the bulk solution, which in turn decreased the amount of substrate that can reach the biofilm surface. Since the rate of biofilm growth is dependent on the rate of substrate consumption which is directly proportional to the substrate concentration on the biofilm surface, it can be concluded that the lower the
substrate concentration on the biofilm surface, the lower would be the biofilm growth rate and thickness.

It was reported that the increase in temperature might increase or decrease the rate of bacterial growth and substrate consumption rates (Cao and Alaerts, 1995). That depends on the optimum temperature for each micro-organism to grow and thrive. Fdz-Polanco et al. (1994) reported that the temperature is a key parameter in the nitrification process producing two opposite effects: bacteria activation and free ammonia inhibition.

Many other researchers (Characklis and Gujer, 1979; Characklis et al., 1990) found that the temperature of a given system influences the rate of chemical and biological reaction processes as well as transport processes taking place in that system. The influence of temperature on biofilm growth processes is much greater when the cells are growing near their maximum specific growth rate (Perry and Green, 1997).

10.7.2.2 Turbulent flow regime

The effect of temperature on the biofilm growth rate and thickness values in the turbulent flow regime were found to be similar to the results found in the laminar flow regime under the same conditions. Figure (10-8) shows that the increase in temperature decreased the steady state values of the substrate concentration along the pipe and on the biofilm surface.

The effect of increasing the temperature from 20 to 30 °C was found to be much bigger than its increase from 30 to 45°C.

By examining Figure (10-8), it can also be concluded that increasing the temperature decreased the difference between the substrate concentration values at $x = 0.25$ and 0.5, but it did not affect the time needed for the substrate concentration to reach steady state values on the biofilm surface.

Figure (10-9) shows that at a given time and location along the pipe, increasing the temperature decreased both the biofilm thickness and the rate of increase in the biofilm
thickness. It was also found that increasing the temperature decreased the difference between the biofilm thickness values at $x = 0.25$ and $x = 0.5$.

From the above discussion, it can be seen that the results and findings for the turbulent flow regime were similar to those for the laminar flow regime, under the same conditions. It is worth mentioning here that the effects of temperature on the biofilm growth and thickness were more pronounced in the turbulent flow regime than those in the laminar flow regime. These findings and results can be explained by the same facts mentioned in the previous section.

10.8 Conclusions

The model presented in this study was successful in estimating the biofilm thickness, the biofilm growth rate, and the steady state substrate concentration values on the biofilm surface at any point along the pipe. That was the main objective of this investigation.

There are many other findings and conclusions that resulted from solving the model under different conditions. They can be summarized as follows:

1. The biofilm thickness and the rate of increase in the biofilm thickness were found to decrease along the pipe.

2. Increasing the fluid flow velocity in the pipe in the laminar regime, decreases the residence time of the flowing fluid which contains the substrate, and that in turn reduces the consumed amounts of the substrate in the pipe.

3. The turbulent flow produces eddies that increase the rate of substrate mixing and lead to higher mass transfer in the pipe and towards the biofilm surface which in turn results in higher substrate consumption rates in the pipe.
4. It was observed that increasing Re number in the laminar flow regime increases both the steady state values of the substrate concentration on the biofilm surface and the biofilm thickness at any point along the pipe.

5. It was found that increasing Re number in the turbulent regime highly decreased the substrate steady state concentration values on the biofilm surface and decreased the biofilm thickness at any point along the pipe.

6. Increasing the operating temperature was found to decrease the steady state values of the substrate concentration and to decrease the biofilm thickness along the pipe.

7. It was observed that, the effect of temperature on the biofilm growth rate and thickness was more pronounced in the turbulent flow regime compared to the laminar flow regime, under the same other conditions.

10.9 References


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Figure (10-1) (a) A schematic diagram of the coordinate system and the control volume chosen for the biofilm-model development (b) mass balance on the control volume chosen for the biofilm-model development.
Figure (10-2) The effect of Reynolds number on the substrate concentration values on the biofilm surface under laminar flow conditions and an operating temperature = 30 °C
Figure (10-3) The effect of Reynolds number on the biofilm thickness under laminar flow conditions and an operating temperature = 30 °C
Figure (10-4) The effect of Reynolds number on the substrate concentration values on the biofilm surface under turbulent flow conditions and an operating temperature = 30 °C
Figure (10-5) The effect of Reynolds number on the biofilm thickness under turbulent flow conditions and an operating temperature = 30 °C.
Figure (10-6) Effect of the operating temperature on the substrate concentration values on the biofilm surface under laminar flow conditions (Re = 1000)
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CHAPTER 11

MODELING AND SIMULATION OF THE PITTING MICROBIOLOGICALLY INFLUENCED CORROSION IN DIFFERENT INDUSTRIAL SYSTEMS

11.1 Abstract

A very high percentage of the corrosion failures in the industry are caused or accelerated by the effects resulting from the activities of specific microorganisms. In aqueous environments, microorganisms tend to attach themselves to metal surfaces and form biofilms. These biofilms create nonuniform surface conditions, leading to severe localized corrosion, usually in the form of pitting. This type of corrosion is called microbiologically influenced corrosion (MIC), and it causes substantial environmental problems and huge economic losses worldwide.

One of the most dangerous and widely recognized types of microorganisms in MIC are the sulfate-reducing bacteria (SRB), which are known to be responsible for the microbial fouling in many industrial systems. SRB are strict anaerobes with the capacity to reduce sulfate to sulfide, which is a corrosive product to most of the metallic structures used in the industry.

In this study, a comprehensive mathematical model was developed in order to predict the shape and growth rate of the anaerobic pitting-MIC of steel in SRB environments. The transient two-dimensional model in cylindrical-coordinates was solved using the finite difference techniques, employing the alternating direction implicit (ADI) method. The SRB cathodic depolarization theory was adopted as the SRB-influenced corrosion mechanism.

The pitting-MIC model was applied to marine, waste, and freshwater environments. The effect of sulfate (substrate) concentration and SRB kinetic parameters on the growth rate and magnitude of the pitting MIC were also investigated.
The model was very successful in estimating and predicting the pitting MIC growth rate and the pit depths in different industrial applications. The model results were also found to be in good agreement with the experimental data found in the literature under the same conditions.

11.2 Introduction

Microorganisms tend to attach themselves to solid surfaces, colonize, proliferate, and form biofilms (Beer et al., 1994; Lee and Beer 1995). Biofilms on metal surfaces produce an environment at the biofilm/metal interface that is radically different from that of the bulk medium in terms of pH, dissolved oxygen, organic and inorganic species (Little et al., 1992; Lewandowski et al., 1997). Since the biofilm tends to create nonuniform surface conditions, localized attack might start at some points on the surface leading to localized corrosion, usually in the form of pitting (Lee and Beer 1995).

The term microbiologically influenced corrosion (MIC) is used to designate corrosion due to the presence and/or activities of specific microorganisms (Videla, 2001). These microorganisms can accelerate the rates of key reactions in the corrosion processes or even alter the corrosion mechanism (Hamilton, 1985). The MIC is becoming an increasing industrial problem at water-handling, crude oil industries, seawater systems and under marine fouling (Biezma, 2001). MIC can cause severe localized attack under biofilms leading to accelerated metal failures (Lee et al., 1992).

Industrial systems are likely to contain various structures where the MIC and biofouling can cause problems. The parts of the industrial system frequently suffering from such hazards are: open or closed cooling systems, water injection lines, storage tanks, residual water treatment systems, filtration systems, different types of pipes, reverse osmosis membranes, and potable water distribution systems (Videla, 2002).

Sulfate-reducing bacteria (SRB) are anaerobic microbes and they are known to be responsible for the microbial fouling on various surfaces such as: oil production facilities,
ship hulls, heat exchangers and wastewater pipelines (Cord-Ruwisch et al., 1987; Bryant et al., 1991; Odom, 1993). They use inorganic sulfate as a final electron acceptor in their respiration (Lee and Characklis, 1993).

SRB are known to be the most widely recognized microorganisms in the MIC (Hamilton, 1985). The anaerobic SRB-influenced corrosion of iron and alloys in contact with water is a conspicuous problem which brings about considerable economic losses in industry. According to Werner et al. (1998) SRB are considered to have at least two potentially corrosive effects: they create a biofilm having a crevice like geometry on the metal surface and they produce hydrogen sulfide.

Rainha and Fonseca (1997) studied the influence of the SRB *Desulfovibrio desulfuricans* on the anaerobic corrosion of mild steel in a lactate/sulfate medium. They confirmed that the presence of SRB induces changes in the kinetics and also in the mechanism of the anodic dissolution of iron in the lactate/sulfate medium.

Many researchers (Salvarezza and Videla, 1980; Hardy and Bown, 1984; Ringas and Robinson, 1988; McKenzie and Hamilton, 1992; Lee and Characklis, 1993; Starostetsky et al., 2000) observed the pitting-MIC of iron and steel in SRB media. Angell and Urbanic (2000) supported the hypothesis that an active SRB colony sustaining a source of metabolic sulfide is required to maintain pitting. They also concluded that a correlation exists between SRB activity and the initiation of pitting.

A high rate of pitting-corrosion (1 cm per year) in a 16-year-old subsea line that transported sour oil in the Gulf of Guinea was detected by Crolet and Magot (1996). This severe pitting-corrosion was attributed to the SRB and thiosulfate-reducing bacteria (Magot et al., 1997). Another flow line of an Indian offshore plant failed due to leakage after 6 years of continuous service. The damaged flow line exhibited localized corrosion resulting from the activity of SRB (Samanet et al., 1997). Thus, in studies of the SRB-influenced corrosion, it is very important to quantify the pitting corrosion.
Current conceptual models concur that there are three stages in the pitting corrosion: initiation, metastable pitting, and active pitting (Frankel, 1998). When microorganisms are involved in the corrosion of metals, the situation is more complicated than it is in an abiotic environment, because microorganisms not only modify the near-surface environmental chemistry via microbial metabolism but also may interfere with the electrochemical processes occurring at the metal-environment interface (Szklarska-Smialowska, 1986).

There are many factors and parameters that affect the SRB growth rate and the associated MIC. Fonseca et al. (1998) studied the influence of the media on the corrosion of mild steel by Desulfovibrio desulfuricans bacteria. They found that without sulfate the results obtained in sterile and in the SRB inoculated media are similar, while in the presence of sulfate a strong influence of the SRB on the corrosion process was observed.

It was found that Fe$^{2+}$ availability had a crucial effect on the physiological properties and growth of SRB and its relevance in the process of anaerobic MIC (Postgate, 1984; Marchal et al., 2001). Sulfide is also known to exert an inhibitory effect on the growth of SRB (Okabe et al., 1992; Okabe et al., 1995). The effect of H$_2$S and that of H$^+$ on the growth of SRB and the anodic dissolution of metals have also been investigated and established (Pankhania, 1988; Reis et al., 1992; Cheng et al., 1998).

The anaerobic corrosion of iron was noted in the 19th century and many theories were proposed about its mechanism. Von Wolzogen Khür and van Der Vlugt (1934) proposed the theory of cathodic depolarization to explain the role of SRB in the anaerobic MIC of steel. They reported that SRB, which consume hydrogen in their metabolisms, could shift a possible equilibrium between proton and hydrogen in solution and consequently increase the rate of proton reduction and that should result in enhancing the oxidation of iron. This mechanism has been a matter of controversy for decades (Booth and Tiller, 1960; Tiller and Booth, 1962; Iverson, 1966; Costello, 1974; Hamilton, 1985; Iverson, 1987; Iverson, 2001; Silva et al., 2002).
Many other researchers (Cord-Ruwisch and Widdel, 1986; Daumas et al., 1988; Pankhania, 1988; Bryant et al., 1991) supported the theory of von Wolzogen Kuhr and van Der Vlugt (1934) that microbial hydrogenases can accelerate the corrosion process by consumption of hydrogen arising from the cathodic reaction.

Peng and Park (1994) studied the effects of SRB and anodic or cathodic depolarization on steel corrosion when 1.5 mM of CaCO3 was added to the SRB media. They found that the polarization curves obtained in the SRB-inoculated solution with 0.5 mM sulfate concentration indicated that both anodic and cathodic depolarization occurred on steel coupons. They also noticed that only cathodic depolarization was obtained in the SRB-inoculated solution with 0.1 mM sulfate concentration, due to low hydrogen sulfide production. In an experimental study of the MIC, Peng et al. (1994) studied the effects of sulfate concentration on the pitting-MIC. They found that the corroded pits were deeper at the higher sulfate concentration values.

The overall process which justifies the corrosive attack by the SRB on ferrous alloys can be expressed by the following series of reactions, proposed by von Wolgozen Kuhr and van Der Vlugt (1961):

\[ \text{Fe} \rightarrow \text{Fe}^{2+} + 8e^- \]  \hspace{1cm} (11-1)

\[ 8\text{H}_2\text{O} \rightarrow 8\text{H}^+ + 8\text{OH}^- \]  \hspace{1cm} (11-2)

\[ 8\text{H}^+ + 8e^- \rightarrow 8\text{H}_{ad} \]  \hspace{1cm} (11-3)

\[ \text{SO}_4^{2-} + 8\text{H}_{ad} \rightarrow \text{S}^{2-} + 4\text{H}_2\text{O} \]  \hspace{1cm} (11-4)

\[ 4\text{Fe}^{2+} + \text{S}^{2-} + 6\text{OH}^- \rightarrow \text{FeS} + 3\text{Fe(OH)}_2 \]  \hspace{1cm} (11-5)
11.3 Modeling and Simulation of the Pitting-MIC

11.3.1 Governing Equations

The transport of a given species in a fluid system can be described quantitatively by the mass conservation equation:

$$\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \ u) = 0$$  \hspace{1cm} (11-6)

In the case of incompressible liquids, the local change of the species concentration \( C(x, y, z, t) \) can be calculated from the modified mass conservation equation:

$$\frac{\partial C^*}{\partial t} + (\vec{u} \cdot \nabla)C^* = D \ \nabla^2 C^* + R^* - S^*$$ \hspace{1cm} (11-7)

where:

- \( C^* \) = concentration of species, mol/m\(^3\)
- \( \vec{u} \) = vector mass velocity, m/h
- \( D \) = species effective diffusion coefficient, m\(^2\)/h
- \( R^* \) = reaction rate, mol/m\(^3\)-h
- \( S^* \) = sink term, mol/m\(^3\)-h

In this work the concentration profiles of sulfate (\( SO_4^{2-} \)) inside a corroded pit must be calculated first to be used later in calculating the pit depths, width and growth rates (Figure 11-1). To achieve this, Equation (11-7) can be used but with the convection term \((\vec{u} \cdot \nabla)C^* = 0\) due to the stagnant conditions inside the pit. Both \( R^* \) and \( S^* \) will be joined together in one term to simplify the equation. Therefore Equation (11-7) can be rewritten as:
\[ \frac{\partial C_{SO_4}^{*}}{\partial t} = D_{SO_4}^{*} \nabla^2 C_{SO_4}^{*} + R_{SO_4}^{*} \]  
\[ (11-8) \]

The second term on the right hand side of Equation (11-8) can be calculated using Monod equation (Grady et al., 1999):

\[ R_{SO_4}^{*} = \frac{q X_p C_{SO_4}^{*}}{K_m + C_{SO_4}^{*}} \]  
\[ (11-9) \]

where:

- \( R_{SO_4}^{*} \) = consumption rate of sulfate inside the pit, mol/m\(^2\)-h
- \( q \) = maximum sulfate utilization rate, mol/g-h
- \( X_p \) = SRB concentration inside the pit, g/m\(^3\)
- \( K_m \) = Monod half velocity coefficient, mol/m\(^3\)

For \( \theta \)-independent (axisymmetric) problems, Equation (11-8) can be rewritten in cylindrical coordinates as:

\[ \frac{\partial C_{SO_4}^{*}}{\partial t} = D_{SO_4}^{*} \left[ \frac{\partial^2 C_{SO_4}^{*}}{\partial r^2} + \frac{1}{r} \frac{\partial C_{SO_4}^{*}}{\partial r} + \frac{\partial^2 C_{SO_4}^{*}}{\partial z^2} \right] + R_{SO_4}^{*} \]  
\[ (11-10) \]

11.3.2 Initial and Boundary Conditions

A rectangular micro-pit with a length \( (2r^*) \) and width \( (z^*) \) equal to 2 and 1 \( \mu \)m, respectively, was taken to be the initial condition (at \( t = 0 \)) for the pit model, as shown in Figure (11-2).
The initial concentration profile of sulfate inside the micro-pit was assumed to be:

\[
C_{\text{SO}_4^{2-}}(r^*, z^*, t = 0) = C_0 \left[ \frac{\left( e^{-z^*} - 0.367879 \right) \cos \left( \frac{\pi r^*}{2} \right) \cos \left( \frac{\pi z^*}{2} \right)}{0.632121} \right]
\]  
\[(11-11)\]

The boundary conditions were taken to be (for more details, see Figure (11-1)):

\[C_{\text{SO}_4^{2-}} = C_0 \text{ at } z^* = 0\]  
\[(11-12)\]

where \(C_0\) is the sulfate concentration in the bulk solution.

\[\frac{\partial C_{\text{SO}_4^{2-}}^*}{\partial r^*} = 0 \text{ at } r^* = 0 \text{ (axisymmetric geometry of the pit)}\]  
\[(11-13)\]

\[-D_{\text{SO}_4^{2-}} \frac{\partial C_{\text{SO}_4^{2-}}^*}{\partial n^*} = R_B^* \text{ on the inner surface of the pit (biofilm surface)}\]  
\[(11-14)\]

where \(n^*\) is the unit outward normal to the inner surface of the pit, and \(R_B^*\) is the rate of sulfate utilization by the biofilm formed on the pit inner surface (Peng et al., 1994).

\[R_B^* (C_{\text{SO}_4^{2-}}^*) = \frac{q_B X_f L_f C_{\text{SO}_4^{2-}}^*}{K_m + C_{\text{SO}_4^{2-}}^*}\]  
\[(11-15)\]

where:

\(q_B = \text{maximum sulfate utilization rate by SRB in the biofilm, mol/g-h}\)

\(X_f = \text{SRB concentration in the biofilm, g/m}^3\)
$L_f = \text{biofilm thickness, m}$

$K_{mB} = \text{Monod half velocity coefficient for SRB in the biofilm, mol/m}^3$

11.3.3 Non-Dimensional Form of the Model Equations

To reduce the modeling effort and to make the model applicable to a wide range of cases, it is advisable to transform the model equations into a non-dimensional form. Also to simplify dealing with the dimensionless equations, the superscript (*) and subscript ($SO_4^{2-}$) will be dropped. By defining the following non-dimensional quantities, the non-dimensional model equations can be derived as follows:

$$C = \frac{C_{SO_4^{2-}}^*}{C_0}$$

(11-16)

$$r = \frac{r^*}{L}$$

(11-17)

$$z = \frac{z^*}{L}$$

(11-18)

$$n = \frac{n^*}{L}$$

(11-19)

$$\tau = \frac{t \cdot D_{SO_4^{2-}}}{L^2}$$

(11-20)

where $L$ is the characteristic length. In this study $L$ was taken to be 1 $\mu$m to coincide with the dimensions of the initial pit.
Substituting the above non-dimensional quantities into Equation (11-10) and after rearranging, gives the following non-dimensional governing equation:

\[
\frac{\partial C}{\partial \tau} = \frac{\partial^2 C}{\partial r^2} + \frac{1}{r} \frac{\partial C}{\partial r} + \frac{\partial^2 C}{\partial z^2} + \mathcal{R} \tag{11-21}
\]

where:

\[
\mathcal{R} = \frac{R_{so_i}^* L^2}{C_0 \cdot D_{so_i}^*} \tag{11-22}
\]

The non-dimensional initial and boundary conditions can also be derived using the non-dimensional quantities defined in Equations (11-16 to 11-20), as follows:

At \( t = 0, \ \tau = 0 \) and the non-dimensional initial sulfate concentration profile inside the micro-pit can be written as:

\[
C(r, z, \tau = 0) = \frac{C_{so_i}^* (r, z, t = 0)}{C_0} = \left[ \frac{\left( e^{-z} - 0.367879 \right) \cos \left( \frac{\pi r}{2} \right) \cos \left( \frac{\pi z}{2} \right)}{0.632121} \right] \tag{11-23}
\]

At \( z^* = 0, z = 0 \) and \( C = 1 \) \( \tag{11-24} \)

At \( r^* = 0, \ \frac{\partial C^*}{\partial r^*} = 0 \) (axisymmetry of the pit), therefore at \( r = 0, \ \frac{\partial C}{\partial r} = 0 \) \( \tag{11-25} \)

On the biofilm surface formed on the inner surface of the pit \(- D_{so_i}^* \frac{\partial C_{so_i}^*}{\partial n^*} = R_b^* \) and therefore:
\[- D_{SO_4^2-} \frac{\partial C}{\partial n} = \frac{L R_B^*}{C_0} = R_B \]  \hspace{1cm} (11-26)

11.3.4 Estimating the Pit Depth Growth Rate

The overall reaction of the SRB-influenced corrosion, which is shown in Equation (11-27), can be derived from the series of reactions shown in Equations (11-1 to 11-5).

\[4Fe + SO_4^{2-} + 4H_2O \rightarrow FeS + 3Fe(OH)_2 + 2OH^- \]  \hspace{1cm} (11-27)

From Equation (11-27), it can be seen that the corrosion rate of iron \( CR_{Fe} \), \( mol/m^2\cdot h \) is equal to four times the consumption rate of sulfate by the SRB biofilm. This ratio \( (N) \) can be defined as:

\[ N = \frac{CR_{Fe}}{R_B^*} \]  \hspace{1cm} (11-28)

From Equations (11-28) and (11-26), \( CR_{Fe} \) can be calculated as follows:

\[ CR_{Fe} = N \cdot R_B^* = - N \frac{C_0}{L} D_{SO_4^2-} \frac{\partial C}{\partial n} \]  \hspace{1cm} (11-29)

On the other hand, \( CR_{Fe} \) can also be calculated from the amount of oxidized iron, as follows:

\[ CR_{Fe} = \frac{V \rho}{(MW_i) t A} \]  \hspace{1cm} (11-30)

where:

\[ V = \text{volume of oxidized iron, m}^3 \]
$MWt = \text{molecular weight of iron, g/mol}$

$\rho = \text{density of iron, g/m}^3$

$t = \text{time, h}$

$A = \text{inner surface area of the pit, m}^2$

Since $\frac{V}{A} = \Delta L$  \hspace{1cm} (11-31)

Therefore Equation (11-30) can be rewritten as:

$$CR_{fe} = \frac{\rho}{(MWt)t} \Delta L$$ \hspace{1cm} (11-32)

The change in the pit depth (see Figure 11-1) can be calculated by equating Equations (11-29) and (11-32) to get:

$$\Delta L = -N \cdot \left( \frac{(MWt)D_{so_2^*}}{\rho} \right) \cdot \left( \frac{C_o}{L} \right) \cdot \left[ \frac{\partial C}{\partial n} \right] \cdot t$$ \hspace{1cm} (11-33)

Using Equations (11-15 and 11-26), Equation (11-33) can also be written as:

$$\Delta L = N \cdot \frac{MWt}{\rho} \left( \frac{q_B X f L_f C_{so_2^*}^*}{K_{mB} + C_{so_2^*}^*} \right) \cdot t$$ \hspace{1cm} (11-34)

### 11.4 Model Discretization and Method of Solution

The governing equation of the pitting-MIC (Equation 11-21) with its initial and boundary conditions (Equations 11-23 to 11-26) were numerically discretized using the finite difference techniques, and employing the alternating difference implicit (ADI) scheme. The ADI method is a well known numerical technique that is unconditionally stable regardless
of the step sizes in both the time and the spatial dimensions. It is also a second-order accurate with a total error of the order of \( O[(\Delta \tau)^2, (\Delta z)^2, (\Delta r)^2] \).

In the course of applying the ADI scheme, the finite difference techniques are used to advance the solution in time at each location in the grid. When using the ADI method, each time step is split into two half steps. During the first half time step, the solution at the time level \( n + \frac{1}{2} \) is created by using the finite differences at the time level \( n + \frac{1}{2} \) for all the partial derivatives in \( r \), and the finite differences at the time level \( n \) for all partial differences in \( z \).

Depending on the above discussion and by using the central differences for both the partial differences in \( r \) and \( z \), the governing Equation (11-21) can be approximated by:

\[
\frac{C_{i,j}^{n+\frac{1}{2}} - C_{i,j}^{n}}{\Delta \tau/2} = \frac{C_{i,j+1}^{n+\frac{1}{2}} - 2C_{i,j}^{n+\frac{1}{2}} + C_{i,j-1}^{n+\frac{1}{2}}}{(\Delta r)^2} + \frac{1}{r} \frac{C_{i+1,j}^{n+\frac{1}{2}} - C_{i,j}^{n+\frac{1}{2}}}{2\Delta r} + \frac{C_{i,j+1}^{n} - 2C_{i,j}^{n} + C_{i,j-1}^{n}}{(\Delta z)^2} + \mathcal{R}_{i,j}^{n}
\]

(11-35)

The above finite difference equation can be re-arranged so that all the terms involving the concentration \( C \) at the time level \( n + \frac{1}{2} \) to be on the left-hand side, and all other terms to be on the right hand side. The resulting equation after rearrangement can be written as:

\[
C_{i,j}^{n+1} \left( \frac{\Delta \tau}{4 \cdot r \cdot \Delta r} - \frac{\Delta \tau}{2(\Delta r)^2} \right) + C_{i,j}^{n+1} \left( 1 + \frac{\Delta \tau}{(\Delta r)^2} \right) + C_{i,j}^{n+1} \left( - \frac{\Delta \tau}{2(\Delta r)^2} - \frac{\Delta \tau}{4 \cdot r \cdot \Delta r} \right)
\]

\[
= C_{i,j}^{n} + \frac{\Delta \tau}{2} \left\{ C_{i+1,j}^{n} - 2C_{i,j}^{n} + C_{i-1,j}^{n} \right\} + \mathcal{R}_{i,j}^{n}
\]

(11-36)

Equation (11-36) which is an implicit equation of the concentration \( C_{i,j}^{n+\frac{1}{2}} \) can be rewritten in the form:
\begin{align}
a_{i,j} C_{i,j-1}^{n+\frac{1}{2}} + b_{i,j} C_{i,j}^{n+\frac{1}{2}} + c_{i,j} C_{i,j+1}^{n+\frac{1}{2}} &= r_{i,j}^{n} \\
\end{align}

where:

\begin{align}
a_{i,j} &= \frac{\Delta \tau}{4 \cdot r \cdot \Delta r} - \frac{\Delta \tau}{2(\Delta r)^2} \\
b_{i,j} &= 1 + \frac{\Delta \tau}{(\Delta r)^2} \\
c_{i,j} &= \frac{\Delta \tau}{2(\Delta r)^2} - \frac{\Delta \tau}{4 \cdot r \cdot \Delta r} \\
r_{i,j} &= C_{i,j}^{n} + \frac{\Delta \tau}{2} \left\{ \frac{C_{i+1,j}^{n} - 2C_{i,j}^{n} + C_{i-1,j}^{n}}{(\Delta z)^2} + \mathcal{R}_{i,j}^{n} \right\}
\end{align}

To solve the above implicit equation at \( r = 0 \), the boundary condition shown in Equation (11-25) must be first discretized as follows:

since:

\[ \frac{\partial C}{\partial r} \bigg|_{r=0} = \frac{\Delta C}{\Delta r} \bigg|_{r=0} = 0 \]

then:

\[ \frac{\partial^2 C}{\partial r^2} \bigg|_{r=0} = \frac{\Delta \left( \frac{\Delta C}{\Delta r} \right)}{\Delta r} \bigg|_{r=0} = \frac{\Delta (\Delta C)}{\Delta r} \bigg|_{r=0} = \frac{C_{i+\frac{1}{2}}^{n+\frac{1}{2}} - C_{i-\frac{1}{2}}^{n+\frac{1}{2}}}{\Delta r} - 0 = \frac{C_{i+\frac{1}{2}}^{n+\frac{1}{2}} - C_{i-\frac{1}{2}}^{n+\frac{1}{2}}}{(\Delta r)^2} \]
Using Equation (11-43) and the forward differences for all the locations at the centerline of the pit \((r = 0)\), Equation (11-21) can be rewritten (after rearrangement) in the following finite difference form:

\[
C_{i,2}^{n+1} \left( 1 + \frac{\Delta r}{2 \cdot (\Delta r)^2} \right) + C_{i,2}^{n+1} \left( \frac{-\Delta r}{2 \cdot (\Delta r)^2} \right) = C_{i,1}^{n} + \frac{\Delta r}{2} \left[ \frac{C_{i+1,1}^{n} - 2C_{i,1}^{n} + C_{i-1,1}^{n}}{(\Delta z)^2} + \mathfrak{R}_{i,1}^{n} \right] \quad (11-44)
\]

The implicit Equation (11-44) can also be simplified and rewritten as:

\[
b_{i,1} C_{i,2}^{n+1} + c_{i,1} C_{i,2}^{n+1} = r_{i,1}^{n} \quad (11-45)
\]

where:

\[
b_{i,1} = 1 + \frac{\Delta r}{2 \cdot (\Delta r)^2} \quad (11-46)
\]

\[
c_{i,1} = -\frac{\Delta r}{2 \cdot (\Delta r)^2} \quad (11-47)
\]

\[
r_{i,1}^{n} = C_{i,2}^{n} + \frac{\Delta r}{2} \left[ \frac{C_{i+1,1}^{n} - 2C_{i,1}^{n} + C_{i-1,1}^{n}}{(\Delta z)^2} + \mathfrak{R}_{i,1}^{n} \right] \quad (11-48)
\]

Another finite difference equation must also be derived at the inner surface of the pit (biofilm surface). To achieve this, Equation (11-26) should be discretized as follows:

\[
D_{so_{i-j}} \left[ \frac{1}{2} \left( \frac{C_{i,j_{\text{max}}}^{n+1} - C_{i-1,j_{\text{max}-1}}^{n+1}}{n_{i,j_{\text{max}}} - n_{i-1,j_{\text{max}-1}}} \right) + \frac{C_{i,j_{\text{max}}}^{n} - C_{i-1,j_{\text{max}-1}}^{n}}{n_{i,j_{\text{max}}} - n_{i-1,j_{\text{max}-1}}} \right] = R_{\gamma_{i-j}}^{n} \quad (11-49)
\]
Equation (11-49) can be simplified and rearranged as follows:

\[ a_{i,j} C_{i,j}^{n+\frac{1}{2}} + b_{i,j} C_{i-1,j}^{n+\frac{1}{2}} = r_{i,j}^n \]  \hspace{1cm} (11-50)

where:

\[ a_{i,j} = 1 \]  \hspace{1cm} (11-51)

\[ b_{i,j} = -1 \]  \hspace{1cm} (11-52)

\[ r_{i,j}^n = C_{i-1,j}^n - C_{i,j}^n + \frac{2}{D} \left( \frac{n_{i,j} \max - n_{i-1,j} \max - 1}{r_{i,j}^n} \right) \]  \hspace{1cm} (11-53)

A special tailored subroutine was prepared to account for the boundary condition shown in Equations (11-50 to 11-53). It is also worth mentioning here that \( n_{i,j} \max \) is a point on a line normal to the inner surface of the pit and it is calculated at every time step and at each location \( r \) and \( z \).

For each value of \( i \) the above mentioned implicit equations form a system of linear equations that can be written in a matrix form as follows:

\[
\begin{bmatrix}
  b_{i,1} & c_{i,1} & 0 & 0 & \ldots & 0 & 0 \\
  a_{i,2} & b_{i,2} & c_{i,2} & 0 & \ldots & 0 & 0 \\
  0 & a_{i,3} & b_{i,3} & c_{i,3} & 0 & \ldots & 0 \\
  0 & 0 & a_{i,4} & b_{i,4} & c_{i,4} & \ldots & 0 \\
  \vdots & \vdots & \vdots & \vdots & \ddots & \ddots & \vdots \\
  0 & \ldots & a_{i,j} \max - 2 & b_{i,j} \max - 2 & c_{i,j} \max - 2 & 0 & 0 \\
  0 & \ldots & 0 & a_{i,j} \max - 2 & b_{i,j} \max - 2 & a_{i,j} \max - 1 & 0 \\
  0 & \ldots & 0 & 0 & a_{i,j} \max - 1 & b_{i,j} \max - 1 & a_{i,j} \max \\
  0 & \ldots & 0 & 0 & 0 & a_{i,j} \max & b_{i,j} \max \\
\end{bmatrix}
\begin{bmatrix}
  C_{i,1}^{n+\frac{1}{2}} \\
  C_{i,2}^{n+\frac{1}{2}} \\
  C_{i,3}^{n+\frac{1}{2}} \\
  C_{i,4}^{n+\frac{1}{2}} \\
  \vdots \\
  C_{i,j}^{n+\frac{1}{2}} \\
  C_{i,j+1}^{n+\frac{1}{2}} \\
\end{bmatrix} =
\begin{bmatrix}
  r_{i,1}^n \\
  r_{i,2}^n \\
  r_{i,3}^n \\
  \vdots \\
  r_{i,j}^n \\
\end{bmatrix}
\]  \hspace{1cm} (11-54)
Another similar matrix can be derived for the z-direction in order to solve the model during the second half of the time step. Both matrices were solved using the Gauss elimination and the back substitution methods. A computer program was developed to perform this task.

11.5 Model Input Parameters and Constants

The pitting-MIC model was applied and solved for marine, fresh, and wastewater environments containing different types of SRB and different sulfate concentrations. The sulfate concentration values and the SRB kinetic parameters for each environment are summarized in Table (11-1). The kinetic parameters for SRB in the pit and in the biofilm were assumed to be the same (Okabe, 1992; Ozer and Kasirga, 1995; Chen, 2001).

The biofilm density ($X_f$) and thickness ($L_f$) on the pit inner surface were assumed to be constant and the same in the three studied environments. $X_f$ and $L_f$ were taken to be $48\times10^3$ g/m$^3$, and $239\times10^6$ m, respectively (Nielsen, 1987). The SRB density inside the pit ($X_p$) was assumed to be 1% of $X_f$ (Yu et al., 2001). The sulfate diffusion coefficient ($D_{SO_4}$) in water systems was taken to be $2.98\times10^6$ m$^2$/h (Nielsen, 1987). $C_{min}$ which is the minimum sulfate concentration that can be utilized by SRB was taken into consideration while solving the model. $C_{min}$ values in each environment are shown in Table (11-1). The molecular weight (MWt) and density ($\rho$) of the corroding iron were taken to be 55.845$\times10^3$ g/mmol and 7.86$\times10^6$ g/m$^3$, respectively. The ratio of iron corroded to sulfate consumed ($N$) was taken to be 4 mmol-iron/mmol-sulfate (see Equation 11-5).

11.6 Results and Discussion

Before the comprehensive pitting-MIC model was applied to the marine, fresh, and waste water environments, it was validated using experimental data found in the literature under similar conditions (Peng et al., 1994).
There are many factors and parameters that affect the SRB-influenced corrosion. The SRB kinetic parameters and the sulfate, sulfide and H⁺ concentrations as well as Fe²⁺ availability are all important in determining the severity of the MIC. In this study, the sulfate concentration and the SRB kinetic parameters were taken into consideration.

11.6.1 Marine Water Environment

Marine and seawater environments are known to be very aggressive to almost all metallic materials (Angeles-Chavez et al., 2001; Huang et al., 2004). In this work, the maximum depth of the SRB-influenced corroded pits (z values at r = 0) were calculated in a marine environment at different time intervals and the results are shown in Figure (11-3). It can be observed from Figure (11-3) that the maximum pit depth increased almost at a constant rate from time zero to day 190. After that, the rate of increase in the maximum pit depth with time started to highly decrease until day 785. After day 785, the rate of increase in the maximum pit depth with time reached approximately a constant value. As also can be seen from Figure (11-3), the maximum pit depth did not reach steady state values and kept slowly increasing with time even after 3000 days.

The above findings can be explained depending on the rate of sulfate diffusion inside the pit. At first, and for small pit depths, the concentration of sulfate inside the pit is almost the same as that in the bulk solution (see Figure (11-1)). As time passes and the pit gets deeper, it becomes more difficult for the sulfate to reach the deep biofilm formed on the inner surface of the pit. This in turn reduces the rate of iron corrosion on the pit inner surface, since it is proportional to the amount of sulfate consumed by the SRB biofilm (Mara and Williams, 1971), (for more details see Equation 11-27).

Figure (11-4) shows the shape and growth of the SRB-influenced corroded pits for the marine environment. It is obvious that the pit is growing in both the axial and radial directions. It can be observed from the results shown in Figure (11-4) that the rate of increase in the maximum pit depth is triple the rate of increase in its radius. This result is in agreement with Equation (11-33), which shows that the rate of increase in the pit
dimensions is directly proportional to $\frac{\partial C}{\partial n}$ value which is the highest at $r = 0$ for any $z$ and lowest at $z = 0$ for any $r$.

Figure (11-4) also shows that as time increases, the rate of pit depth growth decreases. Evidently, as the pit depth grows the sulfate availability decreases leading to the slowing down of bacterial growth and sulfate consumption rates. The bacterial growth and sulfate consumption are linked to the pitting-MIC. Consequently, corrosion rate slows down as the pit depth increases.

11.6.2 Wastewater Environment

Wastewater environments are reported to cause severe MIC of steel and many other metals (Englert and Muller, 1996). In this study, and in the case of the wastewater environment, it was observed from the results shown in Figure (11-5) that, the maximum depth of the SRB-influenced corroded pits increased almost at a constant rate from time zero to day 75. After that the rate of increase in the maximum pit depth with time started to slowly decrease until day 160. After day 160, the rate of decrease in the maximum pit depth with time reached a low constant value. At day 210 the maximum pit depth approximately reached steady state values, as can be seen from Figure (11-5). These results can be explained by the same principles discussed in the case of marine water environment.

The shape and the growth of the pit in both the axial and radial directions in the wastewater environment are shown in Figure (11-6). It can be observed from Figure (11-6) that, the rate of increase in the maximum pit depth is twice the rate of increase in its radius. It can also be seen that as time increases, the rate of increase in the maximum pit depth decreases. These observations are similar to what has been observed in the marine water environment, and they can be explained in the same way as was mentioned in section 11.6.1.
11.6.3 Freshwater Environment

The MIC of steel and stainless alloys in freshwater cooling systems and heat exchangers is a well known phenomenon in many industrial applications (Angell and Urbanic, 2000; Rao et al., 2000). Figure (11-7) shows the maximum depth of the SRB-influenced corroded pits at different time intervals in the freshwater environment. From Figure (11-7) it can be observed that the maximum pit depth increased almost at a constant rate from time zero to day 55. After that the rate of increase in the maximum pit depth with time started to sharply decrease until day 210. After day 210 the rate of decrease in the maximum pit depth with time reached a very low constant value, and at day 240 it reached to approximately zero. This means that the maximum pit depth is not increasing anymore beyond a certain maximum value. This is attributed to the sulfate diffusion limitations mainly at low bulk sulfate concentrations, which is the case in the freshwater environment.

Figure (11-8) shows the shape and growth of the SRB-influenced corroded pits in both the axial and radial directions for the freshwater environment. From Figure (11-8) it is obvious that the rate of increase in the maximum pit depth is three and a half times the rate of increase in its radius. It can also be seen from Figure (11-8) that as time increases, the rate of increase in the maximum pit depth decreases until it reaches a low steady state value, after which it goes down to zero. At this point (when the pit stops growing), it can be concluded that the pit has reached its steady state maximum value in the freshwater environment.

11.6.4 Comparison between the Three Different Environments

The comparison between the SRB-influenced corroded pits in the three different environments is shown in Figure (11-9). From Figure (11-9) it can be seen that in the first 290 days, the maximum pit depth values were the highest in the wastewater environment followed by the marine water environment and they were the lowest in the freshwater environment. On the other hand the maximum pit depth growth rate values were observed
to be in the same order as the maximum pit depth values from time zero until day 150. These results can be justified depending on the values of the sulfate concentration and the SRB kinetic parameters in each environment.

As can be seen from Table (11-1), the SRB $K_m$ value in the wastewater environment is 140 times lower than that in the marine water environment. As mentioned before, the lower $K_m$ values produce deeper maximum pit depths up to a certain critical depth, therefore the higher maximum pit depth values in the wastewater environment compared to marine water environments are justified. It is worth mentioning here that because the sulfate concentration in the marine environment was much higher than that in the freshwater environment, the effect of the high sulfate concentration value dominated the effect of $K_m$, and therefore the maximum pit depth values were observed to be lower than those in the marine environment.

As the pit gets deeper, the effect of $K_m$ decreases and becomes less influential on the pit growth comparing to that for the sulfate concentration inside the pit. After a certain time (290 days in this study) the maximum pit depth values were observed to be the highest in the marine water environment which has the highest sulfate concentration, followed by the wastewater environment and they were the lowest in the freshwater environment which has the lowest sulfate concentration. In a modeling study of the MIC conducted by Peng et al. (1994), the effects of the SRB kinetic parameters on the pitting-MIC were investigated. They found that increasing $K_m$ slightly decreases the rate of the MIC up to a certain level, after which the effect of increasing $K_m$ decreases.

By examining Figure (11-9), another interesting result concerning the time needed for the maximum pit depth to reach steady state value was observed. The maximum pit depth values were found to reach steady state conditions, first in the wastewater environment, followed by the freshwater environment, then the marine water environment. As the pit depth increases, the substrate availability decreases, leading to decreasing microbial activities and sulfate consumption rate. Such activities in turn decrease corrosion rate inside the pit, making the pit depth constant (steady state value). Peng et al. (1994) reported that,
the higher $q$ values gave higher steady state maximum pit depths under the same environmental conditions. It is worth mentioning here that, in the case of the marine water environment, the maximum pit depth values did not reach steady state conditions even after 3000 days. This was attributed to the very high values of the sulfate concentration in the marine water environment.

Figure (11-10) shows the steady state values of both the maximum pit depth and radius for the three studied environments after 3000 days. As can be seen from Figure (11-10), the steady state values of the maximum pit depth and radius were the highest in the marine water environment followed by the wastewater environment and they were the lowest in the freshwater environment. These results and findings are in full agreement with what has been mentioned in the previous sections.

11.7 Conclusions

1. The pitting-MIC model was very successful in estimating the corroded pit radius, depths and shapes at any given time, and under different environmental conditions.

2. The depth of the SRB-influenced corroded pits first increased with a constant rate followed by a gradual decrease until it reached to either low values as in the case of the marine water environment or dropped to zero as in both the wastewater and the freshwater environments.

3. During the first 290 days the maximum pit depth values were the highest in the wastewater environment, followed by the marine water environment and they were the lowest in the freshwater environment. After that and until the steady state conditions, the maximum pit depth values were the highest in the marine water environment, followed by the wastewater environment and lowest in the freshwater environment.

4. The ratio between the rates of increase in the maximum pit depth and radius was the highest in the freshwater environment (3.66), followed by the marine water environment (2.84) and was the lowest in the wastewater environment (2.21).
5. The sulfate concentration and the SRB kinetic parameters for each environment were found to have a strong and complex effect on both the shape and depth of the MIC pits.

6. In general, the modeling results showed that the marine water environment caused the highest SRB-influenced pitting corrosion attack, followed by the wastewater environment while the freshwater environment caused the lowest SRB-influenced pitting corrosion attack.

11.8 References


11.9 Appendices

11.9.1 Appendix A. Tables

Table (11-1) Sulfate concentration and SRB kinetic parameters for different environments

<table>
<thead>
<tr>
<th>Environment</th>
<th>( C_0 ) mmol/m³</th>
<th>( C_{\text{min}} ) mmol/m³</th>
<th>( K_m ) mmol/m³</th>
<th>( q ) mmol/g-h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marine water (Ingvorsen et al., 1984)</td>
<td>10000</td>
<td>15</td>
<td>200</td>
<td>4.2</td>
</tr>
<tr>
<td>Wastewater (Nielsen, 1987)</td>
<td>1000</td>
<td>1</td>
<td>1.4</td>
<td>0.75</td>
</tr>
<tr>
<td>Freshwater (Ingvorsen and Jorgensen, 1984)</td>
<td>200</td>
<td>0.5</td>
<td>7.3</td>
<td>0.8</td>
</tr>
</tbody>
</table>
Figure (11-1) Schematic diagram of the pitting SRB-influenced corrosion

Figure (11-2) Schematic diagram of the shape and dimensions of the initial pit assumed to be on the metal surface at $t = 0$
Figure (11-3) The maximum MIC pit depth values in the marine water environment
Figure (11-4) The MIC pit growth rates in both the axial and radial directions in the marine water environment.
Figure (11-5) The maximum MIC pit depth values in the wastewater environment
Figure (11-6) The MIC pit growth rates in both the axial and radial directions in the wastewater environment.
Figure (11-7) The maximum MIC pit depth values in the freshwater environment
Figure (11-8) The MIC pit growth rates in both the axial and radial directions in the freshwater environment
Figure (11-9) Comparison between the maximum MIC pit depth values in the marine, waste and fresh water environments.
Figure (11-10) Comparison between the steady state MIC pit depth values in the marine, waste and fresh water environments
CHAPTER 12

GENERAL DISCUSSION

At this point, it should have become clear that, the research that has been introduced in the previous chapters (2 to 11) has focused on using new materials and novel methods to control and estimate the degradation and corrosion of materials in different environments and applications. Selected materials are environmentally appealing and mostly inexpensive. The following brief discussions re-emphasize this objective of the research.

In Chapter 2, the influence of sulfate reducing bacteria (SRB), grown in a lactate/sulfate culture medium, on the corrosion of both uncoated and coated mild steel coupons was evaluated in the presence and absence of one the natural additives: olive and fish oils. Oil-based coating (alkyd) was used with and without the addition of natural additives to protect mild steel in a SRB environment. Another objective of this study was to investigate the effects of SRB and/or their metabolites on the alkyd coating and the adverse effect of the coating and additives on the SRB-biofilm formation and bacteria growth rates. The natural products were selected based on the environmental appeal of the products.

The selected natural additives were identified for effective MIC protection. Two additives, derived from olive oil and Manhaden fish oil were found to be effective in reducing the MIC. In general, 2-3% of the natural additive (oil in this study) was deemed adequate for effective MIC protection. The black ferrous sulfide detected on the surfaces of some of the tested coupons confirmed the activity of SRB on those surfaces and, as a result, the MIC attack. It is believed that the presence of SRB reduced sulfate to sulfide, which reacted with iron and produced the black ferrous sulfide.

The results also showed that the SRB-biofilms were heavily attached to the uncoated and coated surfaces without the presence of natural additives as discrete colonies rather than
continuous films. This explains the fast propagation rate of the localized (pitting) corrosion on those surfaces. The MIC attack appeared to proceed at a lower rate on the mild steel surfaces coated with the alkyd coating mixed with one of the natural additives. It is believed that this observation came as a result of the marked inhibition of the bacterial adhesion on those surfaces. This result may also be attributed to the fact that, some of the additives may have increased the corrosion resistance properties of the modified alkyd coating.

At the end of these sets of experiments, it was concluded that mixing natural products such as olive and fish oils with oil-based coatings like alkyd, showed positive results towards inhibiting both the biofilm formation, and the microbial influenced corrosion (MIC) effects on mild steel surfaces. The addition of natural additives is also believed to have enhanced the coatings protection efficiency.

In Chapter 3, the successful natural additives (olive and fish oils) as well as mustard oil were tested with another coating in a different environment. In this study, a set of experiments were conducted to investigate the effects of adding any of the previously mentioned natural oils to the enamel oil-based coating. After that, the coating degradation, performance, and protection efficiency were monitored with time. All tests were conducted according to the ASTM standards in a salt fog test corrosion chamber which simulates the corrosive marine environments.

Various parameters such as surface roughness, weight loss, and pit growth rate on the coated mild steel surfaces were measured using different techniques and equipment like the image analyzer, environmental scanning electron microscopy (ESEM) and energy dispersive X-ray analyzer (EDX).

It was noticed that the addition of olive and/or fish oils to the enamel oil-based coating improved its performance in the simulated marine environments. It is inferred that the moisture resistance of the coating increased in the presence of fish or olive oils. The surface roughness and the weight loss factor values for the oil-based coating surfaces were lowered
by 40-50% by the addition of the olive or fish oils to the coating. It was also observed that, the rate of degradation and growth of pits on the coated surfaces decreased in the presence of the olive or fish oils in the enamel oil-based coating. The presence of olive oil in the enamel oil-based coating was found to significantly decrease the coating degradation and loss of the coating from the coated surfaces, and thereby increased the protection efficiency of various coatings. All these demonstrated that the use of olive oil, fish oil, and other selected natural oils can lead to the development of new generations of coatings that are environmentally friendly yet are more resistant to the harsh corrosive environments than conventional coating materials.

Since the previously mentioned natural oils are to be used in different corrosive environments, such as the marine environment, it is vital to study their degradation and stability in such places. Chapter 4 discusses the biodegradability of various vegetable and fish oils under the influence of natural bacteria in seawater. The influence of nutrients and type and number of microbes in the environment on the extent and rate of degradation for various test oils (olive, mustard, canola and cod liver oils) were studied over time. Time-series visual and microscopic observations were made to characterize the physical changes in the residual oils, formation of floating and precipitate particles, oil droplets size and dispersion.

It was found that the biodegradation process was significantly influenced by the environmental conditions. High rates and extents of biodegradation were observed in seawater amended with nutrients and wastewater that contains elevated level of broad microbial cultures and nutrients. It was also observed that different oils responded in different rates and extents to biodegradation depending on their stability, viscosity and composition. Another output from this study (which came from observing the changes in the physical properties of the residual oil) is that, these findings may be important in the context of oil spill response strategies. For example, simple physical recovery methods may be used to recover polymeric lumps at the sea surface.
Because the previous study gave interesting qualitative results about the degradability of different types of oils in seawater, it was followed by a similar but a quantitative study which is presented in Chapter 5. The main objective of this study was to investigate quantitatively the degradability of various vegetable and animal oils in natural seawater, seawater inoculated with wastewater, and seawater enriched with nutrients. To achieve this, the ESEM, EDX and the Iatroscan techniques were used to study and analyze the different oil-contaminated samples.

The results showed that, the addition of any of the tested oils to each environment led to a high increase in the bacteria numbers in that environment. The results also confirmed that, both natural remediation (oxidation) and biodegradation took place in the oil-contaminated samples. Both the addition of nutrients and the elevated level of broad microbial cultures in the wastewater highly increased the degradation process. From all the generated results, it was clear that canola oil underwent the highest degradation, followed by mustard oil then cod oil and finally olive oil.

After the success of some natural oils in being used in the corrosion control applications, it made sense to test the effectiveness of many other natural materials to be used to combat corrosion. Chapter 6 illustrates and discusses the antimicrobial effects of eight natural products (Neem, olive leaves, chamomile, Salvia officinalis, Curcuma longa (Turmeric), Acacia nilotica (black thorn), fresh and dry Allium sativum (garlic), and cactus) on the Shewanella putrefaciens bacteria, which is known to be involved in many MIC problems.

In order to achieve the objectives outlined above, the bacteriostatic effects of various natural materials were investigated. From this list of natural materials, the ones with pronounced positive bacteriostatic results were selected for further test, namely, their bactericidal effects against the Shewanella putrefaciens bacteria. The bacteriostatic tests were conducted using the ditch-plate and Kirby-Bauer techniques, while the time-kill method was used to conduct the bactericidal tests.
The results showed that, only the black thorn and garlic possess pronounced bacteriostatic properties. The minimum inhibitory concentrations for these two materials were estimated using the Kirby-Bauer technique. It was also found that, the black thorn has lower minimum inhibitory concentrations compared to those for garlic.

After that, the apparent death rate and the decimal factor values were estimated for different concentrations of black thorn and garlic, using the bactericidal test method. The apparent death rate values were found to increase with increasing the garlic and/or black thorn concentrations, while the decimal factor values were found to decrease with increasing their concentrations. It was also observed that, at the same additives concentration, the value of the apparent death rate for black thorn was higher than the corresponding value for garlic. This result proved that, the black thorn has a stronger bactericidal effect than garlic. From the previous discussions and findings, it was concluded that both garlic and black thorn are effective to be used as natural bactericides against the *S. putrefaciens* corrosive bacteria.

The discovered bactericidal properties of both garlic and black thorn against *S. putrefaciens* encouraged to test them again in a sulfate reducing bacteria (SRB) environment. The SRB were chosen intentionally because these species are known to be the most dangerous bacteria associated with and involved in the MIC attacks on metallic structures. Chapter 7 contains the experimental work and the results generated from this study. The conducted tests focused on studying the influence of the surface topography of mild steel coupons and the effect of the addition of natural materials derived from garlic and/or black thorn on the biofilms growth and biocorrosion rates on the mild steel coupons immersed in the SRB media.

To accomplish this, a set of experiments was conducted on mild steel coupons with different surface topographies (roughness), immersed in SRB media for three months, with and without the addition of the natural materials. The conducted set of experiments was also used to study the effects of the addition of the selected natural materials on both the SRB-biofilms growth rate and biocorrosion rates on the immersed mild steel coupons.
At the end of these experiments, the coupons were removed from the SRB media and their surfaces were investigated by visual observations and studied using microscopic methods including the ESEM, EDX and the computer image analyzer techniques. The observations and results generated from the different test samples were compared with each other, and with the corresponding control test samples.

It was observed that, lower SRB-biofilm growth rates, and lower biocorrosion rates were detected on the smoother mild metal surfaces compared to the rougher ones under the same test conditions. It was also clear that the addition of the natural materials to the SRB media inhibited both the SRB-biofilms growth and the associated biocorrosion rates on the immersed mild steel coupon surfaces. As a result, it was concluded that both the surface topography of the mild steel coupons and the addition of the selected natural materials were very important in determining both the SRB-biofilms growth rate and the biocorrosion rates and extents on mild steel surfaces.

In the last set of experiments, the human hair waste materials were used as fibers to enhance the properties of cement mortar that make it more efficient to be used either in lining pipelines or as cement slurry to be placed in wellbores to harden into an impermeable mass that seals the annulus from fluid flow and protects the casing from corrosion.

This study, which is presented in Chapter 8, focused on using human hair fibers to reinforce cement mortar and improve its impermeability. The effect of the cement mortar mix proportions on the plastic shrinkage of the hair-fiber reinforced cement mortar has also been studied. The selected approach for this study was based on the factorial design of experiments, in which the considered parameters were cement/sand ratio (c/s), water/cement ratio (w/c) and hair-fibers content (fₕ).

The results showed that the effect of fₕ in reducing the shrinkage cracks of cement mortar is close to the c/s effect. It was also noticed that the effect of w/c on the cement mortar shrinkage cracks was minimal in the presence of hair-fibers. This observation might be
attributed to the ability of natural hair to absorb a substantial amount of water. It is also worth mentioning here that, the previous result is in contrast to the conventional belief, that the w/c is the most important controllable factor that affects the amount of shrinkage cracks in cement mortars.

From the generated experimental results, it was clear that, the natural hair-fibers were very effective in reducing the plastic shrinkage cracks area of cement mortar by a remarkable amount of 92%. The pronounced reduction in the cement mortar shrinkage cracks area helps in highly decreasing its permeability which in turn enhances its protection efficiency when used in lining applications. The other advantage of this study is that, instead of having the human-hair as a waste material that may cause nuisance to the environment, it has been converted into a useful material of economical value.

The other major part of the work focuses on the modeling and simulation of the microbiologically influenced corrosion (MIC). Modeling MIC is very important since it helps in calculating and predicting the corrosion rates and extents quickly, cheaply and accurately. This advantage becomes more important in the situations where it is very difficult or impossible to calculate the corrosion rate experimentally.

To develop a comprehensive model capable of accurately estimating the pitting MIC rates in different industrial systems, the modeling process was divided into three steps. In step one, which is presented in Chapter 9, a convective-diffusion model was developed and solved under various flow conditions using the finite difference technique (FDT), employing the alternating direction implicit (ADI) method. This model is called the substrate-concentration-model and it assumes that, a liquid containing substrate and bacteria is flowing in a pipeline with known inlet concentrations and then predicts the variation of the transient substrate concentrations in both the axial (along the pipeline) and radial (as a function of the pipeline radius) directions.

The substrate-concentration-model was then used to predict and estimate the substrate concentration profiles on the biofilm surface (which was assumed to be formed on the pipe
inner surface), under different operating conditions. A parametric study was also conducted to study the effect of the different parameters influencing the substrate concentration profiles in the system and on the biofilm surface, like the inlet substrate and bacteria concentrations, flow rate, and pipeline dimensions.

All the results generated from the substrate-concentration-model proved that the model is successful in estimating the substrate concentration profiles at any point in a pipeline system. It was observed that increasing the fluid flow velocity inside the pipe, which decreases the residence time of the flowing fluid, reduces the consumed amounts of the substrate inside the pipe. It was also confirmed that, in the turbulent flow regime the resulting eddies increased the rate of mixing and that led to higher mass transfer inside the pipe and towards the biofilm surface. Due to this fact, higher substrate consumption rates were observed inside the pipe.

The sink term (which represents the rate of substrate consumption in the bulk solution) was found to highly affect the resulting substrate concentration profiles on the biofilm surface. Finally, it was concluded that the model presented in this paper can be applied in a wide range of industrial applications either under static or flow conditions.

In the second step of the modeling process, the main objective was to develop a model capable of estimating the biofilm growth rate and thickness along a pipeline at any given time. To accomplish this, the convective-diffusion transient model was extended to include the substrate consumption rate in the bulk flow. Consequently, this model would predict both the sulfate concentration profiles and the biofilm growth rate and thickness in the pipeline system. The governing equations were discretized and solved using the ADI method.

The model was solved for different inlet substrate (sulfate) concentrations and different operating temperatures under various flow conditions (laminar and turbulent). The effect of the turbulence eddies on the substrate diffusion coefficient was thoroughly investigated and discussed. The instantaneous thickness of the biofilm along the pipe was calculated and
presented under various flow conditions. Finally a parametric study was conducted to study the sensitivity of the model to each of the parameters influencing the rate of the substrate consumption, the biofilm growth rate, and the biofilm thickness.

Both the biofilm thickness and the rate of increase in the biofilm thickness were found to decrease along the pipe. Increasing the fluid flow velocity within the laminar regime, which decreases the residence time of the flowing fluid that contains the substrate, was found to reduce the consumed amounts of the substrate inside the pipe. In the turbulent flow regime, the associated eddies were found to increase the rate of the substrate mixing and lead to higher mass transfer in the pipe and towards the biofilm surface. This effect increased the total substrate consumption rates inside the pipeline.

On the other hand, increasing Reynolds number (Re) in the laminar flow regime was found to increase both the steady state values of the substrate concentration on the biofilm surface and the biofilm thickness at any point along the pipe. Contrary to that, it was found that increasing the Re in the turbulent flow regime radically decreased the steady state concentration values of the substrate on the biofilm surface and also decreased the biofilm thickness at any point along the pipe.

It was also noticed that increasing the operating temperature decreased both the steady state values of the substrate concentration and the biofilm thickness along the pipe. The results also showed that, under the same conditions, the effect of temperature on the biofilm growth rate and thickness was more pronounced in the turbulent flow compared to that in the laminar flow regimes.

Finally, and from the results and discussions mentioned above, it was clear that the model presented in this paper is successful in estimating the biofilm thickness, the biofilm growth rate, and the steady state substrate concentration values on the biofilm surface at any point along the pipe. For that, the main objectives of this study were successfully achieved.
In the third and last step of the modeling process, and as can be seen in Chapter 11, the pitting MIC was modeled. In this study a numerical model was developed to study the anaerobic pitting MIC of a mild steel pipe in a SRB environment. The transient two-dimensional model in cylindrical-coordinates was then solved using the FDT, employing the ADI method. The SRB cathodic depolarization theory was adopted as the SRB-influenced corrosion mechanism. The pitting-MIC-model was applied to marine, waste, and freshwater environments. The effects of the sulfate concentration and the SRB kinetic parameters on the growth rate and magnitude of the pitting MIC were also investigated.

The pitting-MIC-model was proven to be successful in estimating the SRB-influenced corroded pit radius, depth and shape at any given time, under different environmental and operating conditions. It was observed that the rate of increase in the MIC pit depth first increased with a constant rate, then gradually decreased until it reached to either a very low value as in the case of the marine water environment, or dropped to zero as in both the waste and freshwater environments.

During the first 290 days of the modeling time, the corroded pit depth values were found to be the highest in the wastewater environment, followed by the marine water environment and lowest in the freshwater environment. After that and until the steady state conditions, the pit depth values were observed to be the highest in the marine water environment, followed by the wastewater environment and lowest in the freshwater environment. The ratio between the rate of increase in the pit depth to the rate of increase in its radius was found to be the highest in the freshwater environment, followed by the marine water environment and lowest in the wastewater environment.

Both the sulfate concentration and the SRB kinetic parameters were found to have great and complex effects on both the shape and depth of the MIC pits. The modeling results showed that the marine water environment caused the highest and most severe SRB-influenced pitting corrosion attack on the inner surface of the mild steel pipeline,
followed by the wastewater environment, and finally came the freshwater environment which caused the lowest pitting corrosion attack.

Finally, it can be concluded that, the main objectives of this thesis (finding and developing new materials and methods to combat, prevent and estimate corrosion in different industrial applications) have been successfully achieved.
CHAPTER 13

GENERAL CONCLUSIONS

From all the results and discussions presented in the previous chapters, it can be concluded that the main goals of the research included in this thesis (finding and developing new materials and methods to combat, prevent and estimate corrosion in different industrial applications) have been achieved.

The following conclusions are in support of the above claim:

1. Both the rotten-egg odor and the black corrosion products (ferrous sulfide) detected on some of the mild steel coupons immersed in SRB environments, confirmed the activity of the SRB and the associated MIC attack on the metal surfaces.

2. The SRB-influenced corrosion attack appeared to proceed at a lower rate on the surfaces coated with the alkyd oil-based coating mixed with one of the natural additives (olive and fish oils).

3. Mixing natural products like olive and fish oils with oil-based coatings like alkyd inhibited the biofilms growth rate on those coated surfaces.

4. The addition of olive and/or fish oils to the enamel oil-based coatings, improved its performance and protection efficiency in simulated marine environments.

5. The vegetable and fish oils biodegradation processes in marine environments were found to be significantly enhanced by the availability of nutrients and the presence of mixed microbial consortia.
6. Both auto-oxidation and biodegradation took place in the oil weathering process. It is believed that the oxidation process either accelerates the biodegradation rates by producing much smaller and easier compounds to be biodegraded, or inhibits the microbial attacks by producing antibacterial products.

7. Taking the effects of both the degradation susceptibility of the oil and the antibacterial effects of the metabolic byproducts of the oil degradation process into consideration, it can be concluded that canola oil has the highest degradation level, followed by mustard oil, then fish oil and finally olive oil.

8. It was found that, the natural products garlic (*Allium sativum*) and black thorn (*Acacia nilotica*) possess bacteriostatic and bactericidal effects against *Shewanella putrefaciens*, which is one of the bacteria associated with the MIC.

9. It was found that the black thorn has a lower minimum inhibitory concentration compared to that of garlic. Also the apparent death rate (*K*<sub>d</sub>) values for black thorn were found to be higher than those for garlic at the same concentration. All of that implied that black thorn has stronger bactericidal effects than garlic.

10. Pitting MIC was heavily detected on the mild steel coupon surfaces immersed in SRB media, which is “usually” a characteristic of the action of the SRB on metals.

11. The SRB biofilms were found to be thicker and more adherent to the surfaces of the mild steel coupons immersed in the SRB media with no added natural materials, compared to the ones containing natural materials (garlic or black thorn).

12. The severity of the SRB-influenced corrosion attack against mild steel coupons was found to highly decrease by the addition of either garlic or black thorn to the SRB corrosive media.

13. The biofilms growth rate as well as the MIC rate and extent were found to be higher on the rough mild steel coupon surfaces compared to those on the smooth coupon surfaces, under the same test conditions.
14. The efficiency of black thorn was observed to be more pronounced than that of garlic in inhibiting both the growth of SRB-biofilms and the associated MIC on mild steel surfaces having the same surface finish, and under the same test conditions.

15. Natural hair, which is a waste material that might be a nuisance to the environment, was converted into a useful material of economic value by using it as a fiber in the cement lining applications and cementing hydrocarbon wells.

16. The novel method of reducing the plastic shrinkage cracks of cement mortar using natural hair-fibers was successful in reducing the total surface shrinkage cracks area by 92%.

17. A transient two-dimensional substrate (sulfate) concentration-profile-model based on first principles and incorporating the substrate transport in a pipe by advection, axial and radial diffusions along with a generalized sink term $R$ (to account for the substrate consumption by suspended bacteria in the bulk fluid) was established and solved for various flow conditions.

18. The sulfate-concentration-profiles model was successful in estimating and predicting the substrate concentration profiles at any point in the pipeline system under different flow conditions.

19. The flow velocity inside a pipeline was found to highly affect the consumed amounts of substrate by the suspended bacteria and the biofilm inside the pipe.

20. The rate of substrate consumption in the bulk flow by the suspended bacteria was found to highly affect the resulting substrate concentration profiles on the biofilm surface and as a result it affects also the amounts of sulfate being consumed by the biofilm formed on the pipe inner surface.

21. A transient two-dimensional biofilm-growth model was developed and proved to be successful in estimating and predicting the biofilm growth rate and thickness at any point along a pipeline, under different operating conditions.
22. When the biofilm-growth model was applied to a pipeline system, the generated results showed that both the biofilm thickness and the rate of increase in the biofilm thickness decreased along the pipe.

23. The solution of the biofilm-growth model showed that increasing Reynolds (Re) number in the laminar flow regime increases the steady state values of both the substrate concentrations on the biofilm surface and the biofilm thickness at any point along the pipe, while increasing Re number in the turbulent flow regime gave opposite results.

24. When the biofilm-growth model was applied to the pipe system and solved for different bulk flow (operating) temperatures, it was found that increasing the operating temperature decreases the steady state values of both the substrate concentration and the biofilm thickness along the pipe.

25. Depending on the results generated from the biofilm-growth model, it was clear that the effect of temperature on the biofilm growth rate and thickness was more pronounced in turbulent flow regimes compared to laminar flow regimes, under the same other test conditions.

26. The established pitting microbial influenced corrosion (pitting-MIC) model proved to be capable of successfully and accurately estimating and predicting the corroded pit radius, depth and shape on mild steel surfaces at any given time, and under different environmental conditions.

27. The output results from the pitting-MIC model showed that the rate of increase in the MIC pit depth was first constant, followed by a gradual decrease until it reached to either low values as in the case of marine water environment, or dropped to zero as in both the wastewater and freshwater environments.
28. When the pitting-MIC model was solved for different corrosive environments, it was observed that, during the first 290 days the pit depth values were the highest in the wastewater environment, followed by the marine water environment and lowest in the freshwater environment. After that and until reaching steady-state conditions, the pit depth values were found to be the highest in the marine water environment, followed by the wastewater environment and lowest in the freshwater environment.

29. The results obtained from the pitting-MIC model showed that, the ratio between the rate of increase in the pit depth to the rate of increase in the pit radius was the highest in the freshwater environment, followed by the marine water environment and lowest in the wastewater environment.

30. Both the substrate (sulfate) concentration and the SRB kinetic parameters, which are input parameters to the pitting-MIC model, were found to have great and complex effects on both the shape and depth of the MIC pits.

31. The generated results from the pitting-MIC model predicted that the marine water environment is the most corrosive and dangerous environment in causing the SRB-influenced pitting-corrosion attack on mild steel surfaces, followed by the wastewater environment, and finally comes the freshwater environment.
CHAPTER 14

LIST OF ALL REFERENCES


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