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Evaluation of Sessile Microorganisms in Pipelines and Cooling Towers of Some Iranian Industries

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Microbiologically influenced corrosion (MIC) is a kind of electrochemical corrosion that is enhanced by the effect of certain microorganisms including sessile bacteria. In this investigation, more than 200 samples collected from different systems of Iranian refineries have been examined (by culturing methods and observations) for corrosion-enhancing, biofilm-producing microorganisms such as sulfate-reducing bacteria (SRB), iron-oxidizing bacteria (IOB), heterotrophic biofilm-forming bacteria (HBB), and some eukaryotes such as fungi. This study showed the presence of microorganisms, such as SRB, HBB, thermophillic HBB, and yeasts, except for IOB. It was also revealed that biocides are used to reduce the number of planktonic (floating) bacteria, instead of the number of sessile bacteria, that form biofilms. Using surfactants, or washing with chemicals like chlorine or organic acids in overhauls, may destroy the biofilm and free the residential bacteria into the bulk solution, thus exposing them to the biocide. For thick biofilms, a chlorine or acid wash may also yield the same results.

Keywords corrosion, heterotrophic biofilm-forming bacteria (HBB), iron oxidizing bacteria (IOB), microbiologically influenced corrosion (MIC), sessile microorganisms, sulphate reducing bacteria (SRB)

1. Introduction

Microbiologically influenced corrosion (MIC) is believed to account for 20% of the damage caused by corrosion (Ref 1). On the basis of gross national product, the annual MIC-related industrial loss in Australia is estimated to be $6 billion (in Australian dollars) (Ref 2). The MIC has caused a lifetime reduction of flow lines in Western Australia from the designed duration of +20 years to <3 years (Ref 3). In the Iranian petrochemical industry, many cases of MIC occur in gas pipelines (Ref 4), reformer heater tubes (Ref 5), and plant water networks in refineries (Ref 6).

Microbiologically influenced corrosion is the term used for the phenomenon in which corrosion is initiated and/or accelerated by the activities of microorganisms (Ref 7). The microorganisms that are mainly involved in MIC are bacteria (Ref 8). Other organisms that can affect the severity of MIC are either fungi that produce acids (Ref 9) or algae that form local electrochemical cells (Ref 10), both of which enhance corrosion.

This article discusses some groups of corrosion-enhancing bacteria and eukaryotes in systems such as pipelines, water and oil tanks, and cooling towers in Iranian refineries, and addresses how sessile bacteria can act against biocides. The article also addresses methods to reduce the corrosive effects of these microorganisms.

2. Mechanisms of Microbiologically Influenced Corrosion

When bacteria in aqueous environments encounter low amounts of the chemicals that are necessary for their growth (called *nutrients*), they stay in a "planktonic" state. However, if the level of nutrients is high, so that they sink onto surfaces, the bacteria will also try to "stick" on the surfaces and become "sessile." That is, they excrete an exopolymeric substance (EPS) that contributes to the formation of thin biofilms (Ref 11), which can harbor many types of microorganisms as well as inorganic material (Ref 12). More details about nutrient levels and biofilm formation have been discussed elsewhere (Ref 13).

The biofilm consists of cells that are immobilized in a substratum, frequently embedded in an organic polymer matrix of microbial origin (Ref 14). The gradual formation of microbial biofilms can significantly change chemical concentrations at the surface of a metal substrate. The physical presence of the biofilm also exerts a passive effect in the form of a restriction on oxygen diffusion to the metal surface. Active metabolism of the microorganisms, on the other hand, consumes oxygen and produces metabolites. The net result of biofilm formation is that it usually creates concentration gradients of chemical species across the thickness of the biofilm (or tubercle), which is typically between 10 and 400 μm (Ref 15). The influence of biofilm on corrosion can be divided into three general categories (Ref 16-18):

- Production of differential aeration or chemical concentration cells
- Production of organic and inorganic acids as metabolic byproducts
- Production of sulfides under oxygen-free conditions

Under the biofilm, factors such as pH and dissolved oxygen, for example, may be dramatically different from those in the bulk solution, resulting in a shift of the open-circuit potential of passive metals in the noble direction (called *ennoblement*).

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This has been well documented for a range of metals and alloys (e.g., stainless steel) at various salinities (Ref 11, 14, 19). In the presence of certain iron-oxidizing bacteria (IOB), as well as iron-reducing bacteria (Ref 20, 21), the local biofilm environment may become very acidic due to the combining of anions such as chlorides with the ferric ions that are produced by the bacteria. This combination forms an acidic ferric chloride solution inside the tubercle that is highly corrosive (Ref 11). More details have been discussed in other studies (Ref 21, 22).

Sulfate-reducing bacteria (SRB) derive their energy from organic nutrients. They are anaerobic (i.e., they do not require oxygen for growth and activity), and, as an alternative to oxygen, they use sulfate with the consequent production of sulfide (Ref 23). The ways in which SRB affects corrosion can be ranked as follows (Ref 24):

- Generation of sulfides by their growth
- Regeneration of fresh iron sulfide (FeS), enabling it to remain cathodic to the iron beneath
- Depolarization of the FeS cathode. It is easier to depolarize FeS than the steel because atomic hydrogen is usually quite strongly adsorbed onto steel surfaces. Thus, fresh surfaces are constantly contacted with steel by their movement

More details about SRB and the way they can affect corrosion have been discussed in Ref 23.

Almost all types of engineering materials have been reported to undergo MIC by SRB (e.g., copper and copper alloys; nickel; zinc; aluminum; titanium and its alloys [Ref 25-27]; mild steel [Ref 28-30]; and stainless steels [Ref 30-45]). More examples have been given in other references (Ref 16, 23). Duplex stainless steels such as SAF 2205 can corrode and undergo pitting due to the presence of SRB after immersion in seawater for >1 year (Ref 38).

The term iron bacteria (IB) is mainly used to describe IOB. For example, ASTM standard D932-85 defines IB as a general classification for microorganisms that use ferrous iron (Fe^{+2}) as a source of energy, and are characterized by the deposition of ferric (Fe^{+3}) hydroxide.

3. Materials and Experimental Methods

3.1 Sampling

Using sterile containers, about 200 samples were taken from oil and water pipes in desalination units, and also from the cooling towers of industrial units. Samples were taken from internal precipitates of the pipes, the biofilm formed on the leakages, and the precipitates formed at the bottom of tanks (under sterile conditions). Some of the preserved samples (i.e., frozen samples) were taken to the laboratory for investigation, while other samples were examined in the field.

3.2 Culture Media

To investigate bacterial species, the following culture media were used: IOB, consisting of *Sphaerotilus natans* and *Thiobacillus ferroxidance* 9K (Ref 46) and BOD (Ref 23); sulfate-reducing bacteria in API medium (Ref 24); biofilm-forming bacteria in BOD-diluted water (Ref 25); and plate count agar (Merck), fungi (Eukaryotic microbes), and potato dextrose agar (PDA) (Merck). Microscopic examination of the biofilms and microorganisms was carried out on an optical microscope at magnifications of 400× and 1000×.

4. Results

In the samples investigated, the existence of all types of main-group microorganism autotrophic IOB were observed (Table 1).

However, sheath-bearing heterotrophic IOB was found in a limited number of samples taken from the pipes. In almost all samples (~95%), and especially from the cooling tower samples, SRB were observed.

For biofilm-forming bacteria, it was found out that in cooling towers under chemical treatment by biocides and inhibitors, and with <1 ppm of free chlorine, there was far fewer *Pseudomonas* species and enterobacteriacea than in cooling towers with poor chemical control. Also, these bacteria were "permanent residents" in the oil and/or water pipes. The study showed that these bacteria were even present at a depth of about 3000 m in injected wells (Fig. 1).

Thermophillic, biofilm-forming *Bacillus* species were also isolated. In pure cultures, they produced huge amounts of EPS as a measure of biofilm formation. Application of the aforementioned treatments had little effect on their numbers so that they were actually present everywhere.

Enterobacteriacea (known widely as *intestine bacteria* and mainly found in rivers) were isolated from almost all samples, ranging from cooling towers to the bottom of deep injection wells. The fact that these bacteria were omnipresent despite all remediation treatments proved that they were quite resistant to adverse environmental factors. These bacteria were observed to

Table 1 Frequency of Fe-oxidizing bacteria as a percentage of the examined samples

Fe-oxidizing bacteria in samples	%
Sphaerotilus natans	5%
Thiobacillus ferroxidance	Nil
Gallionella ferroginea	Nil

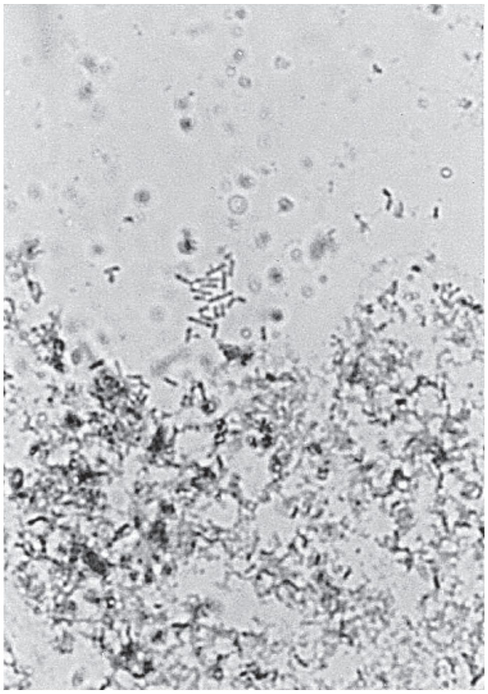

Fig. 1 Some examples of biofilm bacteria observed in the samples

Fig. 2 Algae cells observed in the samples

Fig. 3 Example of biofilm-forming bacteria

produce copious amounts of EPS, which probably protected them from harmful environmental effects.

By providing growth conditions for eukaryotes, even thermophillic fungi (i.e., misselial and yeast forms) were observed (Fig. 2). Remarkable amounts of mold were observed in cooling towers (i.e., they were found in ~70% of sludge samples taken from the cooling towers). Algal cells were observed in the makeup water used in the refinery, in the cooling tower samples, and in the biofilm formed within oil and/or water piping networks (Fig. 2).

Table 2 Presence of test microorganisms in industrial units

Microorganisms	Site of sampling	Positive cultures, %
SRBs	Pipelines	98
	Cooling towers	75
IOBs	Pipelines	5
	Cooling towers	80
BFBs	Pipelines	85
	Cooling towers	2
Eukaryotics	Pipelines	68
	Cooling towers	?

By using the special media mentioned earlier for growing biofilm-forming bacteria and by analyzing these samples, it was revealed that the biofilms were actually "harbors" for host sessile microorganisms that had not been observed as planktonic (Fig. 3).

During the investigation, surfactant was injected into the system with a thin layer of precipitates. A sudden increase in the number of SRB was then observed. This increase can be interpreted as the release of the bacteria that were harbored under the biofilm that were freed by the elimination of the biofilm. It seems that using surfactants can be a good solution for destroying the sessile bacteria that are hidden under the biofilm when it is not very thick. Washing of the system during overhaul periods with chlorine or an organic acid (e.g., acetic acid) will have the same effect.

This investigation also showed that the biocides used were not very effective in killing the sessile bacteria hidden in the precipitates or within the biofilm. This occurred because the biocides had been mainly designed to attack and reduce planktonic bacteria. The floating bacteria formed about 10% of the total population of microorganisms (Ref 25). Table 2 summarizes the findings of this investigation.

5. Discussion

If there is a chance for water stagnancy in a system so that the planktonic bacteria can transit into the sessile phase (on the biofilm), then the chance of success for the use of the biocides normally used in industrial systems will be very slim. The formation of biofilms can then lead into MIC. Two ways to combat MIC are:

- Physical removal, such as with "pigging," of dirt and debris to achieve a cleaner environment
- Chemical purification, involving the use of chemicals such as chlorine or aldehydes for either killing or immobilizing the bacteria (biocidal or biostatic effects). Pickling is also recommended

The main points for utilizing these methods are either to remove the bacterial films or to prevent the bacteria from forming them. It was found that if the biocide used in the system is only targeted at planktonic bacteria, its effect will be quite limited. It was also found that using surfactants on thin biofilm layers destroyed them, and the resident bacteria and other microorganisms were then freed into the bulk solution. A chance exists for applying biocides more effectively because the number of the sessile bacteria will be largely reduced. Washing of the system with chlorine or organic acids, instead of surfactants, at intervals may give approximately the same results for biofilm removal.

6. Summary and Conclusions

The results of these studies have shown that environmental and operational conditions such as water stagnancy in the system can favor conditions for the growth of biofilm-forming bacteria. This will lead, in the case of pipe weldments, to corrosion of the weldments and to the preferential attack of the austenite phase.

Sulfate-reducing bacteria, heterotrophic biofilm-forming bacteria, IOB, and eukaryotics (i.e., algae, molds, and yeasts) have been known for their corrosive effects, which are carried out either by contributing to tubercle formation and by producing organic acids (fungi) (Ref 10) or by forming aeration differential electrochemical cells (Ref 9).

- Various sessile microorganisms were detected in cooling towers and pipelines of some Iranian industries.
- Environmental and operational conditions such as water stagnancy in the system and continuous leaching of ferrous ions into well-aerated water favor conditions for the growth of many corrosion-enhancing bacteria, including IOB and biofilm-forming bacteria.
- Eukaryotes, such as thermophillic fungi (misselial and yeast forms), were also observed.
- The biocides studied were not very effective in killing the sessile bacteria that were hidden in the precipitates, and the biofilm formed as a result. This occurred because the biocides had been mainly designed to affect and reduce planktonic bacteria.

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