

# The Involvement of Bacteria in the Failure of a Carbon Steel Pipe

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**Abstract:** *In the raw water a lot of bacteria who play an important role in the corrosion of steels are present. These bacteria may initiate or accelerate corrosion processes because they are able of inducing localized changes in the aqueous environment, in terms of pH and oxygen concentration, destroying protective layers or creating corrosive deposits. This paper presents a study on the involvement of bacteria in the degradation of a pipe from raw water cooling system. Microbiological analysis of sampled sludge from internal surface of pipe emphasized the existence of some microorganisms: heterotrophic aerobic bacteria, iron oxidizing bacteria and sulphate-reducing bacteria. Investigation techniques of microbiologically influenced corrosion included metallographic analysis of the surface and X-ray diffraction analysis of samples taken from existing deposits. The results emphasized that the change of environmental chemistry under the developed biofilm by the bacteria, together with the corrosive products as a result of the metabolic activity of the bacteria, influenced the perforation of the pipeline.*

**Keywords:** *microbiologically influenced corrosion, bacteria, corrosion products*

## 1. Introduction

The raw water from some cooling systems contains a lot of microorganisms who attach to inner site of the pipes and interact in complex ways to form biofilms and 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.

Microbiologically influenced corrosion is the corrosion which involves the action of bacteria on metal surfaces. It is established that the most aggressive microbial corrosion takes place in the presence of microbial consortia in which many physiological types of bacteria, including metal-oxidizing bacteria, sulfate-reducing bacteria (SRB), acid-producing bacteria (APB), and metal-reducing bacteria (MRB) interact in complex ways within the structure of biofilms [1].

There are numerous forms of localised corrosion which can be promoted by the interaction of microorganisms with metals and that can conduct to the loss of the metallic component integrity. For example, pitting, crevice attack, stress corrosion cracking, augmentation of corrosion fatigue, intergranular cracking and hydrogen embrittlement and cracking can be initiate and develop under the biofilms [2].

This paper presents a study regarding the implication of bacteria in the degradation of a pipe from a raw water cooling system.

## 2. Materials and methods

In our work was investigated an OLT35 carbon steel pipe part. The pipe had a 6" internal diameter.

The diagnosis of microbiologically influenced corrosion requires, besides microbiological analyses, a series of metallographic and physicochemical analyses . The steps for investigation were:

- Visual evaluation of the degraded pipeline part.
- Slam sampling from inside of the pipe for microbiological analysis.

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- Sampling of corrosion product from different areas of the pipe for X-ray diffraction analysis with the aim to identified and quantified the corrosion products.

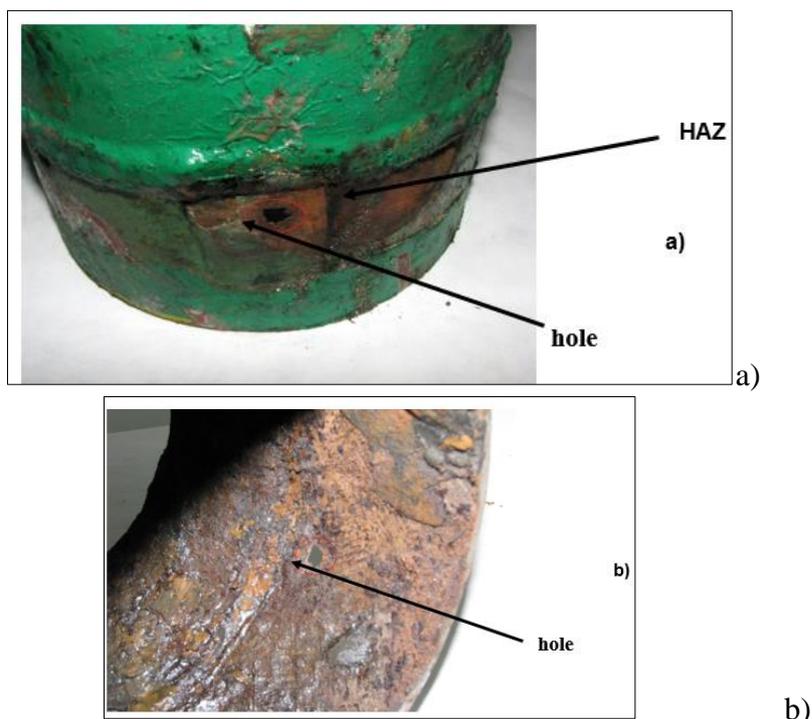
- The cutting of some samples from the pipe for metallographic analysis.

X-Rays diffraction analyses were performed using an X'Pert Pro MPD diffractometer equipped with a Cu LFF (Long Fine Focus) X rays tube ( $\lambda_{K\alpha 1}=1.5418\text{\AA}$ ). It was worked in Bragg –Brentano geometry at 1800W tub power. The XRD spectra were recorded in the range  $20\div 100$  degrees in  $2\theta$ , with a step of 0.01 degrees and 0.5s/step. X'Pert High Score Plus software was used to analyse the XRD spectra [3]. The crystallographic compounds were identified using the ICDD PDF 4+ data base [4].

An OLYMPUS GX 71 Inverted System Metallurgical Microscope was used to perform the metallographic investigation of the pipe samples. This method is appropriate for put in evidence the morphology of corrosion products film and to determine its thickness [5].

### 3.Results and discussions

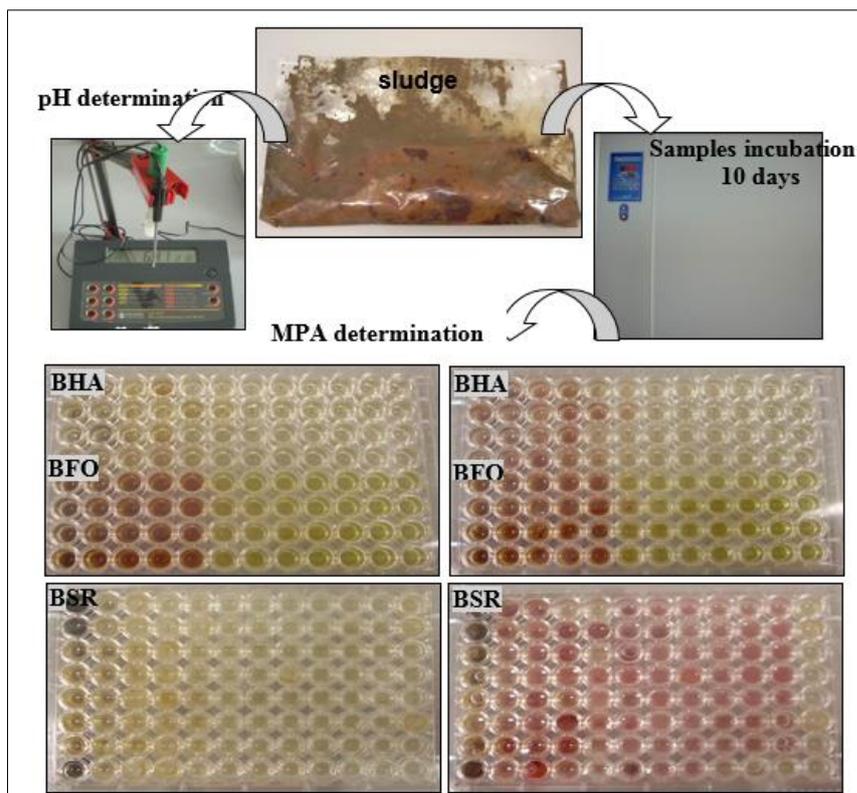
On the outside (Figure 1a), the corrosion protection of the pipe was made by coating, but there are some areas where the paint was exfoliated. However, the pitting corrosion was initiated from inside of the pipe (Figure 1b), because in the inner the pipe was completely covered by uneven and bulky deposits, adhering to its surface. The structure of the deposit is specific to the mode in which the microorganisms form their biofilms on metal surfaces. On the welding joint, in the heat-affected zone (HAZ), the pitting corrosion led in time to its perforation - hole size - about -10mm.



**Figure 1.**  
Aspect of failure pipe.  
*a) outside of pipe,*  
*b) inside of pipe*

Microbiological analyses of sampled sludge from internal surface of pipe emphasized the existence of some microorganisms: heterotrophic aerobe bacteria, bacteria iron oxidizing bacteria and sulfate-reducing bacteria.

The steps of microbiological analyses are presented in Figure 2.



**Figure 2.** The steps of microbiological analyses of sampled sludge  
MPN = Most Probable Number; BHA- heterotrofe aerobic bacteria; BSR- sulphate-reducing bacteria; BFO- iron –oxidizing bacteria

The first step was to determine the *pH* value of the sludge sample, because the culture media for bacteria must have a *pH* value close to the *pH* value of the sample.

The sludge sample taken from the pipe had a *pH* value of 6.5.

Several miniaturized systems [6] were used to determine the number of bacteria (MPN = Most Probable Number) in the analyzed sample. From the sludge sample, serial dilutions ( $10^{-1}$ - $10^{-11}$ ) in saline phosphate buffer were made. These dilutions were inoculated in the LB medium to determine the number of aerobic heterotrophic bacteria, in the Postgate medium to determine the number of sulphate-reducing bacteria and in the Vinogradski medium for the determination of the number of iron-oxidizing bacteria.

LB medium : tryptone 10 g; yeast extract 5.0 g; NaCl 10 g; 1000 mL distilled water ; *pH*=7.0.

Vinogradski medium:  $\text{NH}_4\text{NO}_3$  0.5 g;  $\text{NaNO}_3$  0.5 g;  $\text{K}_2\text{HPO}_4$  0.5 g;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.5 g;  $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$  0.2 g; ferric-ammonium citrate 10g, 1000 mL distilled water; *pH*=6.0.

Postgate medium:  $\text{KH}_2\text{PO}_4$  0.5 g;  $\text{NH}_4\text{Cl}$  1 g;  $\text{CaSO}_4$  1g;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  2 g; calcium lactate 3.5 g; 1000 mL tap water.

After inoculation, the plates were incubated at 30°C for 10 days.

The growing of aerobic heterotrophic bacteria is evidenced by the opalescence of the environment in consequence of changing its turbidity degree.

The growing of sulphate-reducing bacteria is evidenced by the blackening of the environment in consequence of the massive deposition of iron sulphide resulting from the metabolic activity, and the growing of iron-oxidizing bacteria by the opalescent of the environmental and the appearance of a red-brown deposit that contains iron hydroxide.

After 10 days of plate incubation at 30°C, the presence of the following groups of bacteria was found: sulfate-reducing bacteria ( $2.5 \times 10^{10}$  UFC/ml), iron oxidizing bacteria ( $2.5 \times 10^4$  UFC/mL) and heterotrophic aerobic bacteria ( $9.5 \times 10^3$  UFC/mL) (Table 1).

UCF/mL = Bacterian cells/ml liquid

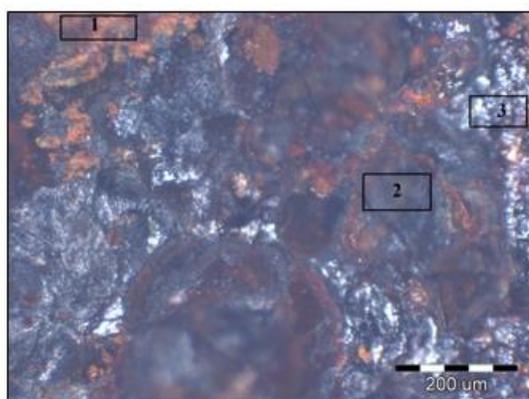
**Table 1**  
The results of microbiological analyses of sampled sludge

Bacteria	Culture medium	Bacterian cells/mL liquid UCF/mL
Heterotrofe aerobe bacteria	LB	$9.5 \times 10^3$
Iron –oxidizing bacteria	Vinogradski	$2.5 \times 10^4$
Sulphate-reducing bacteria	Postgate	$2.5 \times 10^{10}$

The higher number of sulphate-reducing bacteria in comparison to the number of iron-oxidative bacteria and aerobic heterotrophs is explainable, because sludge often creates anaerobe conditions that favor the development of bacteria capable of growing and multiplying in the absence of oxygen.

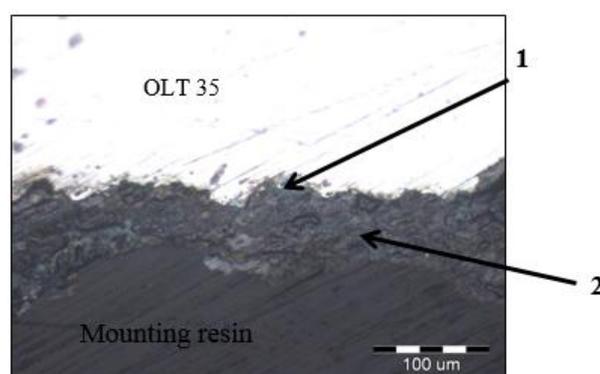
Metallographic analysis [7] of some pipe areas obtained at a magnification of 100 times (Figure 3), emphasized the deposits of corrosion products with different colors, such as, red-brown as a result of heterotrofe aerobe and iron –oxidizing bacteria (zone 1) and black-as a result of sulphate-reducing bacteria (zone 2).

Also, uncovered areas of pipe (such as zone 3) are observed, because microorganisms do not develop uniform biofilms on metal surfaces.



**Figure 3.** Surface morphology of a pipe sample

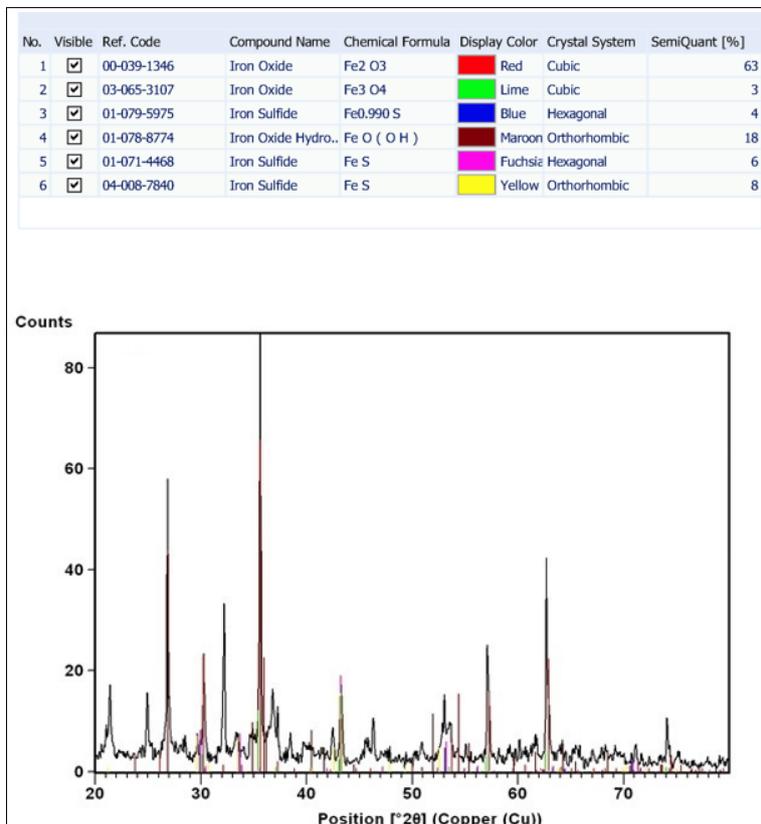
By analyzing the cross-section (Figure 4) were observed the non-uniform corrosion of the steel (zone 1) and the bulky deposition with different thicknesses (34-151 μm) in zone 2.



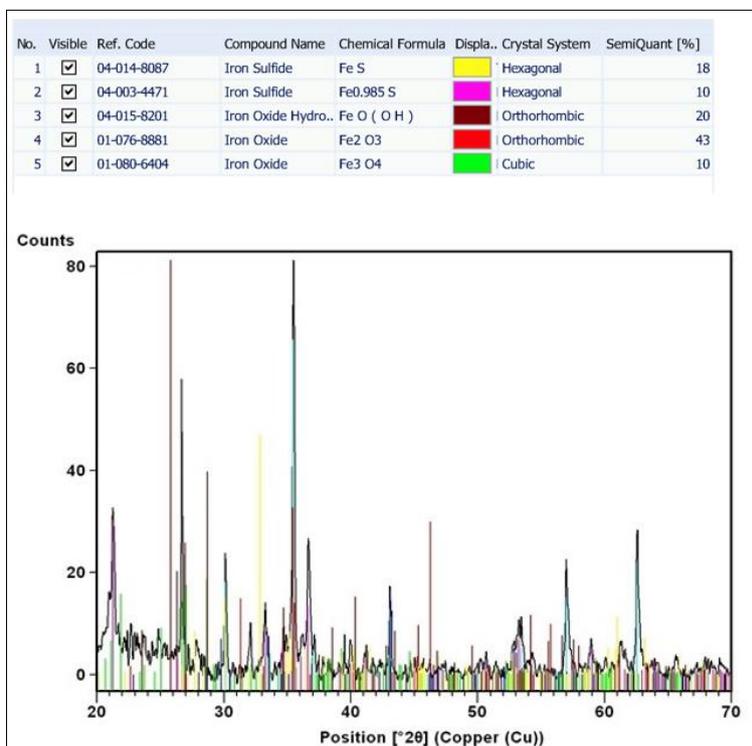
**Figure 4.** Microscopic examination of the cross-section of the pipe sample [x100]

X-ray diffraction analysis of the corrosion products emphasized in zone 1 (Figure 5) the following compounds:  $\text{Fe}_2\text{O}_3$  - 63%,  $\text{FeO}(\text{OH})$  -18%,  $\text{FeS}$ -18%,  $\text{Fe}_3\text{O}_4$ -10% .

In zone 2 (Figure 6) the corrosion products were: Fe<sub>2</sub>O<sub>3</sub> -43%, FeS -28%, FeO(OH) -20% and Fe<sub>3</sub>O<sub>4</sub> -10%.



**Figure 5.** X-ray diffraction analysis of a sample from pipe-zone 1



**Figure 6.** X-ray diffraction analysis of a sample from pipe-zone 2



The important information provided by X-ray diffraction analysis is the emphasizing to of corrosion products specific to both aerobe bacteria (iron –oxidizing bacteria) and anaerobe bacteria (sulphate-reducing bacteria). This demonstrates the fact that in natural conditions, conspecific biofilms are relatively rare, most of them being composed of a mixture of microorganisms.

#### 4. Conclusions

In the sludge sampled from inside of pipe, the presence of a microbiological charge was identified. It consist of sulphate-reducing bacteria ( $2.5 \times 10^{10}$  UFC/ml), iron –oxidizing bacteria ( $2.5 \times 10^4$  UFC/ ml) and heterotrofe aerobe bacteria ( $9.5 \times 10^3$  UFC ml).

Optical microscopy analysis revealed different thickness deposits with bulky appearance inside the pipe (34-151 $\mu$ m), as well as its areas of different coloration (black, red-brown) specific to biofilms developed by bacteria.

X-ray diffraction determined the presence in the deposits of corrosion products specific to the identified bacteria, namely  $\text{Fe}_2\text{O}_3$ , FeS, FeO(OH); these corrosion products produced by both aerobe and anaerobe bacteria demonstrate that under natural conditions, conspecific biofilms are relatively rare, most of them being composed of a mixture of microorganisms.

Microbiological analyses, optical microscopy and X-ray diffraction, show that the microorganisms present in the supply water contributed to the perforation of the pipeline; these microorganisms have influenced physicochemical parameters (e.g. oxygen concentration, pH value, redox potential, conductivity) existing at pipeline / supply water interface.

#### References

1. PEABODY, A.W., Control of Pipeline Corrosion, 2<sup>nd</sup> Edition, NACE International The Corrosion Society, edited by BIANCHETTI, R., Houston, Texas, 2001, p.275.
2. BEECH, I.; GAYLARDE, C., Revista de Microbiologia, **30**, nr.3, 1999, p.177.
3. X'Pert High Score Plus software v4.1, PanAlytical BV 2014.
4. ICDD PDF-4+ data base 2016, JCPDS-International Centre for Diffraction Data, FPLD080169-4382.
- 5.\*\*\* SR EN ISO 1463 / 2004, Metallic and oxide coatings. Measurement of coating thickness. Microscopical method.
6. STANCU, M., GRIFOLL, M., Multidrug resistance in hydrocarbon-tolerant Gram-positive and Gram-negative bacteria. J. Gen. Appl. Microbiol., **57**, 2011, p.1.
7. TUNARU, M., VELCIU, L., DINU, A., POPA, L., Internal Report RATEN ICN, The microbiological corrosion investigation of the buried pipes of cooling water system from Cernavoda NPP, **11059**, 2016, p.18.

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