Bacterial Activity in a Deposit from a Residual Injection Water Pipeline

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This research was conducted on a deposit sampled from a residual injection water pipeline, where corrosion problems identified on metallic equipment have been suspected to be caused by microbiological activity. The residual injection water was also analyzed to confirm the bacterial activity inside the pipeline. Microbiological activity is internationally recognized as a significant contributor to corrosion problems of oilfield equipment, being characterized by rapidity, severity and localized nature. The commonly encountered deposits in water injection pipelines include salts, corrosion products, microbiological mass, suspended matter and water. MIC mitigation methods, as biocide treatment, cannot be implemented without evaluation and confirmation of bacterial activity in the deposit. Several techniques were performed, as SEM and EDS analysis of the deposit to characterize the morphology and elemental composition and microbiological analysis and microscopic examination of the biofilm contained by the deposit, which demonstrated the presence of bacteria communities responsible for MIC.

Keywords: bacteria, biofilm, deposit, microbiologically influenced corrosion

The reliability and safety of industrial equipment in the petroleum industry are substantially influenced by degradation processes such as corrosion, erosion, deposits and blocking of pipes.

A deposit is an accumulation of transported or corroded materials on the wall or pipe bottom [1]. The commonly encountered deposits in water injection pipelines include carbonates, sulfates, phosphates of alkaline earth metals, silica, corrosion products, microbiological mass and suspended matter [2]. Usually, accumulated deposits may create conditions favorable to extremely destructive microbiologically influenced corrosion [1].

Microbiologically influenced corrosion (MIC) is defined as a form of corrosion caused and promoted by microorganisms [3] and represents a very complex and challenging subject in nowadays oil industry. Microbiological activity is internationally recognized as a significant contributor to corrosion problems and this is valid for water injection pipelines, where features of this phenomenon have been identified.

Presence of bacterial population produces an acidic environment helping to corrode the metal piping at highly accelerated rates [4].

Possible MIC sites are often identified by the rapidity, severity and localized nature of the corrosion, resulting in sharp-sided terraced pitting.

The microorganisms that are mainly involved in MIC are bacteria [5]. Bacteria generally exist in one of two types of population, planktonic, freely existing in the fluid, and sessile, as a unit attached within a biofilm and responsible for MIC [6].

Biofilms are densely packed communities of microbial cells that grow on living or inert surfaces and surround themselves with secreted polymers. Many bacterial species form biofilms and their study has revealed them to be complex and diverse [7].

The predominant types of bacteria associated with MIC are Sulfate Reducing Bacteria (SRB), Acid Producing Bacteria (APB) and Iron Oxidizing Bacteria.

MIC has the potential to seriously affect oilfield metallic equipment, therefore, it is essential to correctly diagnose the presence of bacteria in deposit as to interrupt and curtail the development process of the microorganism community [8].

Experimental part

Materials and methods

Sampling location

The experimental study was carried out on a deposit and water from a pipeline transporting residual injection water, located in a Romanian oilfield. This location was selected for the study due to corrosion problems identified on metallic equipment, suspected to be caused by microbiological activity, a fact sustained by analysis background, which revealed the presence of planktonic bacteria in the water.

Residual injection water sampling

Residual injection water sample was collected from the pipeline using sterile bottles.

Deposit sampling

The deposit was sampled using an in-house manufactured device, which was installed as side stream of the pipeline and allowed the accumulation of deposit on metallic disk coupons. The installation point of the device is presented in figure 1.

The device had removable disk coupons exposed internally, similar in composition to the pipeline, which were mounted in *12, 3, 6*, and *9 o'clock* positions on the circumference of the device in order to study the spatial distribution of bacterial activity. The device was exposed for a specific period of time and transported, maintaining

Fig. 1 Installation point of the device

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the same anaerobic conditions, from in-situ location to laboratory for deposit analysis. Four sampling campaigns were performed. The exposure periods are presented in table 1.

Table 1
EXPOSURE PERIOD OF THE DEVICE FOR DEPOSIT ACCUMULATION

Sampling campaign no.	Exposure period
1	2nd of November, 2016 - 12th of December, 2016 (1.5 months)
2	12th of December, 2016 - 9th of February, 2017 (2 months)
3	9th of February, 2017 - 27th of April, 2017 (3 months)
4	27th of April, 2017 - 26th of June, 2017 (2 months)

SEM and EDS analysis of the deposit

The scanning electron microscope (SEM) provides the morphologies of the sample, as particle size, shape, crystal habits, packing tendencies and the degree of agglomeration. Energy Dispersive X-Ray Spectrometry (EDS) analysis is used to obtain compositional information on deposits [2].

Each disk coupon with the deposit was removed from the device using gloves. The deposit morphology was obtained using SEM analysis of the samples in *environmental* mode, which allowed imaging hydrated and uncoated sample at 5,000X magnification, in low-vacuum and at room temperature. EDS analysis in the SEM provided the elemental composition of the deposit.

Microbiological analysis of the deposit

The deposit samples were microbiologically analyzed in order to confirm the presence of biofilm containing bacteria. The following types of bacteria were determined: Aerobic Bacteria, Anaerobic Bacteria - Clostridium, Sulfate Reducing Bacteria (SRB), Acid Producing Bacteria (APB) and Iron Oxidizing Bacteria.

The method used to culture the aforementioned bacteria groups was the most probable number (MPN) method, using specific culture media whose salinity is brought to a level similar to the water. MPN is a statistical analysis which estimates microbial population sizes in a substrate, by dilution and incubation of replicated cultures across several serial dilution steps, until theoretically no bacteria is found in the last dilution [9].

The biofilm growth was performed at room temperature (22-24°C), for a period of about 14 days.

Fig. 2 Disk coupons with the deposit

Bacteria number was calculated for deposit sample considering the exposed surface (63.62 mm^2) of disk coupons.

Bacteria type and number were determined by microbiological analysis of the deposit sampled from different positions on the circumference of the device, in order to compare biofilm development.

Microscopic examination of the biofilm

Microscopy is most commonly used to examine liquid or sludge samples directly to determine the overall numbers of microorganisms or to identify different species by their shape.

The procedure of biofilm examination involves placing a few μL of sample on a glass slide, preparing the slide for examination using various staining techniques and examining the slide with a light at magnifications from approximately 500X to 1,500X [3].

The biofilm in diluted form was examined using the optical microscope at magnification of 400X.

Results and discussions

Characterization of residual injection water

Residual injection water sample was analyzed to determine the chemical indicators for bacterial activity. The characteristics of the residual injection water in the pipeline are presented in the table 2.

Metabolic products of bacterial activity include sulfides, organic acids and various types of organic and inorganic substances [10].

The residual injection water analysis indicates the presence of indicators for bacterial activity. H_2S content is the most important indicator of microbial activity in an oilfield system. Organic acids, propionic and acetic, are by-products of microbial metabolism. Planktonic population of SRB and APB was found. Also, the acidic pH indicates the presence of bacterial activity in the water.

Parameter	Measured Units	Values	Analysis Method
pH	-	6.05	4500-H + B
Electrical conductivity	mS/cm	118.40	2510 B
Suspended solids	mg/l	13	STAS 6953/81
Dissolved free CO_2	mg/l	145	SR EN ISO 9963/2002
Total sulfides as H_2S	mg/l	1.90	SR 7510/97
Total Fe	mg/l	12.00	SR EN ISO 11885/2009
Ba	mg/l	21	SR EN ISO 11885/2009
Ca	mg/l	2 915	SR EN ISO 11885/2009
Dissolved Fe	mg/l	11	SR EN ISO 11885/2009
Mg	mg/l	688	SR EN ISO 11885/2009
Na	mg/l	28 610	SR EN ISO 11885/2009
Sr	mg/l	173	SR EN ISO 11885/2009
Chloride	mg/l	54 440	SR EN ISO 10304-1:2009
Sulfate	mg/l	85	SR EN ISO 10304-1:2009
Alkalinity (bicarbonate)	mg/l	461	SR EN ISO 9963/2002
Acetate	mg/l	193	OMV Petrom internal methodology
Propionate	mg/l	54	OMV Petrom internal methodology
Planktonic SRB	MPN/ml	10^2	AWWA 2012
Planktonic APB	MPN/ml	10^2	AWWA 2012

Table 2
CHARACTERISTICS OF RESIDUAL INJECTION WATER SAMPLE

Characterization of deposit morphology

The morphological characteristics of the deposit samples from every position on the circumference of the device are presented in figure 3-6.

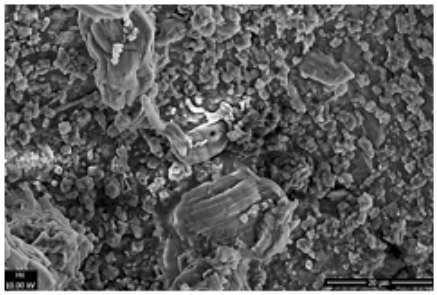


Fig. 3 Scanning electron micrograph of the deposit from 12 o'clock position, 5,000X

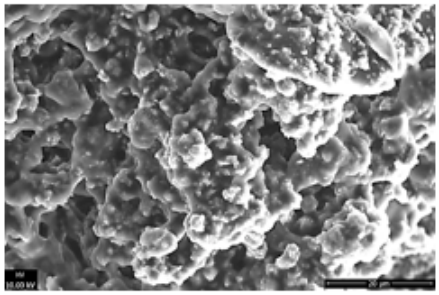


Fig. 4 Scanning electron micrograph of the deposit from 3 o'clock position, 5,000X

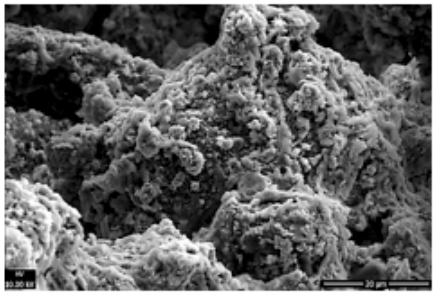


Fig. 5 Scanning electron micrograph of the deposit from 6 o'clock position, 5,000X

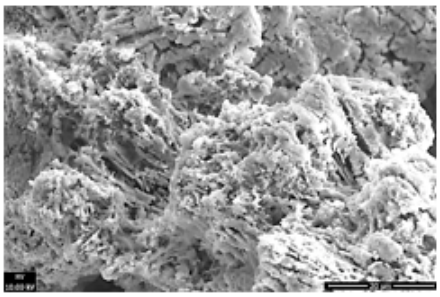


Fig. 6 Scanning electron micrograph of the deposit from 9 o'clock position, 5,000X

The micrographs revealed crystalline morphologies typical of scale deposits, containing inorganic particles, mostly salt crystals, in different hydration phases. The organic matter could not be distinguished, excepting in micrograph of deposit from *12 o'clock* position, where bacteria lookalike shapes may have been identified.

Characterization of deposit elemental composition

The elemental composition of the deposit is presented using EDS spectrum accompanied by SEM image of the selected area in figure 7.

The elemental composition of the deposit corresponds to scale type, containing high amount of salts, represented by Na and Cl compounds. Zn is naturally present in water and, therefore, in the hydrated deposit. Also, silica, oxides and carbonates are present, represented by Si, O and C. The corrosion products identified are Fe, Mn and Cr. Ca presence in the spectrum can classify the scale as calcium carbonate type.

Characterization of the biofilm

In order to characterize the biofilm, bacteria number was classified as:

- high, above 10^5 MPN/exposed surface;
- medium, between 10^2 and 10^5 MPN/exposed surface;
- low, below 10^2 MPN/exposed surface.

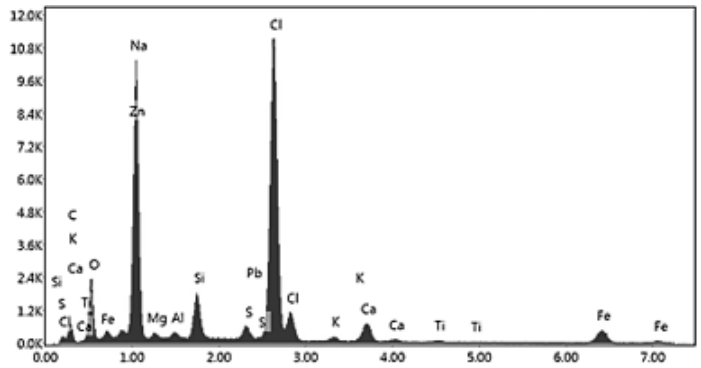
A diversity of bacterial communities in biofilm samples from each position on the circumference of the device was observed. Bacteria number cannot be directly correlated with exposure period or sample position.

Bacteria associated with MIC were identified in all biofilm samples. The biofilm samples contain medium or high population of SRB, medium population of APB and low population of Iron Oxidizing Bacteria. Other bacteria types, aerobic and anaerobic, were identified, in various amounts.

The bacteria type and number in biofilm samples for all sampling campaigns are presented in tables 3-6.

Microscopic examination of the biofilm

Specific bacteria shapes were distinguished in the biofilm (fig. 8-10). SRB, APB and Iron Oxidizing Bacteria were recognized in all biofilm samples from every position on the circumference of the device.



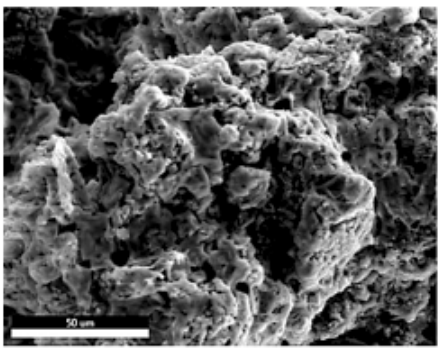


Fig. 7 EDS spectrum of the deposit and SEM micrograph of sample from which spectrum was generated

Biofilm sample		Bacteria number, MPN/exposed surface				
		Aerobic Bacteria	Anaerobic Bacteria–Clostridium	Acid Producing Bacteria	Sulfate Reducing Bacteria	Iron Oxidizing Bacteria
Biofilm from 12 o'clock	1	10^5	10^3	10^2	10^3	10^3
	2	10^4	10^2	$<10^2$	10^3	$<10^2$
	3	10^4	10^2	$<10^3$	10^3	$<10^2$
Biofilm from 3 o'clock	1	10^4	10^2	10^2	10^4	$<10^2$
	2	10^5	10^3	10^2	10^3	$<10^2$
	3	10^4	10^4	$<10^2$	10^2	$<10^2$
Biofilm from 6 o'clock	1	10^5	10^4	10^2	10^4	10^2
	2	10^5	10^4	10^2	10^4	$<10^2$
	3	10^5	10^3	$<10^2$	10^3	$<10^2$
Biofilm from 9 o'clock	1	10^5	10^4	10^3	10^6	$<10^2$
	2	10^4	10^3	10^2	10^4	$<10^2$
	3	10^7	10^4	10^3	$<10^2$	10^2

Table 3
BACTERIA TYPE AND NUMBER IN BIOFILM SAMPLES IN SAMPLING CAMPAIGN NO.1

Biofilm sample		Bacteria number, MPN/exposed surface				
		Aerobic Bacteria	Anaerobic Bacteria–Clostridium	Acid Producing Bacteria	Sulfate Reducing Bacteria	Iron Oxidizing Bacteria
Biofilm from 12 o'clock	1	10^3	$<10^2$	10^3	10^4	$<10^2$
	2	10^3	$<10^2$	10^2	10^4	$<10^2$
	3	10^2	$<10^2$	10^3	10^5	$<10^2$
Biofilm from 3 o'clock	1	10^3	10^3	10^3	10^4	$<10^2$
	2	10^3	10^3	10^3	10^6	$<10^2$
	3	10^5	10^4	10^3	10^4	$<10^2$
Biofilm from 6 o'clock	1	10^4	10^3	10^3	10^5	$<10^2$
	2	10^3	10^3	10^3	10^6	$<10^2$
	3	10^4	$<10^2$	10^2	10^5	$<10^2$
Biofilm from 9 o'clock	1	10^3	10^3	10^3	10^6	$<10^2$
	2	10^3	10^2	10^3	10^6	$<10^2$
	3	10^3	10^2	10^2	10^4	$<10^2$

Table 4
BACTERIA TYPE AND NUMBER IN BIOFILM SAMPLES IN SAMPLING CAMPAIGN NO.2

Biofilm sample		Bacteria number, MPN/exposed surface				
		Aerobic Bacteria	Anaerobic Bacteria–Clostridium	Acid Producing Bacteria	Sulfate Reducing Bacteria	Iron Oxidizing Bacteria
Biofilm from 12 o'clock	1	10^3	$<10^2$	$<10^2$	$<10^2$	<10
	2	10^3	$<10^2$	10^2	10^2	<10
	3	10^5	10^4	10^5	10^4	10
Biofilm from 3 o'clock	1	10^3	10^2	10^4	10^3	10
	2	10^5	$<10^2$	10^4	10^3	<10
	3	10^5	10^3	10^5	10^4	10
Biofilm from 6 o'clock	1	10^5	10^3	10^4	10^3	10
	2	10^5	10^3	10^4	10^3	10
	3	10^5	10^3	10^4	10^3	10
Biofilm from 9 o'clock	1	10^3	10^2	10^3	10^3	10
	2	10^3	$<10^2$	10^4	10^2	10
	3	10^4	$<10^2$	10^3	10^3	10

Table 5
BACTERIA TYPE AND NUMBER IN BIOFILM SAMPLES IN SAMPLING CAMPAIGN NO.3

Biofilm sample		Bacteria number, MPN/exposed surface				
		Aerobic Bacteria	Anaerobic Bacteria–Clostridium	Acid Producing Bacteria	Sulfate Reducing Bacteria	Iron Oxidizing Bacteria
Biofilm from 12 o'clock	1	10^5	10^5	10^3	10^3	10^2
	2	10^4	10^5	10^4	10^3	$<10^2$
	3	10^4	10^3	10^3	10^2	10^2
Biofilm from 3 o'clock	1	10^5	10^5	10^3	10^4	$<10^2$
	2	$>10^6$	10^4	10^4	10^4	$<10^2$
	3	10^5	10^2	10^2	10^2	10^2
Biofilm from 6 o'clock	1	10^5	10^2	$<10^2$	10^2	$<10^2$
	2	10^5	10^4	10^3	$<10^2$	10^2
	3	10^3	$<10^2$	10^2	10^3	$<10^2$
Biofilm from 9 o'clock	1	10^5	10^5	10^4	10^4	$<10^2$
	2	10^5	10^4	10^2	10^5	$<10^2$
	3	10^4	10^2	$<10^2$	10^3	$<10^2$

Table 6
BACTERIA TYPE AND NUMBER IN BIOFILM SAMPLES IN SAMPLING CAMPAIGN NO.4

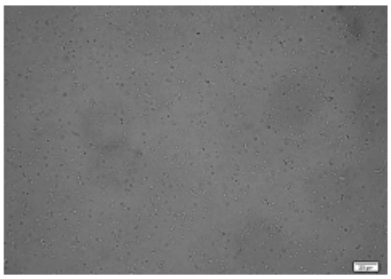


Fig. 8 Photomicrograph of SRB in biofilm sample, 400X

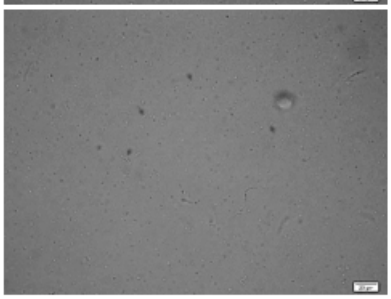


Fig. 9 Photomicrograph of APB in biofilm sample, 400X

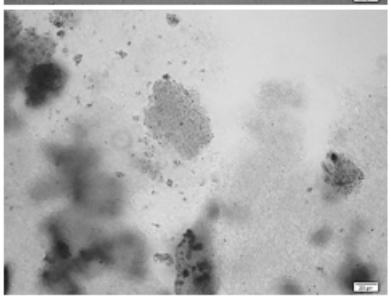


Fig. 10 Photomicrograph of Iron Oxidizing Bacteria in biofilm sample, 400X

Conclusions

Analysis of the deposit sampled from a residual injection water pipeline showed bacterial activity, confirmed by residual injection water analysis.

Characterization of the residual injection water indicated the presence of microbiological indicators, as acidic *p*H, H_2S content, organic acids concentration and planktonic population of Sulfate Reducing Bacteria and Acid Producing Bacteria.

The morphology and elemental composition of the deposit were typical of scale deposits, containing inorganic particles, mostly salt crystals, in different hydration phases. Due to high amount of salts, the microbiological mass could not be distinguished, excepting in deposit sampled from *12 o'clock* position, where bacteria lookalike shapes may have been identified.

Therefore, the deposit was microbiologically analyzed in order to confirm the presence of biofilm containing bacteria. In all sampling campaigns, the microbiologically analysis revealed the presence of all bacteria responsible for MIC and the diversity of bacterial communities in biofilm samples from different position on the circumference of the device.

Also, bacteria shapes were distinguished in the biofilm using the optical microscope.

The confirmation of bacterial activity in the deposit allows the deployment of MIC control methods specific to this location (e.g. biocide treatment), in order to prevent or decrease MIC harmful effects on oilfield metallic equipment.

Acknowledgement: We thank Ramona Visoiu, the microbiology specialist of ICPT Campina, for expertise that greatly assisted this research.

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Manuscript received: 17.08.2018