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Corrosion of low carbon steel by microorganisms from the 'pigging' operation debris in water injection pipelines

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ABSTRACT

Present in all environments, microorganisms develop biofilms adjacent to the metallic structures creating corrosion conditions which may cause production failures that are of great economic impact to the industry. The most common practice in the oil and gas industry to annihilate these biofilms is the mechanical cleaning known as "pigging". In the present work, microorganisms from the "pigging" operation debris are tested biologically and electrochemically to analyse their effect on the corrosion of carbon steel. Results in the presence of bacteria display the formation of black corrosion products allegedly FeS and a sudden increase (more than 400 mV) of the corrosion potential of electrode immersed in artificial seawater or in field water (produced water mixed with aquifer seawater). Impedance tests provided information about the mechanisms of the interface carbon steel/bacteria depending on the medium used: mass transfer limitation in artificial seawater was observed whereas that in field water was only charge transfer phenomenon. Denaturing Gradient Gel Electrophoresis (DGGE) results proved that bacterial diversity decreased when cultivating the debris in the media used and suggested that the bacteria involved in the whole set of results are mainly sulphate reducing bacteria (SRB) and some other bacteria that make part of the taxonomic order *Clostridiales*.

1. Introduction

Microbial development occurs in almost all environments throughout biofilm formation and may be responsible for microbial influenced corrosion (MIC), also known as microbially influenced corrosion or biocorrosion which can be defined as the enhancement or acceleration of corrosion by the presence of bacteria [1]. MIC is not a new corrosion mechanism but it integrates the role of microorganisms in corrosion processes. Thus, an inherently abiotic process can be influenced by biological effects [2].

Corrosion represents a considerable economic stake projected between 1 and 4% of the gross national product (GNP) of developed countries [3]. Fleming et al. [4] estimate that 20% of corrosion problems are linked to the presence of microorganisms and more over Jack et al. [5] estimated that 34% of the corrosion damage experienced by oil companies was related to MIC.

Oil and gas industries battle MIC problems and its potential damages in pipelines by different methods such as the use of biocides, coatings, corrosion inhibitors and different chemicals that could reduce or control bacterial growth and/or reduce corrosion rates but probably the best way to avoid microbial influenced corrosion is an appropriate design and operation to keep the systems clean combined with regular mechanical cleaning [6].

Mechanical cleaning involves any method capable of the physical removal of deposits formed on the surface. It includes brushing, pigging and the use of cleaning spheres or water jet, and it is applied to remove sludge, scale, and encrustations as well as the biomass associated with these deposits [7]. Mechanical cleaning is mainly performed in water injection and production pipeline systems of oil and gas industries by the use of mechanical pigs.

Pipeline operators now describe any device made to pass through a pipeline for cleaning and other purposes with the word pig. The process of driving the pig through a pipeline by fluid is called a pigging operation [8]. Operators need to run cleaning pigs to dewax and descale the inside surface of the pipe and remove debris (corrosion products and biofilm), which help improve pipeline performance [9]. This debris collected in the receivers of the pipelines may be used to analyse not only corrosion products, organic matter, oil and water residues but also biofilm and bacterial presence.

Depending on the operating system, oil and gas companies combine different methods for the prevention and/or protection of corrosion of their pipelines. For instance, pigging operations are combined with biocide treatments and/or corrosion inhibitor treatments in order to reduce bacterial development. However, it is known that bacteria can adapt to drastic conditions posed by these treatments and resist rough

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environmental conditions still causing a threat for the materials. For example sulphate reducing bacteria (SRB) show considerable adaptability to extreme conditions making it possible to isolate active cultures from sites where bacteria are exposed to O_2 regardless of the anaerobic nature of this group [10–12].

One of bacteria's defensive mechanisms against corrosion treatments is precisely the secretion of slime or extracellular polymer substances (EPS) that leads to the formation of biofilms forming a gel matrix on the metal surface that not only may enhance corrosion rates but also protects bacteria from biocides [12]. It also may aid to the entrapment of corrosion inhibitors such as aliphatic amines and nitrites to be later degraded by microorganisms. Biofilms also reduce the effectiveness of corrosion inhibitors by creating a diffusion barrier between the metal surface and the inhibitor in the bulk solution [12–16].

Due to biofilm formation in diverse environments such as pipelines, microorganisms can coexist in naturally occurring biofilms with a wide bacterial community including fermentative bacteria, often forming synergistic communities (consortia) that are capable of affecting electrochemical processes through co-operative metabolisms [1,17]. Nevertheless, most of the research on anaerobic microbially influenced corrosion has focused on SRB primarily for the hydrogen sulphide generation and the fact that there is injection of sulphate-containing seawater into the reservoirs during the secondary recovery of oil which favours the proliferation of these bacteria [18,19]. However, recent studies suggest that SRBs need not be present in abundance in the microbial communities responsible for MIC [20] and that other types of bacteria could be involved, such as metal reducing-bacteria and methanogens [1,19,21]. Thus, studies with other bacterial groups or consortia must be enhanced.

This study has as objective to explore the influence of a consortium of microorganisms present in a sample of pigging debris on the corrosion of low carbon steel and determine its corrosive activity. For this, samples of pigging debris extracted from a water injection system of an oil and gas company located in Norway were used. Electrochemical experiments were performed, simulating some of the field conditions, comparing two different electrolytes as medium: artificial seawater (ASW) and a mixture of water from the field used in the water injection pipelines (MIXED). Furthermore, microbial diversity present in the debris and in the water used for the electrochemical experiments was characterised by Denaturing Gradient Gel Electrophoresis (DGGE) analysis of the Polymerase Chain Reaction (PCR) products for bacterial 16S rRNA genes (550 bp) aiming to comprehend the influence of bacterial consortia involved in corrosion.

2. Experimental procedure

2.1. Sample collection

Pigging debris samples were collected from a water injection pipeline "A" located in the installation "A" of an Oil and gas company located in Norway between November 2010 and June 2011. The pipeline consists of 10–15 km long pipe made of low carbon steel with an average operating temperature of 35 °C. Pig 1 was used for the electrochemical experiments and Pigs 1 and 5 were used for the weight loss experiments. Pig 1 corresponds to the first debris collected after the first pigging operation and Pig 5 corresponds to the fifth debris collected after the fifth pigging operation. Samples were transported to the lab in Schott bottles, flash with an inert gas and sealed.

Water passing through the water injection system is a mixture of 50% aquifer water plus 50% produced water recycled from the production pipelines. This water is re-injected into the oil-bearing formations to maintain pressure and facilitate oil recovery. The mixture of these waters is used for the electrochemical and weight loss experiments and will be called MIXED throughout this paper. Corrosion inhibitor KI-3804, imidazoline type, is added regularly in the production lines. Thus, 4 ppm of corrosion inhibitor is expected to be in the water injection pipelines according to the information provided by this company.

2.2. Inoculum and medium

Two different mediums were tested for the electrochemical experiments: ASW and mixed water from the field (MIXED) previously mentioned. ASW consists of: NaCl 408 mM, Na_2SO_4 28 mM, KCl 9.3 mM, $NaHCO_3$ 24 mM, KBr 839 μM, H_3BO_3 36 μM, $MgCl_2{}^*6H_2O$ 53 mM, $CaCl_2{}^*2H_2O$ 10 mM and it was supplemented with 10 mM of sodium acetate and 25 mM of sodium fumarate. MIXED water was supplemented with 24 mM of $NaHCO_3$ and generally after 300 h of running with 10 mM of sodium acetate and 25 mM of sodium fumarate when indicated.

The supplementation with acetate and fumarate is done to enhance a faster growth of heterotrophic species contained in the consortia. CO_2 added as part of the injected gas and inorganic substances such as iron (metal coupon) and sulphate were also present for the growth of autotrophic and lithotrophic species that may be present in the consortia.

Approximately 10 g of pigging debris was used to inoculate the electrochemical reactors and 3 g for the anaerobic vials used for the weight loss experiments. Inoculation was performed after de-aerating the medium with N_2/CO_2. An approximate number of planktonic cells were evaluated by bacteria counting in a Thoma counting chamber after performing serial dilutions.

2.3. Electrochemical measurements

Anaerobic reactors of 0.5 L were used adjusting the liquid level to 400 mL of total volume. The anaerobic conditions were obtained by bubbling N_2/CO_2 80:20 into the reactors for no less than 45 min before inserting the metal coupons. The flow of N_2/CO_2 was maintained during the whole experiment. Reactors were kept at 35 °C during experiments. pH was measured for all the experiments at the initial time ($h = 0$) and the final time ($h \geq 700$ h).

The working electrodes (WE) were 2 cm diameter cylinders of carbon steel S235 JR. The nominal chemical composition for the steel S235 JR is shown in Table 1. The WE was covered by a polymeric coating (thermo-contractible polyolefin, ATUM ®) leaving uncovered a flat disc surface with a total exposed area of 3.14 cm^2. Connections were made through titanium wire protected with the same polymeric coating. The electrodes were ground with SiC paper using P120–P600 until achieving a 600 grit surface followed by a cleaning with ethanol (70%) and throughout rinsing with sterile deionised water.

For all the experiments, electrochemical measurements were performed using a multipotentiostat (VMP-Bio-Logic) with a platinum grid (Pt, Ir 10%) used as counter electrode (CE) and a silver wire coated with silver chloride (Ag/AgCl 0.5 M) was used as reference electrode (RE).

The open circuit potential (OCP) was measured in function of time for all the experiments. Electrochemical impedance spectroscopy (EIS) was used to obtain information on the interface, using a frequency from 100 kHz to 10 mHz and an amplitude of 10 mV.

2.4. Surface analysis

Scanning Electron Microscopy (SEM) pictures were taken using a TM3000 Hitachi Analytical Table Top Microscope at 7000× magnification working at 15 kV acceleration voltages, immediately after removing the coupons from the solution. The coupons were washed with

Table 1
Chemical composition of AISI S235 JR (wt.%). Main compound: iron.

Alloy	C	Mn	Cu	S	P	N
S235	0.17	1.40	0.55	0.03	0.03	0.01

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distilled water and dried with the gas mixture N_2/CO_2 80:20. Energy dispersive X-ray spectroscopy (EDX) was used for elemental analysis of the coupons.

2.5. Weight loss tests

Weight loss tests were performed following the American Society for Testing and Materials (ASTM) standards D26811 during 3 months. Rectangular sheets with dimensions of 20 mm × 10 mm × 1 mm were used. The sheets were immersed in ASW and MIXED water from the field both supplemented with 10 mM sodium acetate, 25 mM sodium fumarate and 2 g/L of sodium bicarbonate as added buffer to a total volume of 100 mL per test. Fishing thread was used to suspend the coupons into the water mediums served in 150 mL glass anaerobic vials hermetically closed with butyl rubber septums. ASW and MIXED water were flashed with N_2/CO_2 during 1 h previous to the inoculation and 30 min after inoculation.

At the end of the experiment, coupons were cleaned introducing the coupon in a solution of 50% vol HCl concentrated and 2.5 g/L of EDTA ($C_{10}H_{16}N_2O_8$) during 30 s. Weight loss was calculated after the cleaning procedure using the following equation:

$$\text{Vcorr}\ (\text{mm/y}) : \frac{0.456\ X}{tA} \quad (1)$$

where:

0.465: is a factor accounting for the dimensional analysis and the density of the steel.
X: is the difference between "initial weight" and "weight after cleaning" in milligrammes
t: is exposure time in days
A: is total area of the coupon in cm^2.

2.6. Molecular identification of bacteria

Pigging debris from the water injection pipeline "A" and bacterial pellets from the bacterial growth resulting from the electrochemical experiments previously described were analysed in the Microbiology Research Laboratory of the University of Portsmouth (UoP), UK, aiming identification and possible quantification of microorganisms which may be involved in corroding the systems. The samples were analysed using techniques which include, Polymerase Chain Reaction (PCR), Denaturing Gradient Gel Electrophoresis (DGGE), and examination through sequencing.

DNA was extracted using NucleoSpin® (Macherey-Nagel) following the manufacturer's instructions. A NanoDrop ND-1000 spectrophotometer (Thermo Scientific, USA) was used to establish the concentration of extracted and purified DNA.

PCR was performed on DNA extracted from samples in order to probe the presence of gene 16SrRNA. The targeted gene was amplified using the primers 341F+GC: 5′-CGCCCGCCGCGCGCGGGCGGGGCG GGGGCACGGGGGGCCTACGGGAGGCAGCAG-3′ and 907R: 5′-CCGT CA ATTCMTTTGAGTTT-3′ [22] (purchased from Life Technologies, UK). Reactions contained 10× GoTaq® Green Master Mix (12.5 μL, Promega); primers 341F+GC and 907R (each 1 μL, 10 pmol/μL). Reactions were initially denatured at 94 °C for 4 min, followed by a touchdown PCR: 20 cycles of 94 °C for 1 min, 63–54 °C for 1 min and 72 °C for 1 min followed by 15 cycles of 94 °C for 1 min, 53 °C for 1 min and 72 °C for 1 min plus an additional 10 min cycle at 72 °C. Reactions were performed in 25 μL reaction mixtures.

Agarose gel electrophoresis was performed after PCR to determine the size of the PCR products by running beside a DNA marker (1 kb Ladder, Promega). PCR products were run on 0.9% of agarose gel on 150 V for 30 min to 1 h to enable proper separation. Agarose gel was stained with SYBR® Safe DNA gel stain (Invitrogen Corp., USA) and viewed under UV transillumination (Alpha Innotech Corporation, USA) and Digital Camera (Olympus C-4000 Zoom) to ensure that the correct size fragment was amplified.

To separate the PCR-amplified products for bacterial 16S rRNA genes (550 bp), 40 μL of PCR-amplified products was used in an Ingeny Gel Apparatus (Ingeny, Netherlands), at a constant voltage of 90 V for 18 h and a constant temperature of 60 °C, after an initial 15 min at 200 V. The standard gradient was formed of 6% polyacrylamide in 0.5× TAE buffer with between 30% and 90% denaturant (7 M urea and 40% formamide defined as 100% denaturant). After electrophoresis, the gel was stained with SYBR® Safe DNA gel stain (Invitrogen Corp.), viewed under UV transillumination, and a permanent image captured by the Alpha Innotech Gel Documentation System (Alpha Innotech Corporation, USA).

All visible bands were cut from the gel using a sterile scalpel blade and transferred into sterile 1.5 mL microcentrifuge tubes containing 30 μL of ultra-pure water to extract the DNA. Samples were then centrifuged at 13,000 ×*g* for 1 min (Heraeus Fresco 21, Thermo Scientific, UK) and 5 μL aliquots of supernatants were used for PCR re-amplification as described above. PCR products were purified using the NucleoSpin® Extract II PCR purification kit (Macherey-Nagel, UK) and sent to the GATC Biotech UK DNA sequencing service.

3. Results and discussion

3.1. Open circuit potential results and observations

Low carbon steel coupons were immersed in ASW and MIXED water from the field during at least 600 h. The metallic coupons were immersed in the reactor medium 24 h before inoculation. The systems were kept under continuous N_2/CO_2 (80/20) flow during the whole time of the experiments. At the 24th hour, the reactors were inoculated with approximately 10 g of pigging debris obtained from the water injection pipeline, under a continuous inert gas flow. In the cases of the control reactors, the pigging debris was sterilised by autoclaving it before introducing it to the reactor. In this way both systems, control and inoculated, were identical to one another and the only variant element was the presence of microorganisms. The electrochemical behaviour of the system was tested recording OCP in function of time.

3.1.1. Open circuit potential results with ASW

Fig. 1 describes the OCP behaviour of low carbon steel submerged in ASW supplemented with acetate and fumarate in 4 different experiments running in parallel: two control systems (curves A and B) and two systems with microorganisms (curves C and D). OCP remained stable along the time in the control reactors having an average increment of no more than 150 mV vs. Ag/AgCl during the whole experiment

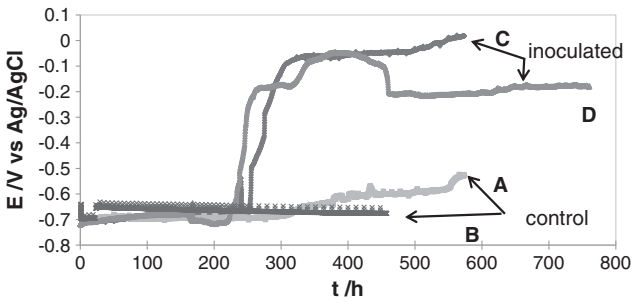

Fig. 1. Variation of OCP (E/V vs t/h) of low carbon steel S235JR in the presence of approximately 10 g of pigging debris in artificial seawater. The ASW contains 0.5 M of chlorides and a supplement of 10 mM of sodium acetate (electron donor) and 25 mM of sodium fumarate (electron acceptor). A and B: control system with pigging debris autoclaved; C and D: inoculated system with pigging debris.

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(600 h). In contrast, a potential increment of over 500 mV vs Ag/AgCl in the inoculated reactors (curves C, D) after t = 200 h was observed.

Note that the results of the experiments stated in this paper were carried out 4 times for the systems with bacteria and 3 times for the control system finding high reproducibility amongst them. In all the cases, increment of the OCP on the inoculated systems was observed whereas none or very little increment was observed in the control systems. The increments on OCP observed in the systems with bacteria were observed between t = 200 and 300 h of the experiment (see Table 2).

The increment in OCP and corrosion potential indicates that there is higher corrosion risk with an incremented probability for pitting and crevice corrosion [21,23,24] which in this case has been enhanced by the presence of microorganisms in the system. The absence of such an increment in the control reactors leads to think that the absence of bacteria posed conditions less aggressive than in the presence of microorganisms.

Macroscopic precipitates observed in the abiotic systems reveal mainly yellow rust looking-iron oxides whereas black precipitates and high turbidity are observed in the inoculated systems suggesting a high quantity formation of iron sulphide, FeS. These observations were corroborated when observing the metal coupons using SEM microscopy. Fig. 2A shows deposits formed on the surface of the metal in the presence of microorganisms whereas Fig. 2B shows no deposits on the surface of the metal of the control system and only a general corrosion pattern is observed.

Surface analysis using energy dispersive X-ray spectroscopy (EDX) (Table 3A) confirmed that the deposits formed on the surface of the coupon in the presence of microorganisms contained high quantity of sulphur and iron (1:1 %), backing up the argument made after macroscopic observations suggesting that the deposits formed are mainly FeS. Table 3B shows the presence of mainly Fe, O and C suggesting, together with the SEM micrographs, that the type of corrosion of the control system is dominated by general corrosion in the absence of oxygen.

Coupons were also observed after performing chemical cleaning aiming to observe pitting corrosion (see Section 2.6) using a 100× lens magnifier (Fig. 2). In Fig. 2C (inoculated system) general corrosion attack with numerous small pits is observed. In contrast, Fig. 2D (control system) shows less general corrosion compared to the inoculated system, to the point that after removing the iron oxide layer, grinding lines may still be observed.

Corrosion enhanced by the formation of FeS in conditions lacking oxygen has been widely studied. For instance, Mc Neal et al. [25] proposed a thermodynamic model about the susceptibility of metal substrata changed by microbiologically produced sulphides in order to explain ennoblement. This model for predicting SRB-influenced corrosion is based on the likelihood that a metal would react with microbiologically produced sulphide which will attack the oxide layer on the metal (or the metal itself) destabilising it and making it act as a source of metal ions. If the reaction to convert the metal oxide to a metal sulphide has a positive Gibbs free energy under surface conditions, the sulphide will not strip the protective oxide and no corrosion will take place. If the free Gibbs energy for the reaction is negative, the reaction will proceed, sulphide microcrystals will redissolve and precipitate as larger, more sulphur rich crystals, ultimately altering the sulphide minerals stable under biofilm conditions.

In our paper, the ennoblement of OCP is attributed to the acceleration of the rate of the cathodic reaction which is the evolution of H_2 catalysed by the presence of sulphide formed by the microorganisms present in the pigging debris of the water injection system. Indeed, it is acknowledge that other mechanisms (change in the local pH, cathodic depolarisation, increase of the partial pressure by biofilm formation, thermodynamics, among others [23–26]) may play a role along with the described one, in the acceleration of corrosion of carbon steel.

3.1.2. Open circuit potential results with MIXED water from the field

Fig. 3 describes the OCP behaviour of low carbon steel submerged in MIXED water from the field of 4 different experiments running in parallel: two control systems (curves A and B) and two systems with microorganisms (curves C and D). MIXED water from the field has a high quantity of long chain hydrocarbon contaminants due to the water recycling process performed from the production pipelines. These hydrocarbons may be used by microorganisms as carbon source to enhance growth rates but it is a time consuming process [27] whereas short chain volatile fatty acids (VFAs) are consumed in a much shorter period of time. These VFAs are not present in high quantities in this water (see Table 4) thus, acetate was added in order to accelerate bacterial growth to both systems (inoculated and control).

In this MIXED water system, OCP also remained stable along the time in the control reactors having an average increment of no more than 100 mV vs. Ag/AgCl during the whole experiment. In contrast, a preliminary corrosion potential increment of 150 mV vs. Ag/AgCl in the inoculated reactors (curves C, D) between t = 180 and 280 h was observed followed by a larger increment of 350 mV around hour 500,

Table 2
Initial and final potentials of S235 JR steel, pH and planktonic cell concentration at the beginning and at the end of the experiments using ASW and MIXED water from the field. (A) Inoculated systems, and (B) control systems.

A

	Planktonic CFU/mL	pH initial	pH final	Initial E(OCP)/V vs Ag/AgCl	Final E (OCP)/V vs Ag/AgCl	time/h after inoculation
ASW inoculated	60.5×10^6	6.8	7.2	−0.67	−0.25	470
	30×10^6	6.6	7.1	−0.72	−0.18	580
	55×10^6	6.7	7.1	−0.72	−0.09	480
MIXED inoculated	4×10^6	6.5	7.0	−0.72	−0.23	550
	4.2×10^6	6.5	6.9	−0.75	−0.21	550
	3.5×10^5	6.8	7.1	−0.70	−0.43	600
	5.7×10^5	6.6	6.7	−0.71	−0.14	480

B

	pH initial	pH final	Initial E (OCP)/V vs Ag/AgCl	Final E (OCP)/V vs Ag/AgCl	Time/h after inoculation[a]
ASW control	5.9	5.8	−0.73	−0.67	475
	6.1	5.8	−0.69	−0.68	580
	6	6.5	−0.73	−0.65	480
MIXED control	6.8	7.1	−0.72	−0.60	550
	6.4	6.6	−0.72	−0.59	550
	6.7	6.8	−0.72	−0.53	600
	6.5	6.8	−0.71	−0.67	480

[a] Inoculation with sterile pigging debris.

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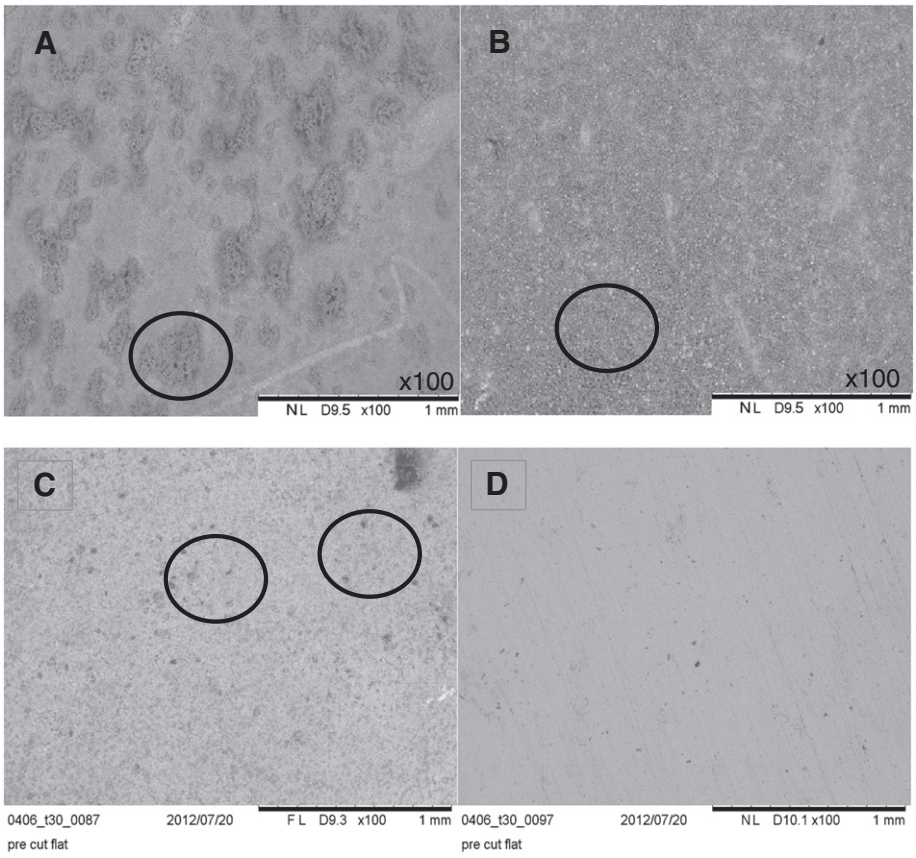

Fig. 2. Micro-photographs of coupons after 600 h of immersion in ASW: (A) SEM image of layer formed in the coupon with bacteria. (B) SEM image of control coupon. Micro-photographs of coupons after 600 h of immersion in ASW and MIXED water (C and D) after cleaning treatment with HCl + EDTA: (C) SEM image of the coupon with bacteria (circles point out zones of pitting corrosion), and (D) SEM image of control coupon.

after the addition of an oxygen free solution of sodium acetate and sodium fumarate between hours 250 and 350. This bigger increment on the corrosion potential is consequently due to a faster development of microorganisms that increased corrosive conditions once in the presence of these short chain carbohydrates (acetate and fumarate).

Deposits macroscopically observed in the abiotic systems (control) with MIXED water reveal that there are also yellow rust looking-iron oxides, whereas in the inoculated ones (with microorganisms) a slight turbidity and light greyish colour deposits in the medium can be observed that are due to their organoleptic properties, which can be inferred that there is a small quantity of FeS. Nevertheless, the colour observed in this system is not as dark as the one observed in ASW. These macroscopic differences between ASW and MIXED water suggest that the corrosion inhibitor and or the presence of hydrocarbon species may be inhibiting not only corrosion but also bacterial growth and/or sulphide production.

Table 2 resumes total bacterial counting in ASW and MIXED water systems at the end of the experiments. At the times considered, a lower concentration of bacteria in MIXED water can be observed compared to bacterial concentration in ASW despite the acetate addition. The low bacterial number at the end of the experiments of MIXED water compared to the counting performed in ASW, supports the argument about bacterial inhibition by the corrosion inhibitor used by the

Table 3
EDX analysis of coupon surface after 600 h of immersion in ASW (see Fig. 3). (A): coupon with bacteria; (B): control coupon.

Element	Weight %	Atomic %
A		
O	15.8	31.0
Na	12.5	17.1
Mg	1.7	2.2
S	24.2	23.7
Ca	0.8	0.6
Fe	45.1	25.4
B		
C	22.2	42.3
O	21.4	30.6
Na	5.4	5.4
S	2.8	2.0
Fe	48.2	19.8

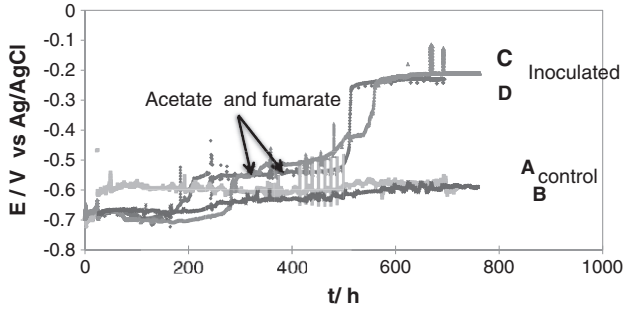

Fig. 3. Variation of OCP (E/V vs t/h) of low carbon steel S235 JR in the presence of approximately 10 g of pigging debris in MIXED water medium. A supplement of 10 mM of sodium acetate (electron donor) and 25 mM of sodium fumarate (electron acceptor) was added. A and B: control system with autoclaved pigging debris; C and D: inoculated system with pigging debris.

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Table 4
Average values of chemical analysis performed on the water injected in the water injection system "A" between 2001 and 2009. Analysis performed and provided by the oil and gas industry which provided the pigging samples analysed in this paper.

Conductivity (mS/cm)	pH	H_2S (mg/L)	Total organic C (mM)	SO^{2-}_4 (mM)	NO_3^- (mM)	PO^3_4 (mM)	NH_4^+ (mM)	Formic acid (mM)	Acetic acid (mM)	Propanoic acid (mM)	Butyric acid (mM)	Pentanoic acid (mM)
38.75	7.45	0.77	3.74	0.42	0.03	0.03	1.33	0.09	0.96	0.08	<0.02	<0.02

company. Due to confidential policies of the company not much information has been provided about the inhibitor.

When observing the metal coupons using SEM microscopy (Fig. 4) the less aggressive corrosion attack is evident compared with experiments in ASW. Fig. 4A shows deposits formed on the surface of the metal in the presence of microorganisms whereas Fig. 4B shows no deposits on the surface of the metal except only for iron oxide.

Surface analysis using energy dispersive X-ray spectroscopy (EDX) (Table 5A) confirmed that the deposits formed on the surface of the coupon in the presence of microorganisms contained low quantity of sulphur (0.7% of the total atomic weight) but high quantities of oxygen (68.6% of the total atomic weight) and Fe (14.5% of the total atomic weight) suggesting that only a small quantity of the deposits precipitated as FeS and instead there is a high quantity of iron oxides. Table 5B shows the presence of mainly Fe, O and C suggesting, together with the SEM micrographs, that the type of corrosion of the control system is dominated by general anaerobic corrosion in the absence of microorganisms.

Surface observations on the clean coupons were also performed aiming to observe pitting corrosion (see Section 2.6) using a 100× lens magnifier (Fig. 4). In Fig. 4C (inoculated system) a general corrosion attack with several irregular pits is observed. Pits seem to be of irregular form but slightly bigger in diameter compared to the ones found in ASW. The reason why these pits seem bigger in diameter might be due to the corrosion inhibitor, which has inhibited further general corrosion inducing a more marked visual effect on the pitting zones. In contrast, Fig. 4D (control system) shows a general corrosion attack that is considerably less strong to the one observed in the inoculated system and to the one observed in ASW. Grinding lines are more evident on the control coupon from MIXED water compared to the control coupon submerged in ASW; once again, it may be due to the presence of the corrosion inhibitor present in the MIXED water from the field.

3.2. Electrochemical impedance measurements

Electrochemical impedance spectroscopy measurements were performed in order to complement OCP results and surface observations to elucidate the effect of the microorganism consortium present in the pigging debris on the corrosion of carbon steel. Two different media were used, ASW and MIXED water; each of them has a control system without microorganisms.

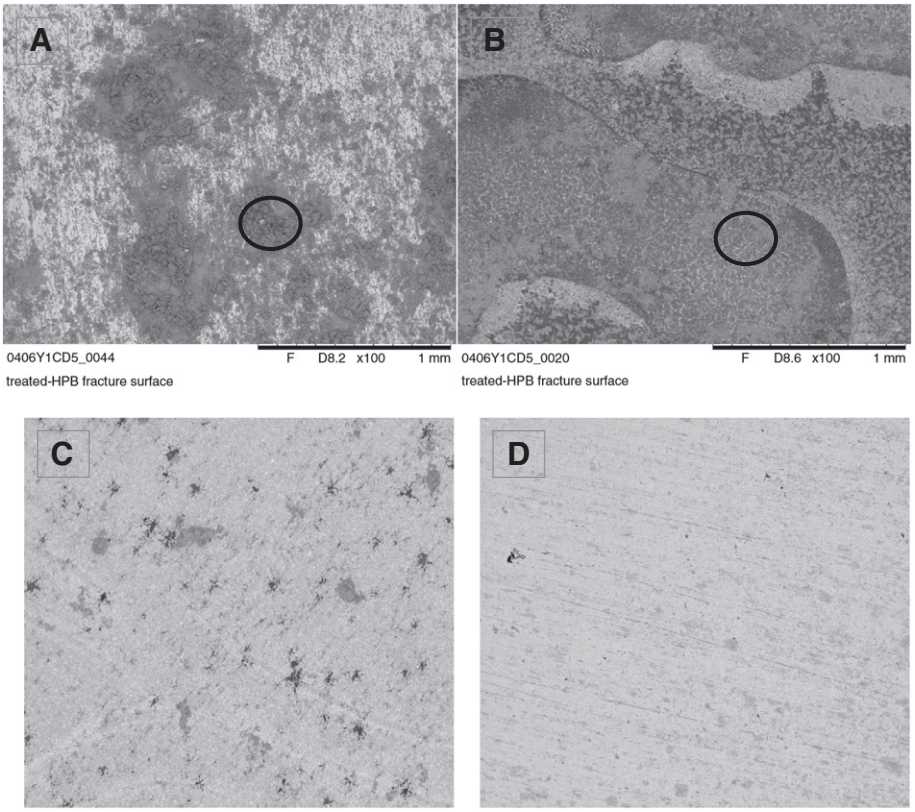

Fig. 4. Micro-photographs of coupons after 600 h of immersion in MIXED water: (A) SEM image of layer formed in the coupon with bacteria. (B) SEM image of control coupon. Micro-photographs of coupons after 600 h of immersion in MIXED water after cleaning treatment with HCl + EDTA: (C) SEM image of the coupon with bacteria. (D) SEM image of control coupon.

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Table 5
EDX analysis of coupons surface after 600 h of immersion in MIXED water (see Fig. 8). (A): coupon with bacteria; (B): control coupon.

Element	Weight %	Atomic %
A		
C	8.6	15.2
O	50.1	68.6
Fe	37.1	14.5
S	3.5	0.7
B		
C	5.4	9.9
O	54.3	74.8
Na	4.0	3.8
S	0.3	0.2
Cl	0.6	0.4
Fe	18.8	7.4

Table 6
Evolution in time for experimental data of R_s, and R_p of the control and inoculated systems in ASW supplemented with 10 mM of acetate and 25 mM of fumarate in the presence of a coupon of S235 JR. Systems kept in anaerobic conditions. (A) control system; (B) inoculated system with pigging debris. NA: not applicable due to linear shape at LF.

Time (h)	R_S (Ω cm^2)	R_p (Ω cm^2)
A		
Initial	13	220
24 h	13	314
72 h	15	3500
144 h	15	5650
216 h	14	5650
288 h	14	NA
360 h	13	NA
432 h	12	270
504 h	12	285
540 h	12	300
B		
Initial	9	270
24 h	10	220
72 h	10	282
144 h	10	452
216 h	8	NA
288 h	11	NA
360 h	10	NA
432 h	9	NA
504 h	9	NA
540 h	9	NA

3.2.1. EIS results with ASW

Fig. 5 shows the impedance response for carbon steel exposed to ASW medium and its evolution with time for the abiotic control system. In the Nyquist diagram, the shape of one semi-circle is observed at all times during the whole experiment, except for times 280 and 360 where a linear shape is observed at low frequencies (LF). The depressed semi-circles and its fluctuating diameters are represented in terms of polarisation resistance (R_p) in Table 6A. R_p corresponds to the real impedance part which crosses the axis at LF. Initially, an increasing tendency of R_p (increase of the semi-circle diameter) is observed between 0 and 216 h (220 to 5650 Ω cm^2, respectively). For times 288 and 360 the phenomena seem to be more complex and R_p cannot be calculated. Beyond 360 h the tendency shifts displaying decreasing R_p values along time.

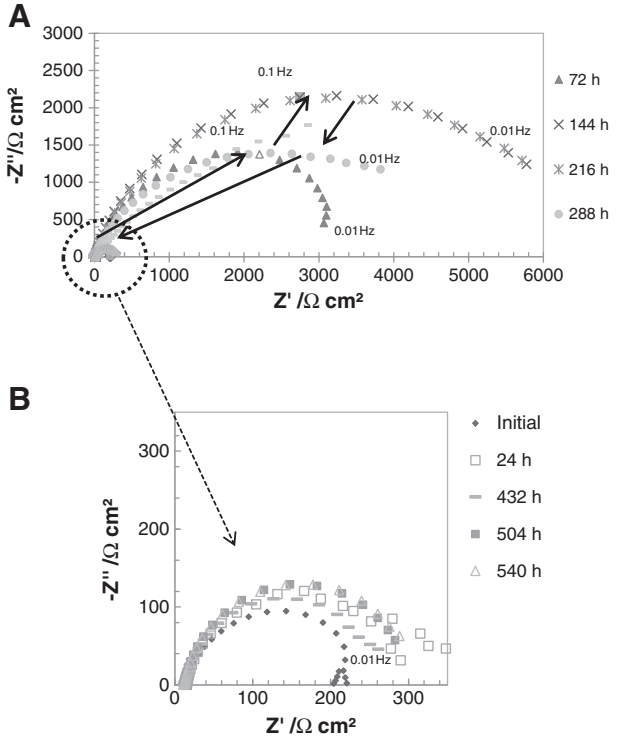

Fig. 5. Impedance spectra of carbon steel S235JR during 600 h of immersion in ASW medium. Control (abiotic) system with pigging debris autoclaved kept in anaerobic conditions. Supplemented with 10 mM acetate and 25 mM of fumarate. (A) Nyquist plot (Z′/Ωcm^2 vs. Z″/Ωcm^2), (B) Zoom-up Nyquist diagram of highlighted zone by a dashed circle.

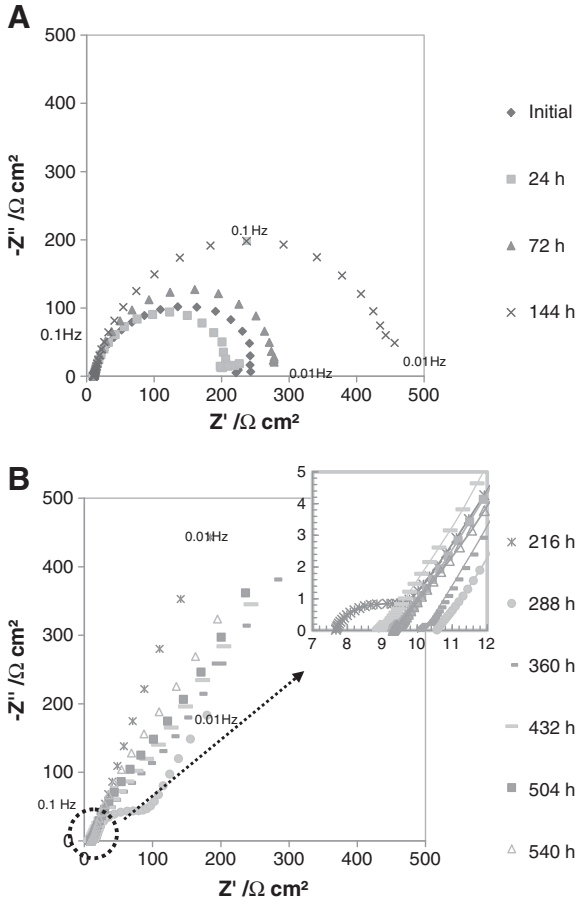

Fig. 6. Impedance spectra of carbon steel S235JR during 600 h of immersion in ASW medium inoculated (biotic) system kept in anaerobic conditions. Supplemented with 10 mM acetate and 25 mM of fumarate. (A) Nyquist plot (Z′/Ωcm^2 vs. Z″/Ωcm^2), and (B) zoom-up Nyquist diagram of highlighted zone by a dashed circle.

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Fig. 6 shows the impedance response for carbon steel exposed to the microorganisms present in the pigging debris in ASW medium and its evolution with time. For the initial times (t = 0 to 144 h), the presence of a depressed semi-circle is observed in the Nyquist diagram, and an increasing diameter is observed during the time which, in terms of R_p, corresponds to an increase in the corrosion protection (R_p from 220 to 452 Ω cm^2, Table 6B), therefore a decrease on the corrosion rate. From t = 216 h the Nyquist diagram shows straight lines at LF preceded by a semi-circle shape at HF for t = 216 and 288 h (Fig. 6B). The linear shapes observed at HF can be linked to mass transfer phenomenon which appears after inoculation with biotic pigging debris when the OCP jumped and the medium turned black.

Due to the so varied impedance shapes and responses exposed by these systems (control and inoculated), in time, a sole model does not achieve to represent the different mechanisms observed. Thus, these systems were now analysed qualitatively, only making allusion to R_p values (that provide us an estimation on the corrosion protection) and/or mass transfer phenomenon.

First, the two systems (control and biotic) exposed the same behaviour between t = 24 and 144 h, i.e. an increase of R_p suggesting the formation of a protective layer. However, it is important to indicate that the magnitudes for R_p in the biotic system are 12 times smaller than in the control abiotic system, showing that the layer in the presence of microorganisms is less protective than the one in the absence of them, at the times considered. The expected growth of bacteria certainly catalysed the formation of FeS, whereas in the control, the formation of an oxide layer is presumed. For the two cases, the following mechanisms are proposed, sustained by macroscopic and surface observations and EDX results.

For the control system:

$$2H_2O + 2e^- \rightarrow H_2 + 2OH^- \quad (2)$$

$$Fe^0 \rightarrow Fe^{2+} + 2e^- \quad (3)$$

$$Fe^{2+} + 2OH \rightarrow Fe(OH)_2 \quad (4)$$

Where Eq. (2) is the cathodic reaction for neutral conditions; Eq. (3) is the anodic reaction in neutral conditions and Eq. (4) is the final corrosion product that precipitated as a yellow rust looking product mentioned in Section 3.1.1.

For the inoculated system:

$$CH_3COOH + SO_4^{2-} \rightarrow 2HCO_3^- + H_2S \quad (5)$$

$$Fe^{2+} + H_2S \rightarrow FeS + 2H^+ \quad (6)$$

Where Eqs. (5) and (6) are reactions catalysed by the presence of sulphate reducing microorganisms (SRM). Reaction (6) is faster mediated by microorganisms than in their absence and also promotes the so-called hydrogen embrittlement of the metal [29].

For times beyond 216, both systems suffer a switch on the impedance behaviour. For the case of the control system, the drastic decrease of R_p observed can be attributed to the decrease of the protection and to a subsequent higher dissolution of the metal. For the case of the biotic system, a mass transfer phenomenon appears, when it is presumed that the rate of the proton consumption increased on the FeS deposit concomitantly with a faster dissolution of iron (anodic reaction) and then a bigger production of FeS is induced [28].: FeS is then found not only on the coupon surface but also as a precipitate in the reactor.

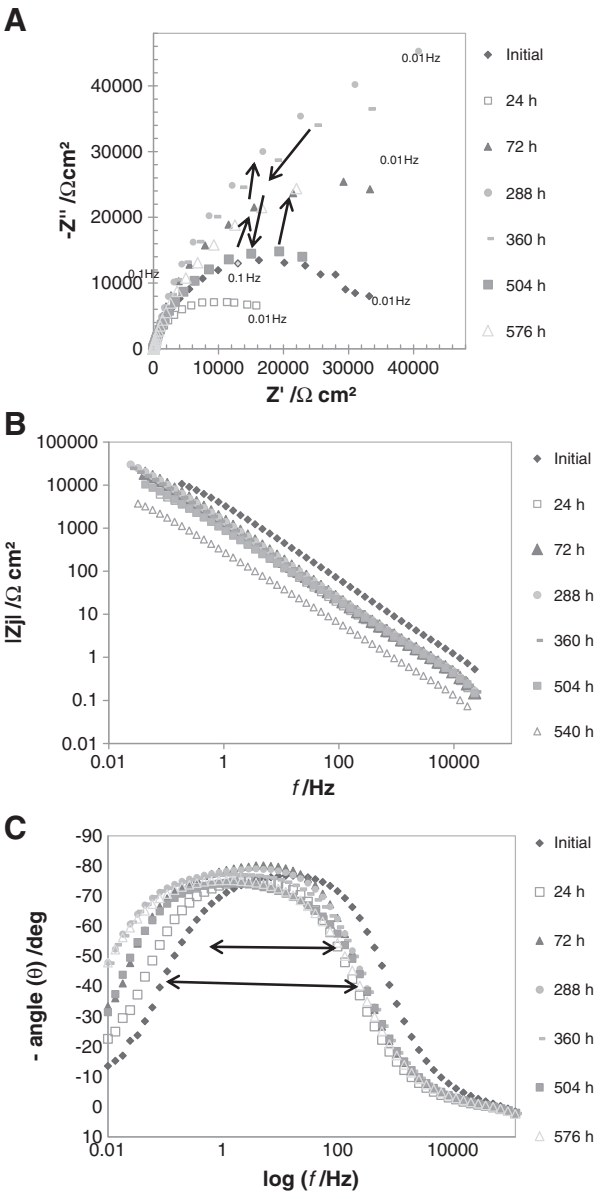

Fig. 7. Impedance spectra of carbon steel S235JR during 600 h of immersion in MIXED water medium. Control (abiotic) system with pigging debris autoclaved kept in anaerobic conditions. Supplemented with 10 mM acetate and 25 mM of fumarate. (A) Nyquist (Z′/Ω cm^2 vs. Z″/Ω cm^2), (B) bode plot of imaginary part of the impedance as function of angular frequency (|Z″|/Ωcm^2 vs. *f*/Hz) and (C) bode plot phase angle vs. frequency (angle (Π)/deg. log(f/Hz)).

3.2.2. EIS results with MIXED water from the field

Fig. 7 (A, B and C) shows the impedance response for carbon steel exposed to abiotic MIXED water medium with autoclaved pigging debris (control system) and its evolution with time. The Nyquist diagram (A) displays the presence of one depressed semi-circle along the different times of measurement. The semi-circles depicted fluctuating diameters. Fig. 8 displays the impedance response for carbon steel in the presence of microorganisms from the pigging debris and its evolution with time. One capacitive loop is observed at all the times tested, exposing depressed semi-circles which grow in size until reaching steady state from around t = 144 h.

Thanks to the apparent simplicity of the MIXED water systems (compared to ASW) the analysis of the impedance response was treated using the approach reported by Orazem et al. [30] plotting the modulus of the imaginary component of the impedance in function of the frequency in logarithmic coordinates, withdrawing the drawbacks of

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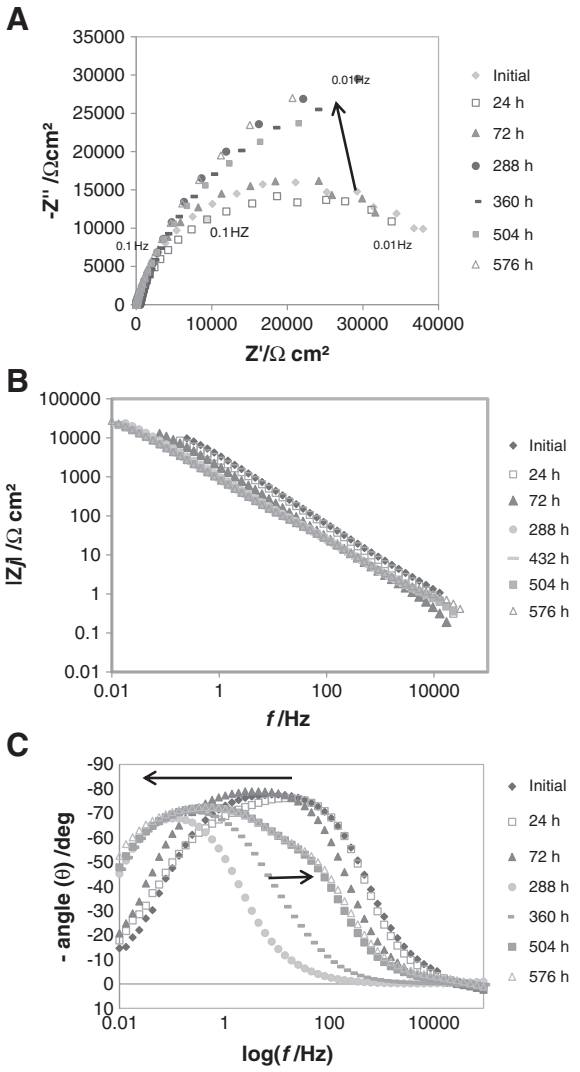

Fig. 8. Impedance spectra of carbon steel S235JR after 600 h of immersion in MIXED water medium. Inoculated (biotic) system kept in anaerobic conditions. Supplemented with 10 mM acetate and 25 mM of fumarate. (A) Nyquist plot (Z'/Ω cm^2 vs. Z''/Ω cm^2), (B) bode plot of an imaginary part of the impedance as function of frequency ($|Z''|/\Omega$cm^2 vs. f/Hz) and (C) bode plot phase angle vs. frequency (angle (Π)/deg. log(f/Hz)).

the influence of the electrolyte resistance on the estimation of time constants (Figs. 7B and 8B). The slope values of the linear parts at high frequencies (HF) were used for the calculation of alpha (α) values to determine whereas the system followed a constant phase element (CPE) behaviour or a pure capacitive behaviour [30]. For both systems, the α values found and displayed in Table 7 were lower than 1 confirming a CPE behaviour, i.e. the system exposed a heterogeneous distribution of time constants. The impedance of a CPE can be described by Eq. (7) [30,31]:

$$Z_{CPE} = 1/Q(\omega)^{\alpha}\tilde{n}[\cos(\alpha\pi/2) - j\sin(\alpha\pi/2)] \text{or} 1/Q(j\omega)^{\alpha} \quad (7)$$

where the coefficient Q was calculated by using the imaginary part of the impedance (Z_j) with:

$$Q = -1/[Z_j(f)(2\pi f)^{\alpha}]\tilde{n}\sin(\alpha\pi/2) \quad (8)$$

Table 7
Evolution in time for experimental data of R_s, R_{ct}, α, Q_{eff} and C_{dl} of the control and inoculated systems in MIX water supplemented with 10 mM of acetate and 25 mM of fumarate in the presence of a coupon of S235 JR. Systems kept in anaerobic conditions. (A) control system; (B) inoculated system with pigging debris.

Time (h)	R_s (Ω cm^2)	R_{ct} (Ω cm^2)	α	Q_{eff}(Ω^{-1} cm^{-2}s$^{\alpha}$)	C_{dl} μF cm^{-2}
A					
Initial	11	32,300	0.86	6.0E−05	18.4
24 h	10	17,900	0.84	2.4E−04	74.7
72 h	9	60,000	0.88	1.3E−04	57.0
144 h	9	100,500	0.89	1.3E−04	56.6
216 h	9	100,000	0.88	1.4E−04	55.9
288 h	9	118,400	0.87	1.4E−04	55.6
360 h	9	100,100	0.86	1.7E−04	60.3
432 h	8	36,800	0.84	2.0E−04	59.8
504 h	8	37,000	0.83	2.4E−04	65.6
576 h	8	72,200	0.82	2.7E−04	69.6
B					
Initial	12	41,000	0.86	5.9E−05	18.2
24 h	12	34,700	0.87	7.1E−05	24.5
72 h	12	41,000	0.89	1.1E−04	49.9
144 h	16	817,000	0.81	2.6E−04	71.7
216 h	17	107,000	0.82	2.1E−04	61.9
288 h	18	109,000	0.80	2.2E−04	53.2
360 h	18	87,000	0.81	2.4E−04	65.4
432 h	19	85,000	0.78	2.5E−04	55.8
504 h	19	84,000	0.77	2.7E−04	55.0
576 h	15	110,000	0.75	2.8E−04	44.8

and

$$\omega = 2\pi f. \quad (9)$$

Table 7 also exposes the Q values (Q_{eff}) corresponding to the experimental parameters of the CPE impedance. The capacitance values were calculated applying the equation derived by Brug et al. [30–32] after assuming that the distribution of time constants was all along the surface:

$$C_{dl} = Q^{1/\alpha}(R_s^{-1} + R_{ct}^{-1})^{(\alpha-1)/\alpha}. \quad (10)$$

For both systems, the C_{dl} values obtained (between 20 and 75 μF cm^2) correspond to typical values for a double layer capacitance [31]. The depressed semi-circle observed is attributed to a resistance to the charge transfer (R_{ct}) that fluctuates along the time (Table 7). These fluctuations provide us an initial estimation on corrosion rates.

For the control system, it is observed that capacitance values do not evolve much along the time but R_{ct} values increase over time until t = 360 h. Beyond this time, R_{ct} values fluctuate between 37 kΩ cm^2 and 100 kΩ cm^2 perhaps reflecting some fluctuations on the activation of the interface. In contrast, for the biotic system a slightly bigger fluctuation is observed on the capacitance values but a lower fluctuation in the R_{ct} values (between 80 kΩ cm^2 and 110 kΩ cm^2 for t = 114 to 576 h, respectively) suggesting that a more stable layer has been formed on the interface. However, the magnitudes for the real impedance are equivalent in both MIXED water systems suggesting that there is probably not much difference in terms of corrosion rate regardless of the presence of microorganisms. Moreover, phase angle plot (Fig. 8C) shows a fluctuating displacement of the phase angle peak between low and middle frequencies, suggesting a continuous fluctuation of the capacitance of the interface with time. These observations are in accordance with capacitance values described in Table 7B.

Furthermore, when the two MIXED water systems (control and inoculated) are compared, it can be inferred that even though the C_{dl} and R_{ct} values are similar, the phenomena occurring at the interface might be different. System with bacteria achieves a steady state after t = 144 h whereas control system R_{ct} fluctuates along time. This divergence might reflect the differences in the interface on

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which the double layer developed. As suggested by micro and macroscopic observations, the layer formed on the coupon surface in the control system is a combination of iron oxides (in the form of magnetite ($FeO^*Fe_2O_3$)) whereas the layer formed in the system with bacteria may contain some deposits of iron sulphide which affected the charge transfer phenomena (R_{ct}, C_{dl}). Moreover, pit areas can be observed on the coupon of the biotic system confirming the idea of this FeS layer formation. Pit formation induced by SRM has been widely studied in the frame of microbial corrosion and its mechanisms have been elucidated by different authors [1,5,10]. Here it can be claimed that even if the bacterial growth was reduced due to the presence of the corrosion inhibitor (previously stated in Section 3.1.2), SRM concentration was sufficient to induce FeS formation and consequently pitting pattern. Iron sulphides could form a continuous film on the surface of the steel whose thickness and structure could confer protective or adherent properties [33,34]. Ruptures in this film could also lead to a system of enhanced galvanic corrosion [34], which may lead to pit formations.

On the other hand, a comparison of the impedance magnitudes in the abiotic systems of the two mediums tested shows that they are smaller in ASW than in MIXED water. The magnitudes of the real part of the impedance in MIXED water are about 200 times higher than in ASW at the initial time (hour zero). The high resistivity observed in MIXED water medium must be due to the presence of corrosion inhibitor and organic acids in the production pipeline water. Thus, this medium could be claimed to be far less corrosive for the carbon steel than ASW. Indeed, the EDX results and macroscopic observations suggest that there was little corrosion in the MIXED water system and only few corrosion products compared to ASW results.

3.3. Weight loss results

Weight loss tests for carbon steel were performed in ASW and MIXED water media using as inoculum two samples of pigging debris (Pigs 1 and 5) collected from the water injection pipeline of system A. Pig 1 corresponds to the first debris collected after the first pigging operation and Pig 5 corresponds to the debris collected after the fifth consecutive pigging operation. Control and blank were run in parallel for the two systems (ASW and MIXED water). The blank consisted on the metal coupon immersed in the respective medium of the system; the control consisted on the previous items plus the addition of autoclaved pig. The mass lost was measured after 3 months of immersion for duplicated coupons. The results obtained from these tests are representative of the complete set of experimental results (Table 8).

In ASW system, the highest corrosion rate observed was in the blank whereas the lowest one observed was in the control system. The corrosion rate of the blank was 90 times higher than in the control system. It is believed that this drastic difference of corrosion rate between the blank and the control systems are due to the organics (in the form of hydrocarbons) and traces of corrosion inhibitor that are attached and released by the debris. Furthermore, when comparing the biotic systems (Pig 1 and Pig 5) a higher corrosion rate in Pig 1 (0.18 mm/year) than in Pig 5 (0.06 mm/year) was observed.

Table 8
Corrosion rate (V(corr)/mm/y) calculations after 3 months of immersion in (A): ASW and (B): MIXED water.

Sample	V_{corr}/mm/y
A	
Pig 1 (biotic)	0.18
Pig 5 (biotic)	0.06
Pigs 1 + 5 (control abiotic)	0.01
Blank	0.95
B	
Pig 1 (biotic)	0.04
Pig 5 (biotic)	0.03
Pigs 1 + 5 (control abiotic)	0.00
Blank	0.04

In MIXED water the results showed that the corrosion rate observed in the blank was 40 times higher than the one observed in the control system. In this case, this difference is mainly attributed to the presence of organics attached to the debris since the amount of residual corrosion inhibitor is the same in the control and the blank of MIXED water. On the other hand, gravimetric measurements of the carbon steel samples indicated that after 3 months of immersion in either ASW or MIXED water, more mass is lost when the carbon steel is immersed in the presence of biotic Pig 1 than biotic Pig 5. However, the relation of mass loss for Pigs 1 and 5 in ASW is (3:1) whereas in MIXED water it is (1.45:1). The explanation for this difference is the higher concentration of corrosion inhibitor in the MIXED water which affects the development of bacteria.

Therefore, the most corrosive media which induced higher mass loss for all the conditions tested was ASW in which a high quantity of corrosion deposits precipitated as biologically induced FeS (Fig. 9C) and planktonic bacterial concentration was greater.

For each condition tested (biotic pig, control autoclaved pig and blank) it was concluded that all of them followed the same trend in both media. The blank was the condition that showed the highest mass loss for both media. This condition did not have inoculated pigging debris, therefore did not have any trace of hydrocarbons. The control for Pigs 1 and 5 which was inoculated with sterile debris was the condition that showed the least mass loss in both media and the pigging debris that induced higher corrosion rates was Pig 1.

Thus, it can be resumed that ASW is the most corrosive system for the immersion of low carbon steel. Furthermore, MIXED water was the least corrosive system for carbon steel and this might be due to the presence of the corrosion inhibitor present in this water. The presence of biotic Pig 1 induced the highest corrosion rates for the biotic systems. In contrast, the presence of autoclaved pigging debris used for the control systems induced the least corrosion rates, probably due to the following reasons (Fig. 9):

(1) the absence of microorganisms, specially the absence of sulphide producers,
(2) the presence of organic acids (in the form of hydrocarbons) attached to the debris,
(3) the presence of corrosion inhibitor traces attached to the debris.

3.4. Bacterial identification

DGGE analysis allows the separation of PCR products of similar size based on the difference of their GC-content in the sequence [19]. DGGE has been applied to the study of microbial community complexity in which the PCR products are variable regions of the 16S rRNA gene, to infer the composition of microbial communities [22].

The DGGE gels in Fig. 10 illustrate the variety of 16S rRNA gene fragments amplified from two sources: 1) from the pigging debris of the water injection system (Fig. 10A) and 2) from the inoculated media used for the electrochemical experiments once these lasts stop (Fig. 10B). Each column in the DGGE gels represents one sample and the number of individual bands obtained is related to the number of bacterial species in the tested samples.

More specifically, Fig. 10 A shows the DGGE community fingerprint of a consortium living in the pigging debris. Out of the 4 samples, a total of 30 strong bands was found; Pig 2 was the sample that exposes the highest quantity of bands (9 bands). However, only 13 bands were taken into account for sequencing assuming that bands that fell in the

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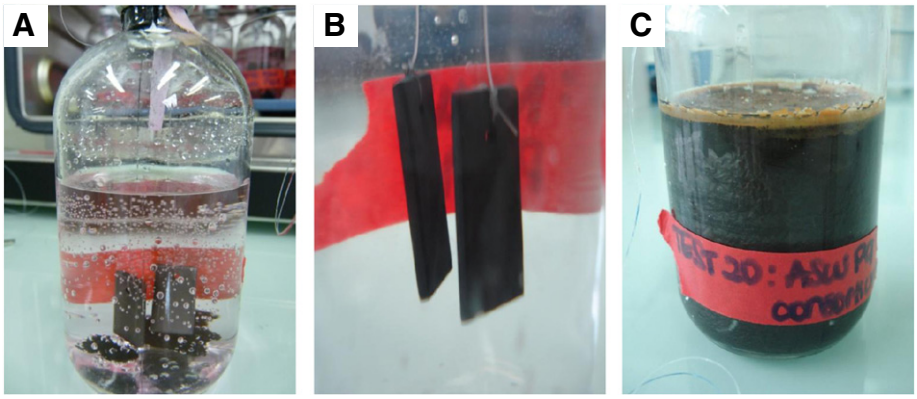

Fig. 9. Weight loss experiment photos for carbon steel S235JR at time zero and after 3 months of immersion. (A): MIXED water with 10 mM acetate and 25 mM fumarate in the presence of pigging debris at time 0; (B): coupons immersed in MIXED water with 10 mM acetate and 25 mM fumarate in the presence of pigging debris after 3 months; (C): ASW with 10 mM acetate and 25 mM fumarate in the presence of pigging debris after 3 months.

same or close-by position in the gel of the different columns were the same repeated DNA sequence or a close polymorphism. Bands marked with "X" (Fig. 10A) did not yield ideal results when sequencing. Likewise, Fig. 10B shows the DGGE community fingerprint of the consortium which developed in the second source (media used for the electrochemical experiments). Out of the 5 samples tested, a total of 16 strong bands was found; ASW was the sample that exposes the highest quantity of bands (6 bands). However, only 9 bands out of the 16 were taken into account for sequencing assuming the same premises mentioned for the previous gel.

Table 9A summarises the bacterial diversity found in the pigging debris extracted from 4 pigging operations performed in the water installation after matching each sequencing result with the most closely related sequence found in the database of the National Centre for Biotechnology Information (NCBI). The obtained sequences were at least 95% identical to already deposited sequences of the GenBank database. Table 9B summarises the species identified that developed in the 2 different mediums used for performing the electrochemical and weight loss experiments after inoculation with pigging debris from the same installation.

A comparison between the bacterial diversity present in the debris and the bacterial diversity that developed in the mediums used for the electrochemical tests was attempted in order to determinate the actual bacteria species that may be involved in the electrochemical and weight loss results in terms of corrosion.

The average of bands found in the gel when amplifying DNA directly from the pigging debris was 7.5 bands. In contrast, the quantity of bands found in the gels containing PCR samples extracted from the media used for the electrochemical tests was 3.2. Moreover, it was observed that only 1 band was found in the 2 samples of MIXED water run in the gel suggesting that bacteria do not develop well in MIXED water and the population diversity gets decreased severely. However, the population diversity does not get as reduced as in ASW, finding in average 5 bands. Overall, it can be said that the quantity of bands detected in the pigging debris was reduced by 33.4% when inoculating the pigging debris in ASW and by 86.7% when inoculating the pigging debris in MIXED water. Thus, the number of bacterial species was reduced when performing the electrochemical and weight loss experiments. These results are in accord with the results obtained with the electrochemical and weight loss experiments.

Among the 11 species identified in Table 9A, 18% of them fall among the SRB group (*Desulfobacter halotolerans* and *Desulfovibrio* sp.). Furthermore, 36% of them are able to produce sulphide as a metabolic

Table 9
Bacterial 16S rRNA sequence results obtained from: (A) pigging debris; (B) developed bacteria in mediums used for electrochemical experiments. Refer to Fig. 10.

ID	Identity (%)	Result
A		
1	95	*Desulfobacter halotolerans* DSM 11383
2	97	*Clostridium halophilum* DSM 5387
3	99	Uncultured Synergistetes bacterium clone NRB29
4	97	*Marinifilum* sp. KYW 585
5	97	*Desulfovibrio* sp. AND1
6	100	*Dethiosulfovibrio russensis* strain WS 100; DSM 12537
7	99	Uncultured *Caminicella* sp. clone TCB261x
7B	99	Uncultured *Caminicella* sp. clone TCB207x
8	100	Uncultured *Thermovirga* sp. clone TCB168x
9	99	Uncultured Synergistetes bacterium clone D010011F15
10	98	Uncultured *Thermovirga* sp. clone TCB8y
B		
12	97	*Desulfovibrio dechloracetivorans* strain SF3
13	99	*Desulfovibrio* sp. Z1 16S ribosomal RNA gene
14	99	Uncultured bacterium 16S rRNA gene, clone Dan_Bac88
15	95	Uncultured *Spirochaetes* bacterium clone D010012E07
16	99	*Dethiosulfovibrio russensis* strain WS 100; DSM 12537
17	100	*Arthrobacter* sp. MDB1-56 16S

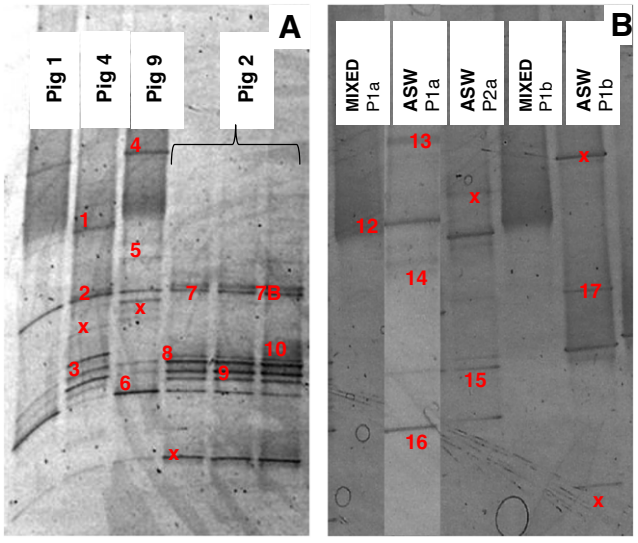

Fig. 10. DGGE gel profile demonstrating diversity of bacterial populations based on PCR products of 550 bp of bacterial 16S rRNA. (A) Profile obtained using directly the pigging debris as samples (Pig 2 in triplicate) (sequenced bands were labelled 1–11; not yield results were labelled "x"), and (B) profile obtained from cultivable bacteria that grew in ASW supplemented and MIXED water supplemented used to perform the electrochemical experiments (bands sequenced labelled 12–30).

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product (*Clostridium halophilum*, *Thermovirga* sp. and the 2 SRB). It was also found that only *Dethiosulfovibrio russensis* is able to reduce elemental sulphur and thiosulphate. Moreover, *C. halophilum*, *Thermovirga* sp. and *D. russensis* share the same taxonomic order; *Clostridiales*. Furthermore, most of the detected bacteria are fermentative bacteria that produce as metabolic products a large variety of organic acids such as butyric, lactic and acetic acids that may affect the local pH accelerating corrosion. SRBs have been widely studied and the danger these bacteria represent to corrosion of metals due to the production of H_2S and FeS catalysed by this group is known. Thus, the presence of these bacteria in pipelines represents the most potential danger in terms of corrosion.

On the other hand, among the 6 bands that yielded acceptable sequences in Table 9B, 3 of them coincided with species found in the pigging debris and 3 with bacteria species that were not detected in the analysis performed to the pigging debris. More precisely, 2 bands resulted to be sequences of bacteria belonging to the SRB group; 1 band belonged to the taxonomic order *Clostridiales* (*D. russensis*), 1 band which sequence corresponds to the typical soil bacteria *Arthrobacter* sp. and 2 bands corresponding to sequences of uncultured bacteria. On the other hand, the detection of the only band in MIXED water yielded the sequence corresponding to the SRB, *Desulfovibrio dechloracetivorans*.

Thus, what was suspected and previously discussed has been confirmed. The corrosion observed in carbon steel S235 JR is enhanced by the presence of SRB and sulphide producers in the 2 media tested. However, the effect of these bacteria is more evident when using ASW due to the better development of bacteria in this medium. The poor development of bacteria in MIXED water medium is attributed to the presence of corrosion inhibitor which reduced the growth of bacteria by nearly 87%.

4. Conclusions

The influence of a consortium of microorganisms present in a sample of pigging debris on the corrosion of carbon steel S235 JR was studied in supplemented ASW and MIXED water media by combining electrochemical measurements, surface analyses and weight loss tests. The bacterial diversity composition of the debris and the resulting growth in the media used were assessed by DGGE analysis of the PCR products for bacterial 16S rRNA genes.

The corrosion potential (OCP) measured in function of time showed a sudden increase of more than 400 mV vs Ag/AgCl in both media tested when the system was exposed to the biotic pigging debris whereas little (less than 100 mV) or none increase was observed in the control systems where the debris was autoclaved. However, it was noticed that this ennoblement occurred later in terms of time and lower in terms of potential increment when MIXED water was used as electrolyte instead of ASW. The increment in OCP or corrosion potential indicates that there is higher corrosion risk with an incremented probability for pitting and crevice corrosion [21,23,24] which in this case has been enhanced by the presence of microorganisms in the system.

In agreement with previous results, EIS measurements show higher impedance magnitudes in MIXED water than in ASW suggesting that MIXED water system is the least corrosive of the two. The high resistivity observed in MIXED water medium must be due to the presence of corrosion inhibitor and organic acids (hydrocarbons) present in the production pipeline where this water has been recycled from. Moreover, in this system, charge transfer governs the interface. In contrast, for ASW, is the presence of FeS catalysed by the microorganisms, the one that gives the characteristics of the interface (mass transfer). In the case of the control systems, it is rather the iron oxides of the type of $Fe(OH)_2$ or $FeO^*Fe_2O_3$ characterising the interface with variations in the quality of protection.

From weight loss tests it can be concluded that results are representative of the whole set of experiments finding higher corrosion rates in ASW than in MIXED water. Moreover, DGGE results proved that bacterial diversity gets decreased when harvesting de debris in the media used and suggested that the bacteria involved in the whole set of results are mainly sulphate reducing bacteria (SRB) and some other bacteria part of the taxonomic order *Clostridiales*.

The combination of electrochemical measurements, surface analyses, weight loss and DGGE tests allow us to conclude that:

- ASW system was more corrosive than MIXED water system.
- Bacteria from the pigging debris grow better in ASW than in MIXED water.
- Bacterial consortium found in the pigging debris altered the electrochemical response of the ASW system tests inducing an increase in the OCP and a decrease in R_p; this is compared to the control abiotic systems. In MIXED water, the presence of microorganisms altered the electrochemical response of the system inducing less fluctuation in R_{ct} along time.
- The bacterial diversity gets reduced when introducing and growing the pigging debris into the 2 water media tested. Thus, it cannot be said that the only bacteria found in the media of the electrochemical tests are representative of the bacteria found in the field. Thus, it cannot be said that only SRB are the ones responsible for the corrosion of carbon steel in the field.

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