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Study of Anaerobic Biofilms from Gasoline Distribution Pipelines

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Petroleum, gas, shipping and water treatment and distribution industries are significantly affected by microbial activity—environments in which biofilms might cause or serious problems such as biofouling, biodeterioration and biocorrosion, which are usually associated with great human, environmental and financial losses. The present work has as a main objective the biological and electrochemical characterization using electrochemical impedance spectroscopy of the anaerobic microorganisms present in gasoline distribution pipelines that might have an influence in corrosion processes, specifically focusing on those biofilm-developers. Several microorganisms have been isolated and their metabolic activities monitored, as well as their electrochemical influence over carbon steel in laboratory conditions.

Introduction

Biofilms are very complex heterogeneous microbial communities attached to solid surfaces or associated with interfaces by a self-produced extrapolymeric matrix (EPM) (1). Biofilm formation is a common survival strategy for the microorganisms embedded within, whose needs consist of a hydrated environment (even poorly hydrated) with a minimal presence of nutrients along with a surface or interface—hydrophobic or hydrophilic, living or inert—on which they can develop (2, 3, 4). This process is of great consequence in many environments, because almost all surfaces are colonizable by the microorganisms forming these biological structures.

Petroleum, gas, shipping and water treatment and distribution industries are significantly affected by microbial activity—environments in which biofilms might cause or serious problems such as biofouling, biodeterioration and biocorrosion, which are usually associated with great human, environmental and financial losses. Petroleum industry has a large metallic infrastructure on which biofilms are able to grow. This occurs in those structures in contact with even very small amounts of water, including pipelines that transport crude oil or refined products. Gasoline distribution pipelines often have small amounts of water associated to the fuel e.g. 0.01%, and this can be an important factor for complex biofilms to develop and influence corrosion of the pipeline, biofouling and biodegradation or contamination of the gasoline compounds.

Thus, the present work has as a main objective the biological and electrochemical characterization of the anaerobic microorganisms present in gasoline distribution pipelines that might have an influence in corrosion processes, specifically focusing on those biofilm-developers. Several microorganisms have been isolated and their metabolic activities monitored, as well as their electrochemical influence over carbon steel in laboratory conditions.

Materials and Methods

13 Samples were obtained from different gasoline distribution pipelines which contained both sediments and gasoline. The samples had a water content of approximately 0.01% and enrichment cultures were performed from them, using the following culture media: (per liter of distilled water): Na_2SO_4 (3.0 g), K_2HPO_4 (0.2 g), NH_4Cl (0.2 g), KCl (0.2 g), $MgCl_2$, $2H_2O$ (0.3 g), NaCl (30 g), $CaCl_2$, $2H_2O$ (0.1 g), sodium acetate (0.5 g), Difco yeast extract (0.5 g), Difco tryptone peptone (0.1 g), trace mineral element solution (10 ml), cysteine-HCl (0.5 g), resazurin 1% (1mg), $NaHCO_3$ 2% (w/v) and final 20 mM concentration of sodium lactate as carbon and organic energy source. The pH of the media was fixed at 7.2 with 10 M KOH solution. The systems were inoculated with 3 mL of sample and incubated at 30 C with no stirring.

Substrate-specific cultures were carried out in a minimal culture media containing (per liter of distilled water): NH_4Cl (0.75 g), K_2HPO_4 (1 g) KH_2PO_4 (2 g), $CaCl_2$ (0.018 g), $MgSO_4$ (0.5 g), $NaCO_2$ (0.02 g), cysteine-HCl (0.5 g), resazurin 1% (1mg) and Fortin and Deshusses trace elements (1 mL). The $CaCl_2$ and $MgSO_4$ are prepared separately using the same procedure and added to the resto f the culture media before cooling, in order to avoid precipitation. The pH of the media was fixed at 7.2 with 10 M KOH solution. The substrates that were tested as sole carbon and energy sources—almost all usually present in gasoline—were benzene, toluene, xilene, pentane, MTBE, TBA, sodium acetate and sodium lacatate. Microbial kinetics experiments (not presented in this work) were performed to quantify the degradation of the recalcitrant compounds and the amount of mineralization.

The anaerobic microorganisms were isolated using the roll-tube technique and working in anaerobic glove box. Biofilm-forming microorganisms were detected and isolated. A microscopic characterization of these microorganisms was performed using a Nikon optical microscope and gram-staining.

With the isolated microbes and artificial consortia, adhesion tests were performed over SAE 1018 carbon steel coupons and the electrochemical influence of the consortia was monitored using Electrochemical Impedance Spectroscopy (EIS) in order to study their possible influence in corrosion processes. In this article the results for just one of these systems is presented.

The electrochemistry experiments included growing biofilms of selected anaerobic consortia. In this work there are just presented the results for a consortium containing anaerobic and aerobic BTEX, MTBE, TBA and lactate-consuming microorganisms. There were used two-electrode electrochemical cells with working electrodes of SAE 1018 carbon steel with a surface of approximately 28.27 cm^2 and counter/reference electrodes Pt/Au (95/5%) electrodes of with surface of approximately 28.27 cm^2.

Only one face of the electrodes was exposed to the culture media, the other faces were covered with Teflon. Prior to immersion, the electrodes were rinsed with distilled water, degreased with acetone, air-dried, sterilized under UV light for 15 min and were maintained under air-drying sterile conditions until their introduction in the described system. The cell was purged with N2 flux and filled with the enrichment culture medium, previously autoclaved at 121 °C, 15lb/in2 for 15 min.

EIS measurements were carried out as a sequence of experiments through time for metal-electrolyte interphase under biotic and abiotic conditions during 120 hours in duplicate experiments, by using a potentiostat coupled with a frequency response analyzer (FRA). EIS measurements (carried out at OCP) were performed with an amplitude of the sine wave of 10 mV. The frequency range used was 10 kHz to 10 mHz and 5 points per decade were used.

Results and Discussion

142 microorganisms which are able to growth in anaerobic conditions were isolated from the enrichment cultures—from which 50% formed biofilms, and 92 microorganisms were found to use specific substrates used as sole carbon source as presented in the table I.

Table I. Microbial growth in the tested organic compounds as sole carbon source. = Planktonic Groth, B = Biofilm, N = No growth.

Compuestos	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S12
Benzene	PG	PG	PG	PG	B	PG	PG	N	PG	PG	N	PG	N
Toluene	B	PG	PG	PG	PG	N	N	N	B	PG	PG	PG	N
Xylene	PG	N	PG	B	B	PG	PG	PG	N	PG	PG	PG	N
TBA	B	PG	B	B	PG	B	PG	N	PG	PG	N	PG	N
MTBE	PG	PG	PG	B	B	N	PG	N	PG	PG	PG	PG	N
Pentane	PG	N	PG	PG	PG	N	PG	N	N	PG	PG	PG	N
Lactate	PG	PG	PG	N	N	N	PG	PG	N	N	N	N	N
Acetate	B	PG	B	PG	N	N	PG	PG	N	PG	N	PG	N

Pure cultures and consortia of the microorganisms that formed biofilms were placed in the presence of SAE 1018 carbon steel coupons to observe if the biofilms were able to adhere the coupons surfaces, and qualitatively determine if they were suitable to participate in corrosion processes (Table II).

Electrochemical Impedance Spectroscopy

The obtained impedance spectra for times between 0 and 120 hours of batch exposition in sterile culture media (figure 1) and in the culture media with the biofilm consortium (figure 2) are shown.

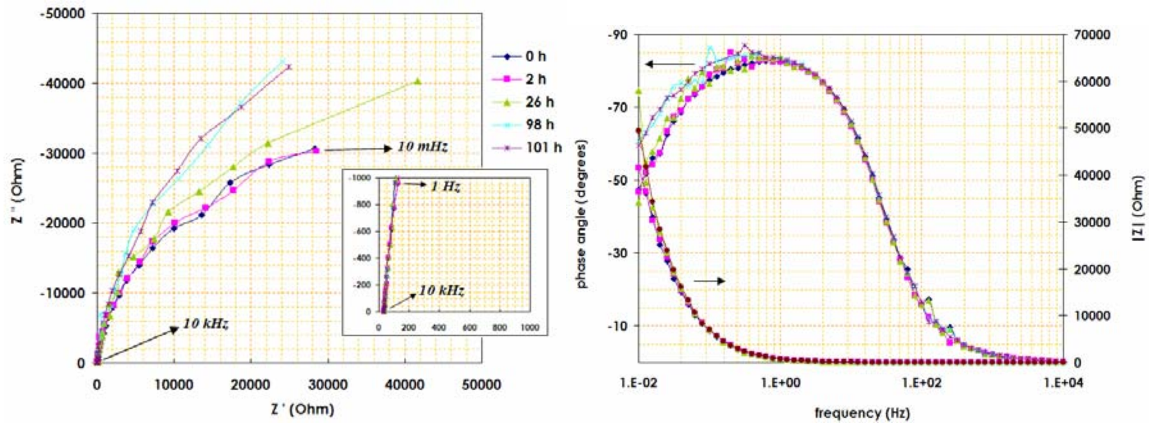
For the abiotic system (sterile culture media) there is observed the presence of one semicircle. This semicircle is associated to an active behavior over the metallic surface which can be due to the dissolution of the metal through time but also to the appearance of corrosion products such as chlorides, sulfates and phosphates.

TABLE III. Adhesioin tests for pure cultures and microbial consortia. The sample numbers refer to isolated microorganisms that have not been yet characterized but that grow with lactate as carbon and energy source. The following sample names refer to the following artificial consortia: AnL = Anaerobic consortia with 24 bacteria that use lactate as carbon and energy source in enrichment culture media, AnSE = Anaerobic consortia with 18 bacteria that use BTEX, MTBE or TBA as carbon and energy source in mineral culture media, AL = Aerobic consortia with 3 bacteria that grow in enrichment culture media with lactate, ASE = Anaerobic consortia with 6 bacteria that use BTEX, MTBE or TBA as carbon and energy source in mineral culture media, AAL = Aerobic/Anaerobic consortia with 27 bacteria that use lactate as carbon and energy source in enrichment culture media, AASE = Aerobic/Anaerobic consortia with 24 bacteria that use BTEX, MTBE or TBA as carbon and energy source in mineral culture media, AASE = Aerobic/Anaerobic consortia with 24 bacteria that use BTEX, MTBE or TBA as carbon and energy source in mineral culture media in the presence of lactate.

Sample	**Adherence to carbon steel coupons**	**Black precipitates formation**	**Biofilm formation**
93-1	++	+++	+
86-2	++	+	**N**
78-1	++	+	**N**
63	++	+	**N**
66-P	++	++	+++
61-4	++	++	++
96-2	++	+++	++
193-1	++	+++	+
89-P2	++	++	+
82-1	++	**N**	**N**
87-1	++	**N**	**N**
94-2	++	**N**	++
64-2	+++	++	+++
92-2	+++	++	+
92-3	+++	++	+++
92-1	+++	++	+++
AnL	+++	+++	+++
AnSE	++	**WY**	+++
AL	+++	+++	+++
ASE	+++	**WY**	+++
AAL	+++	+++	+++
AASE	++	**WY**	+++
AASEL	+++	+++	+++

+++ = Very abundant, ++ = Abundant, + = Reduced, W = White/Yellow structure, N = No formation.

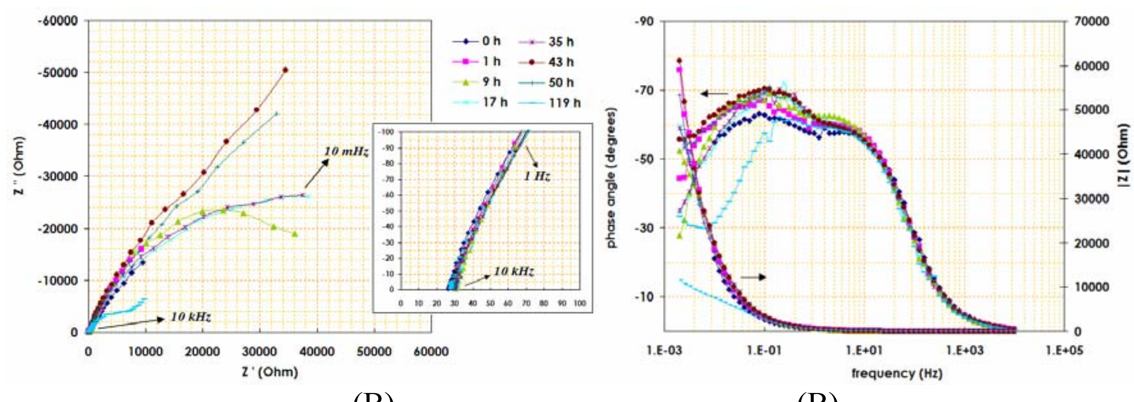
An increasing tendency on the impedance magnitudes of the imaginary part of the semicircles is observed, which indicates that the dissolution process (activation) rate decreases through time, in this case due to the growth of the corrosion products layer formation which decreases the interaction between the metal and the electrolyte species (figure 1A). There is no detectable diffusion limitation due to the corrosion products layer. In the bode diagrams presented for the abiotic system (figure 1B) it is observed that the phase angle has just one maximum value (1 Hz) which is around 80°, and that it increases slightly through time. These contributions in the phase diagram exhibit a very symmetric shape which might be attributed to the presence of a highly homogeneous interphase formed by the corrosion products.



(A) (B)

Figura 1. (A) Nyquist diagram for sterile culture media (with zoom for high frequencies). (B) Bode diagrams for sterile culture media.

For the initial times, the presence of one semicircle is observed for the system in the presence of the biofilm consortium (figure 2); however, the magnitudes for the impedance in the biotic system are smaller than in the abiotic one. This behavior might be attributed to the presence of the microorganisms which favor an increase in the conductivity of the electrolyte. In subsequent periods, the impedance magnitudes become higher and a diffusional process is observed this implies that the activation process is not anymore controlling the electrochemical processes occurring at the interface, but rather the presence of the growing biofilm which has a high diffusional influence. In the highest measured time (119 h) the impedance magnitudes dramatically decrease but still maintain the diffusional influence; these phenomena might be due to the influence of the biofilm species in the corrosion process which rate becomes much higher than in the presence of the abiotic culture media.



(B) (B)

Figura 2. (A) Nyquist diagram for media with biofilm consortium. (B) Bode diagrams for media with biofilm consortium.

In the bode diagrams (figure 2B) a second maximum value is observed at lower frequencies (1E-01 Hz) than in the abiotic system; this contribution is related to the presence of the biofilm, and a contribution due to the corrosion products still remains and it can be detected at higher frequencies (1E+01 Hz). The presence of the latter contribution shifts to lower frequencies when the biofilm is present, which can be due to the interaction with the corrosion products with the biofilm polymers that make this interface more conductive.

Microbial complexity in gasoline distribution pipelines

According to the obtained results previously presented (and to kinetic characterizations not shown in this work, as well as the work with the microbial aerobic counterparts) we were able to determine some possible interactions that might be present in microbial communities of gasoline pipelines.

From figure 3 it can be observed that a highly complex microbiological network is possible to be established inside the gasoline pipelines as long as the presence of small amounts of water exists.

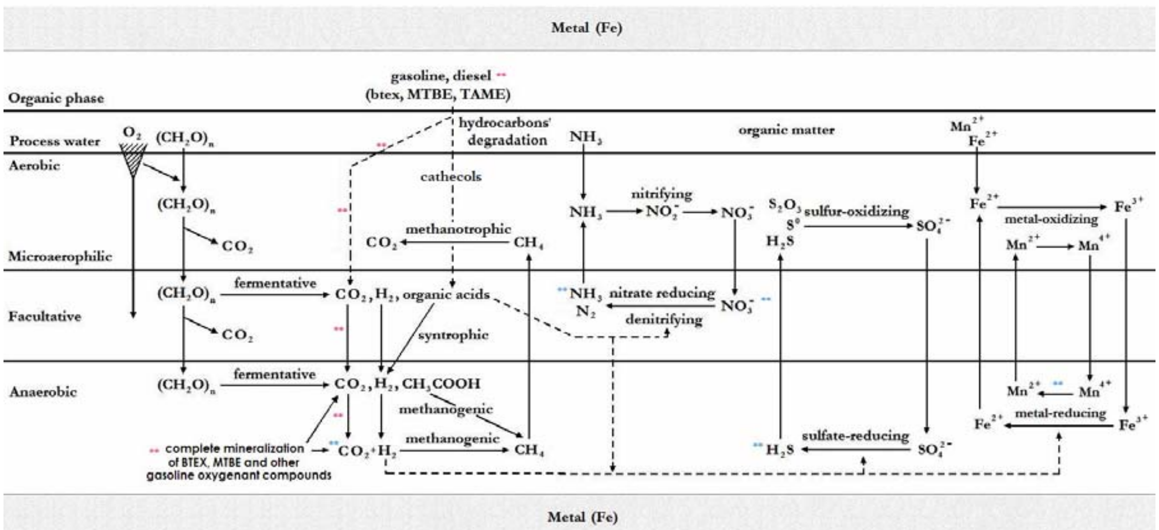


Figure 3. Some of the possible microbial interactions that might occur in gasoline pipelines. The products marked with blue asterisks are the ones which can have influence in corrosion. The products marked with pink asterisks are susceptible to complete mineralization processes by the cultured microorganisms.

The microorganisms present in this network can find the nutrients for their growth and due to the presence of the extreme conditions inside the pipelines (shear stress, low water concentration, poor dissolved oxygen, etc.) it is very likely that these microbes form highly complex biofilms that can enhance an increased corrosion process, due to the biofilm heterogeneity, concentration cells establishment and aggressive corrosion products formation as part of their metabolic activity.

The most important activity in the proposed mechanism is the capacity of the aerobic and aerobic cultured microorganisms to achieve a complete mineralization of BTEX, MTBE and TBA. The CO_2 production was followed using gas chromatography and the BTEX, MTBE and TBA consumption by HPLC; however, the results for these kinetic studies will be presented in a further article.

Conclusions

There is a very diverse microbial flora present in gasoline distribution pipelines that has not been widely studied. This microbial flora might have an influence in corrosion phenomena and might have applications in bioremediation of gasoline-polluted sites.

A great amount of the cultured microorganisms are capable to form biofilms, more than 50% of these microorganisms are capable to form biofilms in the presence of lactate as carbon source, which might influence in biofouling, biocorrosion, biodegradation in gasoline pipelines.

The possible metabolic interactions among all these microorganisms can lead to products that usually have an influence in localized corrosion, such as hydrogen sulfides, nitrates and nitrites, CO_2, organic acids, phosphates, etc.

Differences in the electrochemical response with EIS are evident when comparing the abiotic system against the system with biofilm influence. There is an activation control of the corrosion process for the abiotic system, while in the presence of the biofilm the process becomes diffusion-controlled.

Acknowledgments

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