Corrosive biofilms formed by self-immobilized bacterial cells on metal surfaces of various objects in petroleum industry

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Introduction

For a long time it was considered, that corrosion processes have the chemical nature, but recent results of multiple investigations of different corrosion objects confirmed that various microorganisms provoke up to 30% of all corrosive situations (Flemming, 1991; Da Silva, 2002; Dubey, 2001).
The term biocorrosion or corrosion provoked by microorganisms refers to the accelerated deterioration of metals due to the presence of biofilms on their surfaces. Usually, self-immobilized bacterial cells form biofilms (Beech, 2004; Lee, 2006). The corrosive biofilms have heterogeneous microbial content (consortia of mixed microbial species) but always contain sulphate-reducing bacteria (SRB).
It was established that SRB are the key cells provoking development of biocorrosion processes (Beech, 2004; Beech,2005; Santegoeds, 1998).The primary role of SRB comprises the production of sulphide from sulphate usually present in various wastewaters (Efremenko, 2006). The iron dissolution takes place at the electrochemical anode. It combines with biogenic sulphide to give precipitated iron sulphides as corrosion products.
The natural immobilization of cells during the formation of corrosive biofilms can be divided into following stages:

- adhesion of mixture of planktonic bacterial cells present in the flow of wastewaters on a metal surface;
- appearance of oxic/anoxic interface among various cells;
- accumulation of sulphide and polysaccharides, excreted by heterotrophic cells, in the frame of biofilm;
- mechanical strengthening and metabolic stabilization of immobilized biosystem followed by acceleration of biocorrosion processes.

Evidently, that type of cell immobilization is absolutely undesirable, since then, the main target of researches is to prevent the development of such immobilized systems or to guarantee their effective destruction under environmental conditions.
So, there is a serious research problem which aim is opposite to common one: do not develop a new immobilized biosystem similar natural one, but fight with natural immobilized biosystems. To prevent the formation of corrosive biofilms, it is necessary to enter into mechanism of biofilms formation and destroy it via elimination of its crucial elements or changing conditions of its realization.
Despite considerable research efforts aimed at the investigation of biocorrosion phenomenon the detailed mechanisms of biocorrosion developed under the action of biofilms are still poorly understood. The information concerning factors provoking formation of corrosive biofilms is of a high importance, since it helps to found effective approaches to the prevention of corrosion development.
According to recently obtained data (Efremenko,2006), the corrosive biofilms appear more quicker on the metal surfaces already covered with iron sulfide as compared to “clear” metal surfaces. The presence of sulfide on a surface mainly influences on a log phase of biofilm growth, and specific rate of biofilm growth increases concurrently with increase in sulfide concentration on a metal surface. The influence of sulfide, precipitated on a metal surface, on the growth of corrosive biofilms appeared to be more pronounced at low concentrations, approximately up to 1 $\mu g/cm^2$.
It was shown that porous sulfide layer formed anoxic zone even under aerobic conditions, which accelerates formation of heterogeneous biofilm (Hamilton, 2003). So, metal surface covered by sulfide is more accessible for adhesion (physical immobilization) of bacterial cells from various wastewaters to form anaerobic part of biofilms.

Since then, to avoid the development of corrosive biofilms it is necessary to prevent the precipitation of sulphide, mostly producing by planktonic cells in the wastewater systems, on the surface of metal pipelines and equipment.

Material and methods

Natural association of corrosive cells, isolated from the wastewaters of Almetevsk oilfield (Tatarstan, Russia) composed of SRB and heterotrophic aerobic cells was used in this investigation. To grow the biofilm of natural association cells, the following medium was used (g/l): lactate – 4.0; yeast extract – 1.0; ascorbic acid – 0.1; $MgSO_4$ x $7H_2O$ – 0.2; K_2HPO_4 – 0.01; $Fe(SO_4)_2(NH_4)_2$ x $6H_2O$ – 0.2; NaCl – 10.0; pH 6.8-7.0. Before the inoculation of cells, 1% of oil was added to the medium as additional substrate for the cells.

Oil sample was taken from Surakhani oilfield of Baku region. The chemical content of the oil was characterized by the prevalence of branched alkanes and low concentration of aromatic hydrocarbons. To eliminate the presence of indigenous microorganisms, the oil was pre-heated at 272^0C for 1 h before introduction to the medium.

The cultivation of biofilms was carried out at 28-30^0C in the presence of metallic coupons, purchased from Standard Metal Incorporation (Moscow, Russia). The metal coupons were pretreated with acetone for 10 min, then with 0.1 M HCl for10 min, washed with tap water and dried in air at room temperature prior to be used.

To analyze the formation of biofilms, the differentiated bioluminescent analysis of intracellular ATP concentration in the cells, being part of bacterial systems with mixed cultures, previously developed in our laboratory (Efremenko, 2005a), was used herein.

To extract ATP from cells inside of biofilms, the following conditions were used: the ratio of DMSO volume to square of treated biofilm surface was 0.5 ml/cm^2; the ATP extraction was carried out for 10 min. An aliquot (50 μl) of cell extract was added to the luciferase reagent (50 μl) and the intensity of bioluminescence was measured by Microbioluminometer 3550i (New Horizons diagnostics Co., USA). The luciferase reagent purchased from Lumtek (Russia) was used in the work. To quantify the cells in the biofilms and planktonic cultures the calibrations establishing the dependence of intracellular ATP concentration on cell concentration were used. The precise cell concentrations used for these calibrations were determined by microbiological method (Efremenko, 2005b).

The sulphide determination was based on the detection of methylene blue formed as product of reaction between dimethylparaphenylene diaminesulphate (DMP) and iron (III). To measure sulphide concentration in media, 2 ml zinc acetate was poured to 10 ml volume test-tube, then fixed volume of sample, 2-5 ml distilled water and 1 ml DMP was added. Then 50 μl iron (III) ammonium sulphate was introduced to the mixture, distilled water was added to reach the final volume 10 ml and the concentration of methylene blue was measured spectrophotometrically (Agilent 8453 UV-visible spectrometry system, Agilent Technologies, Germany, 660 nm) after 30 min. The sulfide concentration was identified from calibration graph, plotted using sodium sulfide.

The flow system, used in the work, contained: 3 peristaltic pumps, 2 hermetic chambers used for mixing of medium with biocide and corrosion coupon treatment, filtration column filled with Megasorb, 2 reservoirs for cultural media and biocide solution. Peristaltic pumps according to the requirements of each experiment regulated flow rates and related dilution rates.

The used sorbent, Megasorb (Incomcenter, Russia), being non-woven fibrous material, was made from combinational polymeric fibers. Sorbent material was cut and modified as required for filter filling. Before using, the bioactivity of Megasorb by bioluminescent method was tested. Sorbent material was treated with 10 ml distilled water per 1 g sorbent for 20 min, and then ATP concentration was measured in washed water. There was no any bioactivity in sorbent material.

Corrosion inhibitor (CI), SXT 1003 (Nalco, USA), was used as biocide in this work. To identify Minimal Inhibitory Concentration (MIC) of CI, the optimized version of previously developed method was used (Efremenko, 2005). MIC for the all systems was identified as the minimal continuously

injected concentration of CI, completely preventing biofilm formation on the surface of metal coupons. Each concentration of CI was injected for 64 h into the flow system before cell concentration was analyzed in formed biofilm.

Results and Discussion

Several approaches were offered to partially or completely prevent formation of corrosive biofilms in the flow system:
- the sulphide present in the medium was separated by filtration with hydrophobic sorbent Megasorb,
- the cells were eliminated from the flow by filtration with hydrophobic sorbent Megasorb,
- the cells were treated with corrosion inhibitor possessing biocide activity.

The effectiveness of elimination of sulphide from oil-cell-containing medium by Megasorb was investigated (Fig.1).

It was shown that after the first filtration of tried medium, modeling industrial wastewater from oilfields, through Megasorb (1 L medium via 5 g sorbent) it was possible to completely eliminate the sulphide, accelerating development of biofilms, from the flow. It was established, that the capacity of tested Megasorb was 1 mg of sulphide per 1 g of sorbent.

The removal of cells from the flow by filtration of same medium through supermacroporous Megasorb was investigated in the next set of experiments (Fig.2).

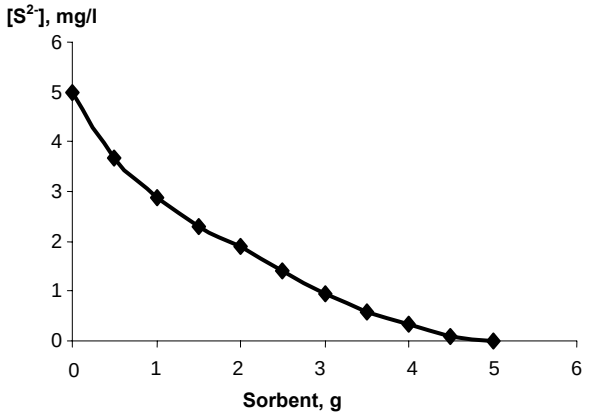

Fig. 1. The dependence of residual amount of sulphide in the medium (1 L) in flow system on the amount of applied sorbent.

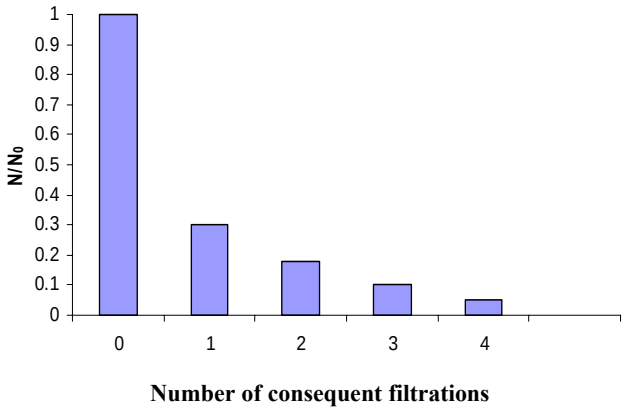

Fig. 2. The influence of consequent filtrations of oil- cell-containing medium through the Megasorb on the efficiency of cell elimination from the flow, where No and N are initial and residual concentrations of cells in the flow, respectively.

It was established that 90% removal of cells, provoking formation of biofilms, could be reached after filtration of 1L of medium through 20 g of Megasorb. The further investigation of kinetics of biofilm formation allowed to reveal the 4-fold decrease in velocity of growth of self-immobilized cells after the treatment of tried medium with Megasorb. The influence of combination of medium treatment by Megasorb with introduction of CI with biocide activity into the medium on the biofilm formation was investigated at the next stage of the work. It should be noted that introduction of CI into the flow of industrial wastewater from oilfields is widely used in practice (Ramesh, 2003, 2005).
It was shown, that such complex approach enabled the 30%-decrease in MIC of tried biocide. It means that the amount of applied CI could be considerably reduced. Additionally, it was shown, that Megasorb could be used up to 15 times for the medium filtration.

Conclusions

The investigation of formation of corrosive biofilms on the metal surfaces allowed to disclose several main factors provoking biocorrosion development in the pipelines and storage reservoirs used in petroleum industry. Effective approaches to the prevention of cell immobilization inside of biofilms and to the removal of biofilms from the metal surfaces were revealed.

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