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Studies of Initial Stages of Biocorrosion of Steel

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Abstract—Initial stages of corrosion of mild steel induced by *Klebsiela rhinoscleromatis* BO2 were studied in various media. The effect of the microorganism was detected 8–10 h after inoculation. The number of viable cells was virtually unchanged over one month in all media, but the corrosive activity of the strain decreased. The corrosive activity of microorganisms can be determined by spectrophotometry even after incubation for only 24 h. At a low level of organic substrate, even strong colonization with microorganisms does not inevitably result in significant damage to metals.

The microbe-induced damage to metals is usually determined quantitatively by weighing [1]. But weighing methods yield significant results only in experiments of at least one week's and sometimes one month's duration. Obviously, this method is inappropriate for studies of the initial stages of biological corrosion. In some cases, metal losses caused by biological damage are assessed by microphotography of corrosion pits [2]. However, this method is also inappropriate for analyzing initial stages of biological corrosion, because pitting is a long process. In theory, the rate of biological corrosion at any moment can be calculated from polarization curves [3], but practical interpretation of the findings is difficult because of the complexity of the systems under study. Moreover, polarization studies are often associated with considerable changes in the potential and current that can produce irreversible changes in the system. Therefore, studies of the dynamics of biological corrosion require many repeated experiments, which considerably increases the expenditure of reagents. The corrosion losses of metal are sometimes assessed by atom absorption [4]. This method is highly sensitive but requires expensive equipment.

Unlike the above-mentioned methods, spectrophotometrical determination of iron ions in solution characterizes corrosion without expensive equipment or great expenditure of reagents [5]. The experimental conditions can be adjusted to determine changes in iron ion concentrations within minutes after placing the steel plates in the solution studied. This method has been insufficiently used in studies on biological corrosion.

This work was designed to study the possibilities of spectrophotometry for determining the initial stages of microbial corrosion of mild steel.

MATERIALS AND METHODS

Corrosion of mild steel induced by *Klebsiella rhinoscleromatis* BO2 was studied in the following media:

Medium 1 (glucose–mineral medium with peptone): 1 g/l $(NH_4)_2SO_4$, 6 g/l K_2HPO_4, 3 g/l KH_2PO_4, 0.2 g/l $MgSO_4$, 0.5 g/l nutrient broth (Difco, USA), and 5 g/l glucose.

Medium 2: medium 1 diluted tenfold.

Medium 3: tenfold lower contents of mineral constituents compared to medium 1 with the same contents of organic constituents.

Medium 4: tenfold lower contents of organic constituents compared to medium 1 with the same contents of mineral constituents.

NB: Difco nutrient broth, 8 g/l.

Ps medium: 2 g/l $(NH_4)_2SO_4$, 4.3 g/l K_2HPO_4, 3 g/l KH_2PO_4, 0.2 g/l $MgSO_4$, 0.5 g/l Difco nutrient broth, and 5 g/l glucose.

E8 medium: 1.5 g/l $(NH_4)_2HPO_4$, 0.7 g/l KH_2PO_4, 0.5 g/l NaCl, 0.8 g/l $MgSO_4 \cdot 7H_2O$.

K. rhinoscleromatis BO2, as well as other cultures used (*Pseudomonas fluorescens* G-1, *Serratia marcensces* SG, and *P. marginata* SOV-6), was isolated from a diesel fuel tank.

Biological corrosion was assessed by spectrophotometry, weighing, and electrochemical methods. For spectrophotometry, mild steel St. 3 (Russia) coupons ($8 \times 4 \times 1$ mm) were scraped with emery paper, degreased with ethyl alcohol, and placed in test tubes. The tubes were closed with cotton plugs and subjected to dry-heat sterilization. After sterilization, 5 ml of sterile nutrient medium was added to each tube, which was then inoculated (except control tubes) with 50 μl of a washout from a 24-h slant culture of *K. rhinoscleromatis* (10^9 CFU/ml) and maintained in a thermostat at 28°C.

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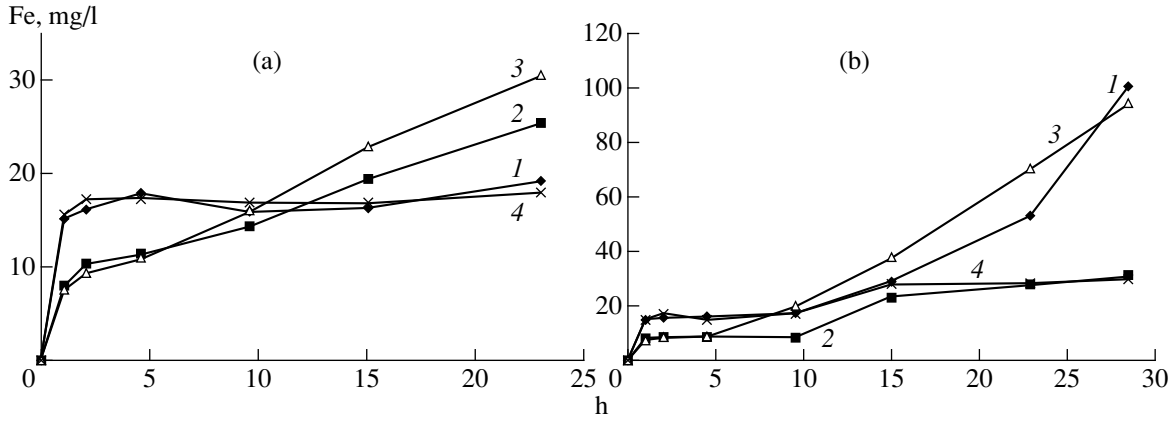

Fig. 1. Accumulation of iron ions during the first day in sterile media (a) and in various media in the presence of *K. rhinoscleromatis* (b): (*1*)–(*4*) the media.

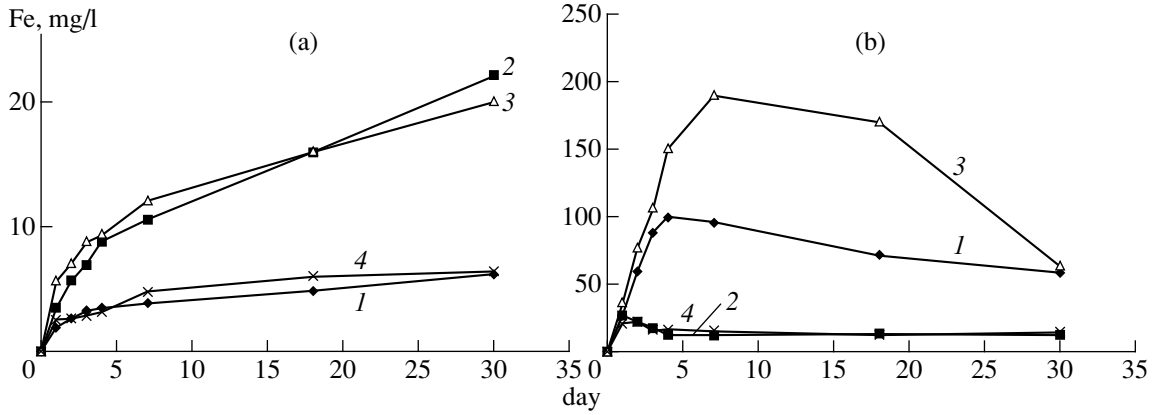

Fig. 2. Accumulation of iron ions over 30 days in sterile media (a) and in various media in the presence of *K. rhinoscleromatis* (b): (*1*)–(*4*) the media.

The content of iron ions in the solution was determined with sulfosalicylic acid [6]. The optical density was determined at 430 nm in an SF-26 spectrophotometer (LOMO, Russia). Each experiment was performed in triplicate. The rate of corrosion was assessed by the content of soluble iron per unit of the coupon surface area per unit time.

For the weight method, experimental conditions were the same as in the previous case. After exposure, the coupons were washed with ethyl alcohol, water, and 1 N HCl for 10–30 s. Such a treatment rapidly removed the corrosion products without changing the weight of metal. The specimens were weighed on a BP210S balance (Sartorius, Germany).

For electrochemical determination, the degrader strain was cultured at 28°C in 750-ml flasks on a shaker at 150 rpm. After a certain period, the contents of the flasks were transferred to an electrochemical cell with the anode and cathode separated by a glass membrane. Platinum was used as a counter electrode in the cell. Working electrodes were made of cylindrical specimens of St. 3 steel embedded in Teflon holder in order to expose only their base surface of 0.5 cm^2. Before the experiment, these electrodes were polished and degreased with alcohol.

Polarization curves were obtained potentiodynamically at the potential sweep rate of 0.2 mV/s by using an IPC-4 potentiostat connected to a computer. The potentiostat was designed and manufactured at the Institute of Physical Chemistry, Russian Academy of Sciences. Potentials were measured against an Ag–AgCl reference electrode. The polarization was started 15 min after the cathodic activation at 850 mV and subsequent establishment of a quasistationary potential. The corrosive activity of microorganisms was assessed by an increase in the curve slope in the region of active dissolution.

RESULTS AND DISCUSSION

Curves of accumulation of iron ions in solutions of four pure and inoculated media during the first day of the incubation are shown in Figs. 1a and 1b. In all cases, the content of iron ions increased during the first hour.

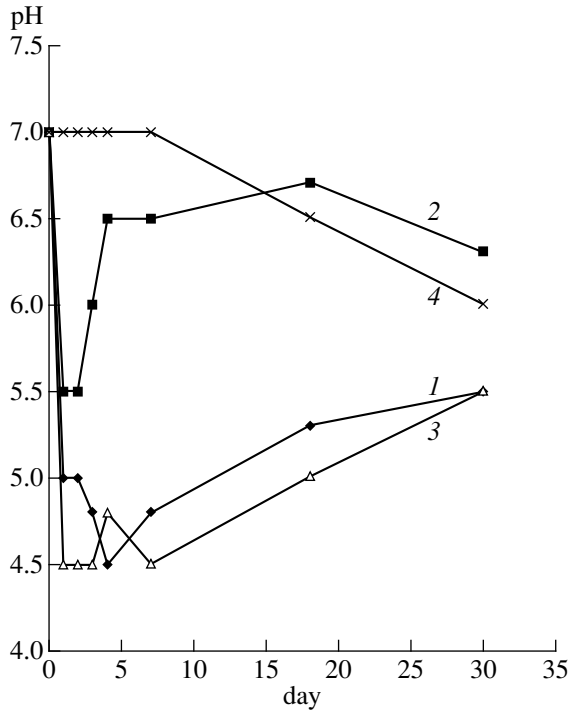

Fig. 3. pH changes in various media during *K. rhinoscleromatis*-induced corrosion: (*1*)–(*4*) the media.

The authors of [5] explained such a sharp initial increase in the corrosion rate by the specific effect of acetone as a corrosive agent. In our opinion, the highest rate of corrosion at the beginning of the experiment was associated with predominant dissolution of defect regions on the metal surface. A comparison of Figs. 1a and 1b shows that the effect of microorganisms on corrosion was detectable only after 10 h of incubation.

Curves of accumulation of iron ions in solutions of four pure and inoculated media over 30 days are shown in Figs. 2a and 2b. Nearly equal corrosive activities were found in pure media 1 and 3, as well as in media 2 and 4 (Figs. 1a, 2a). A comparison of Figs. 1b and 2b shows that the biocorrosive activities of *K. rhinoscleromatis* BO2 in media 2 and 4 were equal during the whole experiment, while in media 1 and 3 the activities were equal during the first three days.

A comparison of Figs. 1 and 2 suggested that in this case the rate of electrochemical corrosion was determined by inorganic constituents of the medium, most likely mainly by phosphates that facilitated metal passivation [7]. In turn, the rate of biological corrosion was determined by organic constituents of the medium. The maximal quantity of viable cells in all media (about 10^9 CFU/ml in media 1 and 3 and about 10^8 CFU/ml in media 2 and 4) was reached within one or two days, remained virtually unchanged for 2.5–3 weeks, and then began to decrease. The corrosive activity of *K. rhinoscleromatis* BO2 began to fall after five to ten days in the organic-enriched media 1 and 3 and sooner (on the third day) in organic-limited media 2 and 4 (Fig. 2b).

In all cases, changes in corrosion rates correlated with changes in pH (Fig. 3), except in medium 4, where a higher content of phosphates (compared to medium 2) prevented a decrease in pH during the first days of incubation. The strain produced a biofilm that covered the whole surface of the metal plate and became visible to the naked eye on the second or third day.

It was interesting to compare these findings with data obtained by weighing and electrochemical methods. The corrosion rates determined by weighing over 30 days and calculated from results of spectrophotometry after incubation for 1, 7, and 30 days are shown in Table 1. Even after one day of incubation, the rate of corrosion calculated from spectrophotometric data was similar to that obtained by weighing over 30 days; the rate of corrosion calculated from spectrophotometric

Table 1. Corrosion rate in four media determined by various methods

Corrosive medium	Corrosion rate, g/m^2 per day			
	weighing, 30 days	spectrophotometry		
		one day	seven days	30 days
Medium 1	0.07 ± 0.01	0.11 ± 0.04	0.011 ± 0.002	0.005 ± 0.001
Medium 2	0.36 ± 0.08	0.13 ± 0.02	0.041 ± 0.01	0.022 ± 0.002
Medium 3	0.26 ± 0.05	0.18 ± 0.04	0.047 ± 0.006	0.017 ± 0.004
Medium 4	0.08 ± 0.01	0.09 ± 0.02	0.014 ± 0.003	0.005 ± 0.001
K. rhinoscleromatis BO2 in medium 1	1.0 ± 0.2	0.8 ± 0.2	0.36 ± 0.06	0.05 ± 0.01
K. rhinoscleromatis BO2 in medium 2	0.42 ± 0.08	0.6 ± 0.1	0.033 ± 0.007	0.021 ± 0.003
K. rhinoscleromatis BO2 in medium 3	1.4 ± 0.3	1.0 ± 0.2	0.7 ± 0.2	0.051 ± 0.006
K. rhinoscleromatis BO2 in medium 4	0.26 ± 0.05	0.33 ± 0.06	0.04 ± 0.01	0.02 ± 0.004

Table 2. Corrosion rates in various media determined by two methods

Corrosive medium	Corrosion rate, g/m^2 per day	
	weighing, over 26 days	spectrophotometry, one day
E8	0.12 ± 0.03	0.095 ± 0.003
Ps	0.14 ± 0.06	0.11 ± 0.01
Nutrient broth	0.49 ± 0.07	0.47 ± 0.04
E8 + *K. rhinoscleromatis* BO2	1.8 ± 0.1	1.7 ± 0.2
Ps + *K. rhinoscleromatis* BO2	1.38 ± 0.05	1.29 ± 0.07
Nutrient broth + *K. rhinoscleromatis* BO2	0.09 ± 0.03	0.4 ± 0.1

Table 3. Corrosion rates in Ps medium determined by two methods

Corrosive medium	Corrosion rate, g/m^2 per day	
	weighing, 27 days	spectrophotometry, one day
Ps	0.11 ± 0.032	0.08 ± 0.02
Ps + *K. rhinoscleromatis* BO2	0.77 ± 0.09	0.92 ± 0.05
Ps + *P. fluorescens* G-1	0.49 ± 0.07	0.80 ± 0.2
Ps + *S. marcescens* SG	0.31 ± 0.06	0.6 ± 0.2
Ps + *P. marginita* SOV-6	0.22 ± 0.05	0.23 ± 0.03

data after 30 days of exposure was nearly one order of magnitude lower than the rate obtained by the weighing method. This was probably due to losses of the metal's weight caused not only by the release of its ions into solution, but also by generation of insoluble corrosion products whose level increased with increasing pH of the solution.

Similar results were obtained from experiments with this microorganism in other media and with other microorganisms responsible for steel destruction in the Ps medium (Tables 2, 3). Thus, spectrophotometry allowed us to assess the corrosive activity of microorganisms even after one day of incubation, and this is very important for rapid selection of biodegraders.

Polarization curves obtained at the initial stages of biological corrosion of steel induced by *K. rhinoscleromatis* BO2 are shown in Figs. 4a and 4b. During the first hours after inoculation, the corrosive activities of media 1 and 2 decreased. Then, by the end of the first day, the activity was higher than in pure medium. During the second day, the corrosive activity was increasing in the organic-enriched medium 1 and was gradually decreasing in the organic-limited medium 2.

A comparison of data shown in Fig. 4 with data of Figs. 1 and 2 shows that the potentiodynamic sweep method was more sensitive than spectrophotometry. The polarization curves allowed us to follow the microorganism-induced changes in the corrosive medium even during the first hours of incubation, whereas spectrophotometry detected the corrosive activity of *K. rhinoscleromatis* BO2 culture only after 8–10 h. Nevertheless, changes that occurred at the initial stages of corrosion induced by microorganisms were generally adequately recorded by spectrophotometry. This method is simple, reliable, needs neither expensive equipment nor large amounts of reagents, and therefore

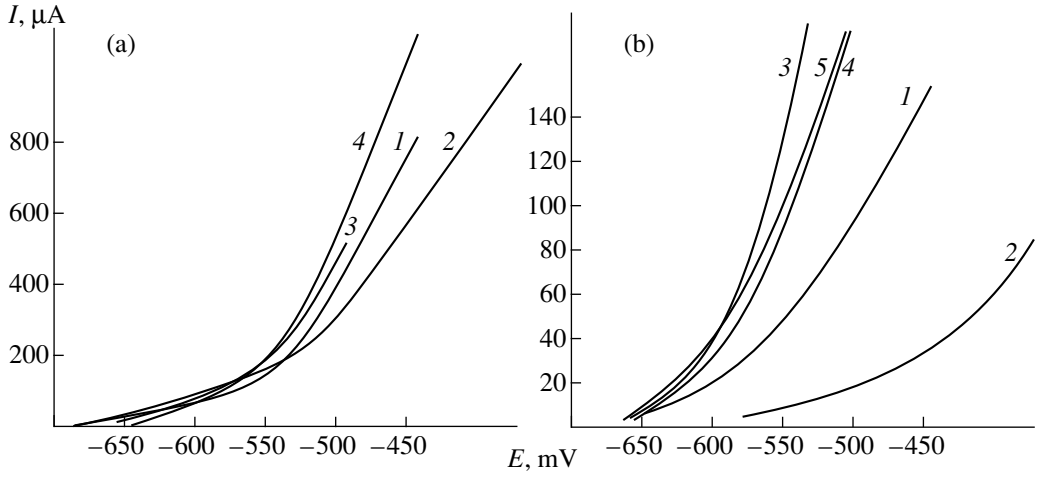

Fig. 4. Polarization curves for various times of *K. rhinoscleromatis* incubation. Medium 1 (a): (*1*) sterile medium, (*2*) 4 h, (*3*) one day, and (*4*) two days. Medium 2 (b): (*1*) sterile medium, (*2*) 2 h, (*3*) one day, (*4*) two days, (*5*) four days.

can be recommended for studies of the initial stages of biological corrosion of steel and also for rapid selection of the most active corrosive microorganisms.

These data also suggest that in the presence of small amounts of organic substrate even strong colonization by potentially active destructors seems to cause no significant damage to metals. In this case, there is no need to use biocides to prevent biological corrosion; it is sufficient to use passivators.

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