Influence of alloying elements on adhesion of corrosion relevant microorganisms

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Abstract

Corrosion relevant microorganisms enhance the rate of corrosion by their presence, by the excreted metabolites and by the exopolymeric substances. Biofilm formed by microbes influences the surface reactions at the metal/biofilm interface. Surface properties (homogeneity of oxide layer, surplus of alloying elements, pH, interference between the exopolymers and the metal ions as well as between the aggressive metabolites and the metal surface) have significant impact on the microbial adhesion and on the biofilm formation. The corrosion relevant microorganisms are mostly dangerous in sessile form, embedded into biofilms, much less in planktonic form. This paper shortly discusses the microbially influenced corrosion (MIC) and its mechanisms and mainly focuses on the influence of the alloying metals on the microbial adhesion, biofilm formation and, as a consequence, on the MIC. Biofilms discussed here are formed either by isolated pure culture (*Desulfovibrio desulfuricans*) or by mixed population of cooling water on iron and on iron alloys with alloying elements: chromium, nickel, molybdenum and ruthenium. The surface with and without biofilms were visualized by light-, fluorescence- and atomic force microscopes. Microbiological techniques helped in enumeration of microorganisms. Correlation was found between the chemical nature/concentration of the alloying elements and the number of microorganisms built in the biofilm.

Keywords: corrosion relevant microorganisms, iron alloys, influence of metal ions on microbial adhesion, microbiologically influenced corrosion, fluorescence microscopy, and atomic force microscopy.

Introduction

Several types of steels are applied at industrial scale due to the passive films formed on their surface that can control corrosion of different types. Stainless steels are produced in different grades; the basic composition, the finishing and the surface treatment influence the surface properties. Alloying elements in steels and their impact on electrochemical/chemical corrosion are well summarized in the paper of Olsson and Landolt [1].

Some important effects caused by the presence of mostly used components in the steel are the follows:1. carbon: its presence is necessary in small quantity for steels; 2. chromium: increases the hardening of steel, the toughness, at 10.5% the resistance to corrosion; 3. manganese: increases the hardening, makes steel more stable in the quench and less susceptible for cracking, neutralizes the negative effect of sulfur; 4. nickel: increases the strength of the steel, reduces the brittleness and the toughness; 5. molybdenum: increases the hardness penetration and, the high temperature, the tensile strength, significantly increases the inhibition of uniform and localizes corrosion. In the last period, when the possibility of microbial corrosion came into sight, the importance of nitrogen and cooper in steels as alloying elements increased as their presence drastically decreases the microbial adhesion [2]. Naturally formed or artificially developed passive oxide layers cover the steel surfaces generally. Alloying elements alter the formation, chemical composition and thickness of passive layer, stabilize the protective oxide film, as well as can alter the susceptibility to MIC. The presence of alloying elements in the stainless steel determines the composition of the oxide films. The corrosion resistance of stainless steels dipped into electrolyte is due to the formation of thin passive Cr-Fe oxide film which consists of a chromium oxide film near to the metal surface and of an iron oxide film developed at the film/electrolyte interface [3].

Steel surfaces are generally resistant to corrosion because of the elemental surface composition and crystalline homogeneity. The most common stainless steels grades are the 304, 3034L, 316, 316L with Cr, Ni; in the last two cases, they contain Mo.

When undesired corrosion relevant microorganisms are present, they enhance the metal deterioration through destroying the passive oxide layer. The microbiologically influenced corrosion (MIC) is not a new type of corrosion but, in this case, the microorganisms enhance the corrosion rate by their presence and by their aggressive metabolites [4]. The MIC is a surface phenomenon; it influences the electrochemical process that affects the metallic surface [5]. The electron exchange between the electrolyte and the metal surface is the key step in corrosion. The MIC mechanism depends on the types of microbes associated with the corrosion, on their metabolites, on the composition of the exopolymeric substances and on the exo-enzymes.

On the role of alloying elements in MIC as well as on the parameters that influence the microbial adhesion there are very good, informative papers [6,7].

From corrosion point of view in cooling water the dangerous microorganisms are mainly anaerobic (first of all sulfate reducers) and there are some metal oxidizers (iron oxidizer, manganese oxidizer), as well as sulfur oxidizer, metal reducers, and acid producers [8]. Among the anaerobic sulfate reducers, the *Desulfovibrios* convert the sulfate ions to sulfide ones that could form blackish, shiny iron sulfide deposits (that can depolarize the anode). The sulfur oxidizers can convert the sulfide to sulfate. The acid producers with their excreted acids are also dangerous because of increased metal dissolution and enhanced cathodic reactions in acidic environment.

There are several publications on the mechanisms of the MIC [9,10]. In the classical mechanism description the sulfate reducer bacteria that, from the point of view of MIC in cooling systems, are more dangerous than the aerobic ones, increase the corrosion rate through the electron transport to the sulfate reduction by hydrogen mediation from the metal surface. When oxygen is present, aerobic microbes produce solid metal oxide/hydroxides.

The surface properties like roughness, oxide coverage, and chemical composition have important impact on microbial adhesion and on the biofilm formation. The communications appeared on influence of surface roughness are sometime contradictory; in some cases they show that with increasing roughness the number of attached bacteria increases but there are some publications that states the opposite [5,11,12]. On the other hand, it is clear and well documented that there is a relationship between the microbial size/morphology/production of exopolymeric substances and the roughness parameters [13]. The metal oxides that have positively charged surfaces enhance the microbial attachment as the cell surfaces are negatively charged.

The presence of water is necessary for microbial growth. As steels are frequently in contact with water, this contributes to the microbial adhesion. The microorganisms that can easily adhere to metal surfaces alter the local physical/chemical conditions that lead to MIC [14,15]. In order to diminish the influence of corrosion relevant microorganisms, it is important to select proper alloys as the alloying elements alter the chemical composition and thickness of the metal surface oxide layer and affect the adhesion of microbes.

When microorganisms start to adhere to a solid surface, the first cells are scattered, than smaller and bigger patches cover the surface. At enough time, which depends on the type of microbes, a homogeneous biofilm will cover the solid. Microbial film develops in consecutive steps (conditioning layer formation, reversible/irreversible microbial adhesion, increase of biofilm thickness with growth and multiplication of microbes) and consists mainly of water (up to 90%), of exopolymeric substances (EPS), of microbes, and of organic/inorganic molecules accumulated from the aqueous environment [16]. As the MIC starts beneath the biofilm, it is important to reduce or inhibit the adhesion of microorganisms and the biofilm formation [17,18]. The microbial biofilms can condition the metal surface, and at the same time, the physicochemical properties of the surface change. This can promote or inhibit the adhesion of microorganisms [19]. On the other hand, biofilms can form a significant diffusion barrier for certain chemical species (e.g. oxygen, nutrients, and aggressive ions).

The microorganisms in biofilms cause mainly pitting type corrosion, in some cases crevice- and stress corrosion.

As it was mentioned earlier, oxide layer covers generally an alloy surface, its chemical composition (that alters the surface passive film) and thickness is important from the point of view of microbial adhesion and of biocorrosion. The existence of biofilms on a metal surface frequently creates new electrochemical reaction pathways, or allows reactions that are normally not favored in the absence of microorganisms, which leads to increased corrosion. The metabolic products of bacteria can also significantly modify the interfacial processes between the biofilm and a metallic sublayer.

MIC may catalyze the oxidation of metals or derive energy from oxidation of metals. Dense deposits of cells and metal ions create oxygen concentration cell that exclude oxygen from the area of deposit. The under-deposit corrosion is important.

The period of the reproduction of microbial life also affects the MIC. In case of the carbon steel, the microbial corrosion is accelerated in the reproduction (lag and logarithmic) phase and is stable during the stationary and decline or death phase. It shows that not only the bacterial number, but also the accumulated metabolic products influence the corrosion rate. Based on bioelectrochemical results, the MIC of carbon steel is mainly due to the effect of biogenic sulfides, which leads to breakdown of the passive layer. This step is followed by the cathodic depolarization by hydrogenase enzymes.

In case of the grade 304 stainless steels under influence of corrosion relevant microorganisms, the most common microbial attack is the localized corrosion (pitting). The presence of *Pseudomonas* bacteria changes the composition of the metal surface, the iron content decreases and the chromium content increases in the surface film. The change in the main alloying element composition on the surface goes parallel with the appearance of the micro-pits. The presence of molybdenum at concentration >6% (316, 316L) the possibility of MIC decreases drastically.

The copper-bearing stainless steels are more often used in the decades because of their microbe repellent activities, which is due to the dissolved copper ions. Comparing the antimicrobial corrosion activity of 304L-Cu with the similar characteristic of 304L stainless steel, not only the corrosion current, but also the sizes of pits significantly decrease in the microbial environment.

When anaerobic bacteria interact with Cr and Ni containing stainless steel surfaces, the passive film weakens and the alloying elements are dissolved. In the case of alloys with higher Ni content, the nickel ions dissolved from steels, negatively influence the multiplication of corrosion relevant microorganisms (*Desulfovibrios*). Nevertheless, Ni ion content below the toxic level has positive effect on the growth of sulfate reducers and increases the metal deterioration. When chromium ions are present, they do not influence the multiplication of bacteria.

In a water piping system, much more microorganisms adhere to the steel surface of 304 than to the grade 316, in few months [20,21]. Another observation is that a negatively charged surface attracts more bacteria than a positively charged one [22].

In addition, the surface energy influences the bacterial adhesion, which depends on the conditioning layer (that forms immediately after immersion into microbial environment). This changes the substrate properties that affect the microbial adhesion [23]. When steel 316 was immersed into solution of metal oxidizing bacteria, the ennoblement of the metal went parallel with localized corrosion [24]. The observations described on the influence of the alloying elements in the literature, clear show that not only their concentration and ratio, but also the quality and quantity of the microbial communities determine the interaction between the microorganisms and the metal surface.

This paper deals with the microbial adhesion of mixed and pure cultures on different iron alloys. The impact of alloying elements on the bacterial adhesion was in the focus of the experiments. Although there are literature data on the influence of Cr, Ni and Mo content on the bacterial adhesion, most papers deal with pure cultures. For us it was important not only to show the influence of concentrations of regular alloying elements (Cr, Ni, Mo) in steels on the change in cell number adhered from pure culture but we wanted to prove the positive, microbial adhesion inhibiting effect of an unusual alloying element, the ruthenium used at low concentration. The other question to answer was how the Cr, Ni and Mo content of stainless steels influences the bacterial attachment of mixed culture in cooling water. In order to learn as much as possible about these problems, different techniques were applied parallel. The influence of alloying elements on the bacterial adhesion was visualized by microscopes (fluorescence, light, atomic force); the number of microbes in biofilms was enumerated by traditional techniques. The deterioration of metal surface beneath the biofilm is also demonstrated.

Experimental part

Materials and methods

Biofilms were developed either from cooling water of mixed population ($7x10^{6}$cell cm^{-3}) or from pure culture of *Desulfovibrio desulfuricans* (ATCC 1249; cell number:10^{7}cell cm^{-3}).

The cell number counting was done by dilution technique or by commercial kits (Easicult TTC for aerobes and Easicult S for anaerobes). After staining the biofilm with acrydine orange (0.1g/100mL ethanol), the microorganisms were visualized by fluorescence microscope.

The microorganisms scattered on solid surfaces were also monitored by atomic force microscope (Digital Instrument, NanoScope 3). The alloy surfaces were identically finished in all cases, first with emery paper and later with diamond past at grain sizes: 16-9-6-3-1.5 μm.

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Metals under investigation were carbon steel (C< 0.5 wt%; balk: Fe), stainless steel with compositions: Cr: 40%; Cr/Ni: 40/6% as well as 18/8%; Cr/Ni/Mo:10/2/2%; Cr/Ni/Ru:10/2/0.1%. In all cases, the balk was Fe.

The length of time the coupons were immersed into cooling water or into broth inoculated by *Desulfovibrio desulfuricans* is indicated at all experiences.

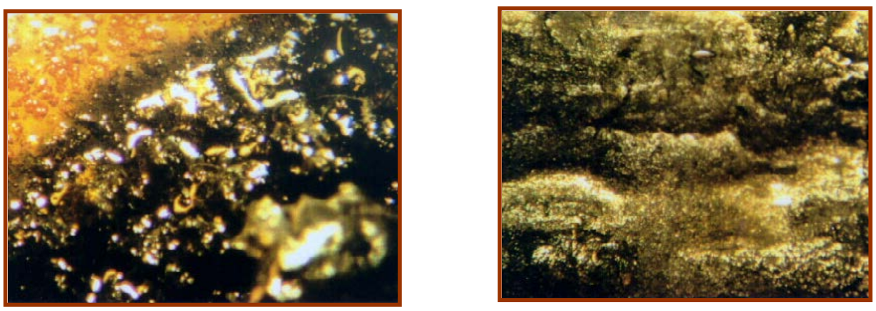
Results and discussion

Taking into account that the chemical composition of the metal surface influences the bacterial adhesion, biofilm formation and the distribution of microbes in biofilms were followed. In the first set of experiments, carbon steel coupons were immersed into inoculated broth of *Desulfovibrio desulfuricans*. The cell numbers of planktonic and sessile populations were measured after two and seven days (Table 1).

Table 1: Time dependent change in the *Desulfovibrio desulfuricans* cell numbers; initial cell number: 10^7cell cm^{-3}; metal: carbon steel.

Desulfovibrio desulfuricans cell number [cm^{-3}]			
Sessile, embedded into biofilm		planktonic	
after 2 days	after7 days	after2days	after7days
$7.3x10^4$	$4.6x10^5$	$3.4x10^7$	$5.0x10^6$

The number of the planktonic cells measured in 2 days show that the bacterial growth continued in the aqueous media, the appearance of iron ions in shorter time did not influence the multiplication of microbes. The number of planktonic cells deceases after 7 days. This shows that the multiplication of bacteria is already in the stationary and decline phase when more cells die than form via multiplication; in other words, the ration of viable and nonviable cell changes, the later one increases. The number of adhered microbes on the metal surface is much less than in the liquid phase. At longer time (7 days) the adhesion of microorganisms continued, however, still are fewer cells in built-in biofilm than in the original inoculated broth. The undesired corrosive deterioration caused by MIC on carbon steel is visible on Figure 1 where the biofilm formed on carbon steel in the presence of *Desulfovibrio desulfuricans* in three months are presented (Fig 1 a), as well as the deteriorated/roughened surface beneath the biofilm is shown (Fig 1 b).



a b

Figure 1 Carbon steel surface immersed into *Desulfovibrio desulfuricans* in ATCC 1249 for three months; a: biofilm formed on the surface; b: the deterioration of the metal surface beneath the biofilm (light microscope)

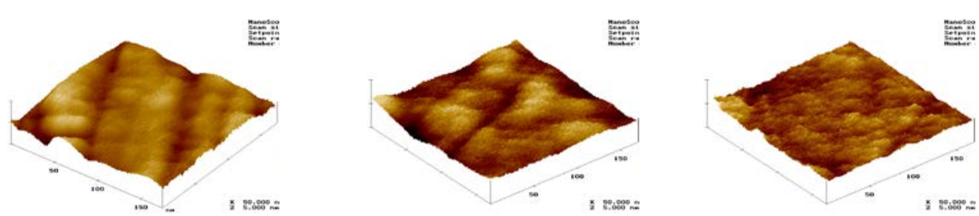
In the following set of experiments, the biofilm formation on iron alloys in the presence of *Desulfovibrio desulfuricans* was followed. The number of microbes in biofilms formed on different steel surfaces in seven days is summarized in Table 2.

Table 2: Influence of the alloying elements on the adhesion of *Desulfovibrio desulfuricans*.

Steels	Carbon steel	Cr (40%)	Cr-Ni (18/8%)	Cr-Ni-Mo (10/2/2%)
[cell.cm^{-3}] in the biofilm	$4.6x10^5$	$0.7x10^5$	$8.2x 10^5$	$0.0041x10^5$

These microbiological data confirm that the presence of nickel enhances the microbial adhesion, vitiates the microbial adhesion inhibiting effect of the chromium. On the contrary, the molybdenum in the surface layer inhibits the adhesion of the microorganisms. It is clear that under identical conditions, the sulfate reducer microbial content in biofilms developed on different metal surfaces is dependent on the alloying elements. These results demonstrate well that the metal surface is generally covered by oxide conditioning films, which is a reactive interface whereto the microorganisms directly adhere; its composition and thickness has important impact on the microbial adhesion, on the biofilm formation and, as a consequence, on the microbiologically influenced corrosion.

The influence of alloying element that alters the formation, thickness, compactness and chemical composition of the surface oxide layer, changes the sensibility to microbial adhesion. The presence of Mo in the steel is a good example. Its presence not only drastically enhances corrosion resistance, but also decreases/inhibits the adhesion of microorganisms. The “porosity” or “compactness” of the oxide layers formed on different iron alloys is visible in Figure 2. The AFM image on Fig 2 a visualizes in 3D the morphology of the oxide layer formed on carbon steel, the Fig 2 b the influence of the Cr/Ni content (18/8) on the surface morphology is visible and on the Fig 2 c demonstrates the influence of the Mo on the oxide layer. The comparative analysis of the images allows drawing the inference that the most compact surface is that one where Mo is in the alloy. This compact layer does allow the interaction with a corrosive environment and inhibits the adhesion of microorganisms.



Carbon steel Cr-Ni (18/8) Cr-Ni-Mo (10/2/2)

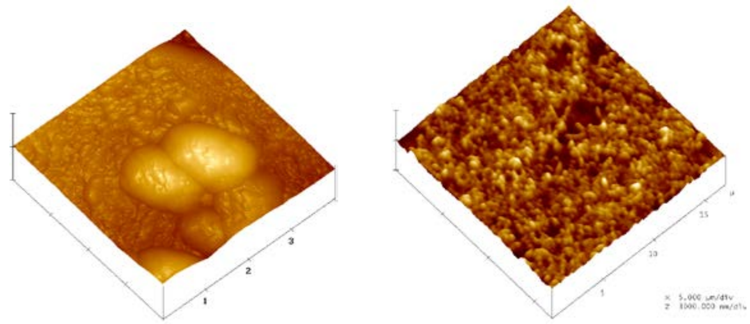
Figure 2 Surface morphology of different iron alloys, visualized by AFM [25].

Additional experiments were planned, when biofilms, developed on carbon steel in cooling water of mixed population, were supposed to show whether the adhesion of mixed population differs from that one observed with pure culture. With other words, will be the biofilm formation influenced only by the metal composition or by the parallel presence of anaerobic and aerobic microorganisms. The first results summarized in Table 2 show the alteration of cell numbers observed on carbon steel in the presence of cooling water with mixed population.

Table 2: Number of sessile and planktonic cells in the presence of carbon steel. The starting cell number: $7x10^{6}$cell cm^{-3}.

Mixed population of cooling water cell number [cm^{-3}]			
Sessile, embedded into biofilm		planktonic	
after 2 days	after 7 days	after 2 days	after 7 days
$3.4x10^{6}$	$2.4x10^{5}$	$5.3x10^{7}$	$2.4x10^{6}$

It is clear that the rate of the attachment is much higher than in the presence of the anaerobic *Desulfovibrio desulfuricans*. Already after two days, the number of the adhered bacteria almost reached the starting planktonic cell number. The decrease in cell number in longer time could be due to fewer nutrients. This could be explained by the inhibited diffusion of molecules into thicker biofilm. The behavior of the planktonic population is similar to that one we have observed with *Desulfovibrio desulfuricans*. Atomic force microscopic images in Figure 3 shows how the microbes attach to the carbon steel surface (as well as the EPS patches that permit of the adhesion of microorganisms, Fig 3 a) and how densely is the metal surface invaded by the microbes after longer time (Fig 3 b).



a b

Figure 3 Microorganisms of mixed population attached to carbon steel surfaces; a: cells scattered on the metal surface, surrounded by EPS patches; after 2 h; b: microorganisms invaded the surface in 24 h.

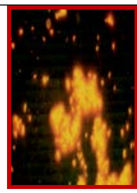
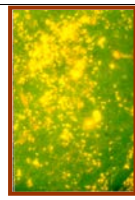
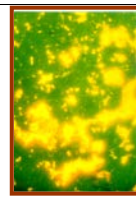
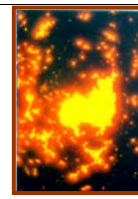
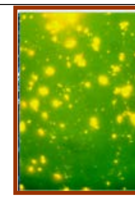
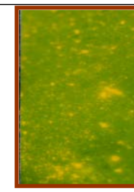
From experiments with mixed population of cooling water, in the presence of different alloys, we can conclude that the results are similar to those observed with pure bacterial culture. The compactness of the carbon steel and different iron alloy surfaces were demonstrated by AFM images in Figure 2 and the influence of the alloying elements on the differences in surface roughness values (RMS, derived from AFM measurements) caused by the microorganisms are summarized in Table 3. The same table shows the fluorescence images of the alloy surfaces covered by biofilms of cooling water mixed population.

As Figure 2 shows, the surface compactness changes significantly by the metal composition. The carbon steel and the Cr/Ni 18/8 are only partly covered by oxide layer (white spots match to the oxide). The surface coverage of Cr/Ni/Mo (10/2/2) seems much more homogenous (the color of the total surface is white-grayish) than in the other two cases.

The compactness of the oxide layer influences/decreases the microbial adhesion; this observation is supported by the fluorescence images.

Comparing the RMS values with the fluorescence images (where the yellow spots represent the microorganisms embedded into biofilms, formed in the presence of mixed population of cooling water) it is clear that the chemical composition of the balk metal and, of course, of its surface layer, has a significant impact on the bacterial adhesion and, as a consequence, on the microbially influenced corrosion. On rougher surface, the microbial population is denser.

Table 3 Alloying-element-dependent biofilms developed on iron alloy surfaces by microorganisms of cooling water; the influence of the metal surface roughness (RMS) on the invasion of alloy surfaces; RMS values were calculated from AFM measurement at 1x1 µm; the microorganisms in the biofilms are visualized by fluorescence microscope after staining by acrydine orange; magnification:1000x.

steels	Carbon steel	Cr [%] (40)	Cr-Ni [%] (40/6)	Cr-Ni [%] (18/8)	Cr-Ni-Mo [%] (10/2/2)	Cr-Ni-Ru [%] (10/2/0.1)
Oxide layer roughness RMS [nm]	31	14	17	28	10	7
Fluorescence images of biofilms formed on different steel surfaces						

The increase in the nickel content enhances the microbiological deposition/adhesion, which ranges with published observations [20,21]. The presence of molybdenum moderates the adhesion of microbes. The best result was observed in the presence of ruthenium alloy, even in a very small concentration, when fewer microorganisms attached to the metal surface. Its much more compact oxide layer (not shown) inhibits/decreases the conditioning layer formation and the irreversible adhesion of microorganisms. This is reflected in the thickness of biofilm characterized with the presence of microbes in colonies.

Summarizing the observations on the microbial adhesion, we can generally mention that the presence of different iron alloying elements affect the susceptibility to MIC as they stabilize the protective oxide films of different composition and surface roughness. Resistance to MIC can be correlated to increased concentration of chromium, molybdenum and ruthenium. The nickel increases the susceptibility to microbial adhesion, both of aerobic and anaerobic microorganisms.

When chromium is the only sub-metal in the iron alloy, in both cases – in the presence of pure *Desulfovibrio desulfuricans* culture as well as of mixed population of cooling water - the adhesion of microbes is limited comparing with values measured on carbon steel. The presence of nickel has an undesired effect as it increases the number of microorganisms and colonies on the surface. When smaller chromium and nickel concentration is accompanied by molybdenum at a few percentages, the invasion by microorganisms was significantly confined. The best microbial adhesion inhibition effect was noticed when, notwithstanding of small chromium and nickel concentration, ruthenium was present in the alloy at a very low concentration. Correlation was found between the surface roughness parameters (RMS) and the microbial adhesion/invasion. The RMS values show the heterogeneity of the metal surface caused by different alloying components. Low RMS values caused by the presence of different metal alloy elements, do not favor the irreversible microbial attachment and the biofilm formation, and the consequence is lower rate of microbiologically influenced corrosion.

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