

INFLUENCE OF SELECTED COMPONENTS OF THE NITE 927 CULTURE MEDIUM ON THE CORROSION OF AZ31 MAGNESIUM ALLOY

¹Leoš DOSKOČIL, ¹Martin BUCHTÍK, ¹Matěj BŘEZINA, ²Nicol HANIŠÁKOVÁ, ^{2,3}Tomáš VÍTEŽ

¹Brno University of Technology, Faculty of Chemistry, Brno, Czech Republic, EU,
doskocil@fch.vut.cz

²Masaryk University, Faculty of Science, Brno, Brno, Czech Republic, EU,
vitez@sci.muni.cz

³Mendel University in Brno, Faculty of AgriSciences, Brno, Czech Republic, EU,
tomas.vitez@mendelu.cz

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Abstract

Microbial activity can represent a significant factor in the degradation of metallic materials. Nevertheless, microbially influenced corrosion of magnesium alloys has received limited attention. This study examines the effect of chlorides, cysteine, and HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) in a methanogen culture medium on corrosion behaviour of the AZ31 alloy (MgAl₃Zn₁). Electrochemical results show the highest corrosion rate in the complete medium (158.5 $\mu\text{A}/\text{cm}^2$). Removing HEPES caused the largest decrease (15.8 $\mu\text{A}/\text{cm}^2$), followed by removal of cysteine (50.1 $\mu\text{A}/\text{cm}^2$), indicating that both strongly accelerate corrosion. Chloride removal had a smaller effect (125.9 $\mu\text{A}/\text{cm}^2$), suggesting that corrosion is not governed by chlorides alone. Corrosion potentials showed only minor changes, indicating kinetic control. Surface analysis revealed severe pitting in the complete medium, while cysteine- and HEPES-free conditions produced smoother surfaces. Fourier-transform infrared spectroscopy (FTIR) confirmed identical corrosion products (Mg(OH)₂, carbonates, phosphates) in all cases. Overall, corrosion of AZ31 is driven by the combined action of medium components, with HEPES and cysteine playing a more significant role than chlorides.

Keywords: AZ31 alloy, culture medium, corrosion, methanogens, HEPES, cysteine

1. INTRODUCTION

Magnesium and its alloys have attracted significant attention in recent years due to their low density, high specific strength, and excellent machinability, making them promising materials for applications in the automotive, aerospace, and biomedical fields [1,2]. However, a major limitation of magnesium-based materials is their high chemical reactivity, which results in poor corrosion resistance, particularly in aqueous environments containing chlorides. Therefore, various strategies have been developed to improve their corrosion performance, including surface treatments (metal-organic framework coatings, layered double hydroxide coatings, plasma electrolytic oxidation coatings, and superhydrophobic coatings) and the use of corrosion inhibitors (molybdate, sodium dodecyl sulphate) [3-9].

While the corrosion behavior of magnesium alloys in conventional environments has been extensively studied, relatively little attention has been paid to microbiologically influenced corrosion (MIC) in these systems. Nevertheless, recent studies have reported that sulfate-reducing bacteria (SRB) can accelerate the corrosion of magnesium alloys [10]. These investigations are typically conducted in culture media that contain high concentrations of inorganic salts, such as chlorides and sulfates, as well as organic components including amino acids, glucose, and vitamins [11-15].

Culture media represent a complex mixture of various components present over a wide concentration range, from $\text{ng}\cdot\text{L}^{-1}$ to tens of $\text{g}\cdot\text{L}^{-1}$. It is well established that certain components, such as chlorides, HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) buffer, and sulfates, can adversely affect the corrosion behavior of magnesium alloys [16-18]. These components are commonly present in culture media at relatively high concentrations. In addition, culture media, particularly those used for anaerobic environments, typically contain cysteine, which may also significantly accelerate the corrosion of magnesium materials [19].

The aim of this work is to investigate the effect of chlorides, cysteine, and HEPES on the corrosion behavior of AZ31 magnesium alloy in a culture medium commonly used for methanogens.

2. EXPERIMENTAL

AZ31 alloy samples (MgAl_3Zn_1 ; $15 \times 15 \times 5$ mm) were used for corrosion tests. The alloy chemical composition was determined using Glow-Discharge Optical Emission Spectroscopy; 3.60 Al, 1.34 Zn, 0.28 Mn, 0.03 Si, 0.002 Fe and 0.01 Sn (all in wt%). The microstructure consists of polyhedral grains of an α -Mg solid solution containing alloying elements, together with Al_xMn_y intermetallic particles. The samples of AZ31 alloy were ground using 320 and 1200 SiC grit abrasive papers, washed in distilled water and isopropyl alcohol and then dried by hot air.

Table 1 Final concentration of components in the culture medium NITE 927

Component	Concentration (g/L)
HEPES	23.8
NaCl	19.0
$\text{MgCl}_2\cdot 6\text{H}_2\text{O}$	2.6
$\text{CaCl}_2\cdot 2\text{H}_2\text{O}$	0.15
Na_2SO_4	4.0
NH_4Cl	0.25
KH_2PO_4	0.2
KCl	0.5
NaHCO_3	2.68
Trace elements solution*	1.0
Wolfe's vitamin solution**	1.0
L-cystein-HCl	1.0
*in 1L of stock solution: 12.8 g Nitrilotriacetic acid, 1.35 g $\text{FeCl}_3\cdot 6\text{H}_2\text{O}$, 0.1 g $\text{MnCl}_2\cdot 4\text{H}_2\text{O}$, 0.024 g $\text{CoCl}_2\cdot 6\text{H}_2\text{O}$, 0.1 g $\text{CaCl}_2\cdot 2\text{H}_2\text{O}$, 0.1 g ZnCl_2 , 0.025 g $\text{CuCl}_2\cdot 2\text{H}_2\text{O}$, 0.01 g H_3BO_3 , 0.024 g $\text{Na}_2\text{MoO}_4\cdot 2\text{H}_2\text{O}$, 1 g NaCl, 0.12 g $\text{NiCl}_2\cdot 6\text{H}_2\text{O}$, 0.005 g $\text{Na}_2\text{SeO}_3\cdot \text{H}_2\text{O}$, 0.004 g Na_2WO_4 , 0.02 g $\text{KAl}(\text{SO}_4)_2\cdot 12\text{H}_2\text{O}$	
**in 1L of stock solution: 2 mg Biotin, 2 mg Folic acid, 10 mg Pyridoxine-HCl, 5 mg Thiamine-HCl, 5 mg Riboflavin, 5 mg Nicotinic acid, 5 mg Ca-pantothenate, 1 mg p-Aminobenzoic acid, 0.01 mg Vitamin B12	

The NITE 927 medium was prepared without the use of microorganisms according to **Table 1**. To investigate the effect of selected components, culture media were prepared in the same manner but without chlorides (2.6 g/L $\text{MgCl}_2\cdot 6\text{H}_2\text{O}$, 0.15 g/L $\text{CaCl}_2\cdot 2\text{H}_2\text{O}$, 0.25 g/L NH_4Cl , 19 g/L NaCl, and 0.5 g/L KCl), without 1 g/L cysteine, and without 23.8 g/L HEPES. The pH of all solutions was adjusted to 7.0.

Potentiodynamic polarization (PDP) was performed in culture medium (CM) at 25 °C. The AZ31 alloy was used as the working electrode and placed in a corrosion cell (exposed area cca 1 cm^2) with a volume of 200 mL. A platinum grid served as the auxiliary electrode and a saturated calomel electrode (SCE) was used as the reference electrode. The open circuit potential (OCP) was recorded for 24 h, after which PDP measurements were carried out. The PDP measurements were performed using a potentiostat (Biologic, VSP-300) at a scan rate of 1 mV/s in the range from

−0.25 V vs. OCP (cathodic branch) to +0.4 V vs. OCP (anodic branch). The corrosion current density was determined from the cathodic branch of the obtained PDP curves.

The morphology after corrosion of AZ31 alloy samples was investigated using a light microscope (Zeiss Stemi 2000-C) equipped with a digital camera (AxioCam ERc 5s). Chemical characterization of corrosion products was performed using Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR; Nicolet iS10) in the spectral range 4000–400 cm^{−1} with a resolution of 4 cm^{−1} and an average of 128 scans.

3. RESULTS AND DISCUSSION

The electrochemical parameters obtained from the PDP curves (**Figure 1**) are summarized in **Table 2**. The corrosion current density (i_{corr}) for the complete culture medium reached 158.5 μA/cm², indicating relatively high corrosion activity. A decrease in corrosion current density was observed for all modified media, suggesting that individual components of the culture medium significantly influence the corrosion behavior. The most pronounced decrease in i_{corr} was observed for the medium without HEPES (15.8 μA/cm²), indicating that HEPES strongly contributes to the acceleration of corrosion. This effect may be related to its buffering capacity, its interaction with Mg²⁺ ions released from the alloy, and the increase in ionic strength, which leads to a reduction in the formation of protective corrosion products [14,18]. Similarly, the removal of cysteine led to a significant decrease in corrosion current density to 50.1 μA/cm², suggesting that cysteine also promotes corrosion. This behavior can be attributed to its reducing properties and ability to complex metal ions, which may destabilize protective corrosion products and facilitate anodic dissolution. Cysteine is added to the culture medium to maintain anaerobic conditions, as it reacts with oxygen to form cystine via disulfide bond formation [21,22]. In contrast, the chloride-free medium exhibited a more moderate decrease in i_{corr} (125.9 μA/cm²). Although chlorides are generally considered aggressive ions that promote localized corrosion, their removal did not lead to a substantial reduction in corrosion rate compared to the complete medium. This suggests that, in the studied system, the overall corrosion behavior is not governed solely by chlorides but rather by the combined effect of multiple components present in the culture medium. The corrosion potentials (E_{corr}) showed only minor variations (−1.49 to −1.55 V), indicating that the thermodynamic tendency for corrosion was not significantly affected by the removal of individual components. Instead, the observed differences in corrosion behavior are primarily related to kinetic factors influencing anodic and cathodic reactions.

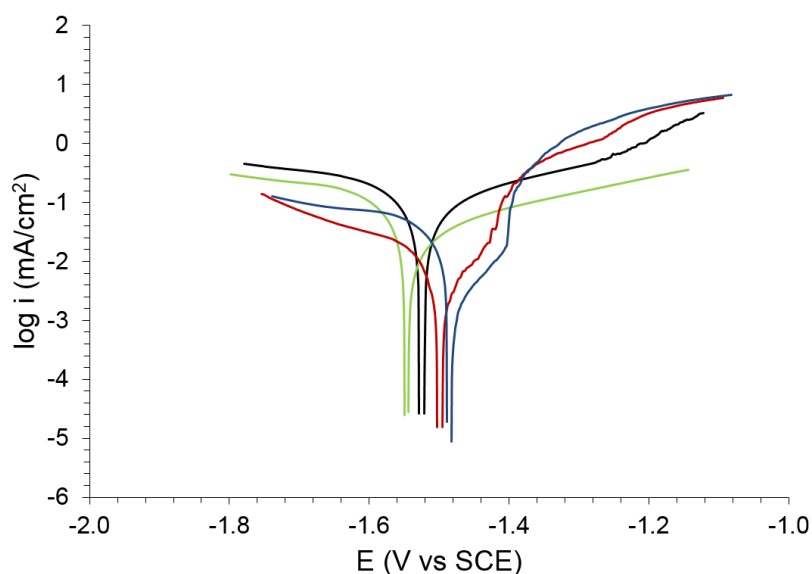


Figure 1 PDP curves plotted as current density (i) versus potential (E vs SCE) for the culture medium (black curve) and the culture medium depleted of chlorides (green curve), cysteine (blue curve), or HEPES (red curve)

Table 2 Electrochemical parameters obtained from PDP measurements

Element	i_{corr} ($\mu\text{A}/\text{cm}^2$)	E (V vs SCE)
Culture medium	158.5	-1.53
Culture medium without chloride	125.9	-1.55
Culture medium without HEPES	15.8	-1.50
Culture medium without cysteine	50.1	-1.49

Microscopic evaluation of the surfaces (**Figures 2A–D**) shows that the composition of the culture medium has a significant influence on both the degradation character and the homogeneity of the studied samples. In the case of the complete medium (**Figure 2A**), the surface is covered with corrosion products and exhibits localized attack in the form of pitting. The presence of all components in the medium evidently promotes the formation of aggressive microenvironments leading to the initiation of pitting corrosion. The absence of chlorides in the culture medium (**Figure 2B**) results in an overall degraded surface; however, pitting is less pronounced compared to the complete medium (**Figure 2A**). This finding suggests that chloride ions are not the only factor influencing the overall degradation of the AZ31 alloy. The surface of the AZ31 alloy in the culture medium without cysteine (**Figure 2C**) is relatively smooth and homogeneous, with a minimal number of observable defects. A similar behavior was observed for the medium without HEPES (**Figure 2D**), where the surface appeared even more preserved than in the case of the cysteine-free medium (**Figure 2C**). Comparison with **Figure 2B**, which shows the surface after corrosion in chloride-free medium, indicates that chlorides are not the primary factor negatively affecting the corrosion of AZ31. These results are consistent with the PDP analysis, which also indicates changes in corrosion activity depending on the composition of the medium.

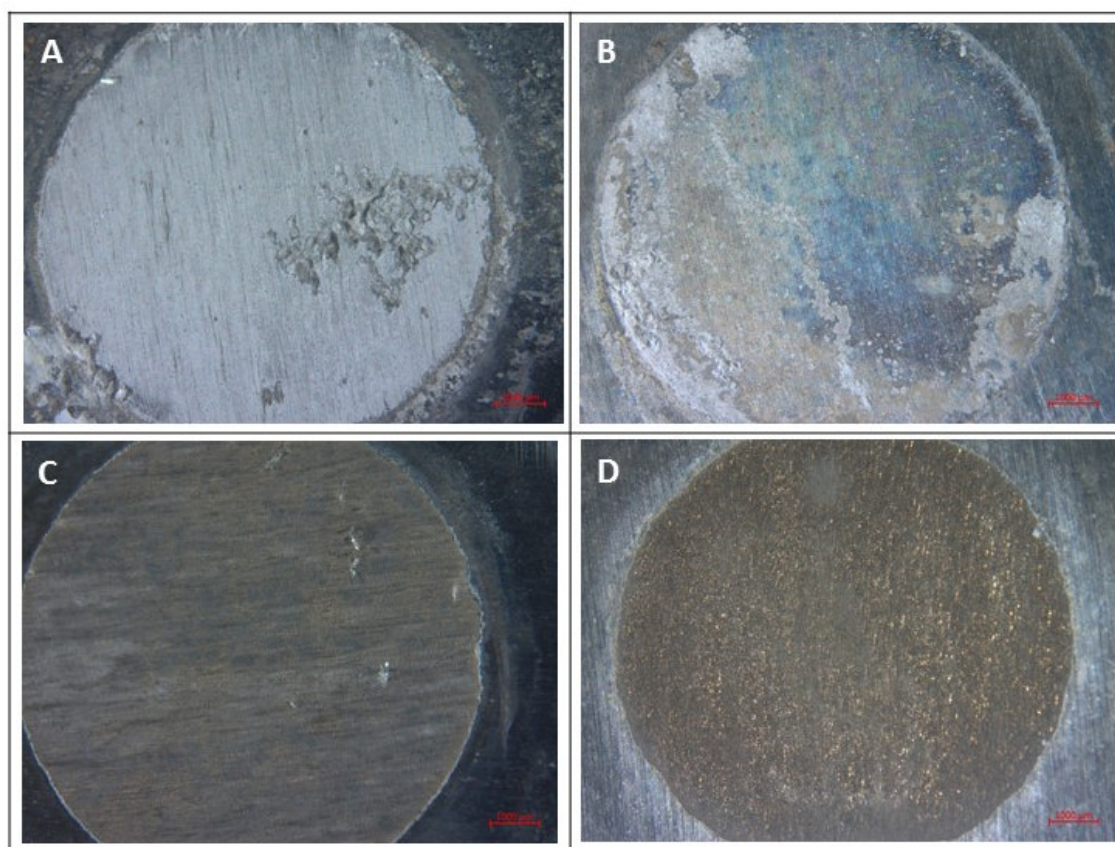


Figure 2 Surface of AZ31 alloy after corrosion in culture medium (A) and in culture medium without chloride (B), without cysteine (C), or without HEPES (D)

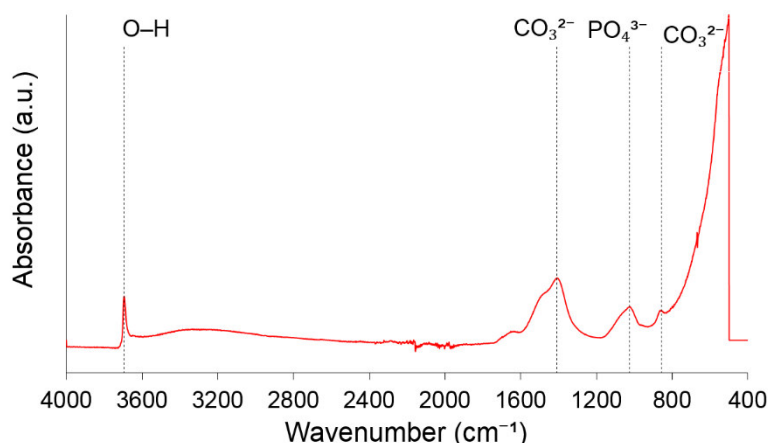


Figure 3 FTIR spectrum of corrosion products

FTIR spectra of corrosion products formed in the culture medium revealed absorption bands characteristic of magnesium alloy corrosion products (**Figure 3**). A sharp band at 3696 cm^{-1} corresponds to the stretching vibrations of hydroxyl groups, confirming the presence of $\text{Mg}(\text{OH})_2$. Additional bands observed at 1409 cm^{-1} and 858 cm^{-1} are

characteristic of carbonate anions, while the band with a maximum at 1024 cm^{-1} is associated with the presence of phosphate anions. Similar FTIR spectra were obtained for all other investigated media (culture media depleted of chlorides, cysteine, or HEPES), with identical corrosion products identified in each case. This suggests that variations in medium composition do not lead to the formation of different corrosion phases, but primarily affect the kinetics of the corrosion process.

4. CONCLUSION

The corrosion behavior of AZ31 magnesium alloy was strongly influenced by the composition of the culture medium. The highest corrosion rate was observed in the complete medium, while the removal of HEPES and cysteine significantly reduced corrosion, indicating their key role in corrosion acceleration. In contrast, the absence of chlorides had a smaller effect, suggesting that corrosion is governed by the combined action of medium components rather than chlorides alone. FTIR analysis showed that identical corrosion products, primarily $\text{Mg}(\text{OH})_2$ with carbonate and phosphate species, were formed in all media. This indicates that variations in medium composition affect mainly corrosion kinetics rather than the nature of corrosion products.

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