Microbiological investigations of corrosion damages in aircraft

Mikrobiologische Untersuchungen von Korrosionsschäden an Flugzeugen

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A joint project of different european aircraft manufacturers involves the investigation of the involvement of microorganisms in corrosion damage in aircraft.

In a first step, the microflora of corrosion failures was investigated. Swab samples were taken from corroded sites and were cultured on different media under different conditions. Media and incubation conditions were suitable for the growth of acrobic and anaerobic heterotrophic bacteria, sulfate reducing bacteria, Thiobacillus (T.) thiooxidans, T. ferrooxidans, fungi, and yeasts.

The isolated microorganisms were identified using standard methods like microscopy, physiological tests and the test systems API and BIOLOG.

From 46 samples, taken from corroded sites of 7 different aeroplanes, a total of 208 microorganisms were isolated (158 bacteria, 36 yeasts, 14 mycelium-forming fungi).

The isolates were examined in the laboratory with regard to their corrosive action towards an aluminium alloy, commonly used in aircraft construction (Al-7075). Material samples were incubated in liquid media, which were inoculated with pure cultures of the respective isolates, over a period of 28 days. The degree of corrosion was determined using quantimetric analysis. Significant damages were examined metallographically.

Results show that the corrosive effect of the isolated microorganisms was quite different. Isolates of the genera Micrococcus, Enterococcus, Staphylococcus, Aerococcus, Bacillus, Aspergillus, and Penicillium provoked strong corrosion towards Al-7075.

Im Rahmen eines von der EG geförderten Verbundvorhabens verschiedener europäischer Luftfahrtunternehmen wird unter anderem untersucht, inwieweit Mikroorganismen bei Korrosionsschäden an Strukturbauteilen von Flugzeugen von Bedeutung sind.

Zunächst wurde die Mikroflora von Korrosionsschäden untersucht. Dazu wurden Abstriche an korrodierten Stellen entnommen und auf verschiedenen Nährmedien unter verschiedenen Bedingungen angelegt. Medien und Inkubationsbedingungen waren geeignet für das Wachstum von aeroben und anaeroben, heterotrophen Bakterien, Sulfatreduzierern, Thiobacillus (T.) thiooxidans und T. ferrooxidans, Pilzen und Hefen. Die isolierten Mikroorganismen wurden mit Hilfe von Standardmethoden wie Mikroskopie, physiologischer Tests und den Testsystemen API und BIOLOG weitgehend differenziert.

Aus derzeit 46 Abstrichen, entnommen in 7 verschiedenen Flugzeugen, konnten insgesamt 208 Mikroorganismen isoliert werden (158 Bakterien, 36 Hefen, 14 myceibildende Pilze).

Die Isolate wurden im Labor hinsichtlich ihres korrosiven Einflusses auf eine im Flugzeugbau verbreitet eingesetzte Aluminium-Legierung (Al 7075) untersucht. Proben dieses Materials wurden in einem mit dem jeweiligen Isolat beimpften Flüssignährmedium über einen Zeitraum von 28 Tagen ausgelagert. Das Ausmaß der resultierenden Korrosion wurde quantimetrisch bestimmt. Bei signifikanten Schäden wurden zusätzlich metallographische Untersuchungen durchgeführt. Es zeigte sich, daß die isolierten Mikroorganismen unter den herrschenden Versuchsbedingungen unterschiedlich stark korrosionsfördernd sind. Starke Korrosion zeigten vor allem Isolate der Gattungen Micrococcus, Enterococcus, Staphylococcus, Aerococcus, Bacillus, Aspergillus und Penicillium.

1 Introduction

In aircraft, microbiologically influenced corrosion (MIC) of fuel tanks made of aluminium alloys is well known since the middle of the sixties [1–3]. These corrosion effects are probably caused by metabolic products of the fungus Hormoconis resinae [4, 5]. On the other hand virtually nothing is known on the involvement of microorganisms in other corrosion damages in aircraft as for example the bilge and the toilet area.

In a common project of different aviation companies it is investigated, how far microorganisms are involved in corrosion damages of structural parts of aeroplanes.

To examine a possible involvement of microorganisms in corrosion, in a first step the respective microflora of corrosion sites is investigated. This is done by isolation and identification of microorganisms from corrosion sites in aircraft. Afterwards it is tested, whether these isolated microbes show any corrosive action towards aluminium alloys, which are frequently used for constructing aeroplanes. If there are any corrosion promoting microorganisms, the mechanisms causing corrosion will be investigated.

The aim of these investigations is to study the involvement of microorganisms in the corrosion of aluminium

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alloys in aircraft. In the end it should be possible to make statements about the practical significance of microbiologically influenced corrosion in aircraft.

2 Experimental

2.1 Rationale of experimental approach

If one wants to evaluate the importance of MIC in aircraft corrosion, the first step is the description of the microflora present at relevant corrosion sites.

This examination of the microflora should by no means be restricted to genera or groups already known to be of importance in MIC in other areas; rather, the selection of isolation methods applied should cover as many different types of microorganisms as possible in order to get a fairly complete picture of the resident microflora. The isolation procedures described below have been chosen, accordingly.

The next step is to answer whether this microflora contributes to corrosion processes or not. This would be performed best using experimental conditions as close to practical conditions as possible. However, in aircraft the relevant parameters (temperature, humidity, pH, availability of organic nutrients etc.) are extremely variable, depending on route of flight, load being transported, momentary flight situation, quality, and frequency of maintenance and numerous others. Therefore, it is virtually impossible to mimic the practical environmental conditions during laboratory experiments or to design a meaningful standard procedure similar to practical conditions.

On the other hand, bearing in mind the broad range of environmental conditions allowing for microbial growth and metabolism, one can undoubtedly state that, at least during certain periods of time, microorganisms will flourish in aircraft in any case.

Another important factor to be considered is time; corrosion promoting activities of microorganisms may happen over long periods of time (months or even years) in aircraft, while experiments for discovering a corrosion promoting potential of microorganisms should take substantially less time.

Based on these considerations, we choose a "worst case"-strategy for screening for a possible corrosion-promoting effect of the respective microbial isolates, i.e., we applied conditions well suited for microbial growth and metabolism.

Al 7075 was chosen as a model material due to its widespread application in modern aircraft.

2.2 Isolation and identification of microorganisms from corrosion failures in aircraft

Swab samples were taken from corroded sites. These were maintained in a transport medium until they arrived at the laboratory. The samples were cultivated on different media under different incubation conditions which were suitable for the growth of a wide range of microorganisms: aerobic and anaerobic heterotrophic bacteria, sulfur oxidizing bacteria, sulfate-reducing bacteria, fungi, and yeasts.

First the swabs were streaked on different solid media, suitable for the growth of heterotrophic bacteria like blood-agar (BA), DEV-agar (DEV), nutrient agar reinforced (NAR), cetrimide-agar (CEMAN), MacConkey-agar (MC), reinforced clostridial agar (RCM), and low nutrient agar (LN). Incubation was performed at 25 °C and 37 °C. For the isolation of fungi and yeasts swabs were streaked on potato-glucose- (KGA), Sabouraud-glucose-maltose- (SGM), and Candida-agar (CA), which were incubated at 30 °C. Then the swabs were washed in sterile 0,85% NaCl solution. With 1 ml of this solution liquid media for the enrichment of aerobic heterotrophic bacteria (NARAB, 30 °C), fungi and yeasts (SGMAB, 30 °C), sulfate-reducing bacteria (SRB, 37 °C, anaerobically), anaerobic heterotrophic bacteria (RCMAB, 30 °C, anaerobically), Thiobacillus thiooxidans (Thth, 30 °C), and Thiobacillus ferrooxidans (Thfe, 30 °C) were inocculated. After growth had occured in the enrichment media, solid media were inocculated. Colonies grown on agar plates were restreaked until pure cultures were obtained. With pure cultures identification was performed using standard methods like microscopy (determination of cell morphology, motility, gram- and spore-staining) [6, 9, 10], physiological tests (e.g. catalase, oxidase, oxidation/fermentation of glucose, test for anaerobic growth, lysostaphin resistance) [6, 9], and different ready to use test systems (API and Biolog).

2.3 Testing for corrosive action

Samples of the aluminium alloy 7075 (15 × 15 × 8 mm) were incubated in PYG-bouillon (PYG), inocculated with pure cultures of the respective microorganisms, isolated from corrosion failures in aircraft. Up to now, testing was performed with aerobic and facultatively anaerobic isolates. The cultures were incubated on a rotary shaker for 28 days at 30 °C and 100 rpm. Sterile PYG-medium was used for control. The 7075-samples were coated at the bottom and the edges with a protective paint (Turco) to leave only the top surface blank. At the end of the test, the sample surfaces were inspected visually. If corrosion was observed, quantimetric analysis (Cambridge Instruments) of the corroded area was performed. Significant corrosion failures were examined metallographically.

2.4 Media

Transport medium, BA, DEV, MC, RCM, KGA, SGM, and CA were purchased from Merck. Thth was made using analytical grade incredients (Merck) according to [7], Thfe according to [8], and SRB according to [11].

NAR: Na-acetate × $3H_2O$ 0.5 g, NaCl 1.0 g, peptone from casein 2.0 g, proteose peptone 1.0 g, meat extract 1.0 g, malt extract 0.5 g, yeast extract 0.5 g, ammoniumcitrate 0.2 g, glucose 1.0 g, K_2HPO_4 × $3H_2O$ 2.0 g, KH_2PO_4 1.0 g, $MgSO_4$ × $7H_2O$ 0.1 g, Tween 80 0.5 ml, agar 15.0 g, aqua dest. 1000 ml, pH 7.0

NARAB: Composition is like NAR without agar.

CEMAN: peptone 0.2 g, $MgCl_2$ 1.4 g, K_2SO_4 10 g, cetrimide 0.3 g, glycerol 5 ml, mannitol 5 g, agar 15 g, aqua dest. 900 ml, 100 ml of a filtersterilized solution of 10 g acetamide, and 12 mg phenol red in 100 ml water, pH 7.0

LN: tryptone 0.1 g, yeast extract 0.1 g, CaCl × $2H_2O$ 0.5 g, $MgSO_4$ × $7H_2O$ 0.5 g, trace element solution (filtersterilized) 1.0 ml, agar 12 g, aqua dest. 1000 ml, pH 7.2

Trace element solution: $MnCl_2$ × $4H_2O$ 0.1 g, $CoCl_2$ × $6H_2O$ 0.037 g, $CuSO_4$ × $5H_2O$ 0.016 g, Na_2MoO_4 × $2H_2O$

Table 1. Results of sampling in 7 different aeroplanes
Tabelle 1. Ergebnisse der Probennahme in 7 verschiedenen Flugzeugen

aircraft	number of samples	number of isolates	sampling area
1. Airbus A 300	3	15	toilet area, pass door area, mid cargo door area
2. Boeing 727	1	9	pass door sill
3. Boeing 727	2	28	foreward cargo compartment
4. Helicopter	3	11	door to fuselage, cabin floor, tail boom
5. Tornado	2	7	left wing
6. Tornado	15	23	wings, fueltank door, fuel tank area
7. Airbus A 300	20	115	forward cargo, toilet area, cargo area, cabin floor
Total	46	208	

Table 2. Results of identification
Tabelle 2. Ergebnisse der Identifizierung

0.01 g, $ZnCl_2$ 0.02 g, LiCl 0.005 g, $SnCl_2 \times 2H_2O$ 0.005 g, H_3BO_4 0.01 g, KBr 0.02 g, KJ 0.02 g, EDTA Disodiumsalt $\times$ $2H_2O$ 8.00 g, Fe(III)-chloride 0.04 g, aqua dest. 1000 ml

RCMAB: meat extract 10.0 g, peptone from casein 10.0 g, yeast extract 3.0 g, glucose 5.0 g, starch 1.0 g, NaCl 5.0 g, Na-acetate 3.0 g, L-cysteiniumchloride 0.5 g, aqua dest. 1000 ml, pH 6.8 ± 0.1

SGMAB: peptone from casein 5 g, peptone from meat 5 g, glucose 10 g, maltose 10 g, aqua dest. 1000 ml, pH 5.4 ± 0.1, 10 ml of filtersterilized solutions: penicillin G 1 g/l, rifampicin 0.2 g/l, nalixidic acid 0.2 g/l

PYG: peptone from casein 5 g, yeast extract 2.5 g, glucose 5 g, NaCl 0,25 g, pH 7,0

3 Results

3.1 Isolation and identification

Up to now, a total of 46 swab samples have been taken from corroded sites in 7 different aeroplanes (Table 1). The places at which the sampling was performed in civil aircraft were predominantly the toilet area and the bilge, but also the cargo area and the cabin floor. In Tornado sampling took place mostly in the tank area and at the wings.

A variety of microorganisms could be isolated from almost all samples investigated. Most isolates have been identified at least to the genus level. The genera found up to now are summarized in Table 2. Totally, 208 microorganisms could be obtained, 158 belonging to bacterial, 36 to yeast,

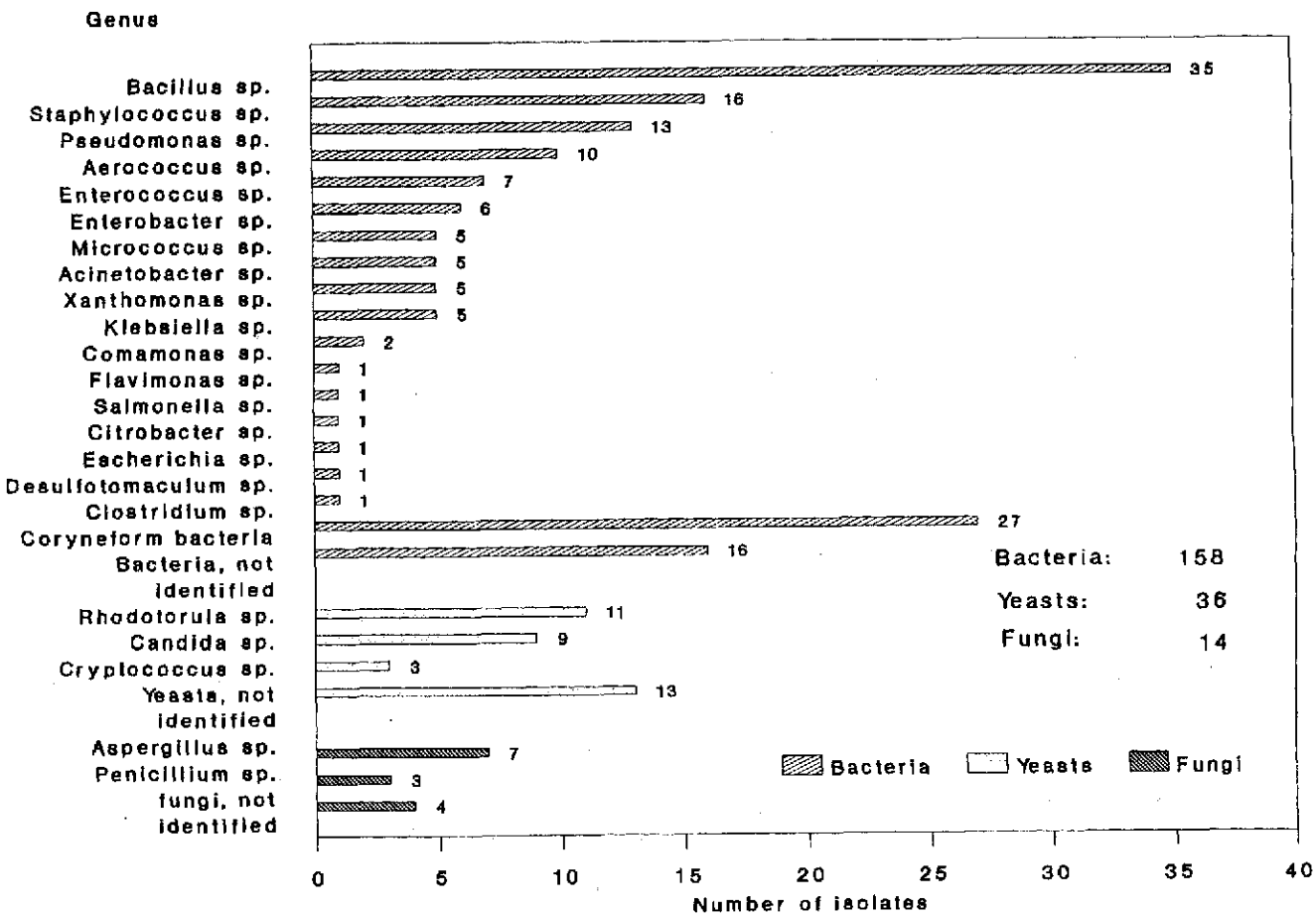

Table 3. Frequency of genera in 7 aeroplanes investigated
Tabelle 3. Häufigkeit der Gattungen in 7 untersuchten Flugzeugen

Genus	1. Airbus A 300	2. Boeing 727	3. Boeing 727	4. Helicopter	5. Tornado	6. Tornado	7. Airbus A 300	total number of aircraft
Staphylococcus sp.	+	–	–	+	+	+	+	5
Bacillus sp.	+	–	+	+	–	+	+	5
Acinetobacter sp.	+	+	+	–	–	+	+	5
Candida sp.	+	+	+	–	+	+		5
Pseudomonas sp.	–	+	+	–	–	+	+	4
Enterococcus sp.	+	+	+	–	–	–	+	4
Xanthomonas sp.	–	+	+	–	–	+	+	4
Enterobacter sp.	–	–	+	–	–	+	+	3
Klebsiella sp.	–	+	+	–	–	–	+	3
Rhodotorula sp.	+	–	–	+	–	–	+	3
Aspergillus sp.	–	–	+	+	–	–	+	3
Micrococcus sp.	–	–	+	–	–	–	+	2
Aerococcus sp.	+	–	–	–	–	–	+	2
Cryptococcus sp.	+	–	–	–	–	–	+	2
Penicillium sp.	–	–	+	–	–	–	+	2
Comamonas sp.	–	–	–	–	–	+	–	1
Escherichia sp.	–	+	–	–	–	–	–	1
Citrobacter sp.	–	–	+	–	–	–	–	1
Desulfotomaculum sp.	–	–	–	–	–	–	+	1
Clostridium sp.	–	–	–	–	–	–	–	1
Flavomonas sp.	–	–	–	–	–	–	+	1
Salmonella sp.	–	–	+	–	–	–	–	1

and 14 to fungal genera. All microorganisms isolated belong to heterotrophic facultatively aerobic or strictly anaerobic microorganisms. Only one sulfate-reducing bacterium was found in the samples, but not Thiobacillus thiooxidans or T. ferrooxidans. It is remarkable that some genera could repeatedly be isolated from different aircraft (Table 3). Within a total of 7 aircraft investigated up to now, Bacillus, Staphylococcus, Acinetobacter, and Candida were found in 5, and Enterococcus, Pseudomonas, and Xanthomonas in 4 cases, respectively.

3.2 Testing of corrosive action

The results of the long time incubation experiments show that the corrosive effect of pure cultures of the isolated microorganisms on Al-alloy 7075 is very different. After an incubation period of 28 days, many of the samples, including the control, incubated in sterile medium showed only slight or no corrosion at all (Fig. 1). On the other hand some isolates provoked strong corrosion damage to the material sample, which could be easily detected by visual

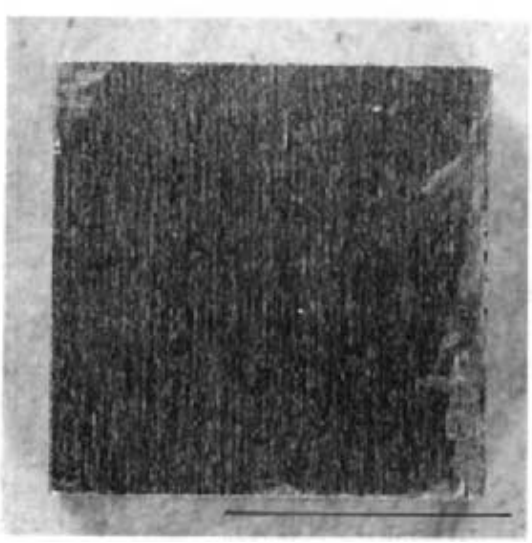

Fig. 1. Sample of Al 7075, incubated at 30 °C in sterile PYG-bouillon for 28 days (control). Bar: 1 cm

Abb. 1. Probe der Legierung Al 7075, welche 28 Tage bei 30 °C in steriler PYG-Bouillon inkubiert wurde (Kontrolle). Maßstab: 1 cm

Fig. 2. Sample of Al 7075 incubated at 30 °C for 28 days in PYG-medium, inocculated with Enterococcus casseliflavus. Bar: 1 cm

Abb. 2. Probe der Legierung Al 7075, welche 28 Tage in mit Enterococcus casseliflavus beimpfter PYG-Bouillon inkubiert wurde. Maßstab: 1 cm

inspection. Mostly, the corrosion failures were covered by white corrosion products (Fig. 2). After removal of the products the actual corrosion sites appeared (Fig. 3). If incubation was performed with corrosive mycelium forming fungi, no visible corrosion products were formed (Fig. 4, 5).

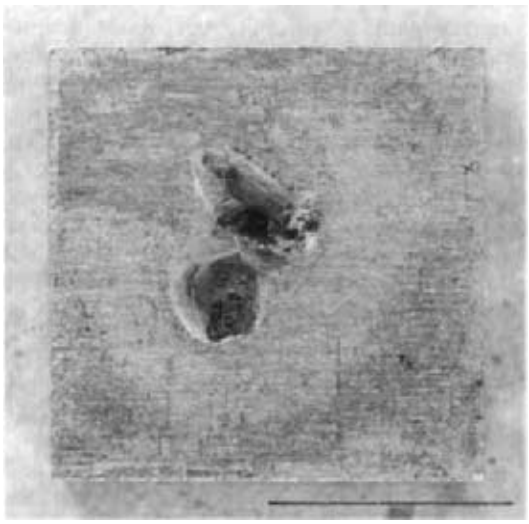

Fig. 3. Sample of Al 7075 incubated at 30 °C for 28 days in PYG-medium, inocculated with Enterococcus casseliflavus after removal of corrosion products. Bar: 1 cm

Abb. 3. Probe der Legierung Al 7075, welche 28 Tage in mit Enterococcus casseliflavus beimpfter PYG-Bouillon inkubiert wurde, nach Entfernung der Korrosionsprodukte. Maßstab: 1 cm

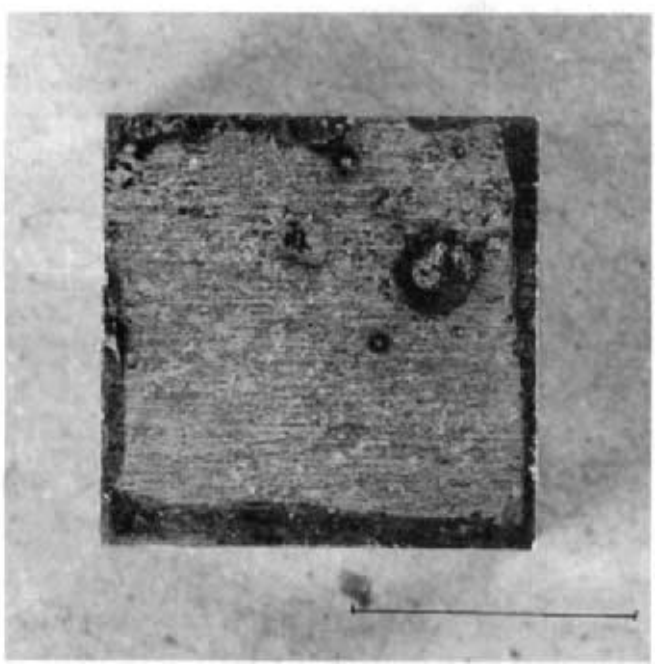

Fig. 4. Sample of Al 7075 incubated at 30 °C for 28 days in PYG-medium, inocculated with Penicillium sp. Bar: 1 cm

Abb. 4. Probe der Legierung Al 7075, welche 28 Tage in mit Penicillium sp. beimpfter PYG-Bouillon inkubiert wurde. Maßstab: 1 cm

Fig. 5. Magnified part of corrosion site of an Al 7075 sample incubated at 30 °C for 28 days in PYG-medium, inocculated with Penicillium sp. Bar: 1 mm

Abb. 5. Vergrößerung der Korrosionsstelle einer Probe der Legierung Al 7075, welche 28 Tage in mit Penicillium sp. beimpfter PYG-Bouillon inkubiert wurde. Maßstab: 1 mm

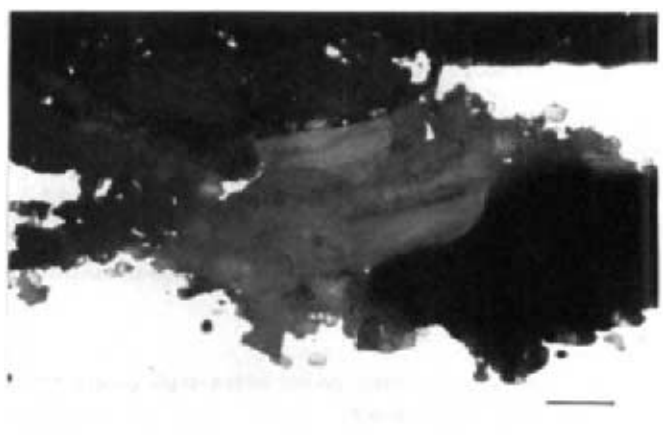

Fig. 6. Cross section of a corrosion site of an Al 7075 sample incubated at 30 °C for 28 days in PYG-medium, inocculated with Aspergillus sp. Bar: 20 µm

Abb. 6. Querschnitt einer Korrosionsstelle einer Probe der Legierung Al 7075, welche 28 Tage bei 30 °C in mit Aspergillus sp. beimpfter PYG-Bouillon inkubiert wurde. Maßstab: 20 µm

Using metallographic investigations, the shape of damages was examined. In cross sections the corroded sites appeared like holes or troughs, which are typical forms of local pitting corrosion (Fig. 6).

Microorganisms, which showed strong corrosive action on Al-7075 alloy, are summarized in Table 4.

It is remarkable, that all tested species of staphylococci, enterococci, and micrococci provoked strong corrosion, whereas only a few of the isolated bacillus, coryneforms, aspergillus, and penicillium species showed corrosive effects (Table 5).

Table 4. Isolates causing strong* corrosion on Al 7075 alloy during incubation in PYG-medium at 30 °C for 28 days
Tabelle 4. Isolate, welche während einer Inkubation von Al 7075 über 28 Tage in PYG Medium bei 30 °C starke Korrosion verursachten

Microorganisms	Total area [mm²]	Corroded area [mm²]	[%]
Micrococcus varians	169	27,0	16,0
Micrococcus roseus	169	1,0	0,6
Enterococcus casseliflavus	169	21,0	12,4
Enterococcus faecium	169	2,0	1,2
Escherichia hermannii	173	21,0	12,1
Staph. capitis	169	20,0	11,8
Staph. sp.	169	16,0	9,5
Staph. sp.	169	15,0	8,9
Staph. sp.	169	10,0	5,9
Staph. cohnii	169	8,0	4,7
Staph. hominis	169	8,0	4,7
Staph. epidermidis	169	6,0	3,6
Staph. capitis	165	5,3	3,2
Staph. warneri	164	5,2	3,2
Citrobacter sp.	169	12,0	7,1
Bacillus sp.	169	10,0	5,9
Bacillus sp.	169	8,0	4,7
Bacillus cereus	169	7,0	4,2
Bacillus sp.	169	2,0	1,2
Bacillus sp.	169	1,6	0,9
Aerococcus viridans	169	7,0	4,2
coryneform bacterium	169	18,0	10,7
coryneform bacterium	169	10,0	5,9
coryneform bacterium	169	10,0	5,9
bacterium, not identified	169	7,0	4,2
bacterium, not identified	169	6,0	3,6
bacterium, not identified	169	4,0	1,8
Aspergillus sp.	164	4,9	3,1
Penicillium sp.	169	n.d.	n.d.
Penicillium sp.	201	7,5	3,7

n.d.: not detectable.
* "strong corrosion": ≥ 1 mm² corroded area after an incubation of 28 days.

Table 5. Corrosion promoting species within single genera
Tabelle 5. Korrosionsfördernde Arten innerhalb einzelner Genera

Genus	total number of isolates	total number of species	number of corrosive species
Micrococcus sp.	5	2	2
Enterococcus sp.	7	2	2
Escherichia sp.	1	1	1
Staphylococcus sp.	16	8	8
Citrobacter sp.	1	1	1
Bacillus sp.	35	27	5
Aerococcus sp.	10	1	1
Penicillium sp.	3	3	2
Aspergillus sp.	7	7	1
Coryneform bacteria	27	26	3

4 Discussion

It can be concluded, that, with a few exceptions, there exists a microflora at corrosion damages in aircraft and that some microorganisms of that flora occur repeatedly. In this group microorganisms like Enterococcus and Staphylococcus occur, which show significant corrosive action during laboratory experiments (applying optimal conditions for microbial growth).

How far microorganisms promote corrosion in aeroplanes in practice is hardly to prove. Simulation is very difficult, since environmental conditions are varying strongly in aircraft.

Our results hint at a possible involvement of microorganisms in corrosion failures in aircraft, since microbial growth cannot be excluded under practical conditions. However, before the importance of microorganisms in general aircraft corrosion can be estimated definitely, more investigations concerning the microflora of corroded sites as well as the underlying corrosion promoting mechanisms have to be conducted.

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