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Aluminum Corrosion Processes in Microbial Cultures

Gordon C. Blanchard

and

Charles R. Goucher

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MELPAR, INC.
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The corrosion of metals by microorganisms has been extensively studied since 1934, when von Wolzogen Kühr and van der Vlugt (1) postulated that the anaerobic sulfate-reducing bacterium, Desulfovibrio desulfuricans, corrodes iron; its metabolism consumes hydrogen produced at cathodic sites for reducing sulfate to sulfide. The role of hydrogenase activity and sulfide production in the corrosion process has subsequently been studied with these organisms (2-7) as well as with other anaerobic bacteria, i.e., Clostridium nigrificans (3,4).

D. desulfuricans has been isolated occasionally from aircraft wing tanks (8) and fuel separators (9) and has been reported by Hedrick et al (10) and Iverson (11) to cause pitting corrosion of aluminum alloys. Hedricks experiments show that aluminum alloy corrosion by D. desulfuricans is more extensive under aerated than static conditions. Corrosion under these conditions was probably not associated with growth (12). Iverson grew D. desulfuricans in stationary tanks in the presence of a mixed culture of fuel isolates. Under stationary conditions the medium was probably anaerobic because the actively growing aerobes in the mixed culture would evolve CO_2 and produce anaerobic conditions in the bottom of the tanks (13,14).

Both autotrophic and heterotrophic bacteria have been demonstrated to cause corrosion in aerobic systems. The autotrophic microorganisms include the sulfur and sulfide oxidizers, Thiobacillus thiooxidans and T. thioparus (15), and the iron oxidizers Gallionella, Ferrobacillus ferrooxidans, and Thiobacillus ferrooxidans (16,17). Heterotrophic species include representatives of the iron

bacteria genera, Siderocapsa, Spheerotilus, Clonothrix, Leptothrix, and Crenothrix (18). Although these aerobic microorganisms are potentially capable of causing aluminum alloy corrosion they apparently do not utilize hydrocarbons or corrode aircraft wing tanks since they have not been isolated from any fuel system water bottom samples (9,19).

The most predominant microorganisms isolated from bulk storage tanks and aircraft fuel tanks were bacteria in the genus Pseudomonas and fungi in the genus Hormodendrum (9,19). These and other microorganisms have been associated with the production of operational difficulties in aircraft fuel systems because they grow in this aerobic environment and oxidize the hydrocarbons in jet fuel to sludges, emulsions and corrosive compounds (20-22). The hydrocarbon-oxidizing microorganisms grow in the aqueous phase of fuel-water systems and synthesize organic materials which are incorporated into cellular materials or accumulate in each phase of the medium (hydrocarbon, interphase, or aqueous). Changes in the composition of the aqueous phase during microbial growth is of utmost importance since corrosion is observed in this phase.

The objective of the work reported herein is to describe some of the mechanisms by which fuel-oxidizing pseudomonad cultures corrode aluminum alloys. The role of nitrate and nitrite ions in the inhibition of aluminum alloy corrosion by microorganisms and by inorganic salt solutions is reported.

Experimental Materials and Procedures

The media used in our studies were modifications of Bushnell and Haas (23) (BH medium); the composition is described in Figure 1. Salt solutions were sterilized by autoclaving. Jet fuel was sterilized by Millipore filtration. Reagent grade chemicals were used in all the studies. The JP-4 jet fuels were supplied free of additives by the Air Force POL Retail Distribution Station, Searsport, Maine.

Both mixed and pure cultures were investigated. Mixed inocula, except where indicated, contained pure strains of jet fuel isolates 96 and 101 tentatively identified as Pseudomonas aeruginosa. The inocula for the corrosion experiments were prepared from 48-hour cultures grown on BH medium. Cells were harvested by centrifugation, suspended in distilled water, and washed three times by centrifugation.

All stock cultures and experimental runs were incubated in 100 ml of medium in 250 ml Erlenmeyer flasks at 28°C on a gyratory shaker (New Brunswick) operating at 100 strokes per minute.

Two types of 1/16 inch aluminum sheet, alloys 7075-T-6 and 2024-T-3 were cut into 4- by 1/4 inch coupons. The coupons were coded at one end with a diamond point, cleaned, and weighed before use. The coupons were cleaned by washing with acetone, wiping with lintless towels, and immersing in 50% HNO_3 for 1 minute. The coupons were then rinsed with flowing tap water, rinsed with distilled water, dipped in acetone, blotted dry on a lintless towel, and stored in a desiccator until weighed.

After weighing, one 2024 coupon and one 7075 coupon were bound together at the upper end by two 1/8 inch strips of tygon tubing. Two pieces of applicator sticks were placed between the two alloys and between the two pieces of tygon tubing to prevent the alloys from touching during incubation. The alloys were prevented from rolling during incubation by spreading the alloys apart to the shape of a wishbone at the upper end just below the tygon strips. The coupons were sterilized by dipping first in methanol and then in acetone. After drying in air the coupons were aseptically placed in the BH medium and incubated at 28°C on the gyratory shaker.

Robertson's procedure (24) was used for cleaning the alloy coupons after test; coupons were treated for 10 minutes at 70°C to 80°C in the cleaning solution (20 g $K_2Cr_2O_7$; 28 ml 85% H_3PO_4, sp. gr. 1.7; and distilled water to 1000 ml) rinsed, dried, and reweighed as described above. Weight loss was calculated. The hydroxyl form of Dowex-1, an anion exchange resin, and the hydrogen form of Dowex-50, a cation exchange resin, were used for determining the ionic characteristics of the corrosion compounds produced by the microorganisms. Sephadex G-25 gel columns were used to separate corrosive compounds on the basis of molecular weight. This gel completely excludes compounds with molecular weights greater than 5000. The excluded compounds occur in the first few fractions.

Dowex-1 and Dowex-50 ion exchange resins* and Sephadex gel G-25 were prepared for use by recommended procedures (25,26).

Experimental Results and Discussion

Blanchard and Goucher (27) have reported that the time required for microbes to make a noncorrosive medium corrosive depends on the nature and concentration of ions in the medium; nitrate is an especially effective corrosion inhibitor. Corrosion in the previous study was determined by visual observation. In the present study the ability of nitrate to inhibit corrosion by young cultures of microorganisms has been confirmed by weight loss measurements. The medium used in this study was more corrosive than the previous medium (27), probably because it contained only one-tenth the phosphate concentration previously used. Mixed cultures of P. aeruginosa (strains 96 and 101) were used. Coupons were incubated for 30 days. The results of this experiment are shown in Figure 1.

The data points in Figure 1 are means of triplicate determinations. The results show that alloy 7075 is more resistant to corrosion by the growth medium and by microorganisms than is alloy 2024. Nitrate inhibited the corrosion of alloy 2024, but had little or no effect on the corrosion of

*Dowex resins were purchased from Calbiochem, 4921 Cordell Ave., Bethesda, Maryland. Sephadex purchased from Pharmacia Fine Chemicals, Inc., 50 Fifth Ave., New York 17, New York.

alloy 7075. Corrosion of alloy 2024 occurred in the control medium (no microorganisms) at 0, 0.2, and 0.4 mmolar KNO_3 but not at 0.6 mmolar KNO_3 or above. In the inoculated medium, corrosion of alloy 2024 occurred between 0 and 0.8 mmolar KNO_3 but not at 12 mmolar KNO_3. The presence of microorganisms in the growth medium caused greater corrosion of both alloys. Nitrate at 12 mmoles/liter inhibited this corrosion. These results agree with the previous corrosion studies where corrosion was assessed by visual examination.

A statistical analysis was performed on the weight loss of alloy 2024 from data obtained between 0 and 0.8 mmolar KNO_3. Corrosion of alloy 2024 incubated in inoculated and sterile low nitrate media (KNO_3 between 0 and 0.8 mmolar) was evaluated for statistical significance using a standard "t-test" on the means. The test was significant at 0.01; this result implies conservatively that inoculated media corrode more than sterile media 99% of the time.

The data in Figure 1 show that high concentrations of nitrate (12 mmolar KNO_3) protected both alloys from corrosion. This agrees with our previous data (27) and with Uhlig and Gilman (28), who found that $NaNO_3$ completely inhibited pitting of 18-8 stainless steel in 1% to 15% $FeCl_3$ solutions. Corrosion in the latter system occurred in a very acidic environment (pH 1.0 to 2.0), whereas corrosion by microorganisms occurred in a neutral (pH 7.0 to 8.0) environment. Thus, the ability of nitrate to passivate metals appears to be a generalized phenomenon independent of the pH of the

corrosive medium. The mechanism by which the passivity is induced is not known.

Previously, visual observation indicated that microorganisms caused corrosion in the high nitrate medium (12 mmolar KNO_3) after 3-month incubations (27). This experiment was repeated using weight loss measurements for evaluating corrosion. The data in Figure 2 show that nitrate concentrations between 0.2 and 0.8 mmolar protect the alloys from corrosion in the absence of microorganisms, but fail to protect the alloy in the presence of microorganisms. The corrosion results between 0.2 and 0.8 mmolar KNO_3 were analyzed statistically by the "t-test" on the means. The test was highly significant at 0.001; this result implies conservatively that, after a 90-day incubation, inoculated media corrode more than sterile media 99.9% of the time.

At the high nitrate concentration (12 mmolar), however, no differences in weight losses were observed between inoculated and control media. The lack of corrosion by microorganisms in the high nitrate media is in disagreement with our previous data, where microbes caused corrosion in 90 days (27). These differences may be due to the use of two different mixed cultures or to the different methods of assessing corrosion. The present experiments used a mixed culture containing two pseudomonads (strains 96 and 101). The previous experiments used a mixed culture containing

strain 101, Cladosporium resinae, Aspergillus niger, and Desulfovibrio desulfuricans; D. desulfuricans did not grow in the aerobic system and the fungi grew poorly. The present method of measuring corrosion was by weight loss, whereas the previous study assessed corrosion by visual observations of pitting and/or blackening.

Effect of Nitrate and Nitrite on $CaCl_2$ Corrosion of Aluminum Alloys

Previous studies have shown that microorganisms reduce the nitrate concentration of the medium from 12 mmolar to 0.1 mmolar during the first 19 days of incubation; nitrite concentration increased from 0 to 2 mmolar during this time (29). Nitrite has been shown to inhibit stainless steel corrosion by $FeCl_3$ (30). It was postulated that the lack of corrosion on high nitrate medium (12 mmolar KNO_3) during the 90-day incubation was due either to nitrite inhibition of corrosion or to the production of products which inhibit corrosion.

Nitrate and nitrite were tested as inhibitors of $CaCl_2$ corrosion. The alloys were immersed in 100-ml mixtures of $CaCl_2$ and KNO_3, or $CaCl_2$ and KNO_2 for 4 days at 28°C on a gyratory shaker. The weight losses observed are shown in Figures 3 and 4.

Three different concentrations of $CaCl_2$ were studied at four different ratios of chloride ion to KNO_3 or chloride ion to KNO_2 -- 0.2, 2.0, 20, and ∞ ratios. The data in

Figure 3 show that nitrate causes a variety of responses in a corrosive system. At a high molar ratio (Cl/KNO_3 = 20) nitrate stimulates corrosion, whereas at lower molar ratios (2 and 0.2) nitrate inhibits $CaCl_2$ corrosion of the alloys. These effects were not observed with KNO_2 (Figure 4). In fact, no inhibition was observed by nitrite even at a molar ratio of 0.2. This indicates that nitrite is not a corrosion inhibitor for these aluminum alloys under the conditions of these tests.

At a Cl/KNO_2 molar ratio of 2, nitrite stimulated corrosion of alloy 7075. This effect was observed initially and the experiment was repeated with 10 coupons in 5 separate flasks. The weight loss observed was 82.2 $\pm$ 27 mg/3.8 cm^2 (means $\pm$ maximum deviation). The reason for this stimulation in corrosion is not known. Thus, KNO_2 appears to be an effective inhibitor only against steel corrosion. Although both nitrate and nitrite are good inhibitors of stainless steel corrosion, it is not possible from the data presented herein or from the literature data cited to determine which is the more effective inhibitor.

Characterization of Corrosive and Corrosion Inhibiting Components in the Aqueous Phase of 90-Day Cultures

The purpose of this phase was to determine if corrosive fractions exist in the noncorrosive medium (12 mmolar KNO_3) after a 90-day growth of strain 101. Six liters of the aqueous phase produced by strain 101 after the 90-day growth on BH

medium (12 mmolar KNO_3) were fractionated. (See Figure 5.) The concentrated filtrate was fractionated by ion exchange chromatography and by Sephadex chromatography. In the ion exchange fractionations, 75 ml of concentrate were placed on the column and eluted with flowing distilled water. The total eluate collected was about 250 ml.

Three ml of concentrate were placed on the Sephadex column and eluted with water. The volume collected in each of the colored fractions is shown in Table 2. The weight losses observed with the concentrate, Dowex-1 eluate, Dowex-50 eluate, and Sephadex fractions are shown in Table 2.

The objective of these experiments was to separate and characterize the corrosive compounds from the growth media, thus no attempt was made to obtain a weight loss balance in the fractionation. The original 90-day sample used in the fractionation study was noncorrosive. The concentrate caused appreciable corrosion of both alloys; alloy 7075 showed about twice as much weight loss as alloy 2024. Both anionic and cationic compounds are produced by the microorganisms, and both fractions caused aluminum alloy corrosion.

The eluate from the Dowex-1, which contains cations, OH ions, and neutral compounds, caused much more corrosion of alloy 7075 than alloy 2024 -- 61.3 mg compared with 5.7 mg by weight loss. The eluate from the Dowex-50 column, which contains anions, H^+ ions, and neutral compounds, like the unfractionated concentrate caused about twice as much weight

loss from alloy 7075 as from alloy 2024. Very little weight loss was observed with the distilled water and sterile medium controls, which were processed in the same manner as the inoculated samples. Neutral compounds, if they existed, did not cause corrosion of either alloy. Very little corrosion was caused by the Sephadex fractions.

The Sephadex experiment was repeated using the previously described conditions except for the following changes: a large column (5 by 65 cm) was used; 300 ml of clear brown filtrate were applied to the column; and 80-ml fractions were collected until it appeared that all materials were removed from the column. The results obtained show that the first seven fractions contained the corrosive components of the medium (Figure 6). No corrosion was observed in any of the fractions containing the pigmented materials. These data indicate that old cultures could cause corrosion by producing large molecular weight components (molecular weight 5000 and above) in the medium. The composition of these large molecular weight materials is presently unknown. Experiments are now in progress to determine (1) the composition of these materials, (2) the presence of these materials in young cultures, and (3) whether any of the other fractions (8 through 24) inhibit corrosion.

SUMMARY AND CONCLUSIONS

The growth of microorganisms for 30 days and 90 days in media containing between 0 and 0.8 mmolar KNO_3 as the only nitrogen source for growth caused significantly greater corrosion of alloys 7075 and 2024 than was observed in controls. Microorganisms failed to cause corrosion, even after 90 days in BH medium (12 mmolar KNO_3), although the nitrate concentration after 20 days had decreased to a noninhibitory concentration (0.1 mmolar) for corrosion. All nitrate concentrations inhibited corrosion by the media alone.

Microorganisms reduced some of the nitrate to nitrite during the first 20 days incubation on 12 mmolar KNO_3 media; nitrite increased from 0 at zero time to 2 mmolar after 20 days. However, chemical corrosion tests showed that nitrite was not an effective inhibitor of aluminum alloy corrosion. Nitrite at 10 times the concentration of $CaCl_2$ caused no inhibition of $CaCl_2$ corrosion of alloys 7075 and 2024. Nitrate, however, was a very effective inhibitor of $CaCl_2$ corrosion in this concentration range.

The 90-day high nitrate growth media were found to contair corrosive anionic and cationic components upon fractionation by ion exchange chromatography. These corrosive components were demonstrated by Sephadex chromatography to be large molecules with molecular weights greater than 5000. The lack of corrosion by microorganisms after the 90-day incubation in BH medium (12 mmolar KNO_3) was believed to be due to the formation of corrosion-inhibiting products.

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TABLE 1

EFFECT OF pH ON CORROSION OF 2024 AND 7075 ALLOYS BY WATER BOTTOM

Run No.	Weight Loss (mg/3.8 cm^2)		Incubation Time (days)	Initial pH*
	Alloy 2024	Alloy 7075		
62	2.28	4.88	30	4.9
66	1.91	3.92	30	4.9
67	1.66	4.31	30	4.9
68	2.81	6.36	30	4.9
69	1.96	5.36	30	4.9
72	3.93	4.40	30	7.0
73	**	3.75	30	7.0
74	4.01	5.14	30	7.0
61	19.59	22.88	90	4.9
63	15.53	16.95	90	4.9
64	19.26	20.29	90	4.9
65	16.72	20.92	90	4.9
70	16.86	16.36	90	4.9
71	10.82	12.80	90	7.0
75	8.36	10.01	90	7.0

* Dilute KOH used for adjustment of pH.

** Flask broken during incubation.

TABLE 2

CORROSIVITY OF FRACTIONS FROM 90-DAY CULTURE OF 101*

	Milli-liters	Weight Loss (mg/3.8 cm^2)	
		Alloy 7075	Alloy 2024
Original Culture	--	0.4	0.5
Concentrate	--	62.9	33.9
Sterile Medium Concentrate Control	--	0.1	0.1
Dowex-1-Distilled Water	--	3.5	0.2
Dowex-1-Sterile Medium Conc.	--	3.4	2.9
Dowex-1-Concentrate	--	61.3	5.7
Dowex-50-Distilled Water	--	0.1	0.1
Dowex-50-Sterile Medium Conc.	--	2.5	2.2
Dowex-50-Concentrate	--	35.7	17.6
Dowex-1-Concentrate-Dowex-50	--	0.1	0.1
Sephadex Fractions			
Void Volume	48	0.6	0.5
Light Brown	24	0.4	0.3
Yellow	38	0.5	0.1
Pale Amber	6	0.8	0.352
Brown	5	0	0
Deep Brown	3	0.1	0.4
Purple	5	0.3	0.4
Yellow-Pink	4	0.3	0.3
Yellow	13	1.5	0.3
Water	10	0.4	0.4

* Incubation was for 8 days at room temperature under stationary conditions.

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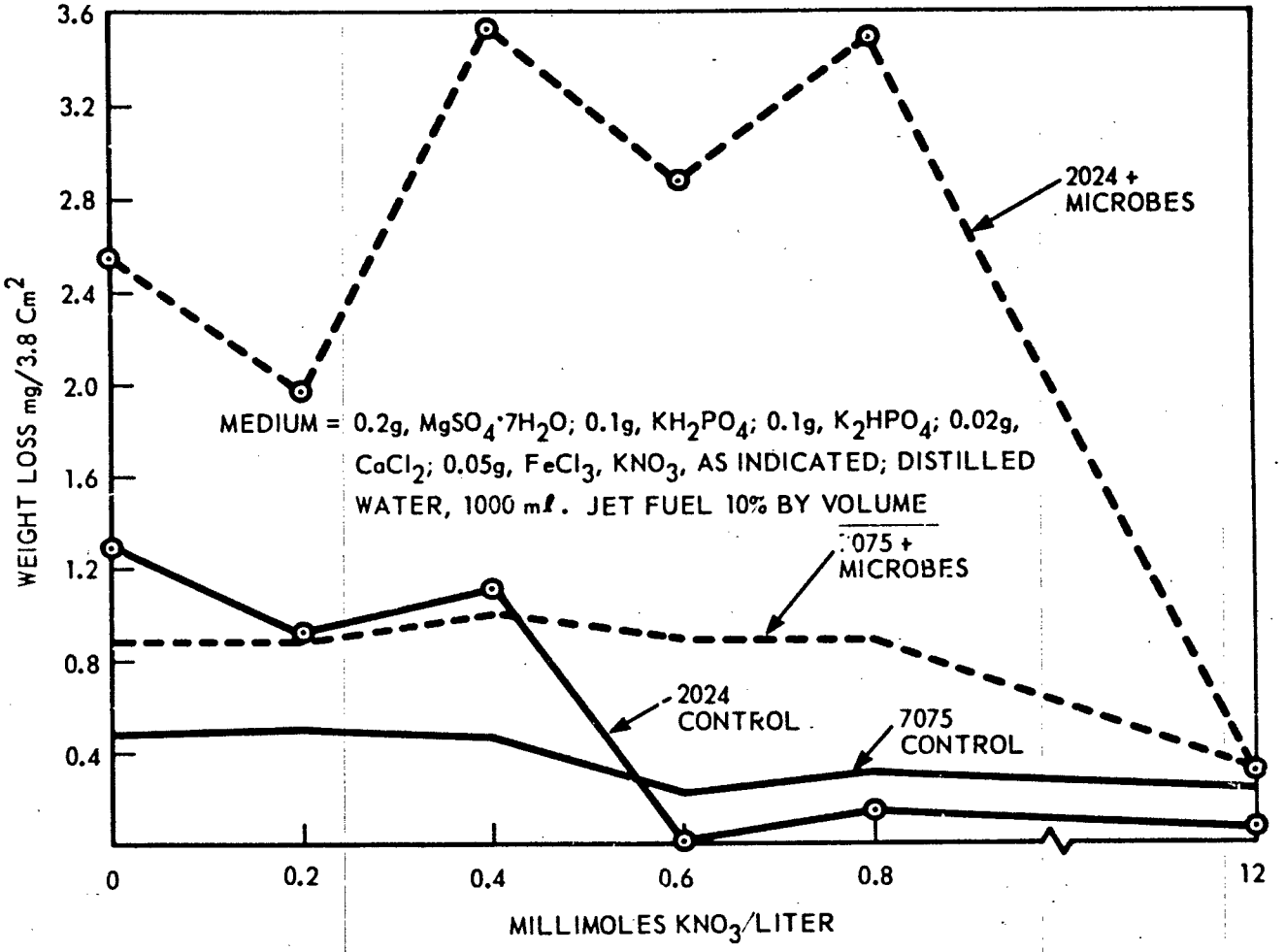

3.6
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
WEIGHT LOSS mg/3.8 C_m^2
2024 + MICROBES
MEDIUM = 0.2g, $MgSO_4 \cdot 7H_2O$; 0.1g, KH_2PO_4; 0.1g, K_2HPO_4; 0.02g, $CaCl_2$; 0.05g, $FeCl_3$, KNO_3, AS INDICATED; DISTILLED WATER, 1000 mℓ. JET FUEL 10% BY VOLUME
7075 + MICROBES
2024 CONTROL
7075 CONTROL
0
0.2
0.4
0.6
0.8
12
MILLIMOLES KNO_3/LITER

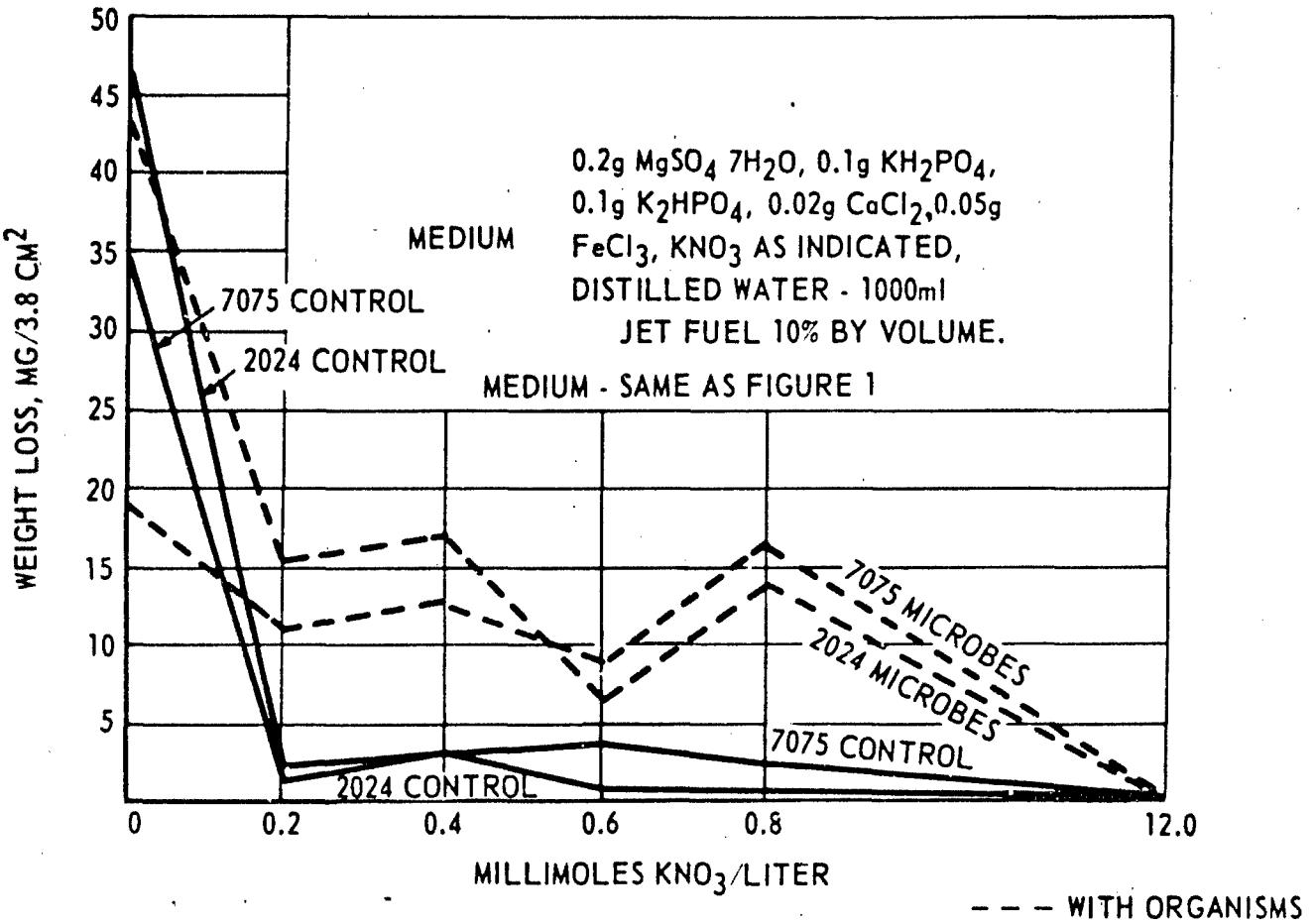
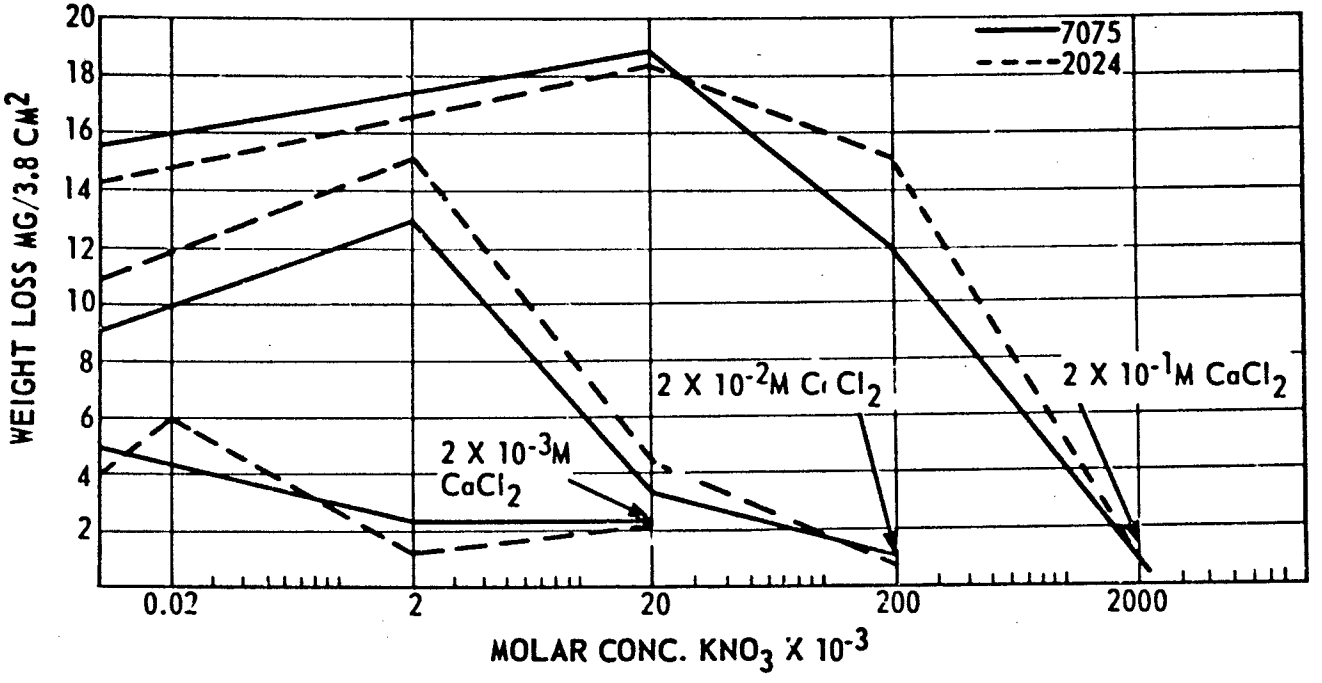


Figure 2. Effect of Nitrate in Microbial Corrosion (90-day Incubation)


7075
2024
WEIGHT LOSS MG/3.8 CM2
20
18
16
14
12
10
8
6
4
2
2 X 10-2M CaCl2
2 X 10-1M CaCl2
2 X 10-3M CaCl2
0.02
2
20
200
2000
MOLAR CONC. KNO3 X 10-3

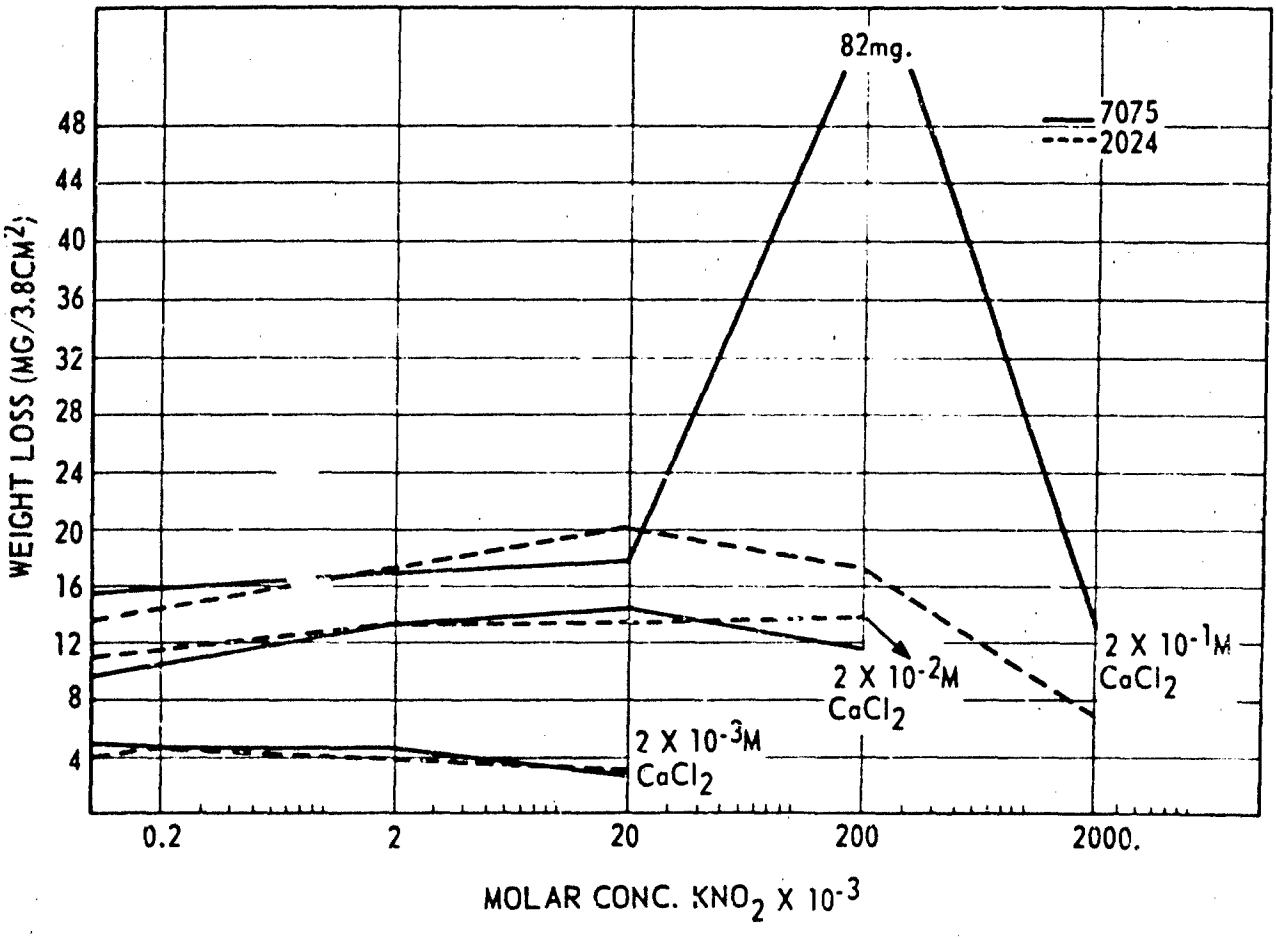


Figure 4. Effect of Nitrite on $CaCl_2$ Corrosion

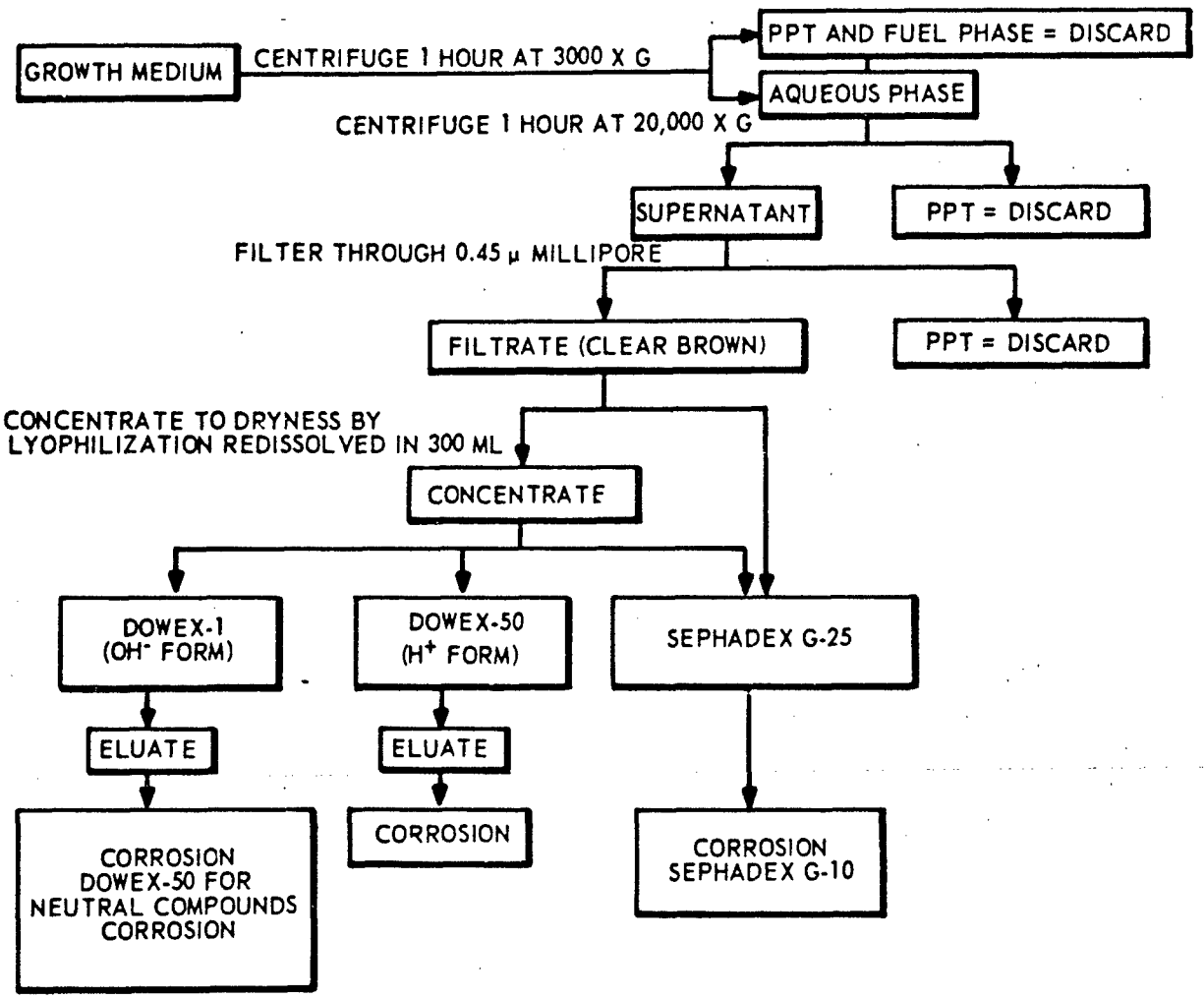


Figure 5. Extraction, Concentration, and Fractionation Scheme

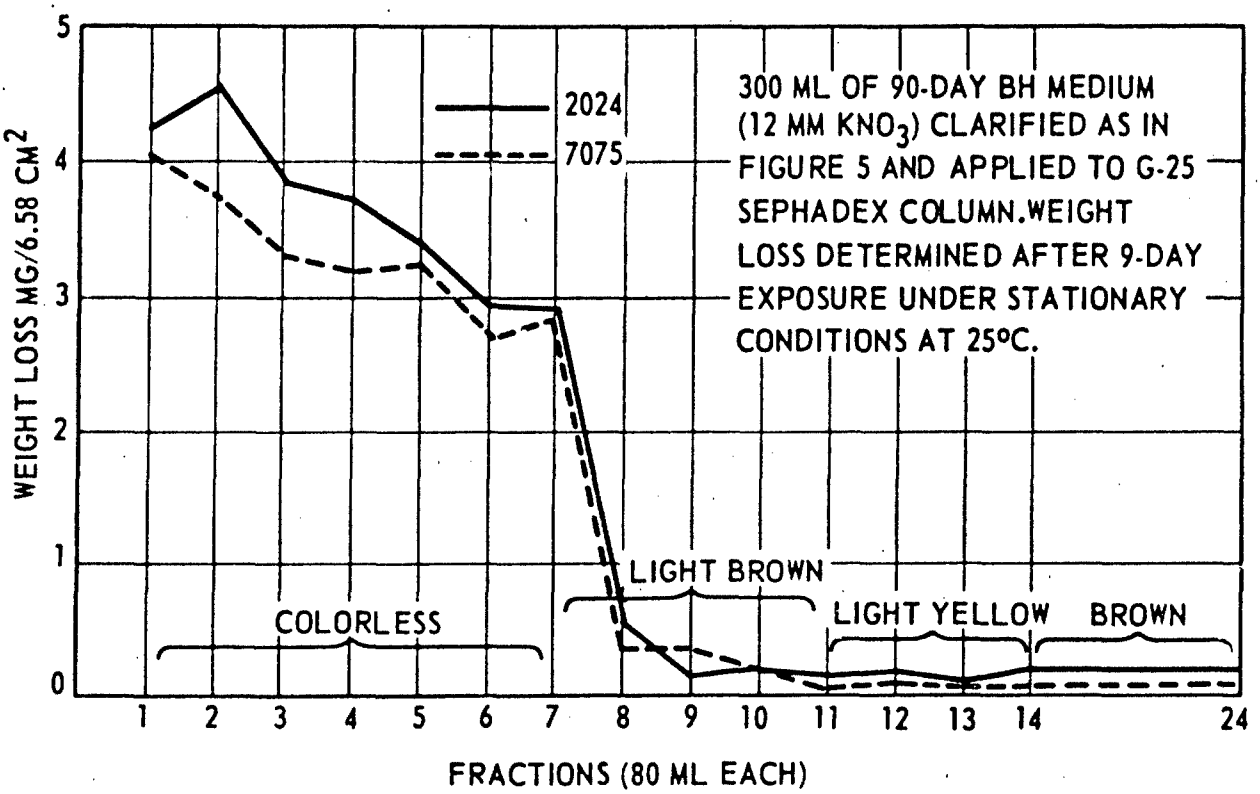


Figure 6. Corrosion by Sephadex Fractions

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13. ABSTRACT

This paper presents data to support the concept that microorganisms can produce corrosion of 2024 and 7075 aluminum alloys by two mechanisms: (1) removal of a corrosion inhibitor, nitrate, from the growth medium and (2) production of metabolic products in the medium.

Results are presented to show that nitrate is a good inhibitor of $CaCl_2$ corrosion of aluminum alloys and that nitrite, one of the metabolic products formed from nitrate by the microorganisms, is not an inhibitor of $CaCl_2$ corrosion of aluminum alloys.

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14 KEY WORDS	LINK A		LINK B		LINK C	
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