ENHANCED DEGRADATION OF ALUMINUM METAL IN THE PRESENCE OF SELECTED MICROORGANISMS

By

John Marion Tennyson

A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in the Department of Microbiology

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JOHN MARION TENNYSON

APPROVED:

Dr. Robert G. Tischer
Professor of Microbiology
(Head of Department)

Dr. Lewis R. Brown
Professor of Microbiology
(Major Professor)

Dr. John C. Mickelson
Professor of Microbiology

Dr. Robert P. Wilson
Associate Professor of
Biochemistry
(Minor Professor)

Dr. Lyell C. Behr
Dean of College of Arts and
Sciences

Dr. J. Chester McKee, Jr.
Dean of the Graduate School

August, 1972

ACKNOWLEDGEMENTS

I would like to express my appreciation to the individuals who made this work a reality. I am indebted to Dr. Lewis R. Brown for his guidance and assistance and to Dr. Robert G. Tischer, Dr. John C. Mickelson and Dr. Robert P. Wilson for their constructive criticisms concerning this thesis.

I am particularly grateful and indebted to the members of the staff of the Agronomy Department and Mississippi Chemical Regulatory Laboratory for their patience and for the use of their equipment.

In addition, I would like to thank Dr. Walter J. Drapala, Dr. Eugene B. Grimley, III, Dr. Harold A. Koelling, and David L. Horton, Jr. for their assistance.

The author is grateful to the National Aeronautics and Space Administration for their financial assistance through grant No. NGR-25-001-32, "Special Support of Saturn-Apollo."

J.M.T.

State College, Mississippi

August, 1972

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INTRODUCTION

From the beginning of time, man has been involved in a continuous endeavor to overcome metallic corrosion. Within this century, for example, the human race has striven to prevent both biological and non-biological disintegration of iron and iron alloy water pipes.

More recently the problems created by the degradation of aircraft fuel reservoirs instigated a tremendous venture by the armed forces to unearth routes and inhibitors of metabolic avenues of destruction especially in aluminum, steel, and their alloys. This research was of the utmost prominence since the invulnerability of our country was directly or indirectly dependent upon our airplane and missile capabilities.

Some of this experimental evidence, with respect to the breakdown of metals, has been released to the general public and other governmental agencies. The National Aeronautics and Space Administration (NASA), for instance, has been interested in this type of material.

The initial journey of mankind to the moon caused a considerable amount of concern among the entire population of the United States. However, now that such trips are commonplace, we have assumed that nothing will go wrong.

At present, NASA has proposed the "Skylab" project and in this planned undertaking, the space vehicle will have people in a closed system for a relatively long period. During this span, it is highly

probable that microorganisms from the skin and other sources would grow under favorable conditions and decompose the metallic interior of the ship.

The role of microorganisms in the deterioration process is unknown. This work, therefore, was an attempt to gain insight into possible mechanisms of metallic corrosion and the objectives of this operation were to determine the consequence of oxygen, pressure, humidity, and microbial growth upon aluminum disintegration. An analysis of several coatings as to their effectiveness as preventers of breakdown was, also, undertaken.

The fulfillment of these goals may bring about prolonged space flights.

REVIEW OF LITERATURE

Corrosion of metals is of two major classes--biological and non-biological. The biodegradation of aluminum probably received the most attention when it was found to be responsible for malfunctions in aircraft. The federal government has spent many man-hours and much money in an attempt to understand this phenomenon.

London, Finefrock, and Killian (1964) tried to determine the course of corrosion with respect to airplane fuel tanks. Their experiments indicated that anaerobic organisms along with fungi were associated with the degradation of aluminum. Although they isolated some iron depositors, the microorganisms did not appear to be true iron organisms, but rather citrate-utilizing organisms. In addition, they did not recover any pigment producing pseudomonads, iron-oxidizers, or sulfate-reducers.

In an attempt to learn where and how deterioration occurred, Wolzogen Kuhr and Vlugt (1953) found that mechanical stresses provided the sight for cathodic and anodic processes on the metallic surface.

Because organisms have been associated with corrosion, four mechanisms were proposed to explain this relationship.

1. Hendrick, Reynolds, and Crum (1966) suggested that microorganisms established microcenters which acted as oxygen concentration cells.

2. Lichtenstein (1968) indicated that the breakdown of metal occurred as a result of indirect action. The oxygen cells produced

produced corrosive compounds such as carbon dioxide, hydrogen sulfide, ammonia, and organic and inorganic acids.

3. Inverson (1968) concluded that cathodic depolarization was responsible for corrosion.
4. Blanchard and Goucher (1964) stated that microbial metabolism produced substances which reacted with the protective oxide films or gas absorbed to the aluminum surface and stimulated the degradation process.

Inverson (1968) explained the oxygen cell theory by stating that when microbiological growth occurred on the aluminum, the concentration of dissolved oxygen was greater at the edge of the mass. Therefore, electrons combined with water and oxygen to produce hydroxyl ions at that point or cathodic area. The metal disappeared at the anodic area of low oxygen concentration under the organic matter and in this case, the deterioration produced aluminum hydroxide and other oxides.

He also suggested that the various types of acids produced by organisms were responsible for the greatest degree of disintegration. Staffeldt and Calderson (1967) had shown earlier that a number of organic acids occurring in the glycolysis or the Krebs cycle corroded aluminum. Wilson (1945) theorized that carbon dioxide dissolved in water had the carbonic acid character and played a role in corrosion.

Zajil (1969) proposed that a series of electrochemical reactions were responsible for aluminum breakdown and if so, the reactions were as follows:

Anodic Reaction:

$$\tfrac{1}{2}O_2 + 2H_2O + Al \rightleftarrows Al(OH)_3 + H^+ + e-$$

$$4OH^- + Al \rightleftarrows AlO_2^- + 2H_2O + 3e-$$

$$3OH^- + Al \rightleftarrows Al(OH)_3 + 3e-$$

$$Al \rightleftarrows Al^{+3} + 3e-$$

$$HSiO_3^- + 2Al + 2H_2O \rightleftarrows Al_2SiO_5 + 5H^+ + 6e-$$

Cathodic Reaction:

$$Al(OH)_3 \rightleftarrows 3/4O_2 + Al^{+3} + 1\tfrac{1}{2} H_2O + 3e-$$

$$AlO_2^- \rightleftarrows O_2 + Al^{+3} + 4\ e-$$

$$Al(OH)_3 + 3Cl^- \rightleftarrows 3HOCl + Al^{+3} + 6\ e-$$

It was further stated that free hydrogen-oxidizing microorganisms containing hyrdogenase enhanced the aluminum degradation by increasing cathodic depolarization. Where trivalent metals were involved, metallic hydroxide, cathodic hydrogen, and electrons were formed.

$$Al + 2H_2O \rightleftarrows Al(OH)_3 + H^+ + e-$$

The depolarization reaction followed with O_2 serving as a hydrogen acceptor with the production of H_2O and the release of energy.

$$2H^+ + O^= \rightarrow H_2O$$

Through microbial metabolism, in the former reaction, hydrogen was removed shifting the equilibrium to the right thus intensifying the rate of corrosion.

Rogers (1948-1949) indicated that the type of film, which formed on the metallic surface and its presence or absence at the point of contact of the corrosive substance, regulated the extent of corrosion.

Inverson (1968) stated that two classes of protective films were in existence:

(a) applied - consisting of organic, inorganic, or metallic coatings.

(b) natural - which are composed of corrosion products.

The resistance of aluminum to breakdown was due to its aluminum oxide film. Therefore, organisms in contact with its metallic surface influenced film formation. If there was interference with formation of the oxide film, pitting occurred. In addition, he suggested that if acids or alkalis were produced, severe disintegration was likely.

In an effort to retard degeneration of metal, Zajil (1969) performed a series of experiments in which he found that the addition of nitrates or phosphates often inhibited aluminum corrosion. However, Inverson (1968) had indicated that nitrates were reduced to nitrites, which were ineffective as inhibitors by hydrocarbon-oxidizing bacteria.

Two rather interesting phenomena have been discovered in regard to aluminum breakdown. Hendrick, Crum, and Reynolds (1968) found a direct correlation between magnesium and zinc content in a aluminum alloy and metal loss due to biodegradation. Rogers (1943) observed that experimental results on metallic decomposition when performed in wax coated glassware were inconsistent and non-reproducible.

In an attempt to develop a simple method for the determination of aluminum, Ramakrishna, West, and Robinson (1967) concluded that the nitrous-oxide-acetylene flame had two big advantages over the air-acetylene flame. These advantages were a lower burning velocity and the decreased danger of flashback. They found that the two absorption lines 3691Å and 3092Å had approximately the same sensitivity. They also studied the possibility of interference with a wide array of ions

and substances having a concentration of 200 mg/ml. The effect of acids was tested by the addition of 5 ml of the appropriate concentrated acid before dilution to 100 ml with distilled H_2O.

None of the solutions decreased aluminum absorption, but two of the mixtures, titanium and acetic acid, exaggerated the actual aluminum content by 25 percent and 10 percent, respectively.

MATERIALS AND METHODS

Metals

The metallic samples used in this work were provided by Mr. Al Krupnick of Marshall Space Flight Center through Dr. Harold A. Koelling of the Materials Engineering Department of Mississippi State University.

In the majority of the experiments aluminum 1100, which contained a minimum specified aluminum content of 99 percent, was examined.

The aluminum 1100 with different color coatings was also used and was designated as follows:

#26314	Medium Gray
#36231	Gray
#37886	Light Beige
#37855	Dark Beige
#25102	Blue
#35231	Blue
#30226	Yellow-Green
#34552	Green
#33538	Yellow
#21105	Red
#37038	Black
-----	Teflon

In addition an 1100 series aluminum foil designated as an alodined 1200 was examined.

Cleaning Procedure

The metal coupons were 1" x 3", the strips were about .003" x 3/8" x 1 1/2" in size and the coated samples were 1" discs. There was one exception. The teflon coat was on a disc; however, it was affixed to the top of a 1" cylinder. Each sample was cleaned by dropping several strips into beakers containing acetone and washing them by means of an ultrasonic cleaner for a period of 10 minutes. Afterwards, the process was repeated except that the acetone was replaced with n-hexane. The solvents were permitted to cool for 20 minutes after each 10 minute run.

Microorganisms

Naturally occurring mixed cultures used in this work were human feces, fingerprints, sputum, and urine.

Pure cultures which were originally isolated from feces and bathwater served for inocula in some experiments and the cultures were Alcaligenes (4), Pseudomonas, Proteus, Bacillus, Aerobacter, Achromobacter (8), Rhizopus, Aspergillus (4), Trichoderm, Mycobacterium, Scopulariopsis, and several unknown ones.

Mr. William Leonard Gibson of the Microbiology Department at Mississippi State University and Dr. Jerome L. Mahloch of the Civil Engineering Department at Mississippi State University had previously identified the pure cultures. In addition, Mr. Gibson provided all the cultures for this work.

Culture Media

The media employed as growth substrates for the cultures under investigation were:

1. Armour Star Beef Bouillon Cubes (Distributed by Armour & Co.). The ingredients listed were Hydrolyzed Plant Proteins, Salt, Sugar, Rendered Beef Fat, Beef Extract, and Flavoring. The material was employed as full strength (4 cubes dissolved in 474 ml of distilled water).
2. Tang Instant Breakfast Drink (General Foods, Corp.). The ingredients listed were Sugar, Citric Acid, Gum Arabic, Natural Orange Flavor, Cellulose gum, Calcium Phosphate, Sodium Citrate, Vitamin C, Hydrogenated Vegetable Oil, Artificial Flavors, Vitamin A, U. S. Certified Color, BHA as a preservative. In general use, 134.8 grams of Tang was dissolved in 1 liter of water.

   Tang and bouillon were examined because they had been chosen as nutrients for the astronauts during the Skylab's mission.
3. Mineral Salts Medium*. This medium was employed by Brown (1958) and consisted of:

NH_4NO_3	4.4 gm
$MgSO_4 \cdot 7H_2O$	2.0 gm
K_2HPO_4	3.8 gm
$FeCl_3 \cdot 6H_2O$	.5 gm
10 liters distilled water	
4.7 ml of 10 percent conc. HCl	

4. In addition, the Difco media employed in phases of this investigation were Nutrient Agar, Nutrient Broth, Plate Count Agar, and Potato Dextrose Agar.

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* It was modified from the original KNO_3 to NH_4NO_3.

5. Micatex, a coating material, was utilized as a substrate for some tests.

Analysis for Aluminum

All analyses for aluminum were conducted in accord with the routine method for determination of aluminum in the "Analytical Methods for Atomic Absorption Spectrophotometry" (1966).

Growth Curve

An unidentified bacterium previously isolated from human feces was used to inoculate two 6 ounce prescription bottles containing nutrient broth. One was capped with a sterile rubber stopper whereas the other had a regular cap. The bottle with the stopper had a vacuum pump connected to decrease the atmosphere to 5 psi. Upon achieving the new pressure, the glassware was disconnected from the pump and placed with the other sample on a rotatory shaker. Both bottles were incubated under shake conditions and at room temperature for 36 hours. During this period samples were withdrawn and standard plate counts were run.

Rhizopus and Scopulariopsis were also examined. Each organism was separately inoculated on the different media listed earlier. Where necessary the pH had been adjusted to 7 and 1.5 percent agar added as a solidifying agent. The only exception being that Scopulariopsis was not tested on the mineral salts medium. The experiment was set up in triplicate. Prior to inoculation, 18 ml of the dissolved agar had been dispersed into the 6 ounce bottles. After sterilization the agar had been allowed to solidify on the flat side of the bottle.

The bottles were placed in an incubator for a period of 24 hours at 30 C. A 1 mm square was marked on the bottle and the organism was inoculated onto the agar surface opposite the square by means of an inoculation needle. Half of the cultures were incubated under ambient pressure; however, those isolates to be incubated at 5 psi were stoppered with sterile serum stoppers and a vacuum was utilized to obtain the correct pressure. Growth was monitored by measuring colony diameter with a ruler periodically throughout a 14 day span. All cultures were incubated at room temperature.

Effect of Oxygen Concentration

The medium used in the study of the pure cultures was composed of Tang Orange Breakfast Drink or bouillon cubes (double strength) at a pH of 7.

The cooled medium was inoculated with the cultures and the inoculated medium was then applied to the metal coupon. The humidity and temperature were ambient. The organisms were grown in an air atmosphere, 50 percent oxygen enriched air, and 75 percent oxygen enriched air. A total of 25 isolates were applied to metal coupons, 5 organisms per plate, inoculated into two separate rows. One line consisted of organisms inoculated into bouillon while the other contained organisms inoculated into Tang. The cultures were incubated for two months at ambient temperatures under static conditions and then were observed for growth and the presence of corrosion with a stereomicroscope (approximately 15X).

The design was triplicated for the 3 different oxygen environments and for the 25 isolates.

The media employed were:

1. Tang - double strength mixed with melted and cooled (45 C) agar in water.

2. Bouillon - double strength mixed with melted and cooled (45 C) agar in water.

The pH of the media was adjusted to 7.0.

The inoculated coupons were washed, dried, and then observed for signs of disintegration.

Effect of Pressure

The cultures were plated on aluminum 1100 on the following types of media:

1. Tang - double strength containing 1.75 percent agar and with an adjusted pH of 7.0.

   The medium was dispensed into test tubes, sterilized, and allowed to cool to 45 C before the addition of organisms. Approximately 0.5 ml of inoculum from the tubes was added to the metals and allowed to solidify.

2. Bouillon - double strength containing 1.75 percent agar and with an adjusted pH of 7.0.

   The medium was treated as above.

The variations in pressure of an atmosphere composed of 50 percent oxygen/50 percent nitrogen were as follows:

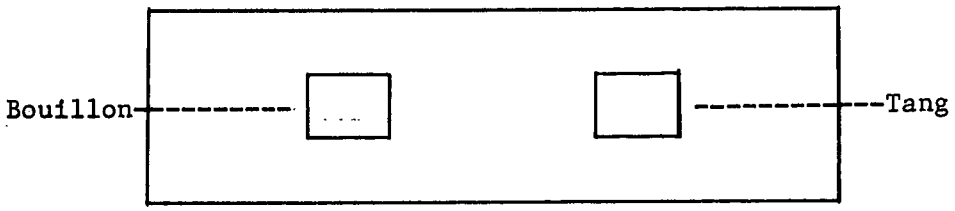
1. approximately 14.7 psi

2. 1 psi

3. 5 psi

These differences were obtained with the aid of 3 pressure cookers.

The metal plates were inoculated in the following manner:



Only one organism was inoculated in the two types of medium per coupon and six controls were used for each variation in conditions.

Effect of Humidity

This experiment was designed as the one in pressure except that the atmosphere was 70 percent oxygen and 30 percent air and the humidity level was varied.

The 10, 50, and 90 percent moisture contents were analyzed for their effect upon aluminum corrosion. These variations were produced by incubating the samples in 1 gallon jars containing 100 ml of saturated solutions of the following:

1. H_3PO_4 - 10 percent humidity
2. $Mg(NO_3)_2 \cdot 6H_2O$ - 50 percent humidity
3. $ZnSO_4 \cdot 7H_2O$ - 90 percent humidity

The appropriate humidity was obtained by using the system described by Hougman (1955) and changes in moisture content were recorded by humidostats.

A rubber diaphragm was used to cover the gallon jar and the vessel was flushed with 22 gauge hypodermic needles with 70 percent oxygen from a gas bomb. After flushing, the surface of the diaphragm was coated with paraffin to seal any possible leaks.

Dissolution of Aluminum

The inocula consisted of Rhizopus, Scopulariopsis, and human feces. Prescription bottles containing nutrient agar were used for the pure culture inocula. Each type of organism was used to inoculate five bottles and was permitted to grow under room conditions for a period of two weeks to insure a large number of spores. Afterwards, 100 ml of distilled water was added to each of the bottles and the agar was washed by a gentle rotating movement in order to obtain as many spores as possible.

The fecal inoculum was prepared by placing 1 g of feces into 100 ml and mixing. Then a 1:10 dilution was made.

To each of eighty tubes containing 2.0 ml of sterile Tang, 0.5 ml of inoculum was injected by means of a sterilized automatic syringe.

The 95 percent humidity was obtained by means of the method stated by Lange (1956).

The pressure chambers were evacuated by using a Precision Scientific Vacuum Pump and were gassed to a gauge pressure of 12 units on the vacuum side (approximately 6 psi). The difference between the lowest gauge reading (about 30 units vacuum side) and 12 was determined and the percentage of gas to be added was calculated to yield the following oxygen-nitrogen ratios of 20/80, 50/50, and 70/30.

The gasses[1] used in this investigation were of commercial grade purity.

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[1] Standard Welders Supply Company, Columbus, Mississippi.

Samples were withdrawn at 0, 11, 25, 32, 39, 46, 53 and 59 days. In addition, at zero time, feces, Rhizopus, and Scopulariopsis inocula samples were taken. After the extraction of the test tubes, the humidity control solution was stirred and checked, before resealing the containers with Dow Corning high vacuum grease and modeling clay. Then the chambers were regassed.

The samples were prepared for analysis by the following method:

The aluminum strip was pulled from the solution with a pair of forceps and each side of the metal was rinsed into the tube with 1 ml distilled water. The sample was then washed with tap water, dried, and checked for corrosion. Due to the limitations of samples size and of analytical balances, the strips were not weighed prior to or following the experiment. Afterwards, the metal was dissolved in 2 ml 6M HCl and diluted to yield a reading between 20 and 80 percent on a Perkin-Elmer 290 Atomic Absorption Sepctrophotometer.[2] The dilution scheme was based upon earlier unreported data.

The Tang solution was mixed and poured into a crucible and 0.5 ml of rinse water from the test tube was added to yield a total mixture of 5 ml. Since the control tubes were not inoculated and were 0.5 ml less in volume, their glassware was flushed with 1.0 ml of distilled water.

After evaporation in a drying oven at 110 C, the samples were ashed in a muffle furnace at 600 C. The remains, which were permitted to stay undiluted for 6 hours after the addition of 0.2 ml of 6M HCl,

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[2] Perkin-Elmer Company, Norwalk, Connecticut.

were filtered through glass-paper and the filtrate was collected in a tube. The crucible and the filter were each washed with 2.0 ml of distilled water and the samples were diluted to 25 ml. The 0 hour controls and samples, however, were treated in a similar manner but instead of being resuspended in 25 ml they were only diluted to a 10 ml volumn. These solutions were analyzed within 36 hours on an atomic absorption spectrophotometer.

The aluminum standard stock solution was made by adding 8.9525 gm of $AlCl_3 \cdot 6H_2O$ (1.0000 gm of aluminum) and 20 ml of 6M HCl to a liter volumetric flask prior to the addition of distilled water. A wide range of dilutions was freshly prepared daily from the stock solution to yield a minimum of 10 ppm and a maximum of 300 ppm. Standard curves were prepared each time prior to analyzing the samples.

Statistical Analysis

Variations in treatment means may have been partly attributable to variation in the initial weights of the aluminum strips. Therefore, a covariance analysis, designed and analyzed as described in Steel and Torrie (1960) by Dr. Walter J. Drapala of Mississippi State University, was used to adjust the treatment means and to control error.

Effect of Coatings on the Corrosion of Aluminum

A series of enrichment tests were designed to utilize 6 ounce prescription bottles consisting of 1 gm of human feces per 100 ml of nutrient broth containing 0.5 ml of micatex, the coating material. The samples were collected after 1 week of incubation at room tempera-

ture on a rotatory shaker and examined for microbial growth by means of the spread plate technique using nutrient agar as a substrate. From plates showing growth, streak plates were performed with the same nutrients. This procedure was repeated until separate colonies were obtained and then the organisms were examined for purity but were not identified. Each culture was used to reinoculate additional bottles as described earlier. If growth reoccurred, then each vessel was rechecked for pure cultures. From those cases where isolates existed, an inoculating loop containing a small amount of suspension was used to inoculate a 6 ounce prescription bottle with 100 ml of mineral salts medium and 0.5 ml of micatex.

In addition, Rhizopus and Scopulariopsis were transferred from nutrient agar slants into the aforementioned bottles and incubated. An experiment was set up to anlayze coated and treated material for corrosion. The samples are listed under the metals entered earlier. The inocula consisted of undiluted human sputum, urine, fingerprints, and a 0.1 percent dilution of human feces. The test conditions consisted of Tang as the substrate at 5 psi in an atmosphere of 70 percent oxygen and 30 percent nitrogen with a humidity of 95 percent and at room temperature for a 4 1/2 week period. In all cases where Tang was used as a substrate, 2 drops of the inoculum were pipetted upon the coated or treated sample. However, where fingerprints were used as an inoculum, none of the substrate was added.

The controls were set up in a separate chamber from the samples and sterilized with ethylene oxide before the addition of the oxygen-nitrogen mixture.

RESULTS AND DISCUSSION

Introductory Studies

Experiments were set up in our laboratory to determine if microbes were capable of corroding the various metal components of the Manned Orbital Workshop. These tests consisted of incubating the metals in solutions of Tang and feces and bouillon and feces. The photographs (Figures 1 and 2) are a graphic display of the effects. It was found that aluminum 1100 and the aluminum alloy 2014-T6 were degraded by the subsequent microbial growth, whereas aluminum alloy 6061-T6 and stainless steel 321 were not attached microbiologically.

Another investigation was set up to determine the effect of various conditions on the growth of microbial enrichments from human feces as follows:

1. Ambient conditions in the laboratory (25 C)
2. 95-100 percent humidity at 28 C (83-85 F)
3. Low temperature of -95 to -100 F

In this analysis of metals, a number of microorganisms were isolated. Of the total of 45 different kinds of organisms, 27 of these cultures were bacteria. Ten were yeasts and eight were molds. The 27 bacterial isolates were represented by the genera -- Achromobacter, Alcaligenes, Escherichia, Pseudomonas, and Mycobacterium. The ten yeasts were of the genus Saccharomyces and the eight molds were represented by the genera Penicillium, Aspergillus, Rhizopus, and Trychophyton.

The effect of unadulterated feces on metal was also examined. The coupons were removed from the humidity chamber and the laboratory shelf and were studied for growth and condition of the metal. Although, the predominant organisms in both cases consisted primarily of fungi, the degree of deterioration in each of the environmental conditions varied. The 95-100 percent humidity resulted in small etch spots on the metal whereas the lower moisture content showed no appreciable change. The amount of corrosion was observably greater using a stereomicroscope (approximately 15X) at the higher humidity level. It has been known for a long time that the desiccation of nutrients (i.e. Tang) would decrease microbial growth and conversely it was expected that an increase in moisture content would enhance the growth of organisms up to a point.

In additional experiments, the effect of a light inoculum of fecal waste upon the breakdown of 1100 was studied in a 95-100 percent humidity in regard to two substrates, Tang and bouillon. After an appropriate incubation period the samples were checked for microbial growth and biodeterioration. The metals were found to have microbiological growth consisting predominantly of molds. Further, examination of the specimens under investigation revealed that Tang caused a much greater corrosion problem than bouillon. As expected, there was a difference in the extent of disintegration with a change of substrates. Bouillon was composed of proteins to a large degree, whereas Tang consisted of carbohydrates.

Pure Culture Studies on the Degradation of Aluminum

Other work in our laboratory with alloys of aluminum and stainless steel had indicated a difference in the rate of deterioration due to the oxygen content of the atmosphere; however, the effects were not as pronounced with aluminum 1100. Since other avenues of study were considered to be more fruitful, this experiment was terminated.

Effect of Pressure and Humidity on the Biodeterioration of Aluminum

The effect of pressures and substrates upon the decomposition of aluminum 1100 by selected microbial species under an atmosphere of 50 percent oxygen and 50 percent air was also tested. The data may be observed in Table 1. The information for the most part, was inconsistent with respect to growth. An appreciable amount of corrosion was noticed with the aid of a stereomicroscope (about 15X) at 1 and approximately 14.7 psi but there was no breakdown recorded in any case at 5 psi. This presented a rather interesting situation since any of these pressures could be used in the space vehicle.

The relationship of the growth of microorganisms to nutrients and humidities may be observed in Table 2. Although the results suggested that no conspicuous change in the rate of growth occurred with bouillon, a slight increase was seen with Tang due to the enlarged moisture content. In addition, it was apparent that the higher humidity level enhanced deterioration. When a stereomicroscope (approximately 15X) was used to analyze the metal, a noticeable difference occurred in the controls and the inoculated samples. The controls had a slight etching of the aluminum, whereas the samples

had either pits or holes. Because of the variations in the degree of disintegration, additional evidence was obtained that the microorganisms not only contributed to decomposition but apparently initiated the process under the test conditions.

Growth Rate

An experiment was designed to compare the growth of organisms at approximately 14.7 and 5 psi. Room temperature and atmosphere were used for the incubation of the samples. The growth over a 14 day period was measured by determining the number of bacteria or the diameter of the colony as was the case with fungi. The microbial count indicated that variations in pressure caused no noticeable change. The growth of fungi, however, was slightly inhibited at 5 psi based upon size of the colonies when compared to the cultures incubated at about 14.7 psi.

Dissolution of Aluminum

An earlier effort to assess weight changes of aluminum, an element highly resistant to decomposition due to an oxide layer, as a result of microbial growth with an analytical balance was a failure. The final analysis indicated that the limitations of the balances and the sample's size caused no differences to be observed.

In an attempt to monitor mass loss from the strip, an experiment was conducted in which an atomic absorption spectrophotometer was employed to determine aluminum concentrations in the test medium. Results of the covariance analysis of the data are reported in Table 3. The significant error mean squares for the 20 percent and 70 percent

oxygen data indicate that real differences exist among the treatment means when adjusted for variation in initial weights of the aluminum strips. Therefore, the treatment means were adjusted. The mean square for the 50 percent data was nonsignificant; unadjusted means were used. After the adjustments, values were plotted in Figures 3-11. It may be clearly noted that with few exceptions observable changes occurred. In most cases, more aluminum was present in solutions containing inoculum than without.

The metal strips employed in the foregoing experiment were examined for observable corrosion with a stereomicroscope (approximately 15X) during the incubation period of the experiment. Figures 12-14 show the types of deterioration that occur with Rhizopus, Scopulariopsis, and human feces. It is of interest to note in Figure 14 that the breakdown was found predominantly along the "rolling marks" of the metal. This is characteristic of all forms of degradation and in all probability stress in aluminum results in a greater susceptibility to microbial assault. The degree of biodeterioration was magnified when one considered the samples having holes (Figure 13) and with few exceptions, pinholes starting with the 32nd day until the termination of the test were found only in aluminum strips inoculated with Scopulariopsis. These results observed in Table 4 suggested that under the experimental conditions, competition among organisms inhibited the microorganisms which produce corrosion.

Coatings

Microbial growth was analyzed using micatex, a coating material, as a possible substrate in a mineral salts medium. The results of

organic growth upon the coating can be seen in Figures 15-18. In these pictures one should note not only the clearing effect but also the small ball-like objects along the bottom. In the bottles containing Scopulariopsis and an isolate assigned the number 10, these objects actually adhered to the side of the glass. The spheres are thought to consist of micatex interwoven with microorganisms. The reason for the cluster and the clearing process that occurred was not understood.

In an attempt to inhibit corrosion of aluminum, a variety of coated and treated samples were examined. Table 5 gives the effects of various inocula upon the samples. With the exception of the teflon and light beige, none of the coatings were satisfactory in preventing degradation. However, alodined 1200 of the aluminum 1100 series showed some promise. One interesting phenomenon that is not evident in the table was that the lighter pigment coatings had a lower degree of breakdown. All the samples were incubated in the dark; therefore, the only apparent reason for this is that the lighter pigments may act as an inhibitor to some degree. The metals are undergoing further study by Dr. Koelling in the Materials Engineering Department at Mississippi State University.

SUMMARY AND CONCLUSIONS

Experiments were performed to determine the effects of oxygen levels, pressures, humidities, substrates, mixed and pure cultures upon aluminum and treated aluminum samples. In addition, studies were conducted to follow the corrosion process through monitoring the quantity of aluminum loss from the metal samples.

There was an observable difference in the degree of corrosion among the 20, 50, and 70 percent oxygen atmosphere and the data tended to indicate that as the oxygen content of the atmosphere increased, the rate of corrosion increased.

Although the fungal growth rate appeared to be slightly inhibited at the lower pressure, little or no change in aluminum degradation occurred with changes in atmospheric pressure.

When variations in moisture levels were tested, corrosion was magnified at the higher humidities.

It can be concluded by the examination of the data that Tang resulted in greater losses from the aluminum strips than those metal samples with bouillon as a substrate. This was not unexpected due to the differences in chemical composition of these two products.

Quantitation of aluminum losses as determined by atomic absorption data on the medium revealed the fact that there was an increased loss of aluminum from strips in the inoculated samples as compared to the aluminum losses from the uninoculated samples. Although it was evident that some corrosion did take place in the absence of microorganisms, the total metal losses and the kind of

corrosion differed. It was particularly significant that disintegration in the presence of Scopulariopsis resulted in holes in the metal and not simply overall aluminum losses.

One of the coating materials, micatex, which has been suggested to prevent deterioration of aluminum 1100, has been shown to serve as a growth substrate for organisms. Aluminum 1100 coated with the micatex underwent decomposition as did alodined aluminum 1200.

Telfon prevented corrosion of aluminum 1100 under the conditions employed in this investigation. Therefore, teflon is recommended for additional tests.

ABSTRACT

John Marion Tennyson, Master of Science, 1972

Major: Microbiology, Department of Microbiology

Title of Thesis: Enhanced Degradation of Aluminum Metal in the Presence of Selected Microorganisms

Directed by: Dr. Lewis R. Brown

Pages in Thesis: 53 Words in Abstract: 161

ABSTRACT

Experiments were conducted to determine the effects of microorganisms, substrates, pressures, humidities, and oxygen concentrations upon aluminum corrosion. In addition, the effects of microbes upon coated and treated aluminum were examined and an attempt to correlate aluminum in solution with degradation of the samples was undertaken.

The organisms, humidities, oxygen levels, and substrates all played a major role in the corrosion of aluminum. However, the effect of pressure and oxygen still leaves some doubt as to their role in the biodegradation of the metal.

Quantitation of aluminum losses indicated that the total metal losses from inoculated samples were significantly greater than those of the uninoculated samples. The inoculated medium, in addition, had a pitting type of corrosion, whereas, the controls were uniformly etched.

Micatex, a coating material that may be utilized by microorganisms as a substrate, and alodined aluminum 1200 were virtually worthless as inhibitors of aluminum corrosion; however, teflon was extremely effective in preventing deterioration under the experimental conditions.

APPENDIX

Table 1. The effect of pressure on the corrosion of aluminum 1100 by selected microbial species under an atmosphere of 50 percent oxygen/50 percent air.

	Substrate											
	Bouillon						Tang					
	Pressure						Pressure					
	1 psi		5 psi		14.7 psi		1 psi		5 psi		14.7 psi	
Corrosion	Gro.	Cor.	Gro.	Cor.	Gro.	Cor.	Gro.	Cor.	Gro.	Cor.	Gro.	Cor.
Alcaligenes	+++	+	+++	o	++	+	+++	o	+++	o	++	+
Pseudomonas	+++	o	++	o	++	o	+++	+	+++	o	++	o
Proteus	+++	o	+++	o	++++	+	+++	o	+++	o	+++	+
Bacillus	+++	o	+++	o	++++	o	++++	o	+++	o	+++	+
Aerobacter	++	o	+++	o	++++	+	++	+	+++	o	+++	+
Archromobacter	+++	o	+++	o	++	+	+++	o	+++	o	++	o
Rhizopus	+++	+	+++	o	+++	o	+++	+	+++	o	++++	+
Aspergillus	+++	+	+++	o	++++	+	++++	+	++++	o	+++	+
Trichoderm	++	o	++	o	++++	o	++	o	++	o	++++	+
Unknown	++++	o	++++	o	++++	o	++++	+	++++	o	++++	+
Control	o	o	o	o	o	o	o	o	o	o	o	o

Gro. = Growth: o = No growth, + = Little growth, ++ = Moderate growth, +++ = Heavy growth, ++++ = Very heavy growth

Cor. = Corrosion: o = No corrosion, + = Corrosion

Table 2. The result of humidity on the corrosion of aluminum 1100 by selected microbial species under an atmosphere of 70 percent oxygen/30 percent air.

	Substrate											
	Bouillon						Tang					
	Humidity						Humidity					
	10 percent		50 percent		90 percent		10 percent		50 percent		90 percent	
Corrosion	Gro.	Cor.	Gro.	Cor.	Gro.	Cor.	Gro.	Cor.	Gro.	Cor.	Gro.	Cor.
Alcaligenes	++	o	++	+	++	+	+	o	+	+	++	+
Pseudomonas	++	o	++	+	+	+	+	o	+	+	+	+
Proteus	++	o	++	+	++	+	+	o	+	+	++	+
Bacillus	++	o	++	+	+++	o	+	o	++	+	++	+
Aerobacter	++	o	++	+	++	+	+	o	++	+	++	+
Achromobacter	+++	o	++	+	+++	+	+	o	+	+	++	+
Rhizopus	+++	o	+++	+	+++	+	+	o	+++	+	++++	+
Aspergillus	+++	o	+++	+	+++	+	+	o	+++	+	+++	+
Trichoderm	+++	o	+	+	++	+	+	o	+	+	++	+
Unknown	+++	o	++	+	+++	+	+	o	+	+	++++	+
Control	o	o	o	o	o	o	o	o	o	o	o	o

Gro. = Growth: o = No growth, + = Little growth, ++ = Moderate growth, +++ = Heavy growth, ++++ = Very heavy growth

Cor. = Corrosion: o = No corrosion, + = Corrosion

Table 3. Covariance analysis for aluminum losses from aluminum 1100 strips during microbial growth.

Table for 20 percent oxygen data

Source	df	$\Sigma y^2 - 2b\Sigma xy + b^2\Sigma x^2$	SS	MS	F
Organism	3	$2.677990-(2)(.006301)(-24.205345) + (.006301)^2(2248.25)$	3.0723	1.0241	20.77**
Period	6	$41.559513-(2)(.006301)(207.304708) + (.006301)^2(3379.97619)$	34.0813	6.5136	132.12**
Organism x period	18	$3.704882-(2)(.006301)(52.847887) + (.006301)^2(4076.833333)$	3.2008	.1778	3.61**
Error	55	$3.187051-(2)(.006301)(75.7505) + (.006301)^2(12022.833333)$	2.7098	.0493	9.68**

Continued

Table 3. Covariance analysis for aluminum losses from aluminum 1100 strips during microbial growth (continued).

Table for 50 percent oxygen data

Source	df	$\Sigma y^2 - 2b\Sigma xy + b^2\Sigma x^2$	SS	MS	F
Organism	3	$2.060083-(2)(.002812)(29.945232) + (.002812)^2(2273.247024)$			
Period	6	$50.156458-(2)(.002812)(70.735) + (.002812)^2(2718.97619)$			
Organism x Period	18	$2.818754-(2)(.002812)(-2.208524) + (.002812)^2(6537.52381)$			
Error	55	$2.194036-(2)(.002812)(41.840667) + (.002812)^2(14881.0)$	2.0764	.0378	3.111

Continued

Table 3. Covariance analysis for aluminum losses from aluminum 1100 strips during microbial growth (continued).

Table for 70 percent oxygen data

Source	df	$\Sigma y^2 - 2b\Sigma xy + b^2\Sigma x^2$	SS	MS	F
Organism	3	.940213-(2)(.004485)(29.657911) + $(.004485)^2$(1590.96131)	.70618	.2354	4.746**
Period	6	51.886718-(2)(.004485)(-20.139167) + $(.004485)^2$(1964.416667)	52.1069	8.6845	175.01**
Organism x Period	18	3.546670-(2)(.004485)(29.999714) + $(.004485)^2$(6358.39285)	3.4055	.1892	3.815**
Error	55	3.085685-(2)(.004485)(79.1845) + $(.004485)^2$(17654.333333)	2.7305	.0496	7.16**

** Significant at 1 percent level

Table 4. The effect of inocula and oxygen content upon aluminum 1100.

Days	Inocula											
	Rhizopus			Scopulariopsis			Feces			Control		
	Atmosphere			Atmosphere			Atmosphere			Atmosphere		
	$20\%O_2$: $80\%N_2$	$50\%O_2$: $50\%N_2$	$70\%O_2$: $30\%N_2$	$20\%O_2$: $80\%N_2$	$50\%O_2$: $50\%N_2$	$70\%O_2$: $30\%N_2$	$20\%O_2$: $80\%N_2$	$50\%O_2$: $50\%N_2$	$70\%O_2$: $30\%N_2$	$20\%O_2$: $80\%N_2$	$50\%O_2$: $50\%N_2$	$70\%O_2$: $30\%N_2$
0	-	-	-	-	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-	-	-	-	-
32	-	-	-	+	-	+	-	-	-	-	-	-
39	-	-	-	+	-	+	-	-	-	-	-	-
46	-	-	-	+	-	+	-	-	-	-	-	-
53	-	-	+	+	-	+	-	-	-	-	-	-
59	+	-	+	+	+	+	-	-	-	-	-	-

+ = Hole(s)

- = No holes

Table 5. Corrosion of coated aluminum disks and alodined aluminum 1200 strips.

Metals	Inocula						
	Control	Feces	Sputum	Urine	Rhizopus	Scopular.	Fgpts.
26314 (Medium Gray)	-	+	+	+	+	+	-
36231 (Gray)	-	+	+	+	+	+	-
37886 (Light Beige)	-	-	-	+	-	-	-
37855 (Dark Beige)	-	+	+	+	+	-	-
25102 (Blue)	+	+	+	+	+	+	-
35231 (Blue)	+	+	+	+	+	+	-
30226 (Yellow green)	+	+	+	+	+	+	-
34552 (Green)	+	+	+	-	+	+	-
33538 (Yellow)	+	+	+	+	+	+	-
21105 (Red)	+	+	+	+	+	+	-
37038 (Black)	+	+	+	+	+	+	-
Teflon cylinders	-	-	-	-	-	-	-
Alodined 1200	-	-	⊕	⊕	-	-	-

Scopular. = Scopulariopsis Fgpts. = Fingerprints

+ = Corrosion of coating and/or metal, - = No observable effect on the sample, ⊕ = Hole

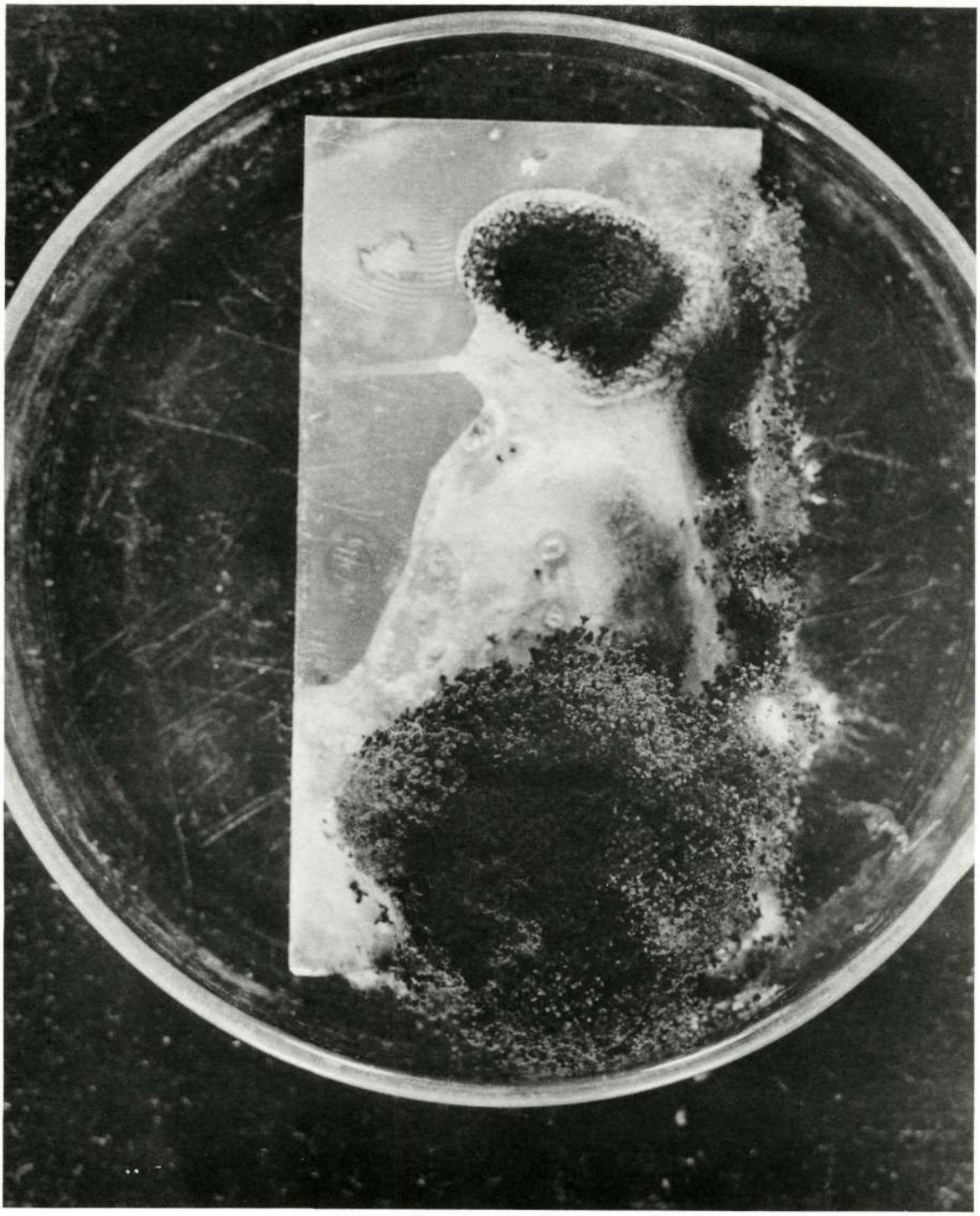

Figure 1. Aluminum coupon covered with microbial growth.

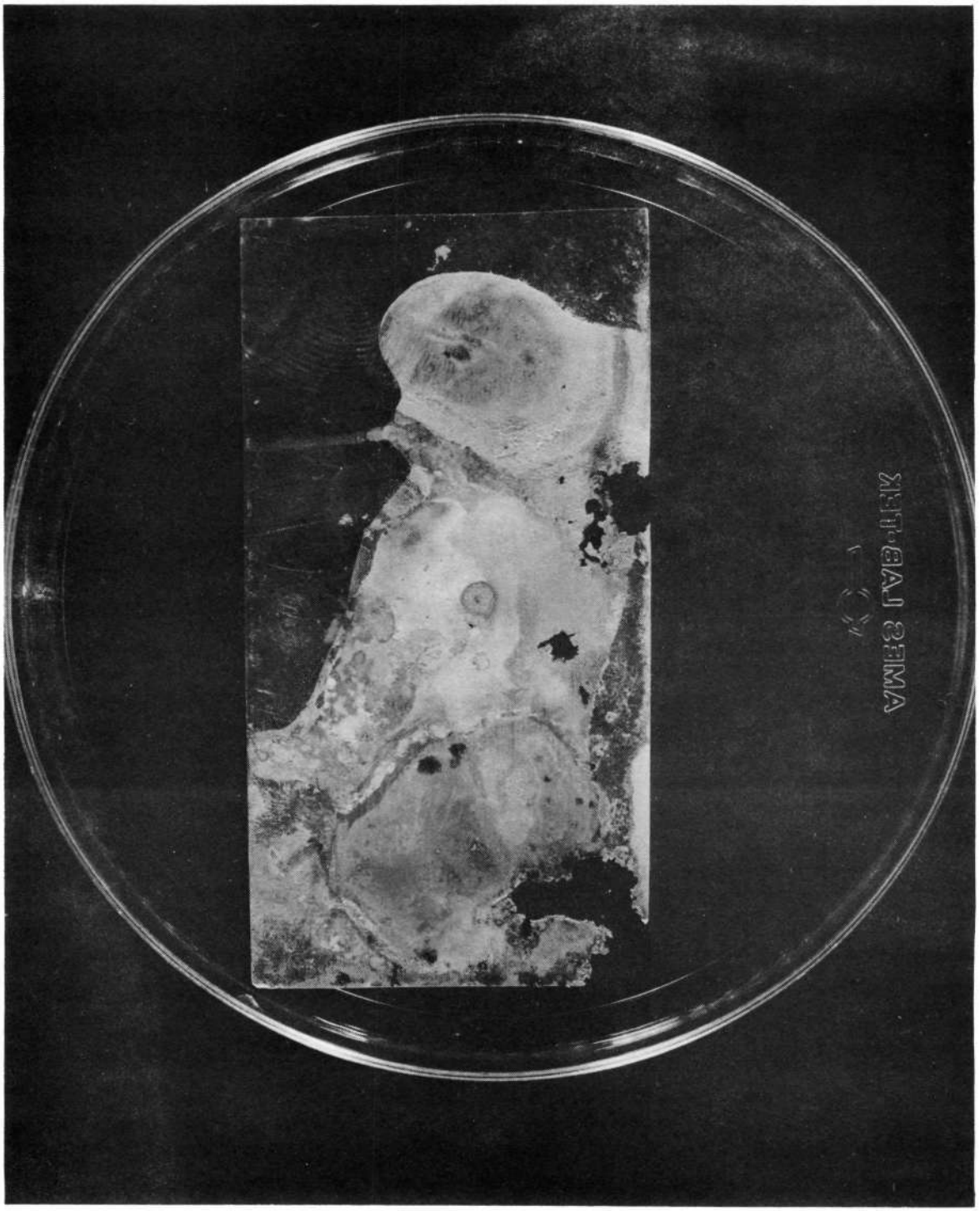

Figure 2. Aluminum coupon after the removal of growth.

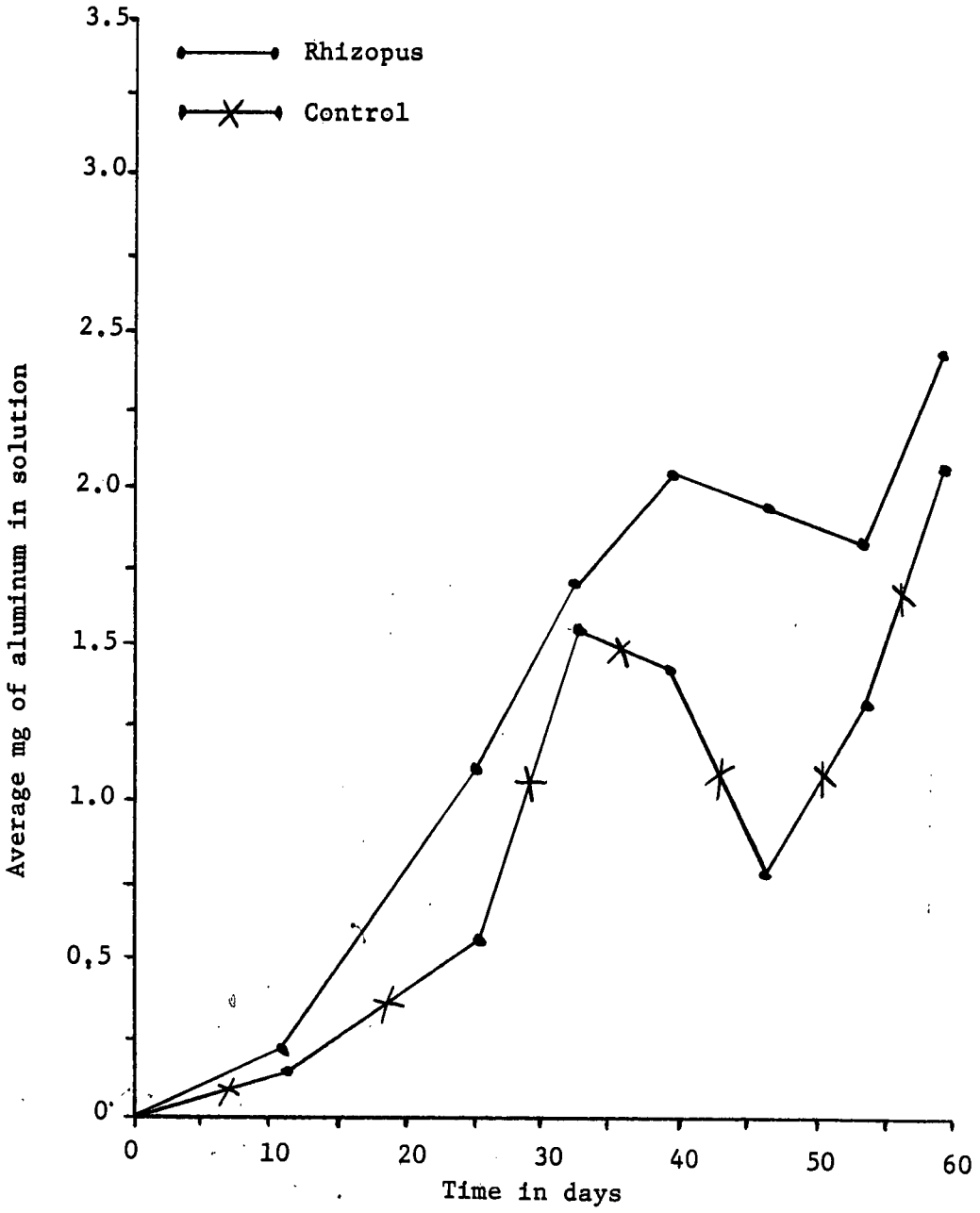

Figure 3. The effect of Rhizopus on the aluminum concentration in Tang under an atmosphere of 20 percent oxygen.

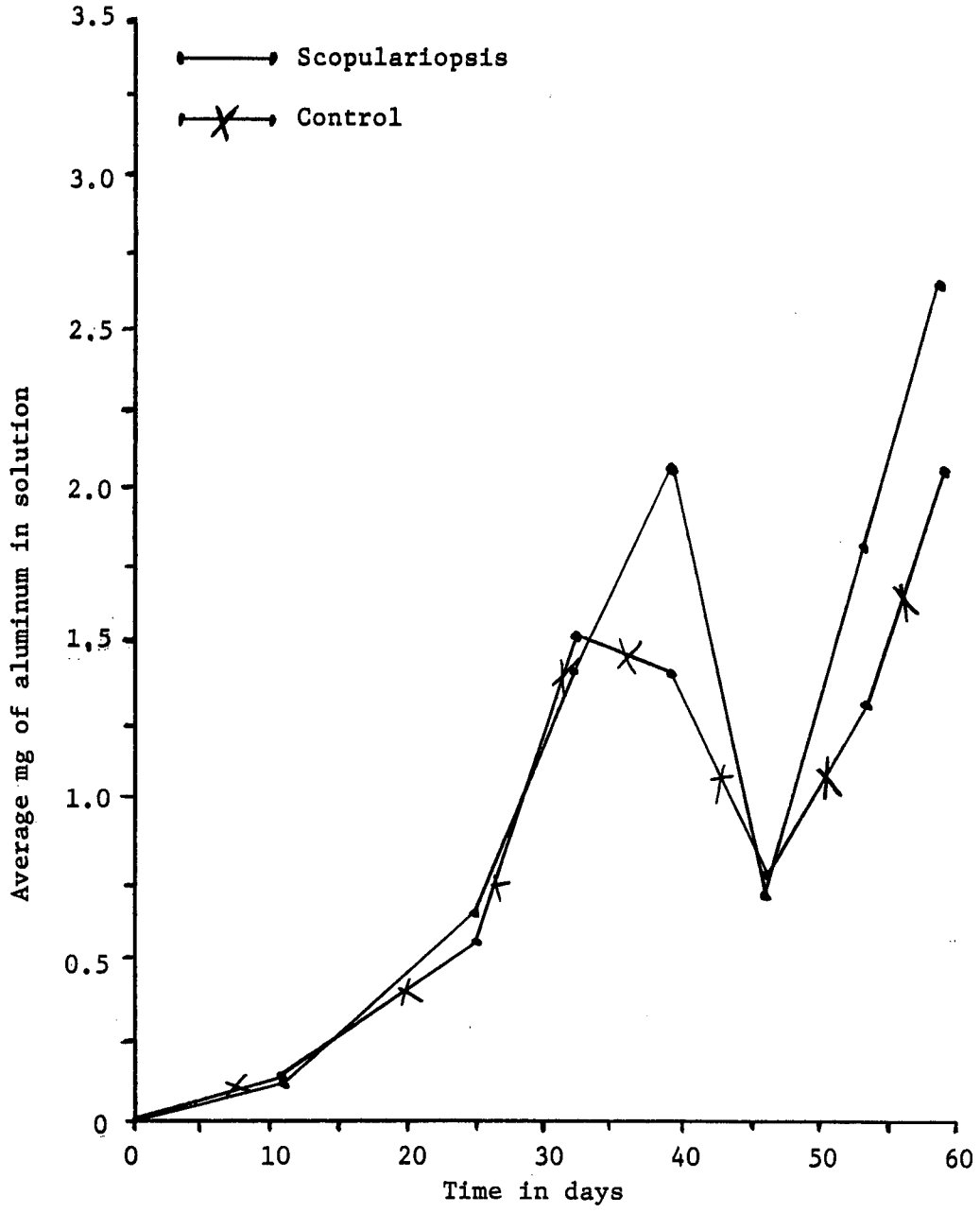

Figure 4. The effect of Scopulariopsis on the aluminum concentration in Tang under an atmosphere of 20 percent oxygen.

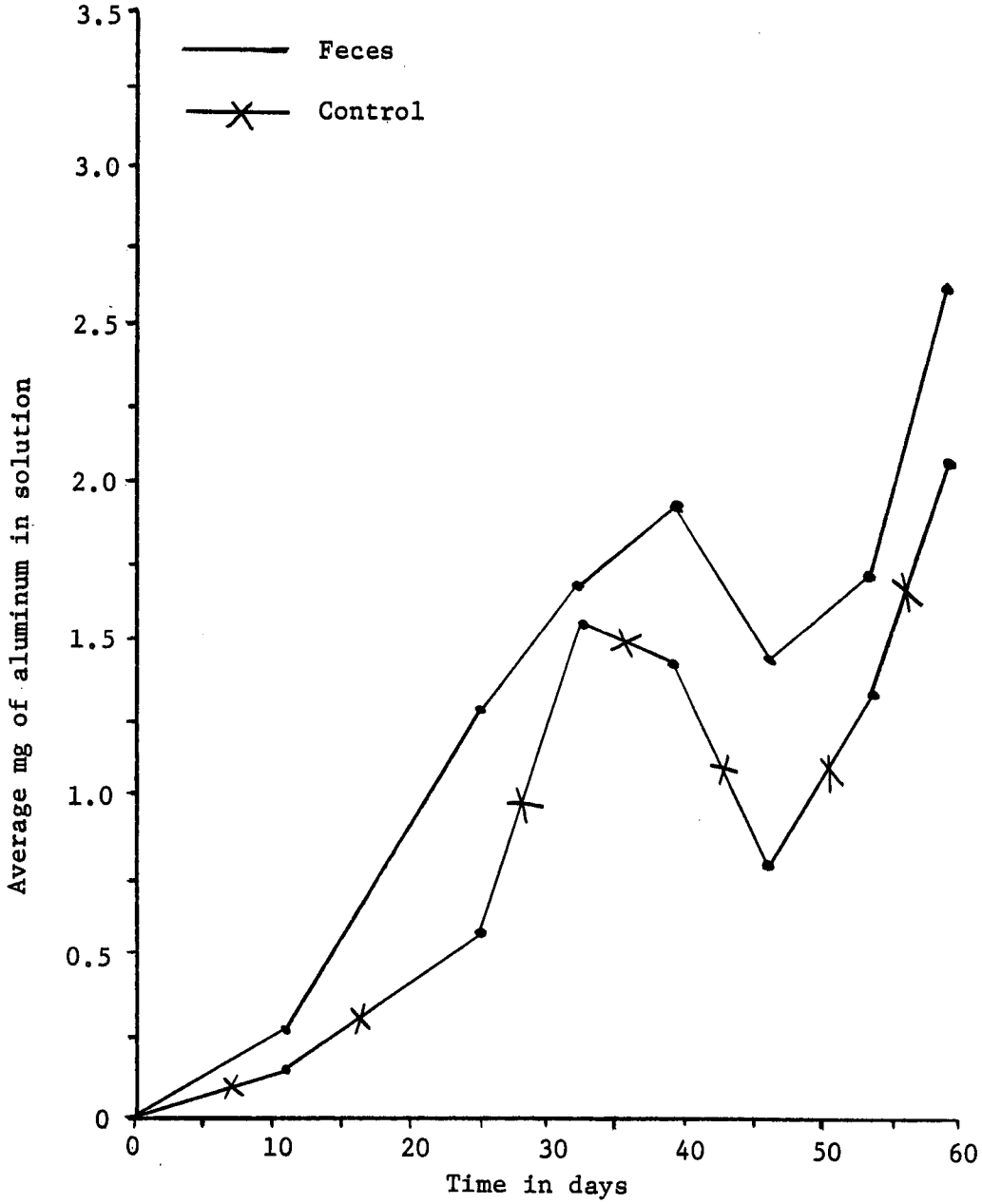

Figure 5. The effect of feces on the aluminum concentration in Tang under an atmosphere of 20 percent oxygen.

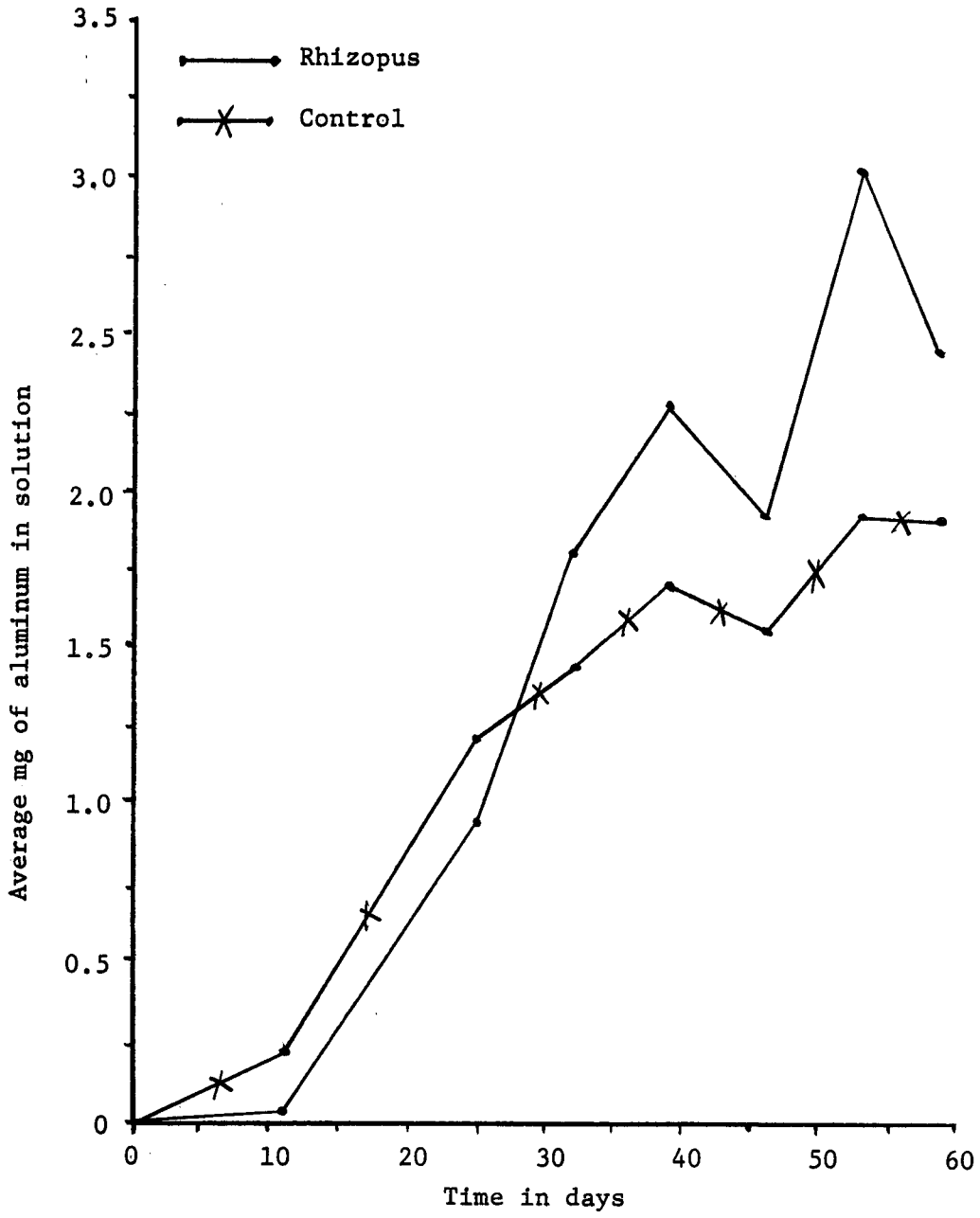

Figure 6. The effect of Rhizopus on the aluminum concentration in Tang under an atmosphere of 50 percent oxygen.

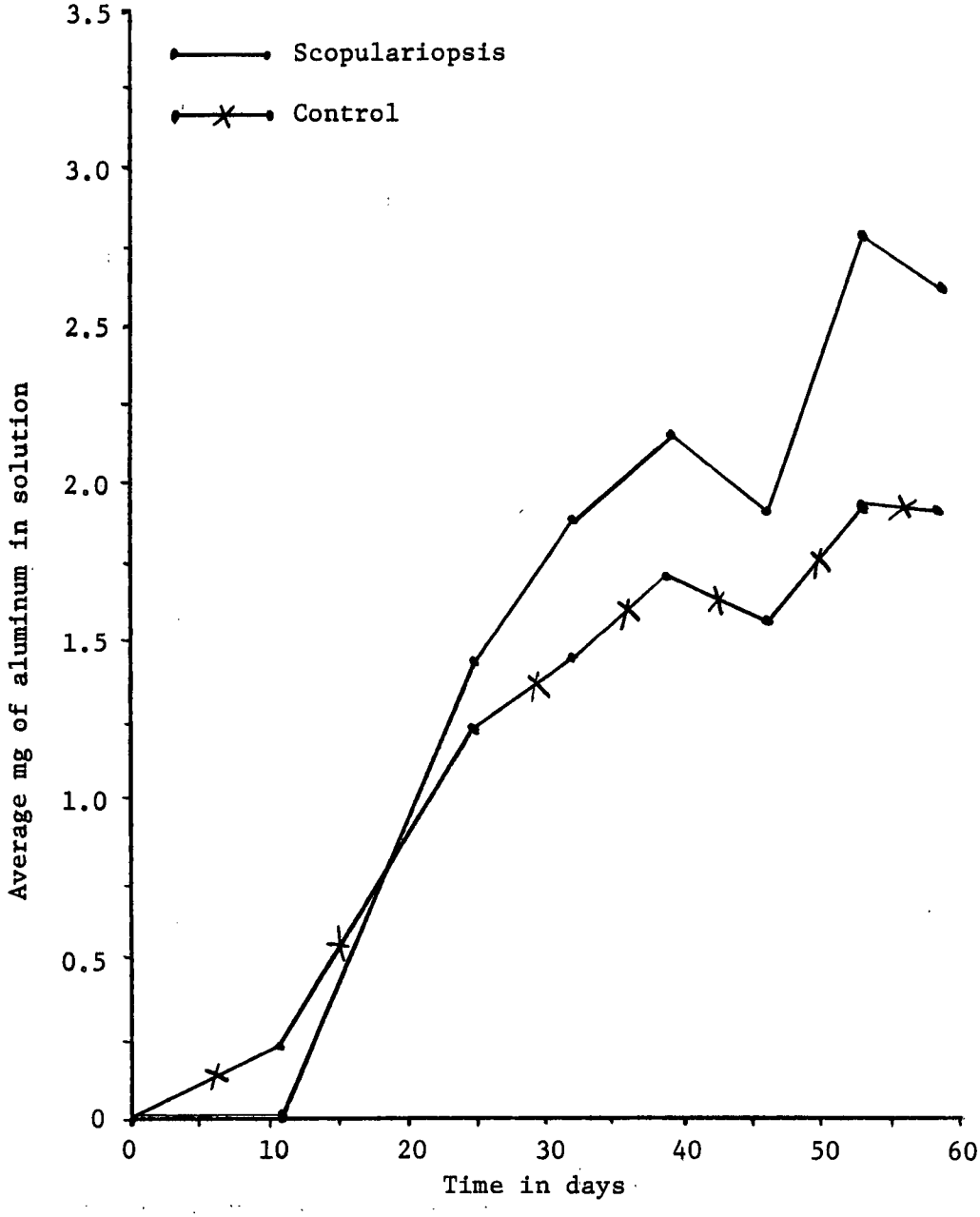

Figure 7. The effect of Scopulariopsis on the aluminum concentration in Tang under an atmosphere of 50 percent oxygen.

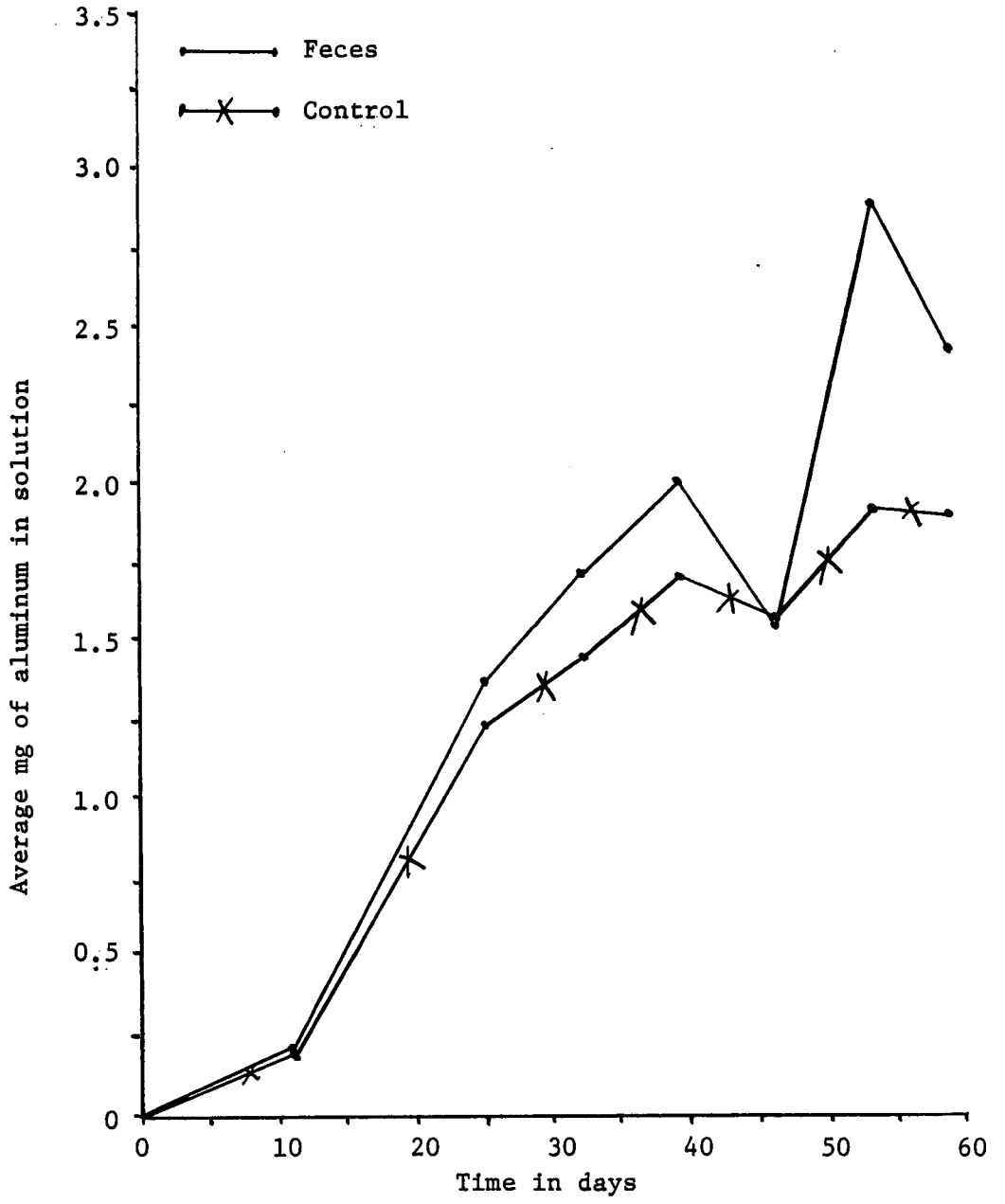

Figure 8. The effect of feces on the aluminum concentration in Tang under an atmosphere of 50 percent oxygen.

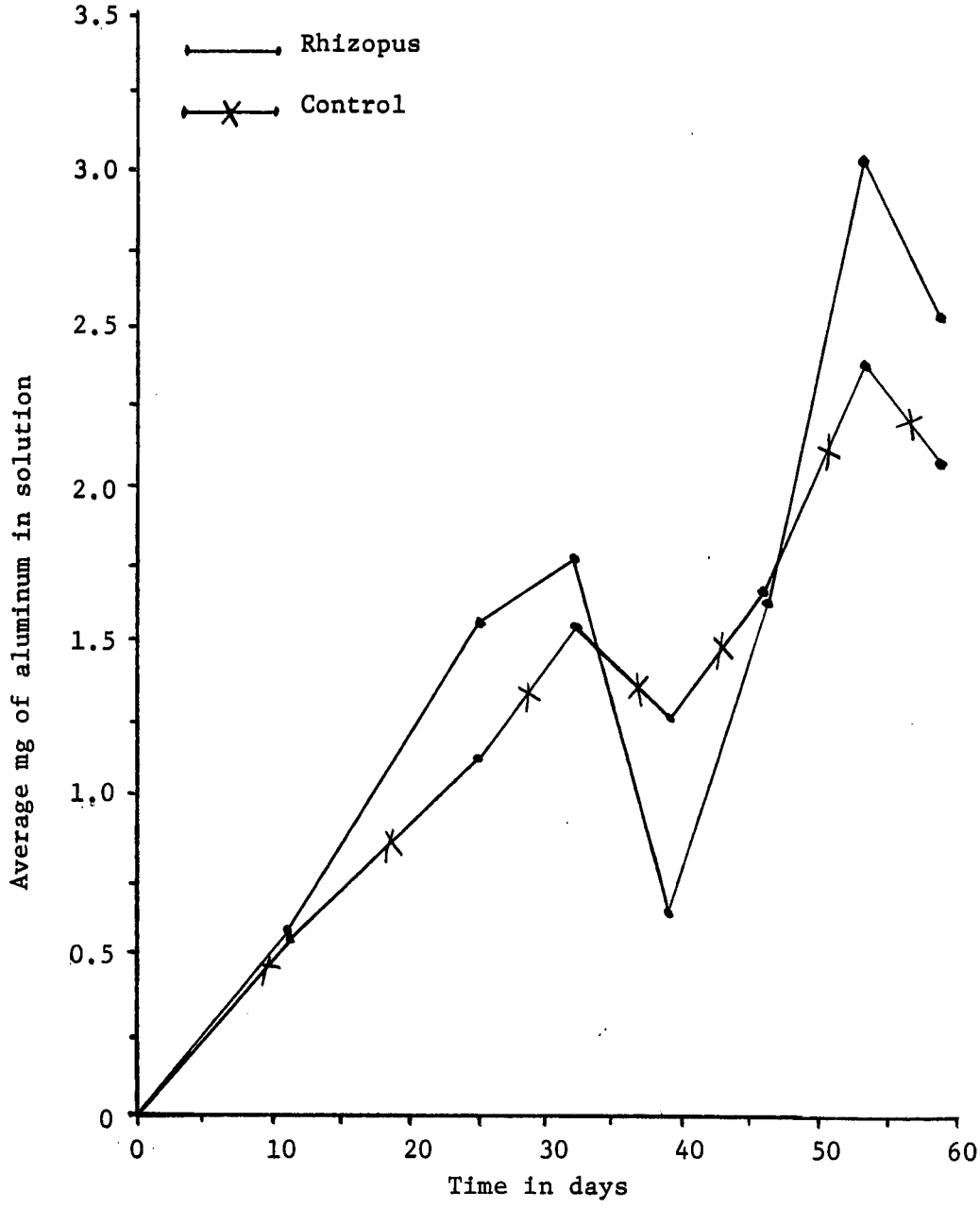

Figure 9. The effect of Rhizopus on the aluminum concentration in Tang under an atmosphere of 70 percent oxygen.

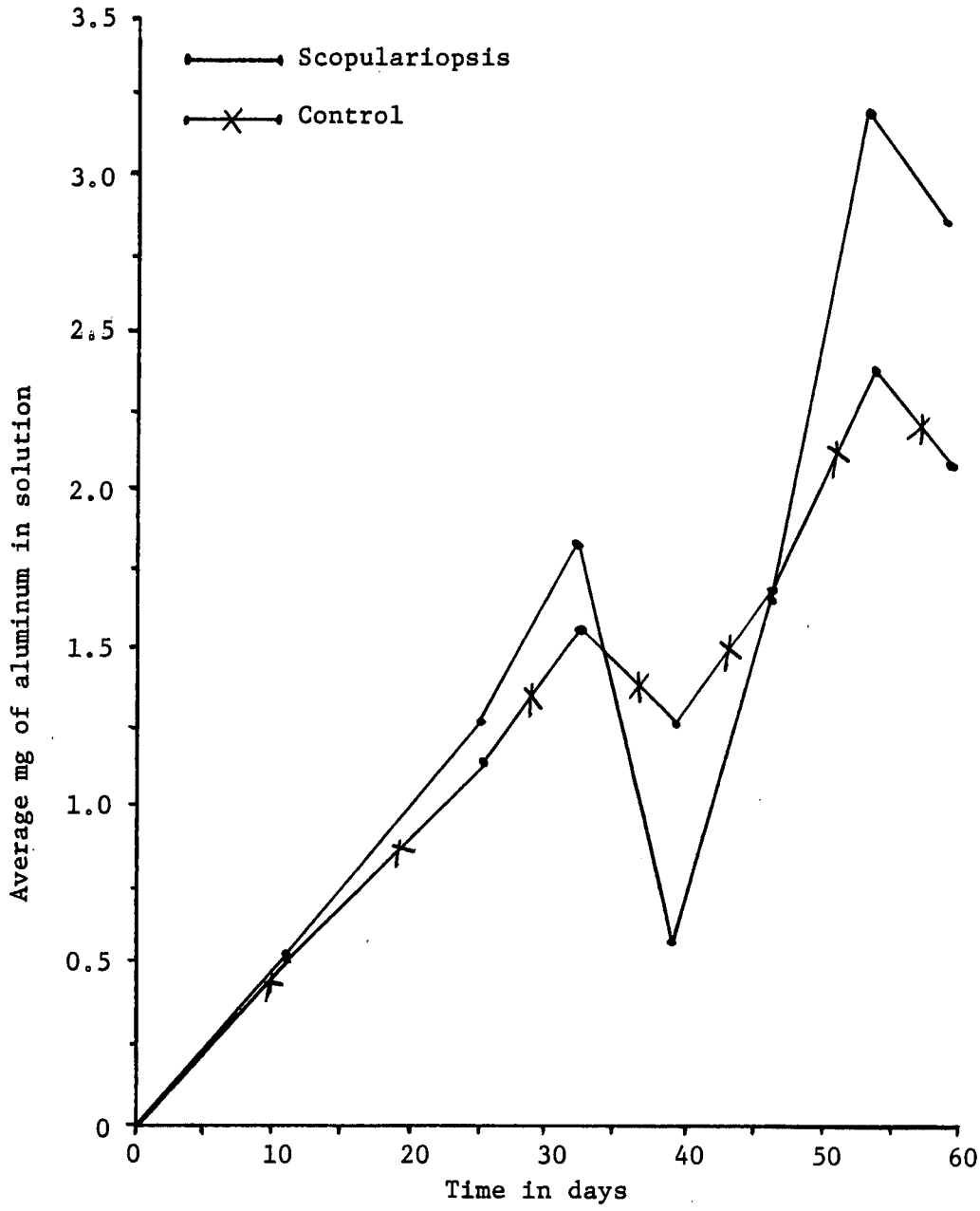

Figure 10. The effect of Scopulariopsis on the aluminum concentration in Tang under an atmosphere of 70 percent oxygen.

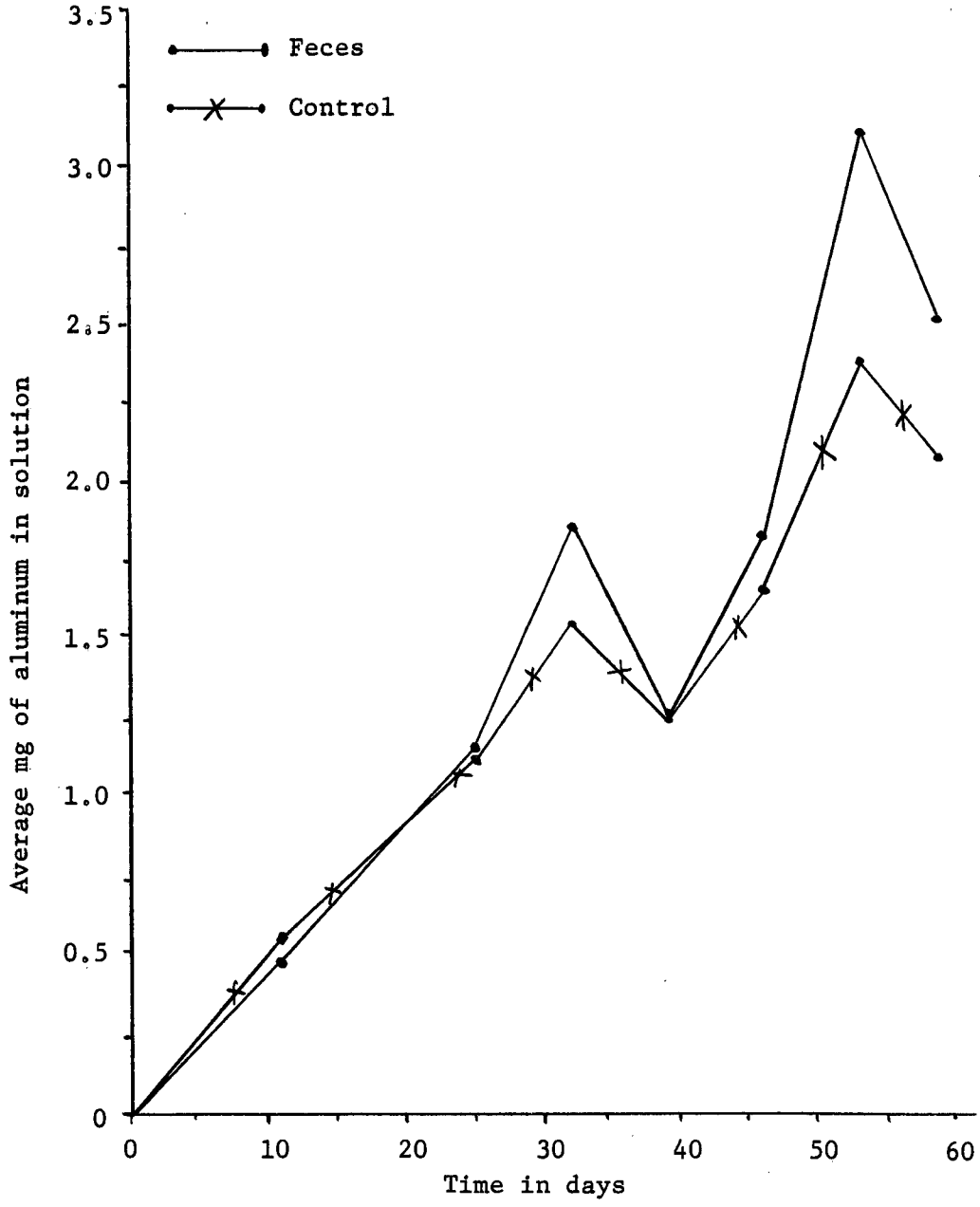

Figure 11. The effect of feces on the aluminum concentration in Tang under an atmosphere of 70 percent oxygen.

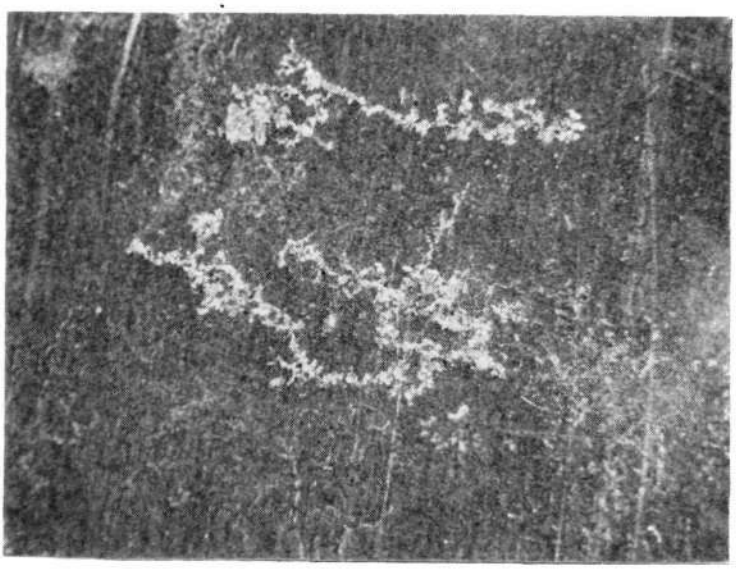

Figure 12. The characteristic type of corrosion of aluminum by Rhizopus (approximately 15X).

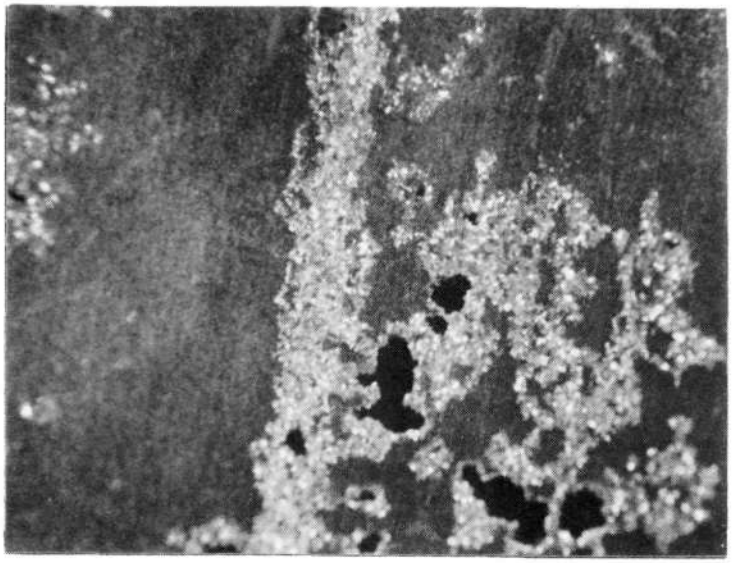

Figure 13. The characteristic type of corrosion of aluminum by Scopulariopsis (approximately 15X). Note: the dark areas in the photograph are holes.

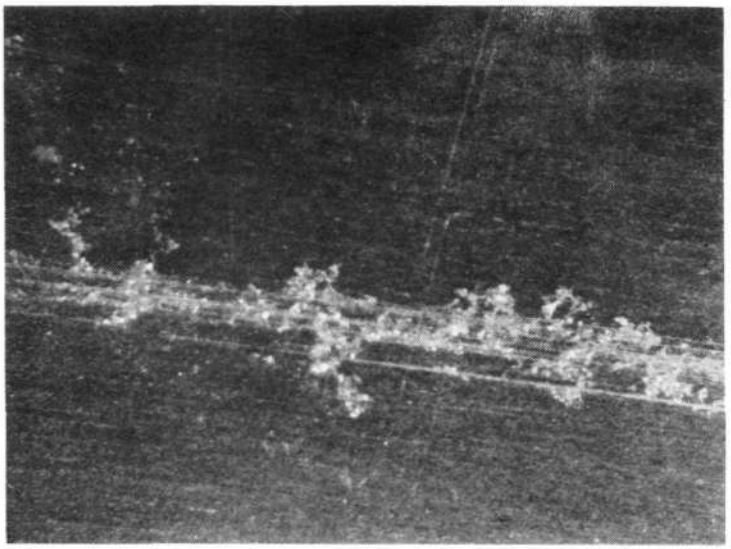

Figure 14. The characteristic type of corrosion of aluminum caused by fecal matter (approximately 15X). Note: the degradation along the "rolling marks" on the aluminum strip.

Figure 15. Growth of Rhizopus (Rhiz) and Scopulariopsis (Scop) in mineral salts medium containing micatex. Note: pellets of Rhizopus (Rhiz) growth present on the bottom of the bottle and the completely clear medium and also the Scopulariopsis (Scop) growth evident on the bottom of the bottle and the partial clearing of the medium.

Figure 16. Growth of Rhizopus (Rhiz) and unidentified bacterial isolate (3) in mineral salts medium containing micatex. Note: heavy growth settled to the bottom and clearing of the medium.

Figure 17. Growth of Rhizopus (Rhiz) and an unidentified bacterial isolate (5) in mineral salts medium containing micatex. Note: pellets of Rhizopus (Rhiz) growth present on the bottom of the bottle and the completely clear medium and also the unidentified bacterial isolate (5) produced no observable change.

Figure 18. Growth of Rhizopus (Rhiz) and an unidentified bacterial isolate (10) in mineral salts medium containing micatex. Note: pellets of Rhizopus (Rhiz) growth present on the bottom of the bottle and the completely clear medium and the unidentified bacterial isolate (10) growth on the bottom of the bottle and the clearing of the medium.

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