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ABSTRACT

Laboratory experiments were conducted to determine the role of microorganisms in the pitting of mild steel. Experimental setups consisting of 4.8 mm (3/16 in.) mild steel plates covered with 12.7 mm (1/2 in.) thick pieces of plywood were exposed to several different aqueous media supplemented with various combinations of a soil suspension and selected inorganic and organic compounds. Half of the replicate metal-wood-water setups were sterilized and aseptically maintained during incubation, after which they were checked for the presence of viable microorganisms and pitting of the mild steel.

Results of the first set of experiments showed that pitting of the mild steel specimens in many of these setups occurred during an

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incubation period of 3 to 6 months. However, the method used to exclude microorganisms by sterilizing the components separately was unsuccessful. In a second set of experiments, setups were sterilized by exposure to gamma irradiation after they had been assembled. The sterilized setups remained sterile throughout the experiment, while those which were not originally sterile still contained viable microorganisms at the end of the experiment. Pitting of the mild steel specimens was more severe when they were exposed to sterile conditions than when viable microorganisms were present. These experiments showed that while microorganisms are known to enhance corrosion processes in some circumstances, their presence can reduce corrosion in others.

INTRODUCTION

It has been well established that microorganisms influence the corrosion of many different alloys.[1] Two classic examples are: 1) corrosion of iron alloys by bacteria, especially sulfate-reducing bacteria,[2-8] and 2) the pitting of aluminum fuel tanks by the action of a bacterial-fungal community.[9-13] However, the exact mechanisms by which microorganisms cause or accelerate corrosion are not well understood. Several mechanisms have been proposed,[14-20] but none have become universally accepted. A problem in many of these investigations is the inability to reproduce, in the laboratory, the severity and rates of attack found in some "field" conditions. There is a need, therefore, to provide laboratory conditions where a reproducible rate of corrosion of a particular alloy simulates "field" situations. Extensive, controlled studies could then be done to determine what role microorganisms have in the corrosion process. This would lead to an understanding of the mechanisms involved and, ultimately, the control of these corrosion processes.

After a brief examination of samples from a case where mild steel flooring which had been overlayed with a fire-retardant plywood became pitted (3 to 6 months), conditions similar to this situation were reproduced in the laboratory. After the basic criterion of a similar rate and severity of pitting was reasonably satisfied, further experiments were conducted to determine what role microorganisms had in the pitting process.

This work showed that the presence of viable microorganisms in the laboratory setups provided mild steel specimens protection from pitting.

MATERIALS AND METHODS

Large Setups: Experiment No. 1

Because pitting in the "field" situation appeared to be random, metal specimens as large as reasonably manageable were used for the initial test. Components of these setups included 216 mm (8-1/2 in.) square, 4.8 mm (3/16 in.) thick, piece of mild steel (A-537 Class B), 203 mm (8 in.) square piece of 12.7 mm (1/2 in.) plywood treated with a proprietary polyphosphate fire-retardant and an aqueous media to which the steel and plywood were exposed. The flat surface of the steel was polished using a 600-grit paper. Pieces of both new plywood and plywood removed from the "field" where pitting had been observed were used. The water was a combination of rainwater (80%) collected on the Savannah River Plant (SRP) site and well water (20%), also from the SRP site. This water was supplemented with either: 1) a suspension of soil (2.0 gm/L) also obtained from the SRP site or 2) the following mixture (in grams per liter): Bacto Peptone (Difco Laboratories, Detroit, Mich.), 1.0; yeast extract (Acumedia, Baltimore, Md.), 0.5; glucose, 0.5; and ammonium sulfate, 1.38. The metal, wood, and medium for each of these setups were contained in individual 254 mm (10 in.) square casserole dishes. A total of 24 setups

were used to provide quadruplicates of six conditions. These six conditions consisted of all combinations of the two aqueous media with three types of wood overlays (old wood, new wood, and no wood).

All components of half of the quadruplicate setups were sterilized separately by autoclaving except the metal specimens were sterilized by soaking them in a 70% ethanol bath. After allowing these metal specimens to air dry, they were immediately placed in the sterile casserole dishes. Enough medium was then added so that when the wood was placed on the metal surface, the level of the medium was just above the wood-metal interface. All these experimental assemblies were incubated at room temperature. One of each of the duplicates was dismantled for examination after one-month incubation, and the other was incubated for 11 months before examination.

Small Setups: Experiments No. 2 Through 4

A second series of experiments were conducted using the same metal and wood components but in petri dishes. The smaller setups were fabricated from the same material and in the same manner as the large setups except they were 57 mm (2-1/4 in.) and 51 mm (2 in.) square, respectively.

The first of three experiments using these setups (Experiment No. 2) was prepared, assembled, and incubated (3 months) in a manner similar to that of the large setups. The second (Experiment No. 3) involved a much larger matrix consisting of 96 setups

designed to test the effect of additions to the water (individually and in combination) of a soil suspension (2.0 gm/L; organic nutrients (Bacto Peptones, 1.0 gm/L; yeast extract, 0.5 gm/L; glucose, 0.5 gm/L) and inorganic compounds (ammonium sulfate, 1.38 gm/L; dibasic sodium phosphate, 3.0 gm/L). All combinations of these three supplements were tested with old wood, new wood, and without wood overlays. Each condition was prepared in quadruplicate, and the edges of all the petri dishes were sealed with Parafilm® (American Can Co., Greenwich, Conn.), which was left in place throughout the incubation period to prevent dehydration. Two of each quadruplicate setup were then sterilized by gamma irradiation (cobalt-60 at 2.5×10^5 rads/hr) for 23 hours. This dose had been previously shown to be effective in sterilizing soil samples.[21] All of these setups were then placed in a psychrotherm incubator (New Brunswick Scientific Co., Edison, N. J.) at 20°C with a 12 h / 12 h light-dark cycle for 15 months' incubation.

The final experiment using the smaller setups (Experiment No. 4) had 32 replicates. The medium used contained (in grams per liter) yeast extract, 0.5; ammonium sulfate, 1.38; dibasic sodium phosphate, 3.0; and a soil suspension, 2.0. A new piece of plywood was used in all these setups, and all were exposed to gamma irradiation for 23 hours after being completely assembled. Half of these (16) were then opened and inoculated with 0.1 mL of a suspension produced from 10 mL of the original unsterilized solution to which 0.1 mL of a *Desulfovibrio desulfuricans* culture and 0.1 mL of a

Desulfotomaculum orientis culture (both sulfate-reducing bacteria) had been added. These inoculated setups were then resealed with Parafilm® and placed, along with the uninoculated setups, in the psychrotherm incubator and incubated for 13 months.

Microbiological Analysis

When a setup was opened, a sample of the bulk medium was aseptically plated directly onto nutrient agar (Acumedia) and Sabouraud agar (Difco Laboratories) to detect viable bacteria, fungi, and yeast. A sample of bulk fluid was also injected into a vial of API broth (Difco Laboratories) to detect viable anaerobic bacteria, particularly sulfate-reducing bacteria.

Pitting Analysis

The smaller metal specimens which had been covered with plywood were examined for the extent of pitting corrosion by ASTM standard practices.[22] The metal surfaces were cleaned by immersing the specimens in Clarke's solution.[23] Pits which had formed on the entire surface in contact with the wood were enumerated with the aid of a dissecting microscope. Since the topography of the individual pits was such that the width and depth appeared to be directly related, the pits were enumerated according to their diameter in ranges as follows: 1) between 0.5 and 2.0 mm, 2) between 2.0 and 4.0 mm, and 3) over 4.0 mm.

RESULTS

The initial attempts to expose mild steel specimens to sterile conditions for an extended period of time were unsuccessful. This occurred with both the large and small setups and was directly attributable to the methods of sterilizing and assembling the components. These two initial experiments were successful, however, in creating conditions that caused pitting of the metal specimens. The metal in the setups without wood displayed extensive general corrosion in most cases but did not have any obvious pits.

The use of gamma irradiation to sterilize samples in experiments No. 3 and 4 was successful in excluding viable microorganisms during the period of incubation. No growth was detected on either of the two solid media or in the API broth inoculated with samples from these sterilized setups.

The metal specimens in the 32 setups of Experiment No. 3 which were not overlaid with wood had no detectable pits. Most of these specimens exposed to aqueous media supplemented with the inorganic salts appeared to be "passivated." There was a yellowish green film on these metal surfaces, and no accumulation of corrosion products occurred in the aqueous phase. The metal specimens in the "no wood" setups not supplemented with inorganic salts suffered extensive general corrosion, and the aqueous phase contained a large amount of corrosion product after a relatively short period of incubation. There was no obvious, consistent difference between the sterile and nonsterile quadruplicate setups without wood

overlays in this experiment either with respect to the final condition of the aqueous media or the metal specimens.

The surface of the other metal specimens which had been overlaid with plywood in this experiment suffered varying degrees of pitting corrosion as well as general corrosion. The most striking difference in the degree of pitting was found between metal specimens from setups which contained viable microorganisms and those which had remained aseptic. The average number of pits found on the surfaces of these two subsets are shown in Figure 1. When the averages of the number of all the pits on the metal surfaces exposed to these two conditions were compared, a highly significant difference ($P<0.01$) was found. There were over 2.5 times as many pits on metal specimens from setups in which no viable microorganisms were present as compared to specimens from setups with viable microorganisms. There was also more severe pitting of specimens from the gamma irradiated setups as can be seen by the larger average number of pits with diameters wider than 4.0 mm (see Figure 1).

When the pitting data were grouped into categories of sterile and nonsterile setups and then further with respect to the various additions to the aqueous media or to the two types of wood used (each of these groupings consisted of four groups of 16 metal specimens), no further significant differences could be shown except between the inorganic supplements to the aqueous media. This comparison, using Student's t-test, is shown in Table 1. As with

all these groupings, the sterile versus the nonsterile conditions had a significantly ($P<0.05$) different number of pits on the metal specimens, but a significant ($P<0.1$) reduction in the number of pits was found on the metal specimens from setups which did not have the inorganic compounds added to the aqueous media as compared to those that had been supplemented with the inorganic compounds. This trend in the susceptibility of mild steel, incubation with plywood, to pitting corrosion was the reverse of the trend in general corrosion of the mild steel exposed to only the aqueous media.

In order to eliminate the effect gamma irradiation might have had on pitting, the third series of small setups was assembled (Experiment No. 4). The degree of pitting found on the surface of the metal specimens in this experiment is shown in Figure 2. In each of the three categories of pits measured, significantly ($P<0.05$) fewer pits occurred on specimens incubated with viable microorganisms. There was also significantly ($P<0.001$) over three times as many total pits on the metal specimens from sterile as compared to nonsterile setups.

DISCUSSION

The major finding of this work is the reduction in pitting observed on mild steel specimens exposed to sterile conditions. This was unexpected since there is an increased awareness that microorganisms in some circumstances enhance the rate of corrosion.

However, the possibility that microorganisms could reduce corrosion rates has not previously received much attention. What is needed is a better understanding of the mechanisms by which microorganisms influence corrosion in a particular system before measures are taken to reduce corrosion processes by controlling microorganism activity.

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FIGURE LEGENDS

Figure 1. Average number (± 1 S. E.) of three ranges of pits on two groups of 32 mild steel specimens from Experiment #3. One group (irradiated) was from laboratory setups which were exposed to gamma irradiation while the other group (non-irradiated) was from setups containing viable microorganisms.

Figure 2. Average number (± 1 S. E.) of three ranges of pits on two groups of 16 mild steel specimens from Experiment #4. Both groups of specimens were from laboratory setups exposed to gamma irradiation but one group (inoculated) was opened after this treatment and an aliquot of a suspension containing microorganisms was added while the other group of setups (uninoculated) were left sealed through the entire incubation period.

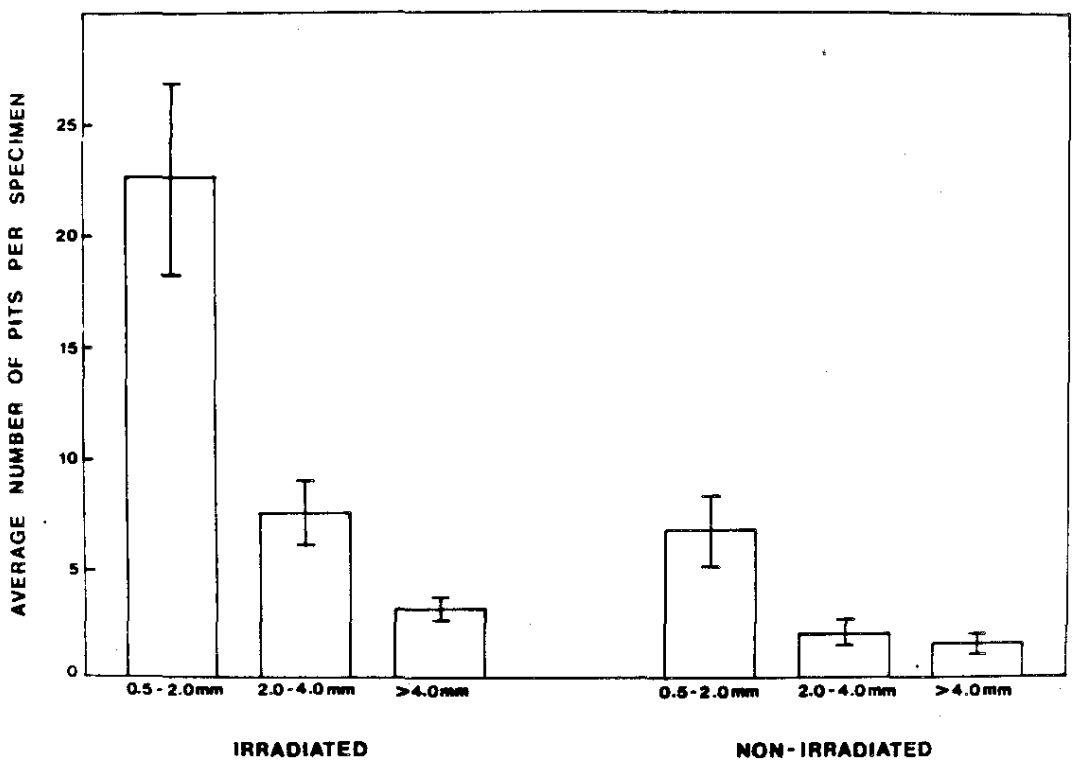
AVERAGE NUMBER OF PITS PER SPECIMEN
25
20
15
10
5
0
0.5 - 2.0mm
2.0 - 4.0mm
>4.0mm
0.5-2.0mm
2.0-4.0mm
>4.0mm
IRRADIATED
NON-IRRADIATED

FIGURE 1.

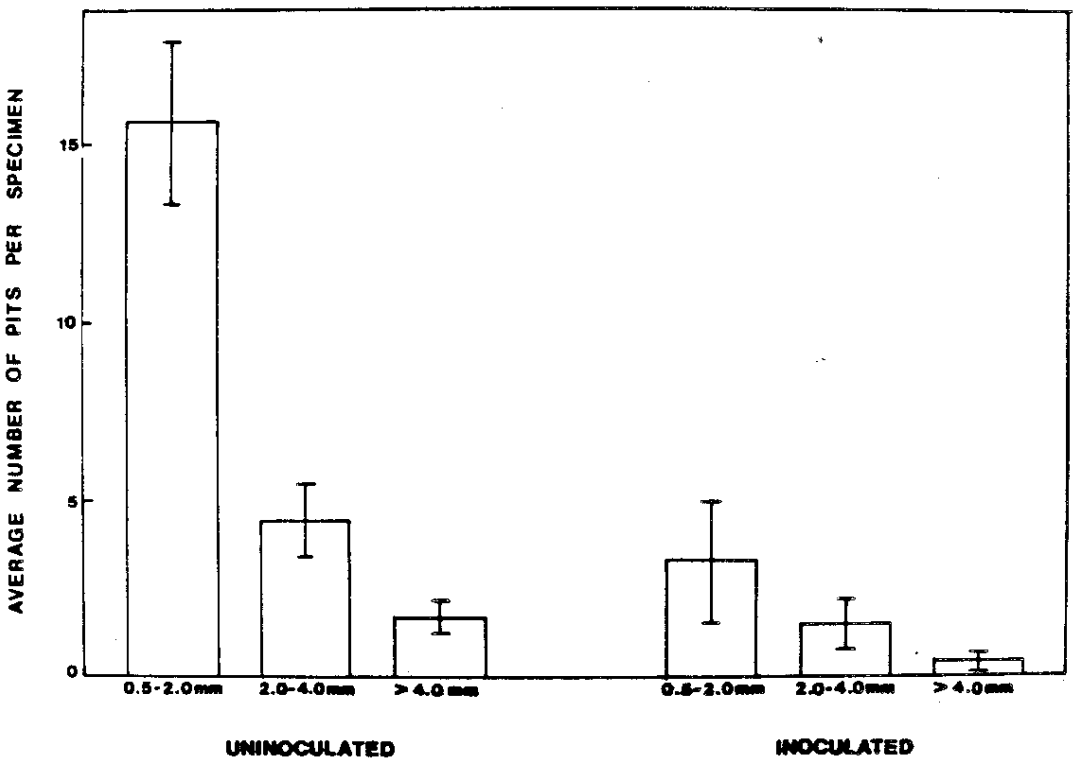
AVERAGE NUMBER OF PITS PER SPECIMEN
15
10
5
0
0.5-2.0mm
2.0-4.0mm
> 4.0 mm
0.5-2.0mm
2.0-4.0mm
> 4.0mm
UNINOCULATED
INOCULATED

FIGURE 2.

TABLE 1. Student's t-values comparing the number of pits on metal specimens incubated with wood. The groups formed were based on addition of inorganic compounds to the aqueous media and whether there were viable microorganisms present.

		Supplemeneted with Inorganic Compounds Non-sterile	Not Supplemented with Inorganic Compounds Sterile
		11.4 ± 4.10[a]	22.9 ± 4.60
Supplemented with Inorganic Compounds Sterile	43.6 ± 9.31[a]	3.170 ***	1.995 *
Not Supplemented with Inorganic Compounds Non-sterile	8.7 ± 2.81	0.546	2.633 **

[a] Average number of pits on 16 metal specimens ± 1 S. E.

The levels of significants are indicated as follows: * P<0.1; ** P<0.05; and *** P<0.01