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CHAPTER 28

The Oxidation of Metallic Iron by *Escherichia coli* and Other Common Heterotrophs

W. W. UMBREIT

Department of Microbiology, Rutgers University, New Brunswick, New Jersey 08903

Under conditions in which nitrate serves as an oxygen acceptor, *Escherichia coli, Bacillus megaterium, Serratia marcescens,* and *Samonella typhimurium* all promote the oxidation (or rusting) of iron. *Enterobacter aerogenes, Pseudomonas fluorescens, Sarcina lutea,* and *Streptococcus faecalis* do not oxidize iron. These reactions take place in a neutral environment, are inhibited by phosphate, and are dependent upon the ability of the organism to reduce nitrate. In three cases a hydrogenase can be demonstrated and it appears that these organisms, including *E. coli,* can use this hydrogen, chemically generated at the surface of the iron, to reduce the nitrate, thus causing iron oxidation. But in *S. marcescens* we have been unable to demonstrate a hydrogenase. Either these strains oxidize the iron by some other mechanism or possess a hydrogenase with features such that it does not register in our assays for the enzyme.

INTRODUCTION

It has been known for many years that the corrosion of iron under anaerobic conditions was caused by sulfate-reducing bacteria (Starkey 1947). These organisms seemed to depolarize the metal surface by removing cathodic hydrogen, although ferrous sulfide seems to be involved (King and Miller 1971). Such corrosive action resulted in deposits of black sulfide and ferric hydrate on the metal surface. The organisms capable of causing such corrosion invariably contained hydrogenase to utilize the cathodic hydrogen and the sulfate reductase system. Under these conditions, sulfate acts as a source of oxygen and the net effect is the oxidation of iron.

Similarly, nitrate can serve as a source of oxygen and it has been suggested that nitrate-reducing organisms could also cause iron corrosion providing they possessed a hydrogenase capable of reacting with the cathodic hydrogen of the metal. Such nitrate-reducing organisms have been implicated in the corrosion of water pipes. Nevertheless, it was somewhat surprising when Mara and Williams (1971) reported that mild steel could be corroded by *Escherichia coli.* The methods used by Mara and Williams were somewhat complex but such complexities proved to be unessential to demonstrating the phenomena. Indeed, *E. coli* and a variety of common laboratory organisms are capable of oxidizing metallic iron under anaerobic conditions in the presence of nitrate.

MATERIALS AND METHODS

The system used initially was similar to that of Mara and Williams (1971). The basal medium consisted of 1% each of yeast extract and tryptone to which was added KNO_3 (usually at 1% final concn) in which strips of sterile polished steel were suspended. After 24 to 96 h of growth at 30 C, the weight loss of the metal was determined.

However, the corrosion is different from that caused by the sulfate-reducing bacteria in that little, if any, hydrogen sulfide is produced and, thus, little "black sulfide" is formed so

the iron oxides produced tend to disperse into the medium. This permitted the use of a very much simpler method. An iron nail was autoclaved with the medium. With a 16 x 125 mm test tube containing 15 ml of medium, there was relatively little corrosion in the uninoculated controls due to diffusion of air. Therefore, after growth for a suitable interval, the iron nail was removed, 1.5 ml of 15% HCl was added per tube, which dissolved the iron oxides, and a suitable aliquot was used to determine the iron dissolved by way of its reaction with potassium thiocyanate.

Hydrogenase was determined by placing 2 ml of a washed cell suspension grown on the basal medium (with and without nitrate, using both aerobic and anaerobic conditions) into the main compartment of a Warburg flask. The suspension contained about 10 mg bacterial protein per ml. The Warburg flask had, in addition to the cells, 0.2 ml KOH in the center well and 0.3 ml 5% KNO_3 in the side arm, with sufficient buffer (pH 7, 0.1 M Tris) so that the total volume was 3 ml. The flasks were gassed with hydrogen either from a compressed cylinder or generated at the desk from zinc and hydrochloric acid. After equilibrating and determining the basic endogenous hydrogen uptake, if any, the nitrate was tipped in and the uptake of hydrogen, if any, followed manometrically at 30 C. In some cases a second side arm containing 0.5 ml of 0.1 M methylene blue was employed which could be tipped in later.

Results and Discussion

Typical data are given in Table 1. Lines 1 and 2 show that iron does not rust in the absence of microorganisms under the circumstances employed even in the presence of nitrate. Line 3 demonstrates that no rusting occurs even if *E. coli* B grows in the medium if no nitrate is

TABLE 1. *Corrosion of iron by common microorganism*

Organism	% KNO_3	Visible Rust 2 Days	Visible Rust 7 Days	Growth μg/dw/ml (7 Days)	μg Fe/ml	NO_3	NO_2 at 7 Days	NH_3
1. None	–	–	–	–	18	–	–	–
2. None	1.0	–	–	–	21	+	–	–
3. *E. coli* B	–	–	–	170	0	–	–	–
4. *E. coli* B	0.1	+	++	205	152	–	–	+
5. *E. coli* B	0.5	++	++	157	515	+	+(70)[b]	++
6. *E. coli* B	1.0	+++	++	198	650	+	+(80)[b]	++
7. *E. coli* B	1.0[a]	–	–	157	17	+	+	++
8. *E. coli* MRE	1.0	++	++	181	335	+	+	++
9. *Bacillus megaterium*	1.0	–	++	54	305	+	+	++
10. *S. marcescens*	1.0	++	++	100	570	+	+	++
11. *Salmonella typhimurium*	1.0	–	++	187	320	+	+	++
12. *Sarcina lutea*	1.0	–	–	57	12	+	–	–
13. *Streptococcus faecalis*	1.0	–	–	74	14	+	–	–
14. *Enterobacter aerogenes*	1.0	–	–	136	29	+	–	–
15. *Pseudomonas fluorescens*	1.0	–	–	45	33	+	–	–

Base medium: 1% each of yeast extract and tryptone autoclaved with iron nail in medium.
[a] With 0.5% K_2HPO_4.
[b] μg NO_2^- per ml.

present. The organism is, of course, growing anaerobically, and is under considerable metabolic hydrogen pressure so that it evidently does not utilize the cathodic hydrogen from the iron nail. In the presence of nitrate, however (lines 4, 5, and 6), there is considerable corrosion roughly proportional to the nitrate present. All cultures show the presence of nitrite except those of line 4, where presumably the level of original nitrate was low enough so that it has all been reduced to ammonia. Line 7 shows that phosphate completely inhibits the corrosion since, except for the phosphate, the conditions are the same as in line 6 in which high corrosion is evident. Metallurgists are evidently familiar with this phenomenon. Phase diagrams are available to explain why phosphate inhibits corrosion, but to a more biologically oriented person the best explanation which can be provided is that ferric phosphate is very insoluble and forms a coat over the metal so that little cathodic hydrogen is released.

Other organisms, lines 8, 9, 10, and 11, are also capable of such corrosion and all of these are capable of nitrate reduction to nitrite, but other common laboratory organisms which lack the ability to use nitrate as a source of oxygen (lines 12 through 15) do not cause iron corrosion.

The rate of iron corrosion is shown in Table 2 for two organisms, *E. coli* and *Serratia marcescens.* In the first experiment, in which the tubes were exposed to air, there was some corrosion in the uninoculated controls, that is, iron can rust in the complete absence of microorganisms. But in the second experiment, in which air was excluded, the uninoculated controls did not corrode, while the microorganisms promoted the corrosion. Of course, since both organisms are facultative, the growth was less in the absence of air but the corrosion of iron was increased by the anaerobic conditions.

TABLE 2. *Time course of iron corrosion by pure cultures*

	Uninoculated	*E. coli* B		*S. marcescens*	
Time (h)	Iron - μg/ml	Growth μg/dw/ml	Iron μg/ml	Growth μg/dw/ml	Iron μg/ml
I. Air diffusion					
24	1	79	8	66	9
48	3	165	19	172	17
72	12	382	62	383	62
120	24	625	110	625	110
II. Anaerobic conditions					
24	1	79	60	115	60
72	1	138	180	220	160
120	1	165	340	238	340

We next looked for hydrogenase among the positive organisms and found hydrogenase present in *E. coli, Bacillus megaterium,* and *Salmonella typhimurium.* However, we could not detect hydrogenase in two strains of *S. marcescens.* There was rapid and extensive hydrogen uptake with *E. coli,* but none whatsoever with *Serratia* (Fig. 1). Since this result was surprising, inasmuch as *Serratia* clearly does corrode the iron in, so far as we can see, the same manner as *E. coli,* one would expect it to possess a comparable type of hydrogenase. It was possible that during the preparation of the cell suspensions, we had inactivated the hydrogenase so we, therefore, prepared suspensions without washing, simply concentrating the cells by centrifugation, but hydrogenase assays were negative. We added extracts of *E. coli*

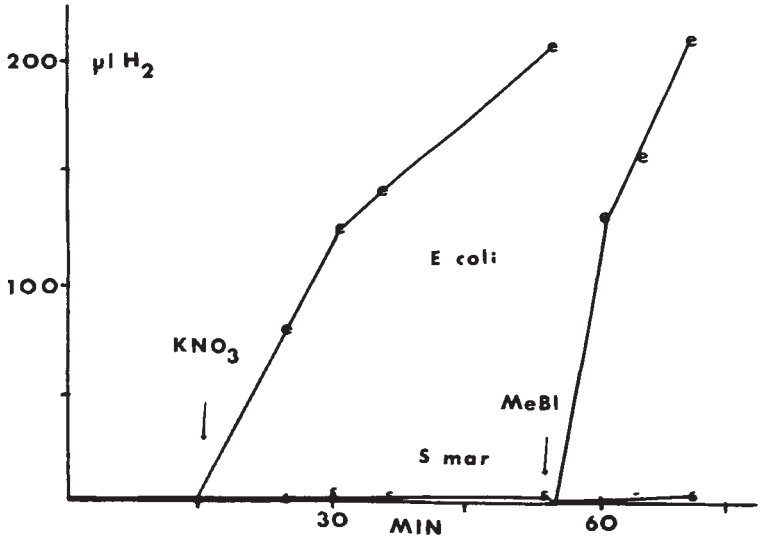

FIG. 1. Uptake of hydrogen by suspension of *E. coli* and *S. marcescens* upon addition of potassium nitrate (0.2% final) and methylene blue (0.17 M). Warburg flasks contained approx. 10 mg cell protein, buffer at pH 7.2, KOH in center well in an atmosphere of hydrogen.

to the *Serratia* cells to possibly supply factors which might be missing in the latter. We even prepared cells of *Serratia* under completely anaerobic conditions, with the air replaced with nitrogen so that exposure to air would be minimal. We have tried reduction of chlorphenol-indophenol. All of these proved to be completely negative for *Serratia* while abundantly positive for *E. coli.* It seems possible that there might be an enzyme which acts upon cathodic hydrogen but will not act upon molecular hydrogen, but we are not aware of any reports in the literature of such an enzyme. As such, therefore, either our methods for determining and detecting hydrogenase are simply inadequate for the enzyme as it exists in *Serratia,* or there is a special and unusual cathodic hydrogenase in *Serratia,* or the iron is corroded by a mechanism not involving hydrogenase when acted upon by *Serratia.* We do not have data which will distinguish between these hypotheses. Whether or not this phenomenon is an important source of corrosion in nature is not at all clear. The presence of decomposable organic matter, the anaerobic conditions, and the presence of nitrate may occur in nature more often than we suppose. It is further possible that the microorganisms can enhance corrosion in air since they are clearly not restricted to nitrate reduction. However, we have no data on natural corrosion caused by these organisms and can report only that it does exist under laboratory conditions and that common laboratory organisms are involved.

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