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Effect of pH on Anaerobic Mild Steel Corrosion by Methanogenic Bacteria

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Methanogens can use H_2 produced by cathodic depolarization-mediated oxidation of elemental iron to produce methane. Thermodynamic consideration of the cathodic depolarization mechanism predicts more oxidation of Fe^0 at lower pH. Methanogenic responses to pH by *Methanococcus deltae*, *Methanococcus thermolithotrophicus*, and *Methanosarcina barkeri* were examined. When grown on H_2-CO_2, these bacteria had pH optima from 6.2 to 7.0, but when all H_2 was supplied from Fe^0, methanogenic pH optima were lower, 5.4 to 6.5. Corrosion was monitored with and without cultures and at various pHs; more corrosion occurred when cultures were present, biologically induced corrosion was greatest at the pH optima for methanogenesis from Fe^0, and corrosion without cultures increased with a drop in pH.

Metal biocorrosion, demonstrated in numerous studies (2–4, 6–8, 11–13, 15, 16, 18–21, 23–28), accounts for a portion of the global corrosion of metal surfaces. There is disagreement over the precise mechanism and what percentage is due to biological rather than chemical factors (3, 7, 12, 15, 16, 18, 24, 27). The most likely mechanisms by which bacteria corrode metal are consumption of electrons from the elemental metal (via cathodic depolarization) and production of corrosive metabolic end products such as H_2S or organic acids; these mechanisms are found in anaerobic ecosystems in association with fermentative bacteria, sulfate reducers, and methanogens. In some cases, a corrosive phosphorous species may be involved as well (15, 27).

Sulfate-reducing bacteria (SRB) have been most thoroughly studied regarding their corrosive properties, and they may act via both cathodic depolarization and sulfide production, as shown in Fig. 1A. Sulfide may cause corrosion chemically by depolarization of the cathode via solid FeS (3). One environment where sulfide production is associated with corrosion is in oil well steel casing, where sulfate-containing waters (e.g., from seawater) induce severe corrosion. SRB populations are thought to be responsible, although clear microbiological proof is lacking; other metal objects exposed to seawater are subject to this type of corrosion (12). In many environments, methanogenic bacteria are found near SRB and may play a role in biocorrosion there, as well as in environments relatively free of SRB. We first demonstrated that cathodic depolarization was a mechanism responsible for corrosion and oxidation of elemental iron and mild steel, as shown in Fig. 1B (11); methane is produced as the result of the chemical production of low levels of H_2 gas. More recently, we have reported that elemental aluminum and zinc function as similar electron donors but that a variety of other metals do not (2). We have been studying the methanogen system, in which methanogens allow for an investigation of the cathodic depolarization reaction in the absence of complications caused by sulfide-induced chemical reactions and in which little drop in pH occurs, since no fermentation products are produced (11). Virtually all methanogens use H_2 as an electron donor, with CO_2 as the sole source of carbon, although some can use other substrates as well (e.g., methanol or acetate). Methane is produced by H_2 reduction of CO_2 in a respiratory process, whereby energy is produced. These properties allow the creation of an experimental system in which metals can be used as the sole electron source for methanogen metabolism and in which methane production can be conveniently followed as a measure of metal oxidation.

As shown in Fig. 1, H_2 formation involves proton consumption. Thus, the thermodynamics of the overall reaction should be affected by the concentration of protons; i.e., at lower pH the reaction should be more favorable. In this paper, we have examined the effect of pH on the ability of methanogens to use elemental iron (mild steel) coupons as the sole electron source for methanogenesis and the role of methanogens in biocorrosion as measured by weight loss of the metal coupons.

Methanococcus deltae ΔLH (5) was obtained from J. Reeve, *Methanococcus thermolithotrophicus* (14) was obtained from K. O. Stetter, and *Methanosarcina barkeri* 227 (17) was a gift from S. H. Zinder. Methanogens were grown with H_2-CO_2 (80:20, vol/vol) at 37°C (*M. deltae* and *M. barkeri*) or 64°C (*M. thermolithotrophicus*) as described previously (10, 22), except that *M. thermolithotrophicus* medium also contained 1.0 μM (each) sodium selenate and sodium tungstate. Effects of pH on corrosion were examined in anaerobic serum tubes (no. 2048-00150; Bellco Glass, Vineland, N.J.) containing 10 ml of medium. Medium pH was adjusted with sodium carbonate or HCl while being gassed with N_2-CO_2 (80:20, vol/vol), made anaerobic in bottles (540 ml, no. 223952; Wheaton Scientific, Millville, N.J.) under the same gas, and autoclaved. Because of the hazard of exploding bottles, a plastic shield was used to cover the bottles during and after autoclaving (9). The effect of pH on methanogenesis in H_2-abundant medium was examined with media prepared under H_2-CO_2 (80:20, vol/vol). Mild steel coupons (XC 18, NFA 35552; 2.3 ± 0.1 g; surface area, 6.8 cm^2; obtained from Centre d'Etudes Nucleaires, Cadarache, St. Paul-Liz Durance, France) were the source of metallic iron. Coupons were treated with 2.0 M HCl for about 2 min to remove surface corrosion products and rinsed immediately with distilled water. Coupons were

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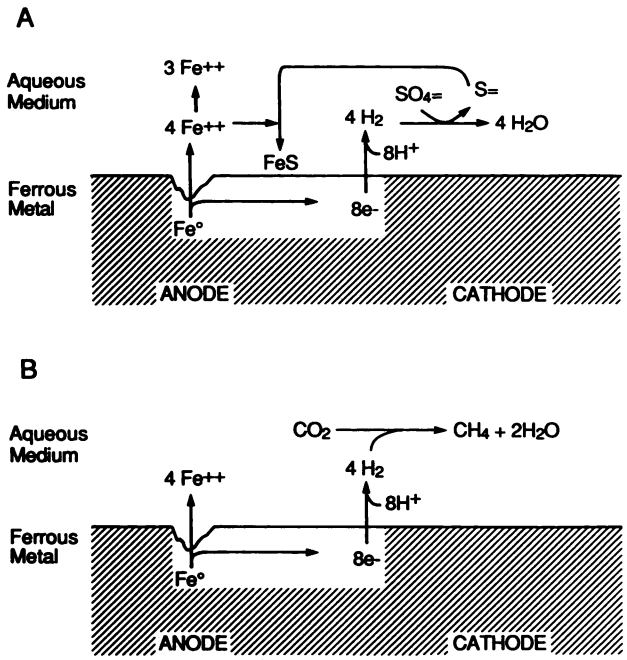

FIG. 1. The mechanism of cathodic depolarization in elemental iron as accelerated by sulfate-reducing bacteria (A) or by methanogenic bacteria (B). This concept was originally described by von Wolzogen Kuhr and van der Vlught (25) and occurs with sulfate-reducing bacteria (23) and methanogenic bacteria (11).

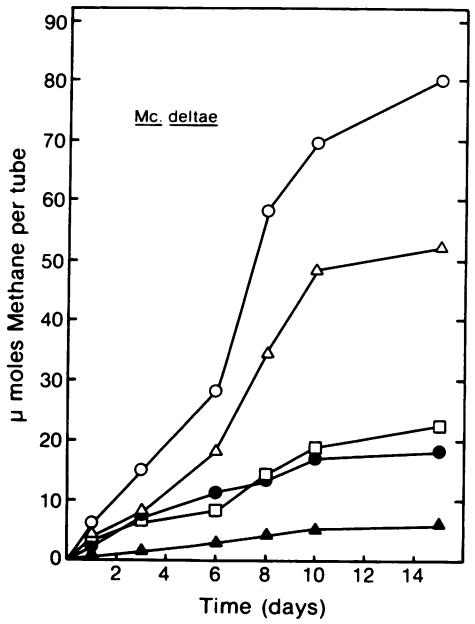

FIG. 2. Methane production by *M. deltae* using elemental iron (mild steel) coupons as the sole source of hydrogen, via cathodic depolarization. Symbols: ○, pH 5.4; △, pH 5.9; □, pH 6.4; ●, pH 6.9; ▲, pH 7.4. The graph indicates starting pH values; because of Fe^0 oxidation, the pHs cited above rose to 6.0, 6.4, 6.8, 7.3, and 7.5, respectively.

then washed with acetone, dried, carefully weighed, and placed in serum tubes. The tubes were made anaerobic by using N_2-CO_2 and autoclaved without medium. Media of various pHs, prepared anaerobically in bottles, were distributed into tubes aseptically, and the tubes were flushed with gas by using sterile hoses and needles. The medium in each tube was then reduced by adding a sodium sulfide solution to a final concentration of 2 mM. Tubes were inoculated (10% volume) with mid-log-phase cultures and incubated without shaking for 15 days. At each pH, there were duplicate tubes with metal and methanogens, metal without methanogens, and methanogens without metal, all with a gas phase of N_2-CO_2. Also, there were duplicate tubes at each pH with H_2-CO_2 and without metal coupons.

Methane production was measured by gas chromatography (1) with a Mininert locking syringe valve (no. 654051; Alltech Associates, Deerfield, Ill.). After the incubation period, metal coupons were removed from the tubes, cleaned ultrasonically (model no. SC 150 TH; Sonicor Instrument Corp., Copiague, N.Y.) in 4% sodium citrate, dried, and weighed (Gramatic type B6 analytical balance; Mettler, Zurich, Switzerland; readability, 0.05 mg; reproducibility, 0.1 mg). Corrosion was expressed as milligrams per day per square decimeter.

All methanogens examined (*M. deltae*, *M. thermolithotrophicus*, and *M. barkeri*) produced methane from elemental iron (mild steel coupons) as a source of electrons, most likely via the same cathodic depolarization mechanism we have previously demonstrated (2, 11). As shown in Fig. 2, *M. deltae* produced methane steadily over 15 days, with higher production rates in the lower pH media; for the entire period, the fastest and greatest methanogenesis occurred at pH 5.4, the lowest pH examined, and the least methane was produced at pH 7.4, the highest pH examined. Tubes with no methanogens produced no methane; tubes under N_2-CO_2 with methanogens but no metal produced an average of 2 μmol of methane per tube.

Methane production as a function of initial pH both with and without additional H_2 was plotted, as shown in Fig. 3. When elemental iron was the electron source available for methanogenesis, the pH optima were 5.4, 5.7, and 6.5, respectively, for *M. deltae*, *M. thermolithotrophicus*, and *M. barkeri*. This was in contrast to the pH optima for cells growing on abundant H_2-CO_2 medium, where maximal methanogenesis was observed at initial pH values of 6.9, 6.2, and 7.0, respectively, for *M. deltae*, *M. thermolithotrophicus*, and *M. barkeri*. The most clear-cut difference was with *M. deltae*, where the pH giving maximal metal-driven methanogenesis was 1.5 pH units lower than when abundant H_2 was present.

These observations are consistent with our hypothesis that chemically mediated production of H_2 via cathodic depolarization (Fig. 1) would be more favored at lower pH. The favorability of this reaction can be expressed by the $\Delta G^{0\prime}$ of equation 1:

$$8H^+ + 4Fe^0 + CO_2 \rightarrow CH_4 + 4Fe^{2+} + 2H_2O \quad (1)$$

At pH 7.0 and 37°C, $\Delta G^{0\prime} = -136$ kJ/mol of methane, which is relatively favorable, comparable to the energy in 4 mol of ATP. When this value is corrected for the changes in H^+ levels, the $\Delta G^{0\prime}$ is -182 and -228 kJ/mol of methane at pH 6 and 5, respectively; i.e., it is increasingly favorable as the pH drops. In contrast, the $\Delta G^{0\prime}$ is -90 kJ/mol of methane at pH 8; i.e., it is less favorable. Thus, even though at lower pH values the cells do not normally produce much methane from H_2-CO_2, with iron-driven methanogenesis there is a balance struck between the thermodynamic ability of the metal to

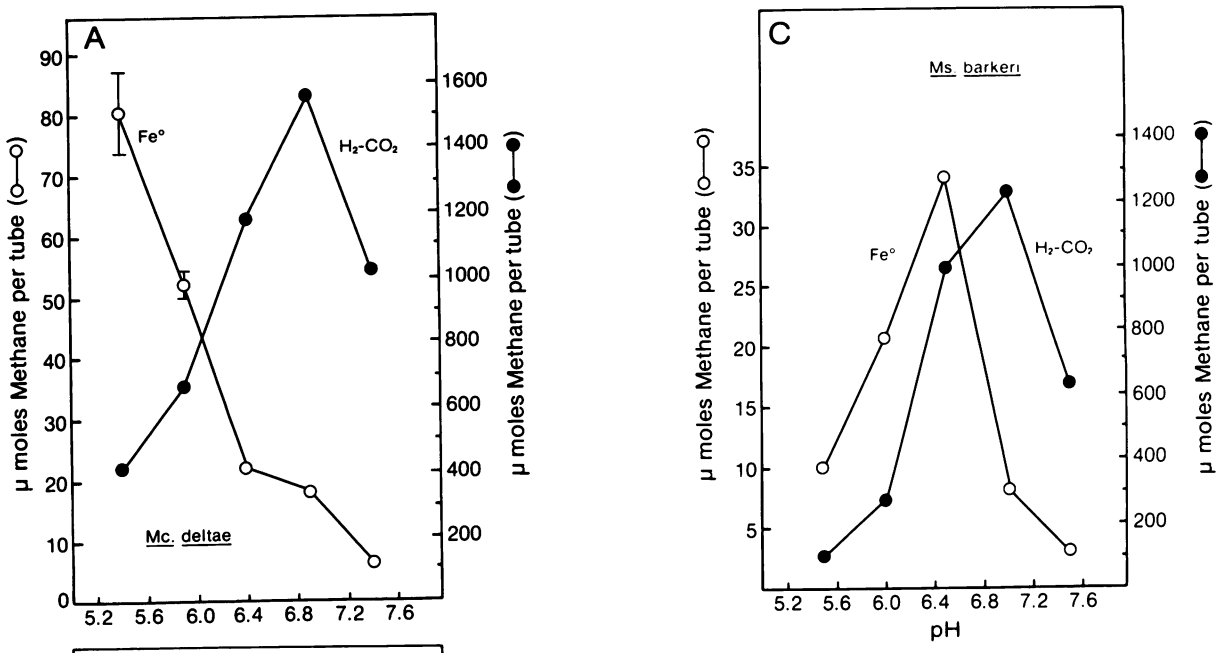

FIG. 3. Effect of pH on production of methane by methanogens. (A) *M. deltae*; (B) *M. thermolithotrophicus*; (C) *M. barkeri*. Symbols: ○, methane production with iron coupons as sole source of H_2; ●, methane production with excess H_2. All data points were taken after 15 days of incubation. The graph indicates starting pH values. Because of CO_2 consumption, the pH for all H_2-CO_2 tubes rose 0.2 to 0.4 pH units by the end of the experiment. Because of iron oxidation, the pH for Fe^0 tubes of *M. deltae* rose to the values given in the legend to Fig. 2. The pH for corresponding Fe^0 tubes of *M. thermolithotrophicus* rose to final pHs of 5.7, 6.1, 6.7, 7.1, and 7.7. The pH for corresponding Fe^0 tubes of *M. barkeri* rose to final pHs of 5.8, 6.5, 6.9, 7.3, and 7.8. Error bars indicate the extremes of duplicates when variation was great enough to be plotted outside of the symbols.

produce H_2 and the ability of the microbes to metabolize in suboptimal pH environments.

We also examined the effect of methanogenesis, in the absence of added H_2 and at various pH values, on the corrosion of the metal coupons as measured by weight loss. Corrosion rates with and without methanogens are shown in Fig. 4. As predicted thermodynamically, iron corroded chemically more at lower pH values; our data show a steady increase in corrosion rates as pH drops from 7.5 to 5.2. More corrosion is seen in the thermophilic and more saline medium of *M. thermolithotrophicus*, both of which conditions may accelerate the reaction nonbiologically. In all cases, the presence of methanogens accelerated corrosion, demonstrating for the first time that methanogens cause metal weight loss. If the corrosion rate with cells is compared with the chemical rate without cells, the maximal corrosion rates with cells are 52, 56, and 110% higher, respectively, for *M. deltae*, *M. thermolithotrophicus*, and *M. barkeri*; this extra corrosion is thus biocorrosion. If the maximal biocorrosion or the total corrosion in methanogen cultures is compared with the methanogenesis rates in Fig. 3, it is clear that the maxima for both are at pH 5.4, 5.7, and 6.5, respectively, for *M. deltae*, *M. thermolithotrophicus*, and *M. barkeri*. Thus, electron loss from the iron is directly responsible for the observed biocorrosion.

When maximal total corrosion rates in the methanogen cultures (10 to 14 mg/day/dm^2) are compared with those from several studies of pure cultures of *Desulfovibrio* spp. (1.3 to 21.0 mg/day/dm^2), they are comparable; however, the corrosion rate of 95 mg/day/dm^2 reported by Wakerly (26) and our methanogen estimate of 77 mg/day/dm^2 based solely upon methanogenesis stoichiometry (11) are considerably higher. On the basis of the methane production reported in this paper of 88 μmol per tube in 15 days by *M. thermolithotrophicus* and the accompanying weight loss of 14.2 mg/day/dm^2 (1.0 mg per tube per day), the amount of Fe^0 oxidation calculated from the methane produced would correspond to 1.3 mg of iron per tube per day; thus, the data are in reasonable agreement. These data suggest that meth-

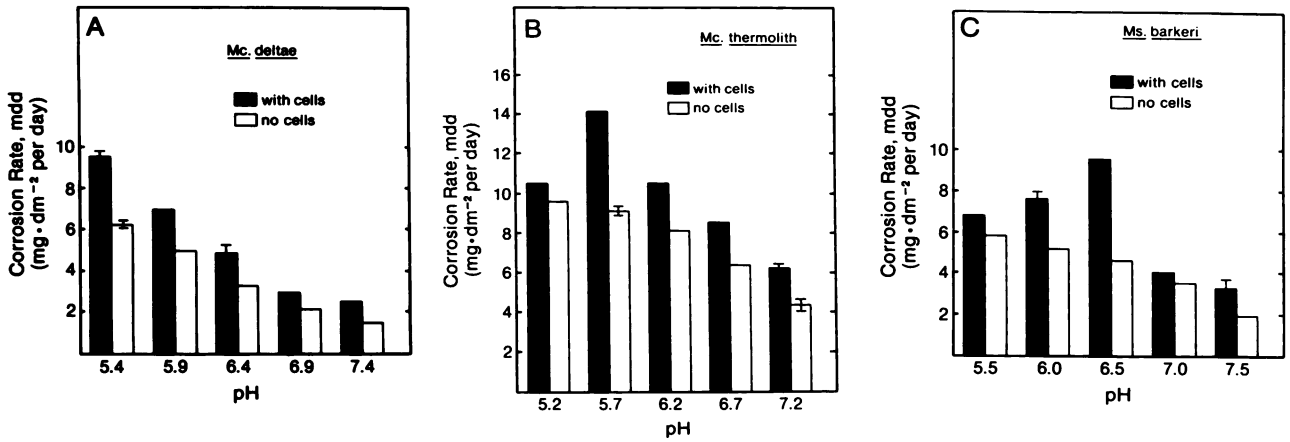

FIG. 4. Effect of pH on corrosion rates of iron coupons in the presence and absence of methanogenic bacteria. (A) *M. deltae*; (B) *M. thermolithotrophicus*; (C) *M. barkeri*. All incubation times were 15 days. Solid bars, media inoculated with cells; empty bars, no cells were present. The pH values at the end of incubation are given in the legend to Fig. 3. Error bars indicate the extremes of duplicates when variation was great enough to be plotted outside of the symbols.

anogens may play a significant role in biocorrosion, even when compared with the SRB.

It is likely that in biofilms where heterotrophs produce acids near methanogens, the resulting lower pH would create an environment favoring biocorrosion by both methanogens and SRB. It is also possible that biocorrosion of aluminum would be similarly accelerated under these conditions, except that the aluminum oxide layer is more cohesive than that of iron, and frictional removal of the oxide might be required to make the process significant. An interesting approach to the study of metal corrosion by methanogens might be to conduct experiments with a defined coculture of a homoacetic acid-producing heterotroph and a strictly hydrogen-dependent methanogen.

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