

## Involvement of Organic Acid During Corrosion of Iron Coupon by *Desulfovibrio desulfuricans*

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**Abstract** Microbiologically influenced corrosion (MIC) is an electrochemical process where the participation of microorganisms initiates, facilitates, or accelerates the corrosion reaction. Sulfate-reducing bacteria (SRB) reduce sulfate to sulfide and are known to be the most destructive microorganisms in anaerobic MIC. Accordingly, the current study attempted to elucidate the mechanisms involved and the relative importance of the corrosive products in SRB-induced corrosion. The measured rate of anaerobic corrosion of iron coupons by *Desulfovibrio desulfuricans* was  $89.9 \mu\text{g cm}^{-2} \text{d}^{-1}$ . Direct contact between the cells and the iron coupon did not seem to be necessary for corrosion to occur, since the corrosion rate was similar ( $100.8 \mu\text{g cm}^{-2} \text{d}^{-1}$ ) when the coupon was enclosed in a dialysis bag. The participation of sulfide in the corrosion process was only marginal, as the specific corrosion rate was 2.5 times higher in a sulfate-free pyruvate medium than in an  $\text{H}_2\text{S}$ -producing lactate medium. Acetate (18.8–22.1 mM), the end-product of pyruvate and lactate metabolism, was identified in the culture medium and thus presumed to play a major role in the corrosion process involving *Desulfovibrio desulfuricans*.

**Key words:** Acetate, biofilm, corrosion, *Desulfovibrio*, organic acid

Biocorrosion is the oxidization of metal resulting from the participation of microorganisms. It is a primary cause of failure in power plant water service systems and a variety of auxiliary coolers. The deterioration in such water system components has significant economic costs and can result

in an ecological impact on the environment if there is a failure at the plant [12]. Sulfate-reducing bacteria (SRB) are one of the best-known causes of microbially induced corrosion and account for 20% of all corrosion [8]. SRB-influenced corrosion is not limited to mild steel, but also affects a wide range of metals, including stainless steel and copper and its alloys [3].

Based on previous studies, corrosive products, such as sulfide, are thought to be responsible for the occurrence of the corrosion induced by SRB. The corrosion of metallic alloys in an aqueous anaerobic environment is an electrochemical phenomenon. SRB are associated with the process of cathodic depolarization, whereby sulfate is reduced to sulfur, which then reacts with available hydrogen and iron to form hydrogen sulfide and iron sulfide. The result is an alkaline environment, and any metal surface that cannot tolerate this environment will corrode [3].

Conversely, the effect of organic acids on corrosion has been demonstrated in many fungi and even in lichens [19]. The organic acids produced by *Fusarium* sp., *Penicillium* sp., and *Hormoconis* sp. have been found to cause localized corrosion and crack of sheathed carbon steel tendons [13]. However, such organic acid-mediated corrosion has not yet been tested with SRB, and the relative contribution between  $\text{H}_2\text{S}$  and organic acid on the SRB-induced corrosion process remains unexplored.

Accordingly, the current study investigated the corrosion of iron coupons by *Desulfovibrio desulfuricans* to elucidate the mechanisms involved. As a strictly anaerobic heterotroph, *D. desulfuricans* is a well-known corrosion-causing SRB that mainly uses low MW organic acids as its carbon and energy sources. The relative importance of the corrosive products in SRB-induced corrosion was also explored.

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## MATERIALS AND METHODS

### Strain and Culture Media

*D. desulfuricans* strain Norway 4 was obtained from the Korea Culture Type Collection (KCTC 1907) and used as the representative SRB species. The cells were grown on either Postgate medium C or D in serum bottles prepared under strictly anaerobic conditions using the Hungate technique [5]. Culture medium C had the following composition in g l<sup>-1</sup>: KH<sub>2</sub>PO<sub>4</sub>, 0.5; NH<sub>4</sub>Cl, 1; Na<sub>2</sub>SO<sub>4</sub>, 4.5; CaCl<sub>2</sub>·6H<sub>2</sub>O, 0.06; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.06; sodium lactate, 6; yeast extract, 1; FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.004. Sulfate-free medium D contained the following in g l<sup>-1</sup>: KH<sub>2</sub>PO<sub>4</sub>, 0.5; NH<sub>4</sub>Cl, 1; CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.1; MgCl<sub>2</sub>·6H<sub>2</sub>O, 1.6; yeast extract, 1; FeSO<sub>4</sub>·7H<sub>2</sub>O, 0.004; sodium pyruvate, 3.5. The cell growth was measured using a Coomassie blue protein assay [18] with a spectrophotometer at 595 nm (UV-1601PC, Shimadzu).

### Assessment of Corrosion

The iron coupons, kindly donated by POSCO (Pohang Steel Co., Korea), were 1 mm thick, 1.2×3.8 cm in size, and over 99% pure. The coupons were polished with SiC grit paper (AA-320) and degreased with a 10% NaOH solution at 80°C. Next, the coupons were rinsed with distilled water and 99% ethyl alcohol, then dried completely by storing in a desiccator overnight before use.

After different time intervals, iron coupons exposed to cells growing in medium C or D were retrieved from triplicate cultures. The coupons were first scraped to remove any biofilm using a cell scraper (Becton & Dickinson Co.), then sonicated in 0.5 N HCl (30 min) and distilled water (10 min) to remove any iron sulfides and hydroxides. Thereafter, the coupons were rinsed with 100% ethyl alcohol, dried with N<sub>2</sub>, and stored in a desiccator overnight before weighing. The iron coupons were also subjected to SEM observation (Model S-4300, Hitachi) after staining with an osmium tetroxide solution and carbon coating, as described by Domingo *et al.* [6]. The weight loss was determined with an analytical balance and used as a quantitative indication of the extent of nonuniform corrosion. The corrosion rate was expressed as a function of the weight loss per unit area with time in µg cm<sup>-2</sup> d<sup>-1</sup>. The corrosion rate divided by the protein yield of the cell cultures provided the specific corrosion rate as an indication of the medium-specific corrosion rate. Iron coupons incubated without cells, yet otherwise treated in the same manner, served as abiotic controls.

### Dialysis Bag Experiments

To determine whether it was necessary for the cells to be in contact with the iron coupon for corrosion to occur, contact was prevented by the use of dialysis tubing. An iron coupon placed inside a dialysis tube (seamless cellulose tubing, MW cutoff 12,000) was transferred to a serum

bottle containing the culture medium and autoclaved. The medium was inoculated with cells and the corrosion rate determined.

### Acetate Analysis

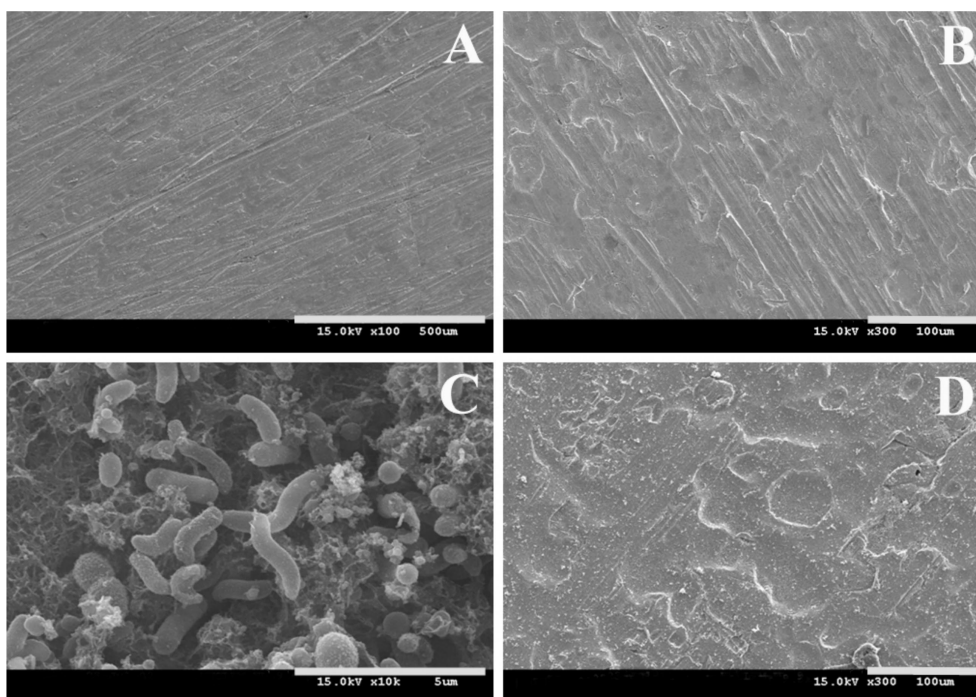
Acetate is the major metabolic end-product of *D. desulfuricans* when grown in medium C or D. Therefore, to assay the amount of acetate produced during the growth of *D. desulfuricans* at certain time intervals, 10 ml of spent medium C or D were transferred into a 160-ml serum bottle and sealed with a Teflon-faced butyl septum. The acetic acid was then volatilized by the addition of 1 ml of 6 N HCl and the bottles were kept in a 50°C water bath. A gas-tight syringe (Hamilton Co.) was used to remove a 100-µl sample of headspace gas, then the acetate concentration was determined using a gas chromatograph (5890 series II plus, Hewlett Packard) equipped with a flame ionization detector and capillary column, 0.53 mm in diameter × 30 m in length (HP-1, Hewlett Packard). The chromatograph was operated at an injector temperature of 210°C, detector temperature of 250°C, and nitrogen carrier gas flow rate of 10 ml min<sup>-1</sup>. The oven temperature was programmed to remain at 75°C for 1 min, then increase to 200°C at 10°C min<sup>-1</sup>, and finally held at 200°C for 3 min.

## RESULTS AND DISCUSSION

### Corrosion vs. Biofilm Formation

When a dialysis bag prevented contact between the *D. desulfuricans* cells and the iron coupon, the corrosion rate was 100.8±2.2 µg cm<sup>-2</sup> d<sup>-1</sup>. The corrosion rate of the control sample, which allowed cell-to-coupon contact, was similar at 89.9±8.9 µg cm<sup>-2</sup> d<sup>-1</sup>. No cells were observed on the surface of the iron coupon placed in the dialysis bag, while many cells colonized the surface of the coupon with no dialysis bag (Fig. 1). Thus, the current results indicate that cell-to-iron coupon contact and biofilm formation were not essential for the corrosion processes. However, this contradicts previous studies, where biofilm formation was identified as the key factor in microbially induced corrosion, as it altered the metal surface and accelerated the corrosion process [3, 7, 11]. In general, biofilm-corrosion product interactions on a corrosion-resistant alloy, such as many stainless steels, can lead to favorable conditions for corrosion initiation, and these conditions do not occur in the absence of a biofilm [19]. It has been postulated that a biofilm is important in corrosion as it absorbs hydrogen from the surface of the steel, thereby accelerating the corrosion process [14].

However, the slightly lower corrosion rate in the control sample (89.9±8.9 µg cm<sup>-2</sup> d<sup>-1</sup>) obtained in the current study would seem to suggest that biofilms actually protect against corrosion. It has already been shown that a protective



**Fig. 1.** Scanning electron micrograph of iron coupon surface before incubation with *D. desulfuricans* (A). When a dialysis bag prevented the cells from coming into contact with the coupon, the surface was similar to that of the uninoculated medium control (B). In contrast, cells heavily colonized the surface after a 21-day exposure (C), and localized pit corrosion occurred (D).

aerobic biofilm decreases the corrosion rate of mild steel by reducing the oxygen concentration at the metal surface [10]. Another recent report also noted a significant decrease in the corrosion rate in the presence of a biofilm [15]. Although these previous results were not obtained under strictly anaerobic conditions, the bacterial production of an unidentified inhibitor seemed to be responsible for protecting the surface of the metal plates from corrosion.

The current results from the dialysis bag experiment also implied that extracellular substances (MW less than 12,000 Da) produced by *D. desulfuricans* played a significant role in the corrosion process. To identify these extracellular substances, the effects of different growth substrates and their metabolites on corrosion were further examined.

### Effects of Growth Substrates

Pyruvate is not oxidized by *D. desulfuricans*, but rather utilized as a fermentative substrate yielding acetate and  $\text{CO}_2$  ( $\Delta G^{\circ} = -32.2 \text{ kcal mol}^{-1}$ ) [16]. There was no sulfate, a

terminal electron acceptor, in pyruvate-containing medium D. Therefore, since no  $\text{H}_2\text{S}$  was produced, the participation of  $\text{H}_2\text{S}$  in the corrosion was excluded when the cells were grown in medium D.

Iron coupons, incubated with cells growing in either medium C or D, were examined by SEM, which revealed localized pit corrosion on both samples, yet not on the control coupon (Fig. 1). When the coupons were weighed, cell growth resulted in a 1.5- to 2-fold higher corrosion rate when compared to the control (Table 1). The net corrosion rate was 30.7 and 64.3  $\mu\text{g cm}^{-2} \text{ d}^{-1}$  for medium C (lactate and sulfate) and D (pyruvate), respectively. Since the growth yield of *D. desulfuricans* was higher in lactate-containing medium C ( $\Delta G^{\circ} = -45.0 \text{ kcal mol}^{-1}$ ) [16], the difference in the corrosion rate increased, when calculated on a protein yield basis: The specific corrosion rate, the corrosion rate divided by the protein yield, was 2.5 times higher for the coupons incubated with the cells grown with pyruvate. Therefore, these results contradict previous

**Table 1.** Corrosion of iron coupons by *D. desulfuricans* growing in either lactate or pyruvate medium.

Tests		Corrosion rate ( $\mu\text{g cm}^{-2} \text{ d}^{-1}$ )	Specific corrosion rate <sup>a</sup> ( $\mu\text{g cm}^{-2} \text{ d}^{-1} \text{ mg}^{-1} \text{ protein}$ )
Lactate medium	without cells	59.2	-
	with cells	89.9	76.3
Pyruvate medium	without cells	57.1	-
	with cells	121.4	189.1

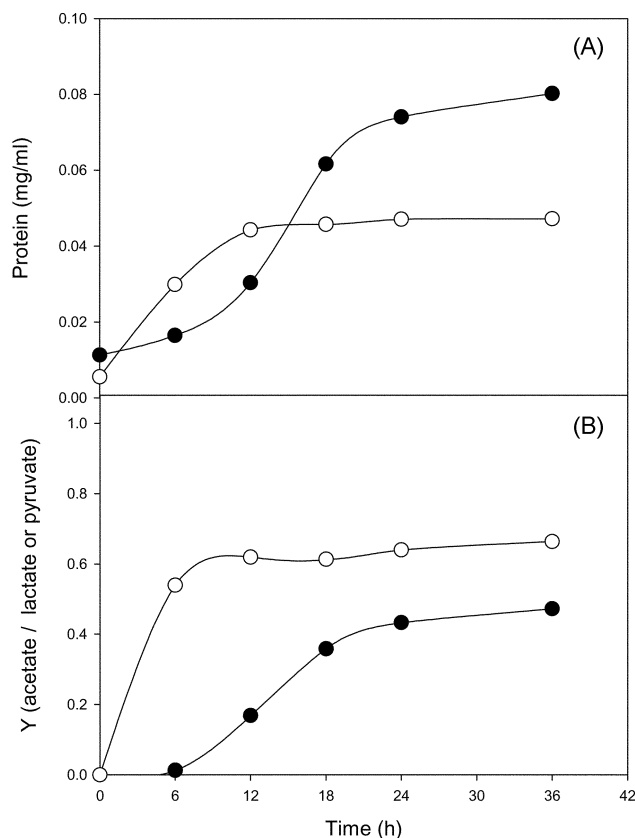
<sup>a</sup>Specific corrosion rate was expressed as a function of the weight loss per unit area with time divided by the protein yield of the cell cultures.

findings that suggest a critical role for biogenic H<sub>2</sub>S during the corrosion process [1, 3, 17]. It has also been reported that the hydrogen sulfide produced by SRB is a pitting (form of localized corrosion) activator and may be subsequently oxidized to thiosulfate, which is an even more aggressive pitting activator [1]. However, in the current study, it is worth noting that any possible carryover of sulfide from the lactate to the pyruvate-grown cells was eliminated by consecutively subculturing the cells several times in medium D. Therefore, it was concluded that sulfide was not absolutely required during the corrosion process, at least with *D. desulfuricans*.

### Involvement of Acetate

Acetate is the final metabolic product of *D. desulfuricans*, when it is grown on either pyruvate or lactate. Since *D. desulfuricans* is an incomplete oxidizer [2, 16], it does not oxidize acetate further into carbon dioxide, even in the presence of sulfate. Therefore, this would seem to suggest that acetate is the most probable corrosion inducer, regardless of the growth substrate.

During the growth of *D. desulfuricans* on lactate or pyruvate, the production of acetate and cell protein was



**Fig. 2.** Cell protein synthesis (A) and acetate production (B) with time caused by *D. desulfuricans* growing on either lactate (●) or pyruvate (○) medium. The acetate production values ( $Y_{\text{acetate/lactate or pyruvate}}$ ) represent the molar yield coefficient for the acetate production.

**Table 2.** Comparative abiotic corrosion assessed with selected organic acids (sodium salts) in absence of *D. desulfuricans* cells.

Organic acids <sup>a</sup>	Corrosion rate ( $\mu\text{g cm}^{-2} \text{d}^{-1}$ )
Acetate	209.0
Formate	57.7
Lactate	59.3
Pyruvate	57.1

<sup>a</sup>Organic acids were added to Postgate medium C, replacing lactate at 53.3 mM.

determined simultaneously (Fig. 2). Although the protein yield was higher on lactate than pyruvate, the molar yield coefficient for acetate production ( $Y_{\text{acetate/lactate or pyruvate}}$ ) was higher on pyruvate. The higher production of acetate in the pyruvate medium was also consistent with the higher net corrosion with the pyruvate-grown cells (Table 1). Similarly, a low pH environment, the major contributing factor for corrosion produced during *Hormoconis resiniae* growth, was previously found to be due to the production of organic acid [19]. Clear correlations between acidification, weight loss in aluminum specimens, and the mean fungal growth have also been reported [19].

In the absence of *D. desulfuricans* cells, the comparative abiotic corrosion was assessed with selected organic acids (sodium salts) added to Postgate medium C, replacing lactate at 53.3 mM. Acetate addition resulted in a higher corrosion rate when compared with formate, lactate, or pyruvate (Table 2). In addition, the added acetate lowered the medium pH to 4.2, while the pH remained at 6.5 with the other acids. Similarly, for metals, such as iron, cadmium, and magnesium, the rate of corrosion is known to increase rapidly with a low pH [4, 9]. Therefore, the acidic environment resulting from acetate production during the utilization of either lactate or pyruvate by *D. desulfuricans* was considered to be the factor that contributed most to the corrosion process.

Accordingly, the current study is the first to show that sulfide is not absolutely involved in anaerobic corrosion by *D. desulfuricans*, while the production of acetic acid plays an important role. As such, the present results should facilitate the development of a new strategy for preventing anaerobic corrosion and help in establishing a paradigm that can reduce the cost of corrosion prevention.

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