Fundamental Studies on Biomachining of Carbon Steel by Iron Oxidizing Bacteria†

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Abstract

Bacteria living in natural environments usually attack metals leaving unacceptable corrosion and causing critical deterioration. This negative phenomena is studied in this paper in a positive sense by means of control of these bacteria for the purpose of biomachining of metals. The main objective of this research is the achievement of a low cost machining process at normal temperature with less energy involvement and more involvement of bacteria in biomachining without straining them. The main focus in this paper is on the effect of Thiobacillus ferrooxidans, iron oxidizing bacteria and their ability to oxidize iron. It has been found that the extent of corrosion of carbon steel in the presence of Thiobacillus ferrooxidans was remarkably large. A tendency towards preferential corrosion of ferrite was also observed. The results of our experiments show that biomachining using Thiobacillus ferrooxidans is possible by controlling the structure of steel.

KEY WORDS: (Biomachining), (Bacteria), (*Thiobacillus ferrooxidans*), (Carbon Steel), (Machining), (Corrosions), (Exposure Test)

1. Introduction

The target of this research is to examine the feasibility of biomachining of metal by using bacteria.

Recently, a corrosion phenomenon caused by some kinds of bacteria has received attention [1,2], and is called microbioligically influenced corrosion (MIC). The influence of metallurgical microstructure on MIC phenomena was also reported [3] as a negative factor.

If a corrosion behavior by bacteria can be applied to a machining process of metal surface as a positive factor, then the process would be environmental friendly, free of residual strain, and offer energy conservation.

A bacteria called *Thiobacillus ferrooxidans* (*T. ferrooxidans*) has an oxidation capability with iron. This bacteria utilizes the energy produced by a process based on the oxidation of Ferrous(Fe^{2+}) to transform into Ferric(Fe^{3+}). This iron oxidation of *T. ferrooxidans* has been reported for metal surface processing [4].However, the biological action of the bacteria is not applied yet as a selective process for machining of metallurgical microstructure. Therefore, it has been examined for the development of bacteria fabrication techniques (Biomachining) for metal.

2. Experimental Method

Experimental Coupons

In our work, the workpiece was a 10 mm thick low alloy steel plate of a ferrite pearlite metallurgical microstructure, as shown in **Fig. 1**. The chemical compositions of this alloy are given in **Table 1**. A coupon for exposure test was cut with a micro cutter to a size of 10-20 mm. The observation surfaces were ground with emery paper and also diamond paste. The coupons were cleaned with acetone using an ultrasonic washing apparatus and sterilized with ethyl alcohol.

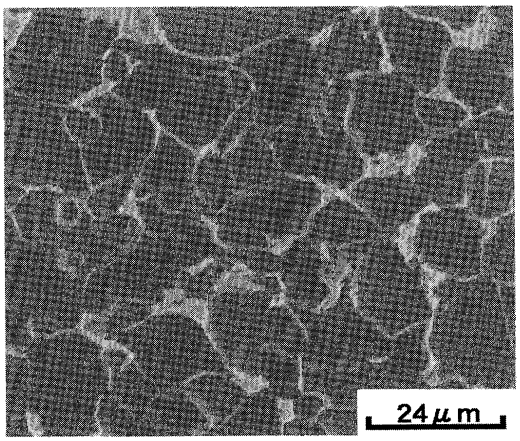

Fig.1 SEM micrographs of low alloy steel showing microstructure

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Table 1 Chemical composition of low alloy steel used (mass%)

C	Si	Mn	P	S
0.090	0.130	0.560	0.011	0.017
Ni	Cr	Cu	Sn	Fe
0.091	0.158	0.116	0.008	Bal.

2.2. Exposure test in Matsuo Mining Water

A high density of natural bacteria usually may be found in mines drainage water. The drainage water used in our study was gathered from a neutral processing facility of Matsuo in Iwate Prefecture, Japan. The chemical analysis of the water is shown in **Table 2**. An Iron oxidation bacteria named *T. ferrooxidans* at high density (10^8cell/ml) exists in the liquid that includes rich chemical content of iron, and sulfur elements etc. as nutrition resources for the bacteria. The acidity was pH2-3.

The water used was mixed with the mining water and distilled water, it inserts in an Erlenmeyer flask, which was put into an incubator, and maintained at a constant temperature of 303K. The exposure test period was 28 days. A half volume of the medium was exchanged every 14days with new liquid with active *T. ferrooxidans*, to hold constant activity condition of bacteria.

The samples were investigated with a scanning electron microscope (SEM) after exposure test completion.

Table 2 Chemical composition of Matsuo-mine drainage water (mg/ℓ)

COD	PO_4^{3-}	Ca^{2+}	Fe^{2+}	Fe^{3+}
1300	<0.04	52	9100	<100
Cd	T-N	SO_4^{2-}	Al	As
<0.005	53	16000	69	1.67

2.3Exposure test in Medium with Pure *T. ferrooxidans*

Pure *T. ferrooxidans* (JCM : No. 7811) was obtained from the Japan Collection of Bacteria (JCM), and cultivated in Silverman9K [2] medium whose chemical composition is given in **Table 3**. An air-purged cultivation method was used with the medium including pure bacteria put into an Erlenmeyer flask as shown in **Fig. 2**.

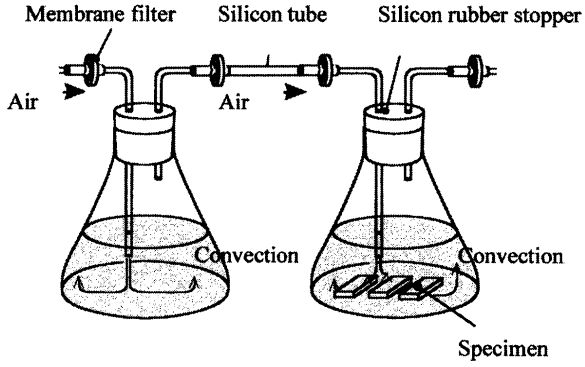


Fig. 2 Appearance of exposure test procedure

Table 3 Chemical composition of 9K medium (g)

$(NH_4)_2SO_4$	KCl	K_2HPO_4	$MgSO_4 \cdot 7H_2O$
3	0.1	0.5	0.5
$Ca(NO_3)_2$	$FeSO_4 \cdot 7H_2O$	Distilled Water	
0.01	20	1000	

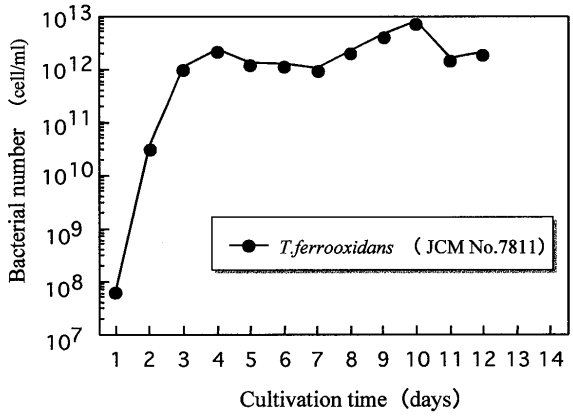


Fig. 3 Effect of cultivation on bacterial number

The bacteria number in the medium was observed and counted directly under a microscope and the images were recorded using a CCD camera. **Figure 3** shows the time variation of the bacteria number in the flask. Exposure tests continued for 5 days while the bacteria density was at a maximum level of 10^{12}cell/ml.

The volume loss of the ferrite microstructure of the tested samples was estimated by an analytical method using an SEM image. The influence of the metallurgical microstructure on the bacteria's corrosion phenomenon was examined.

3. Experimental Results and Discussion

3.1 Case of Matsuo mining water

Figure 4 shows the surfaces of low alloy steel samples after exposure tests in mining water. The coupon surface was widely covered by corrosion waste and corroded remarkably as shown in Fig. 4 (a). Fig. 4 (b) shows corroded naked surfaces, which were exposed at areas where the corrosion waste sloughed off. The exposure face shown in Fig. 4 (c) indicates that a clear lamella structure was formed by cementite. This shows that the ferrite microstructure between the cementite lamella corrode selectively. Furthermore, Fig. 4 (d) shows bacteria having the form of jelly-beans and balls attached to a sample surface exposed in mining water. Therefore, the mining water mainly consists of *T.ferrooxidans* and other kinds of bacteria with different shapes.

T. ferrooxidans is classified as aerobic bacteria, chemoautotrophs. The bacteria utilizes energy that occurring when iron and sulfur are oxidized as a resource needed for a life activity. *T. ferrooxidans* uses three kinds of iron oxidation mechanism, direct oxidation mechanism, indirect oxidation mechanism and also those intermediate

mechanisms[5]. A direct oxidation mechanism require the bacteria cell to contact directly on the metal surface[6] and oxidize and dissolve by means of its own enzyme. On the other hand, an indirect mechanism is produced by the pure chemical reaction when the oxidation of iron occurs by sulfuric acid involving ferric sulfate generated by the bacteria.

The ferrite that existed in a sub-micron scale gap of the steel microstructure was dissolved selectively, and is explained by an indirect oxidation mechanism of *T. ferrooxidans*, because the size of bacteria is bigger than the gap and cannot enter. Ferric (Fe^{3+}) as an oxidizer produced by *T. ferrooxidans* remotely reacts with a ferrite microstructure more than a cementite structure that has carbon connected and no free Fe.

Judging from the exposure tests in the mining water, a bacteria can be applied to a micro-machining process corresponding to the metallurgical microstructure.

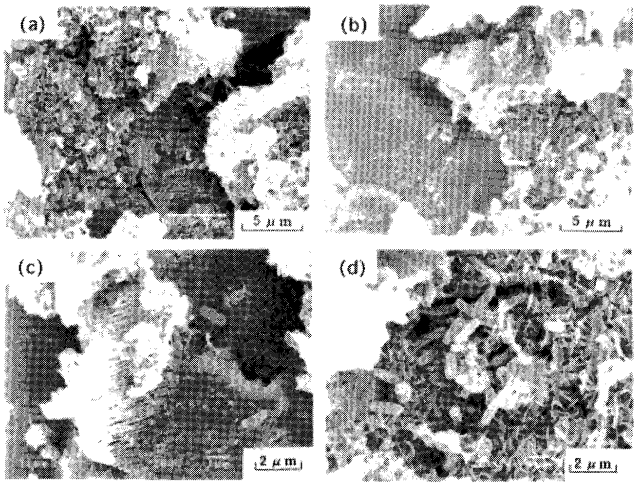


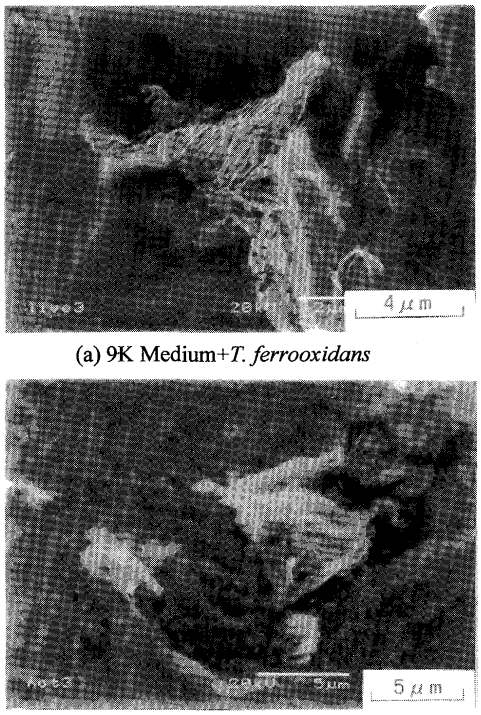
Fig. 4 SEM images of surface condition of low alloy steel after 28 days exposure in Matsuo-mine drainage water.
(a) Appearance of the specimen surface.
(b) The surface where the corrosion product exfoliated.
(c) The surface where cementite remained.
(d) Bacteria adhered on the surface.

3.2 Case of Pure *T. ferrooxidans* Medium

Figure 5 (a), (b) are SEM observations of samples after dipping in the medium and also comparing with one distilled medium. The white microstructure Fig. 5 is pearlite. The surface roughness of the coupon dipped in the medium including the bacteria was remarkable and the lamella structure was observed clearly. Ferric ion (Fe^{3+}) produced by *T. ferrooxidans* easily reacts with iron and produces Ferrous ion (Fe^{2+}) in the ferrite microstructure that has no carbon connection. Then the bacteria uses Ferrous ions as an energy source and again changes them to Ferric.

Figure 6 shows the relationship between the weight loss of the sample and its dipping time in each liquid. A remarkable weight loss by corrosion in the medium with pure *T. ferrooxidans* was observed comparing to a distilled nutrient broth. From this case, the iron oxidization capability of *T.ferrooxidans* will effectively act to increase the corrosion volume of low alloy steel. The relation between the dip time and the area of ferrite are shown in **Fig. 7**. Here, a ferrite area fraction is defined by the following equation.

$$\text{Area of ferrite} = \frac{\text{Total area - Pearlite area}}{\text{Total area}} \times 100\%$$



(a) 9K Medium+*T. ferrooxidans*

(b) 9K Medium

Fig. 5 SEM image of surface condition of low alloy steel after 3 days exposure

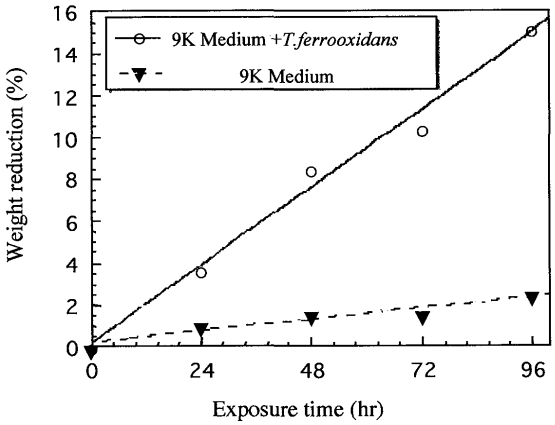


Fig. 6 Relationship between the rate of weight reduction and exposure time

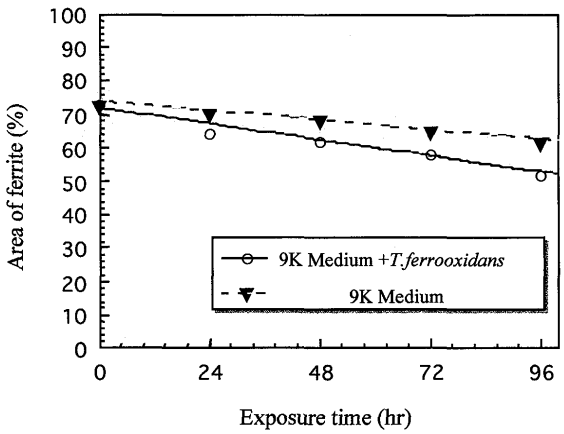


Fig. 7 Relationship between area of ferrite and exposure time

The decrease of an area fraction of ferrite in a live bacteria medium is bigger than in a distilled one. Therefore, the data indicates that *T. ferrooxidans* promotes the dissolution of the ferrite of a sample surface.

4. Result

(1) The ferrite microstructure of steel was subjected to preferential corrosion influence by *T. ferrooxidans*, which acts as an indirect system by means ferric (Fe^{3+}) ions.

(2) The result suggests that the feasibility of micro scale order removal biomachining of controlled microstruc tures in low carbon steels using *T. ferrooxidans* is possible.

Acknowledgement

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Referances

1) Y.Kikuchi, "Microbiologically Influenced Corrosion on Welded Metal", Welding Technology, Vol.47, no.7, pp.119-127, Jul.1999.

2) P.Silverman and D.G.lundgren, "Studies on chemoautotro phic iron bacterium *Ferrobacillus ferrooxidans*", J. Bacteriol., Vol.77, pp.642-647, 1959.

3) D.Walsh, D.Pope, M.Danford and T.Huff, "The Effect of Microstructure on Microbiologically Influenced Corrosion", Joining of Materials, pp.22-30, Sep.1993.

4) Y.Uno and A.Okada, "Biomachining of Metal by Bacteria", Journal of High Temperature Society, Vol27-1, 4-9, Jun. 2001.

5) Y.Konishi, "Recent Developments in Research on Bioleach ing", Journal of High Temperature Society, Vol.27, pp.28-33, 2001.

6) K.Sreekumari, K.Nndakumar and Y.Kikuchi, "Bacterial Attachment to Stainless Steel Welds", Biofouling, vol.17, pp.303-316, 2001