Biomachining of Stainless Steel Using Bacteria†

MIYANO Yasuyuki*, TSUBONUMA Takeshi *, K.R. Sreekmari, OMORI Akira *** and KIKUCHI Yasushi*****

Abstract

Bacteria having the ability to produce corrosion pits on metal surface were detected from a Microbiologically Influenced Corrosion (MIC) failure case analysis. To apply the corrosive ability of these bacteria to material processing is the aim in the present study. This is a new type of machining process utilizing biological principles. Hereinafter this process is expressed as biomachining.

To investigate the possibility of biomachining, the following experiments were planned. In the 1st experiment, Bacillus sp., detected as the cause of the corrosion of stainless steel in an MIC failure case, was selected as the test strain for processing. Also, the optimal conditions for machining in the case of Bacillus sp. were examined. In the 2nd experiment, a prototype of bioreactor for processing was designed. An experiment for biomachining using this reactor was carried out.

KEY WORDS: (Microbiologically Influenced Corrosion (MIC)), (Stainless Steel), (Microorgan-ism), (Micromachining process), (Biomachining)

1. Introduction

Microbiologically Influenced Corrosion (MIC) is caused by various microbes present in the natural environment. Even in mild environments, the generation of severe pitting and high corrosion rates are reported.[1] These are some of the main characteristics of MIC.

From the viewpoint of mechanical engineering or material science, the mechanism of MIC, especially corrosion ability of microbes is interesting. Because, if this ability is applied to material processing, machining of metals using microbes as a tool may be realized. According to this concept, materials are processed under normal temperatures or occasionally in mild environments. This is a novel type of low energy processing which is based on biochemistry. Hereinafter this process is expressed as biomachining.

The purpose of the present paper is to investigate the possibility of biomachining. In the 1st experiment, *Bacillus* sp., one of the bacteria which cause MIC in stainless steel was selected. It was found that the corrosion ability of this bacteria derived from two factors. One is the adhesion to the metal surface, and the other is the metabolic acid production. To explore the optimum conditions for processing, the difference in bacterial adhesion and metabolic reaction according to the nutrient media was examined. In the 2nd experiment, as a result of the former experiment, a prototype of bioreactor for processing was designed, and the processability examined.

2. Experimental Procedures

2.1. 1st experiment

2.1.1 Test solution

Two types of media were prepared. One was Difco nutrient broth (protein base) and the other was PYG medium (carbohydrate base). To investigate optimal medium concentrations, 4 types of concentration profiles were set up as shown in **Table 1**.

Table 1 Chemical composition of medium used (g/l)

(a) Nutrient Broth Medium (g/l)

	NB	NB-1	NB-2	NB-3
Beef extract	3.0	0.3	0.03	0.003
Peptone	5.0	0.5	0.05	0.005

(b) Poly peptone・Yeast extract・Glucose Medium (g/l)

	PYG	PYG-1	PYG-2	PYG-3
Poly peptone	5.0	0.5	0.05	0.005
Yeast extract	1.0	0.1	0.01	0.001
Glucose	5.0	0.5	0.05	0.005

2.1.2 Material used

Coupons used were base metal of SUS304L stainless steel ($20\times10\times10^{t}$mm). Coupons were polished to 1000 grit with emery paper to a uniform surface finish.

2.1.3 Bacterial strain used

An isolate of *Bacillus* sp. from MIC causing water was used. This strain is reported to cause pitting of stainless steel[2].

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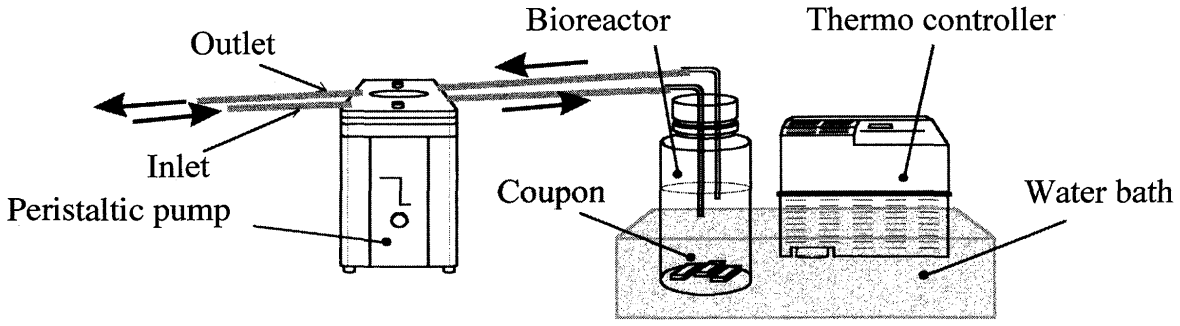


Fig. 1 Appearance of the prototype of bioreactor

2.1.4 Exposure studies

Exposure studies were conducted with the above described media. Media were taken in Erlenmeyer flasks and sterilized. 1ml of inoculum were introduced into each flask. Coupons were introduced into the medium aseptically and horizontally. The bacteria-containing liquids were kept in an incubator set at 298K with shaking (85 rpm). Coupons were retrieved for observation aseptically at 24 hours, 72 hours and 120 hours and observed using a epiflourescence microscope. At the same regular intervals, the change in pH of the medium and the bacterial number in the medium were monitored. In another set of experiments, similar coupon exposure tests were conducted for 14 days. These 14 day coupons were used for SEM observation, ie. to test for a machined area formation by bacteria.

2.1.5 Epiflourescence microscopic observation

Coupons taken out from the medium were air dried inside a sterile chamber and were stained with acridine orange fluorescence dye (0.01 w/v %), prior to observation. About ten different fields were selected randomly and the images were captured through a CCD camera. These images were further analyzed for bacterial adhesion area using image-processing software.

2.2 2nd experiment

To overcome the problems emerging in the 1st experiment, the experimental setup (the prototype of bioreactor) which enable a continuous supply of medium was be designed. (**Fig. 1**)

2.2.1 Medium

Media adjusted to concentrations of PYG-2 in the 1st experiment were used.

2.2.2 Material used

To examine the processability with respect to the metallurgical factors, various type of materials were used. They were SUS304L base metal (austenite single phase structure), SUS304L weld metal (δ -ferrite and austenite dual phase structure), and SUS329J (Duplex stainless steel). Coupons used were of the size $20\times10\times3^{t}$mm. Their surfaces were polished with emery paper to 1000 grid. In the case of SUS304L weld metal, the as-welded condition was used.

2.2.3 Bacterial strain used

An isolate of *Bacillus* sp. the same as in the 1st experiment and a strain of *Pseudomonas* sp. from MIC causing water were used. Both these strains are reported to cause pitting of stainless steel[2,3].

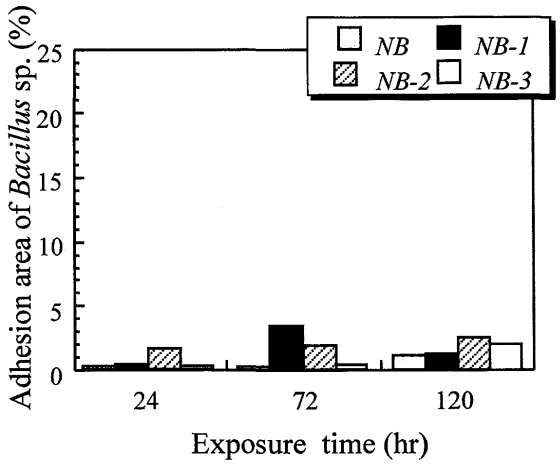
2.2.4 Incubation

The working volume of the culture medium in the bioreactor was 300ml. Isolated strains were used to evaluate their individual processing ability by carrying out processing experiments with a single strain at a time. 2ml of inoculum were introduced into each reactor. To exchange spent medium and fresh medium, a peristaltic pump was used. A constant medium flow rate was regulated at 10ml/h. These reactors were kept in a constant temperature water bath unit set at 298K.

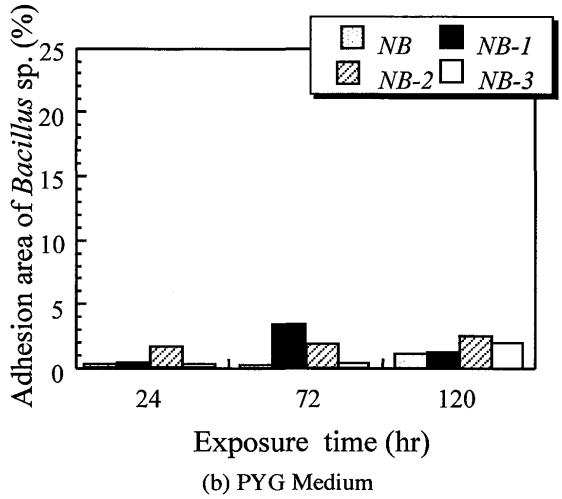
3. Results and Discussions

3.1 1st experiment

Figure 2 shows the result of analysis of percentage area of adhesion using imaging software. It is seen that bacterial adhesion is significantly greater in PYG media compared to NB media.



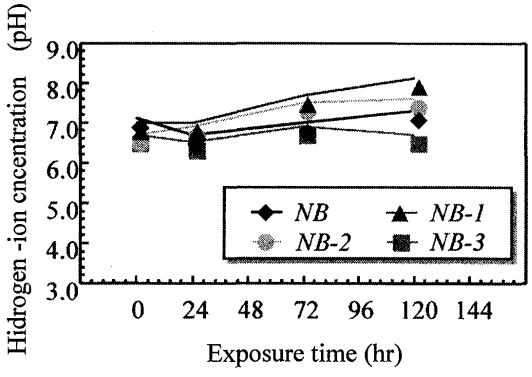
(a) Nutrient Broth



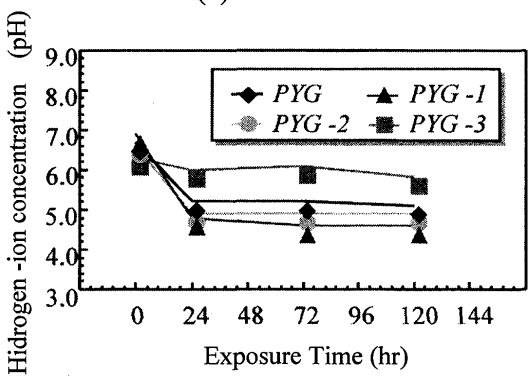
(b) PYG Medium

Fig. 2 Comparison of adhesion area of *Bacillus* sp. on experimental coupon in each media

The variation of pH in various culture media inoculated with *Bacillus* sp. as a function of time is given in **Fig. 3**. In various NB media, no significant change in pH was noticed. On the other hand, in various PYG media (except PYG-1), dramatic falls of pH were observed. It was interpreted that the organic acid production by *Bacillus* sp. was promoted more in PYG than NB.



(a) Nutrient Broth



(b) PYG Medium

Fig. 3 Variation of pH in Various Culture Media Inoculated with *Bacillus* sp. as a Function of Exposure Time

Figure 4 shows the SEM images of processed surfaces of coupons. Processed area was observed near the attached *Bacillus* sp..

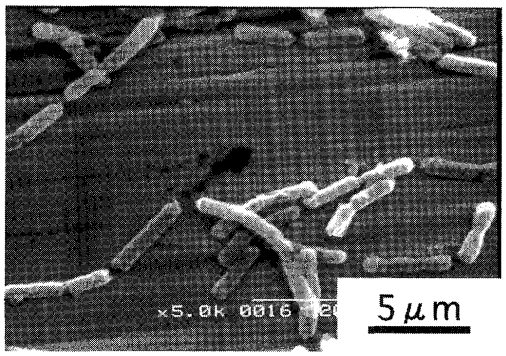


Fig.4 SEM image of processed surface of SUS304L

From the above result, it was clear that microbial adhesion and acid production which are the important processing factor of *Bacillus* sp. are promoted at the medium condition of PYG-2. But the drop of pH of medium derived from metabolic reaction decreased the number of living microorganism (**Fig. 5**).

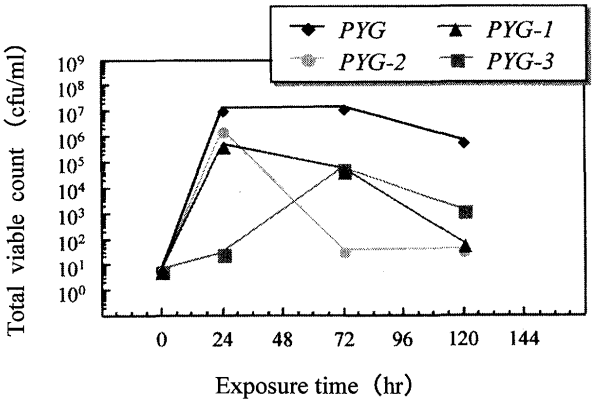


Fig. 5 Variation in Total Viable Count in Various Experimental Media as a Function of Exposure Time

3.2 2nd experiment

The variation of pH of medium inoculated with *Bacillus* sp. during the study is given in **Fig. 6**. At the beginning, a dramatic drop of pH was observed. Thereafter the low pH level was preserved. The total viable count of bacteria in the medium during the study is given in **Fig. 7**. At the beginning, a drastic increase and decrease were observed. But thereafter the bacterial number fluctuated between 10^5~10^6 (cfu/ml) through out the period of experiment. From the above result, it was considered that the continuous culture method succeeded in reconciling both the drop of pH derived from promotion of organic acid production and preservation of the

number of living microorganism.

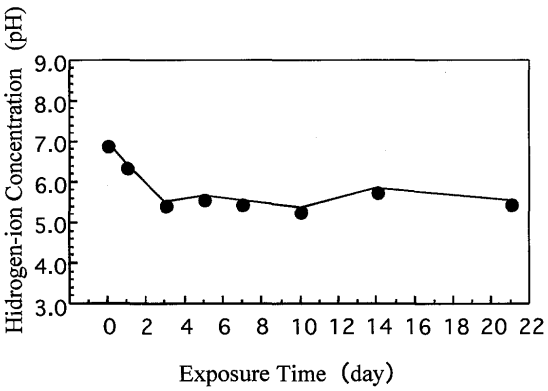


Fig. 6 Variation of pH of media inoculated with *Bacillus* sp. as a function of exposure time

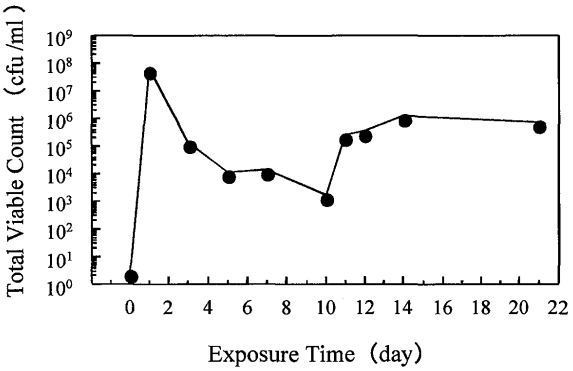


Fig. 7 Variation in total viable count in the experimental medium as a function of exposure time

Figure 8 shows the appearance of the test coupon after the processing. The surface of the coupon with prolific attachment of *Bacillus* sp. observed through environment scanning electron microscopy (ESEM) is shown in
Fig. 8 (a). It was concluded that the condition of the bioreactor is proper for bacterial adhesion or biofilm formation that leads to MIC.

The processed area on each coupon is shown in Fig. 8 (b), (c). In the case of single phase stainless steel, pitting corrosion was observed (Fig. 8 (b)). From this result, it was considered that there is a possibility of biomachining to make a micro bore or a micro groove in stainless steel. On the other hand, in the case of the dual phase material, spots of initiation of preferential attack were observed (Fig. 8 (c)). In fact, preferential attacks are frequently reported in MIC case analysis (**Fig. 9**)[2].
From these results, it was concluded that there is a possibility of processing based on controlling of metallurgical microstructure. From this concept, biomachining toward three-dimensional structure can be expected.

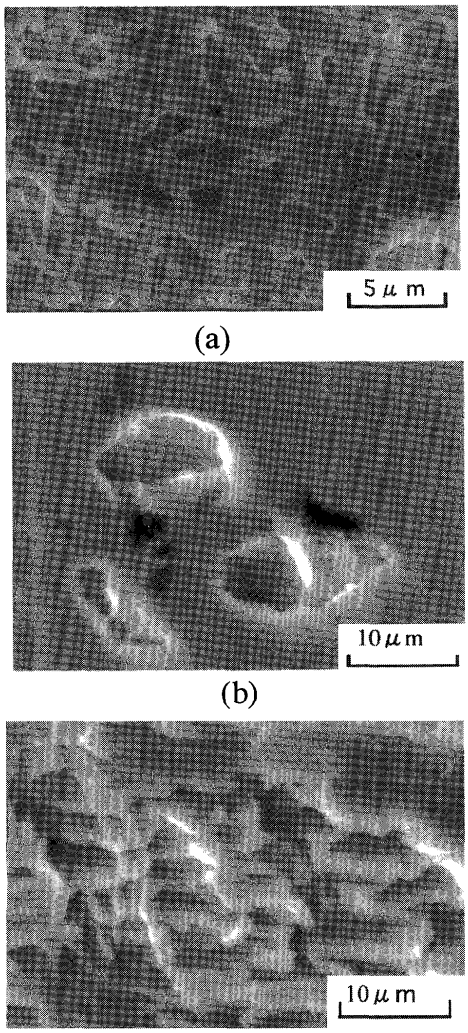


Fig. 8 SEM microphotograph of processed surface of stainless steel
(a) Surface stainless steel with prolific attachment of *Bacillus* sp. observed through ESEM
(b) Initiation of pitting on single phase stainless steel exposed to *Bacillus* sp. (7days processing)
(c) Initiation of pitting on dual phase stainless steel exposed to *Pseudomonas* sp. (21days processing)

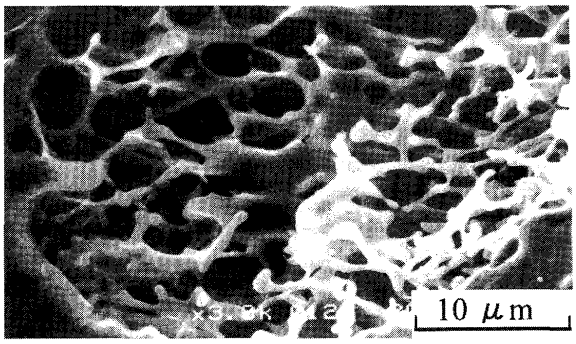


Fig. 9 SEM photomicrographs of preferential attack against austenite

6. Conclusions

The conclusions in the present paper are summarized as follows.

(1) Adhesion area of *Bacillus* sp. was significantly higher in PYG media compared with NB media.
(2) In PYG-2, the largest percent area of adhesion was detected.
(3) In various control media (without *Bacillus* sp.), pH remained constant.
(4) In various NB media, no significant change in pH was noticed.
(5) In various PYG media (except PYG-1), dramatic drops of pH was observed.
(6) PYG-2 medium was presumed one of the suitable media for biomachining using metabolic reactions.
(7) Processed area was observed near attached *Bacillus* sp..
(8) The continuous culture method succeeded in reconciling both the drop of pH derived from promotion of organic acid production and preservation of the number of living microorganism.
(9) Pitting area was observed in single phase and preferentially attacked area was observed in dual phase stainless steel.

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