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Autotrophic growth of *Acidithiobacillus ferrooxidans* by oxidation of molecular hydrogen using a gas–liquid contactor

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Abstract

The iron-oxidizing bacterium, *Acidithiobacillus ferrooxidans*, was cultivated on a medium without ferrous iron. Molecular hydrogen and air were supplied to the medium. It was found that *A. ferrooxidans* could grow with hydrogen in the pH range between 2.0 and 3.5. A trickle-bed contactor was used to increase the dissolution rate of hydrogen. The doubling time was increased and the cell concentration reached 4.0×10^9 cells ml^{-1} after 6 days. When the cells taken from the hydrogen medium were transferred back into the medium containing ferrous iron, the growth rate and the iron-oxidizing ability were the same as the predictions assuming that the microorganism grown with hydrogen was *A. ferrooxidans*.

Keywords: Biotechnological leaching; *Acidithiobacillus ferrooxidans*; Ion-oxidizing bacterium; Hydrogen bacterium

1. Introduction

The iron oxidizing bacterium, *Acidithiobacillus ferrooxidans*, has been used for enhancing the extraction rates of copper and uranium from mining wastes and low-grade mineral resources (Deng et al., 2000 and Nasernejad et al., 1999), the treatment of acid mine drainage (Umita, 1996 and Jensen and Webb, 1995) and purification of gases containing sulfur compounds (Malhotra et al., 2002, Pagella et al., 1996 and Jensen and Webb, 1995). In addition, this bacterium has been used for the desulfurization of coal containing pyretic sulfur (Pandey et al., 2005), the extraction of vanadium from spent catalysts (Bredberg et al., 2004) and to the amendment of an alluvial saline solodic soil (Stamford et al., 2002). *A. ferrooxidans* is acidophilic, aerobic, chemolitho-autotrophic and Gram-negative. It gains energy for growth by the oxidation of a proton to H_2O in a sulfuric acid solution. Ferrous iron is used as the electron donor and is oxidized to ferric iron.

Beside the oxidation of ferrous iron, *A. ferrooxidans* oxidizes soluble or mineral sulfides and inorganic sulfur compounds (Mason and Rice, 2002, Picher et al., 2002, Suzuki, 2001 and Kai et al., 2000). Since it is an autotrophic bacterium, the growth rate is significantly lower than that of heterotrophic bacteria. In addition, the maximum cell concentration is restricted to about 2×10^8 cells ml^{-1} in the usual culture method using ferrous iron due to the presence of an optimum ferrous-iron concentration. Recently, Kawabe et al. (2003) have studied the inhibitory effect of ferric ions on the activity. In order to expand the application of *A. ferrooxidans*, it is important to increase the maximum cell concentration. By applying the electrical reduction of ferric iron produced by the bacterial oxidation (Matsumoto et al., 1999, Nakasono et al., 1997, Blake et al., 1994, Taya et al., 1991 and Yunker and Radovich, 1986), the cell concentration can be enhanced even at a low total iron concentration.

Drobner et al. (1990) have found that *A. ferrooxidans* can grow by the oxidation of molecular hydrogen. They reported that the strain was less acidophilic during the growth on sulfides when cultivated on hydrogen. Hydrogenase was induced during hydrogen cultivation. The cells of *A. ferrooxidans* grown on hydrogen and on ferrous iron showed a 100% DNA homology. Fischer et al. (1996) have successfully purified the hydrogenase of *A. ferrooxidans* from the cells grown with hydrogen. In this case, the final cell concentration reached 4×10^9 cells ml^{-1}. This result indicated that the maximum cell concentration could be enhanced by hydrogen cultivation.

In this study, the gas–liquid contact was studied in order to enhance the hydrogen dissolution rate by using a trickle-bed type contactor at ambient pressure, and a new cultivator using molecular hydrogen was proposed.

2. Methods

2.1. Microorganism and medium

The strain of *A. ferrooxidans* used in the present study was isolated from the acid mine water of the Yanahara Mine (Japan). The organism was routinely maintained in 9 K medium (Silverman and Lundgren, 1959) with ferrous iron in shake flasks. The liquid medium contained (in kg m^{-3} water): $(NH_4)_2SO_4$, 3.0; KCl, 0.1; K_2HPO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 0.5; $Ca(NO_3)_2 \cdot 7H_2O$, 0.01. Ferrous iron was also contained and the initial concentration was adjusted to 8.0 kg m^{-3}. The pH of the medium was adjusted to 2.0 using sulfuric acid and cultures were incubated at 30 °C.

2.2. Growth media

In this experiment, the cultivation was carried out in a 300-ml

Erlenmeyer flask containing 100 ml of liquid medium. Hydrogen and air were passed through a humidifier then into the medium (flow rates 0.58 and 0.058 ml s^{-1}, respectively). Bacterial cultures were grown in 9 K medium without ferrous iron. The pH of the medium was controlled with 10 N sulfuric acid during the cultivation. The flasks were placed in a water bath at 30 °C. The initial concentration of cells was adjusted to 3.0×10^6 cells ml^{-1}. The cell numbers during growth were counted using a microscope at 600× magnification and a Thoma counting chamber. The flow rates of gases were controlled by mass flow controllers.

A trickle-bed contactor was used to increase the rate of hydrogen dissolution in the medium. Fig. 1 shows the system. Glass beads of 2.5 mm diameter were used as packing materials in the trickle-bed. The inner diameter of the column was 25 mm. Air containing carbon dioxide was supplied to the cultivator controlled at 30 °C at a flow rate of 0.075 ml s^{-1}. The medium lifted up by a pump was supplied to the trickle-bed from the upper side. The total liquid volume was 250 ml. Hydrogen gas was supplied at the flow rate of 0.083 ml s^{-1} from the bottom of the contactor. The medium that absorbed hydrogen was then returned to the cultivator at the flow rate of 0.25 ml s^{-1}.

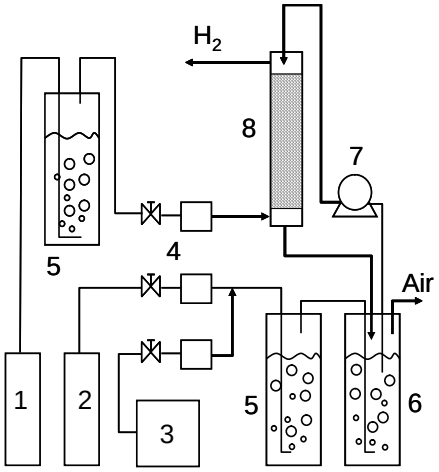

Fig. 1. Experimental system for hydrogen cultivation. Numbers symbolize: (1) hydrogen cylinder, (2) carbon dioxide cylinder, (3) air compressor, (4) mass flow controllers, (5) humidifier and (6) cell generator.

2.3. Test of iron-oxidizing ability

In order to examine the oxidizing ability of the cells cultured by molecular hydrogen, they were transferred back into the 9 K medium containing ferrous iron. The initial concentration of ferrous iron and the microorganisms were adjusted to 8.0 kg m^{-3} and 3.0×10^6 cells ml^{-1}, respectively. The concentration of ferrous iron was colorimetrically analyzed by the *o*-phenanthroline (1,10-phenanthroline) method (APHA, 1992).

3. Results and discussion

A. ferrooxidans grew with molecular hydrogen under certain conditions. During the cultivation, the pH slightly decreased. Fig. 2 shows the change in the cell concentration vs. culturing time when the gases were supplied by bubbling. In this case, the ratio of carbon dioxide to air was changed while the ratio of hydrogen to air was fixed at 10. The pH was controlled at 2.5. The initial cell concentration was 3×10^6 cell ml^{-1} increased with culturing time and reached 5×10^8 cells ml^{-1} after 250 h. In the case of the ordinary iron medium, the cell concentration reached about 1×10^8 cells ml^{-1} after a 70 h cultivation. Therefore, the growth rate was about 1/3 for the hydrogen cultivation, while the maximum cell concentration was higher for the hydrogen cultivation. The growth rate was slightly higher when the ratio of the partial pressure of carbon dioxide to air was 0.2. However, the concentration of carbon dioxide was not considered to be the rate determining step.

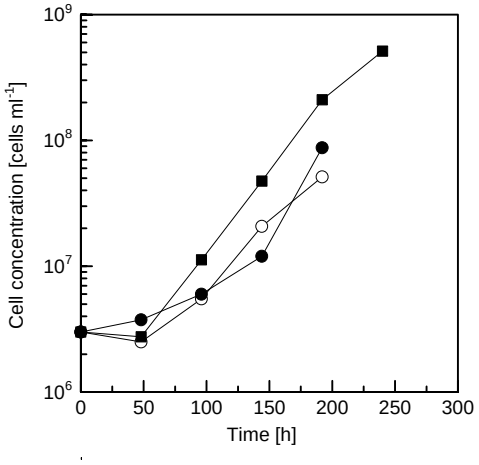

Fig. 2. The change in the cell concentration with time for the gases mixed with carbon dioxide. The symbols represent the ratio H_2:air:CO_2: (○) 10:1:0; (●) 10:1:1; (■) 10:1:0.2.

Fig. 3 shows the relationship between the ratio of hydrogen to air and the cell concentration at 200 h when hydrogen was supplied with air by bubbling. When the ratio was low, the supply of hydrogen was not sufficient. On the other hand, the concentration of dissolved oxygen and carbon dioxide were low when the ratio was high. Although Drobner et al. (1990) did not observe growth below pH 2.5, it was found that *A. ferrooxidans* could grow on hydrogen even at pH 2.0. However, the conditions suitable for growth were limited when the pH was 2.0.

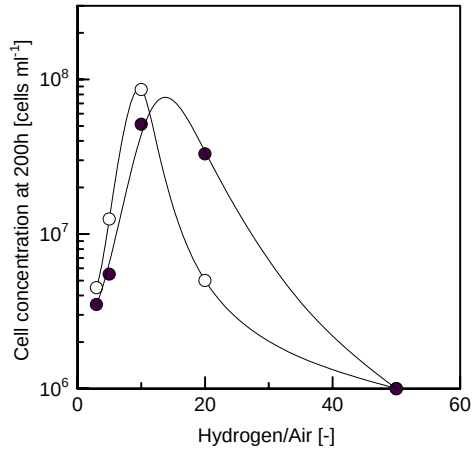

Fig. 3. The effect of the ratio of hydrogen to air on the cell concentration at 200 h. The symbols represent pH: (○) 2.0; (●) 2.5.

The final cell concentration reached 2.4×10^9 cells ml^{-1} after a 200 h cultivation when the ratio of hydrogen to air was 10 and the pH 2.0. Fig. 4 shows the change in cell concentration and the change in the concentration of ferrous iron when the microorganisms were transferred back into the iron medium from the hydrogen medium. The medium containing the microorganism cultured on hydrogen was diluted 800 times, and then transferred back to the medium containing ferrous iron. The dashed lines in this figure were the calculations based on the following

assumptions: (1) the microorganisms grown in the hydrogen medium were hydrogen bacteria and they did not have any iron oxidizing ability, (2) *A. ferrooxidans* did not grow during the hydrogen cultivation. In this case, although the initial apparent concentration of the cells was 3.0×10^6 cells ml^{-1}, the concentration of *A. ferrooxidans* was only 4.3×10^3 cells ml^{-1}, (3) only the iron oxidizing bacterium grew after they were transferred back to the ferrous-iron medium, and (4) the number of all microorganisms was counted.

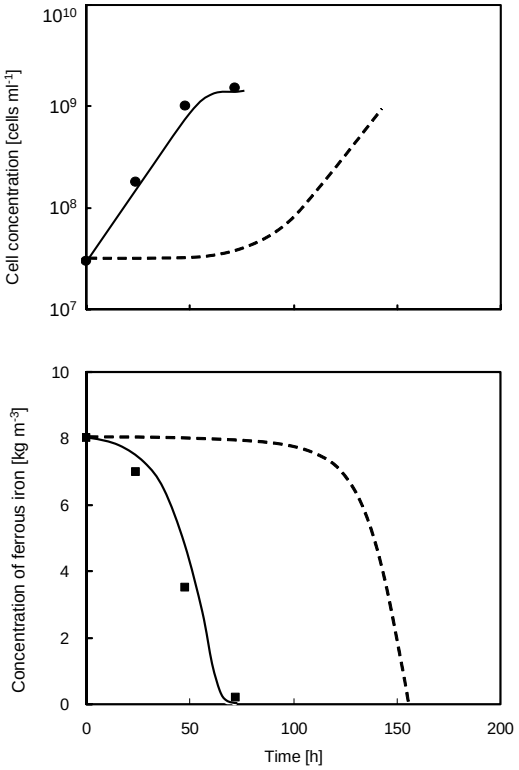

Fig. 4. Growth curve and consumption of ferrous iron the cells transferred back into the iron medium from the hydrogen medium. The lines represent the calculations based on the assumption that the cells grown with hydrogen are: (—) iron-oxidizing bacterium; (- - -) not iron-oxidizing bacterium.

The solid lines were obtained by assuming that the cells grown in the hydrogen medium were the iron-oxidizing bacterium. In this case, the value of the growth yield of the bacterium on ferrous iron was 1.9×10^{13} cells kg^{-1} and maximum specific growth rate of bacteria was 0.086 h^{-1}. The experimental data agreed with the values shown by the solid line. This result indicated that the cells transferred back to the iron medium were the iron oxidizing bacteria. Therefore, *A. ferrooxidans* could grow on molecular hydrogen. In addition, the ability to oxidize ferrous iron by the cells grown on molecular hydrogen was not reduced. Also, the cell yield was not influenced by the hydrogen cultivation.

The cultivation of *A. ferrooxidans* in a hydrogen medium has some advantages. The iron precipitate, jarosite $[KFe_3(SO_4)_2(OH)_6]$, is not produced. The formation of jarosite causes some problems such as plugging of an incubator. The maximum cell concentration increased in the case of hydrogen cultivation and it reached 2.4×10^9 cells ml^{-1}. This was almost the same as that obtained by Fischer et al. (1996), 3×10^9 cells ml^{-1}. These values were also almost the same as the maximum value obtained by the electrical reduction of ferrous iron. Generally, hydrogen bacteria have a comparatively large growth rate among the autotrophic bacteria. Therefore, the growth rate of *A. ferrooxidans* would be enhanced by optimizing the hydrogen cultivation conditions. Drobner et al. (1990) observed the doubling time of 5 h between pH 3.0 and 5.8. This value was much higher than that for the cultivation on an iron medium.

In order to investigate the effect of the gas composition on the cell growth, a batch cultivation was carried out. A 30 ml aliquot of the inoculated medium was poured into a 100-ml vessel. The initial cell concentration was adjusted to 3.0×10^6 cells ml^{-1}. A Tedlar gas bag of 1 L was connected to the vessel. After adjusting the gas composition, the bag was filled with the mixed gas of H_2–air–CO_2 and it was sealed. Since the concentration of CO_2 in the used air was less than 0.04%, its effect was insignificant. The cell concentration was measured after an 8-day cultivation at 30 °C. Although the growth rate was slower for this method than for the bubbling method, the effect of the gas composition on the cell growth could be studied. Fig. 5 shows the effects of the gas composition on the growth rate. The ratio of the cell concentration at 8 days to the initial cell concentration is shown in this ternary diagram. Apparently the growth rate increased with the increasing hydrogen concentration in the gas and the microorganism did not grow when the hydrogen concentration was less than 50%. The optimal concentration in the feed gas was 70–80% for H_2, 5% for air and 15–20% for CO_2. This result indicated that the growth rate would be enhanced by increasing the dissolution rate of hydrogen into the medium.

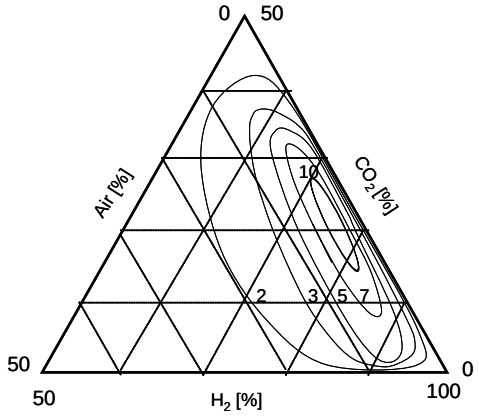

Fig. 5. The effect of gas composition on the contour line showing the relative cell concentration after 8 days.

Generally, the longer bed length is preferable for increasing the hydrogen concentration in the medium. Therefore, the effect of the bed height on the growth rate was studied. The bed height was changed from 75 to 225 mm. The other experimental conditions were not changed. Fig. 6 shows the relation between the cell concentration and culturing time. The cell growth rate was higher when the bed height was higher. When the bed height was 75 mm, the cell growth rate was only slightly higher than that when hydrogen was supplied by bubbling. The result for the bed height of 225 mm was almost the same as that of 150 mm. This result indicated that it was important for enhancing the growth rate to increase the dissolution rate of hydrogen in the medium and that the cell growth rate could not be further increased even if the bed height was higher than 150 mm. The cell concentration reached 1.0×10^9 cells ml^{-1} after 72 h under the best conditions. In this case, the doubling time was about 5 h and this value was almost the same as that obtained by Drobner et al. (1990).

It is known that ferrous iron oxidation by *A. ferrooxidans* decreases at a pH higher than 2.5. This is partially due to the reduction of the mass transfer on the cell surface by the formation of ferric iron precipitates (Meruane and Vargas, 2003). However, in the case of the ferrous iron oxidation by *A. ferrooxidans*, the

proton is one of the reactants. Therefore, the decrease in the growth rate with an increase in pH cannot be avoided. On the other hand, in the case of the oxidation of molecular hydrogen, the proton is not involved in the apparent reaction equation. Therefore, the effect of pH was supposed to be small and was studied in the present study. Results on the relation between pH and the cell number after 72 h showed that the microorganism grew at a pH between 2.0 and 3.5. The growth rate decreased with the increasing pH value (data not shown). This tendency was different from the result of Drobner et al. (1990). It has been reported that *A. ferrooxidans* strains grew on hydrogen between pH 3.0 and 5.8, and not at pH 2.2. The reason for this difference was not clear, but the main difference in the experimental condition was the gas phase pressure. The cultivation in the present study was carried out at ambient pressure, while the pressure used by Drobner et al. was 250 kPa.

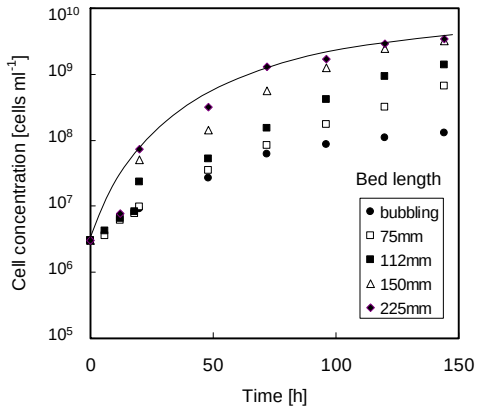

Fig. 6. Cell growth when a trickle-bed contactor was used for the hydrogen dissolution.

4. Conclusions

It was concluded that *A. ferrooxidans* grew on molecular hydrogen at a pH between 2.0 and 3.5 and the iron-oxidizing ability was not changed during the growth. The new cultivation system using a trickle-bed contactor increased the hydrogen dissolution. By using this system, the maximum cell concentration was 20-fold and the growth rate was two times higher than that of the conventional ferrous iron medium.

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