J. Jpn. Soc. Soil Phys.
土壌の物理性
No. 102, p. 71～78（2006）

Population Dynamics of *Thiobacillus ferrooxidans* in Different Soil Moisture Conditions of a Pyritic Soil

Kaoru UENO*, Tadashi ADACHI** and Hajime NARIOKA***

* Research Institute for Biological Functions, Chubu University,
1200 Matsumoto-cho Kasugai, Aichi 487-8501, Japan
** Faculty of Environmental Science and Technology, Okayama University,
3-1-1 Tsushima-naka, Okayama 700-8530, Japan
*** Faculty of Bioresources, Mie University, 1577 Kurimamachiya-cho,
Tsu-shi, Mie 514-8507, Japan

Abstract

Iron-oxidizing bacteria (*e.g. Thiobacillus ferrooxidans*) are known as an influential catalyst of soil acidification in primary oxidization of pyritic soils. We have reported that multiplication rate of the bacteria is influenced by soil moisture. In this paper, we tried to estimate the configuration of the microhabitat of *T. ferrooxidans* under three different soil moisture conditions (−3.5 kPa, −35 kPa, and −3000 kPa) to look into the mechanism of bacterial oxidation in detail measuring the number of adsorbed and free form of the bacteria (AFT and FFT) using incubation and centrifugation method. At the beginning of the soil incubation, most *T. ferrooxidans* were counted as AFT in every soil moisture condition. Multiplication rate of both forms of bacteria under the condition of −35 kPa was highest of all conditions. Under the condition of −3.5 kPa, the survival rates of both forms of bacteria were higher especially in AFT. And under the condition of −3000 kPa, FFT could not be counted and a small number of AFT remained at the first stage of incubation, which did not live long. These data indicate that population dynamics of *T. ferrooxidans* in soil matrix is influenced by soil moisture conditions.

Key words : pyrite, soil moisture, bacterial oxidation, *Thiobacillus ferrooxidans,* population dynamics

1. Introduction

Bacterial activity is an important factor to control natural soil conditions, and this is the same in pyritic soils. Pyritic soils are one of the worldwide problem soils because of its sulfur production in the process of water drainage. It is difficult to control acidification without using a large amount of water, and the soil improvement method washing out the acid from the soil causes water pollution. *Thiobacillus ferrooxidans* which is an iron-oxidizing bacterium is known as an influential catalyst of pyrite oxidation (Silverman and Ehrlich, 1964 ; Singer and Stumm, 1970). Pyrite in sediments is produced and piled on the bottom of sea coast or lakes with brackish water. The sediments contain a large amount of 2 : 1 type clay minerals (Yoneda, 1958 ; Fujii and Yasuda, 1971 ; Attanandana *et al.,* 1981 ; Dixson *et al.,* 1982), and during the drainage, they generate many cracks and aggregates. These soil structures improve gas exchanges and promote pyrite oxidation, because the bacteria catalyzing the oxidation are aerobic and chemoautotroph.

Degree of bacterial oxidation in pyritic soils is influenced by soil moisture condition (Adachi *et al.,* 1992 ; Ueno *et al.,* 2002 a b). A soil moisture condition of −35 kPa is suitable for

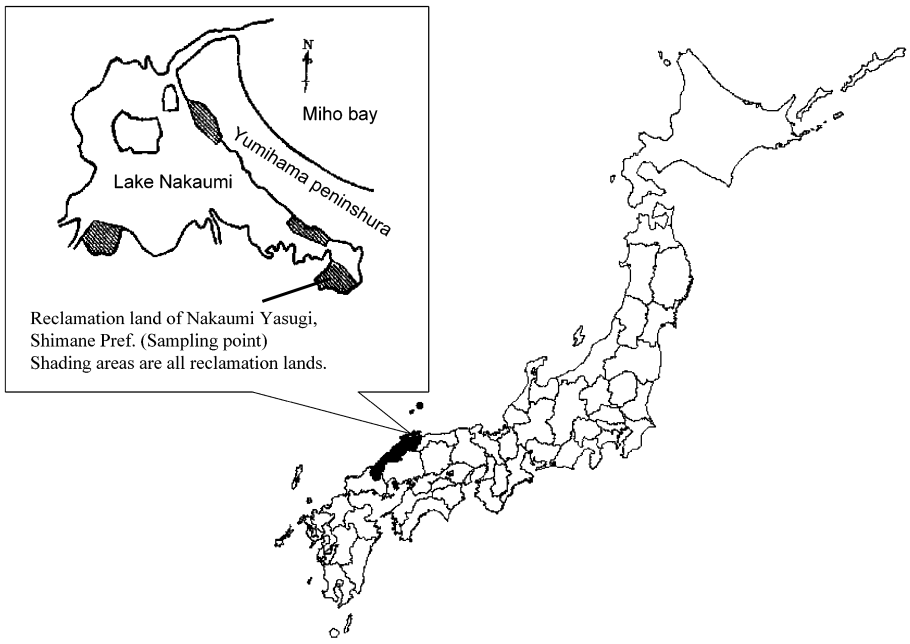

Fig. 1 Outline of sampling site.

multiplication of *T. ferrooxidans* (Ueno *et al.,* 2003). Bacterial activity in soil is also influenced by soil moisture conditions (*e.g.* Tanaka and Sakamoto, 1972 ; Mielnich and Dugas, 2000), but details about the movement of bacteria or their microhabitats in soil matrix have not been elucidated. Hattori (1976) suggested that bacterial activity results in a big difference in the adsorption of soil particles. Soil moisture condition is assumed to have effective influences on the adsorptions of bacteria to the soil. Understanding on the mechanism of bacterial multiplication and their movement in soil matrix under drying condition contribute in controlling pyritic soils. We examined changes in the adsorption and multiplication rate of *T. ferrooxidans* under different soil moisture conditions, and also the effects of soil moisture conditions on bacterial microhabitat.

2. Materials and Methods

2.1 Soil

Nakaumi-Yasugi soil, a redoxed and a potential acid sulfate soil in Shimane Prefecture, was used (Fig. 1). The area of the collection was a reclamation area, but the sampling point had never been used as an agricultural field but as a material yard. The soil was collected from a depth of 185 to 210 cm. Sampling and treatment methods were the same as in the previous study (Ueno *et al.,* 2003). Physical and chemical soil properties are shown in Table 1. As mentioned above, most pyritic soils in Japan contain 2 : 1 type clay minerals. We did not analyze the type of clay minerals in the sampling soil, but have presumed to contain them because 40% of the soil was clay and showed a character of swelling and shrinkage. In addition, Fujii and Yasuda (1971) analyzed sediments of Lake Nakaumi and indicated that the soil include high rates of montmolilonite and kaorinite. Our sampling point was in a reclamation area of Lake Nakaumi, and the sampled soil showed similar characteristics as the sediments.

2.2 Experiments

2.2.1 Soil Moisture Conditions

Table 1 Fundamental physical and chemical properties of sample soil.

soil texture[1)]	SiC (Clay 40%)
density of soil particle (ρ_S, Mg m^{-3})[2)]	2.58
natural soil moisture content (kg kg^{-1})	1.32
liguid limit (w_L, kg kg^{-1})	1.59
plastic limit (w_P, kg kg^{-1})	0.56
shrinkage limit (w_S, kg kg^{-1})	0.32
plasticity index, Ip	102.44
pH (H_2O ; 1 : 5)	6.25
pH (H_2O_2)[3)]	2.05
$EC_{1:5}$ (mS cm^{-1})	4.41
pyrite (mg kg^{-1})[4)]	49.9
Feo (mg kg^{-1})[5)]	0.9
Fed (mg kg^{-1})[6)]	23.9
sulfate ion (mg kg^{-1})	7.2

[1)] Hydrometer-method (international system), [2)] Picnometer-method, [3,4)] Murakami (1961), [5)] Tamm (1922), [6)] Mehra and Jacson (1960).

Three moisture conditions were prepared by drying the soil at 30℃ in an incubator.

1) Matric potential −3.5 kPa, water content 1.38 kg kg^{-1}, wet condition over liquid limit, pasted.

2) Matric potential −35 kPa, water content 0.70 kg kg^{-1}, near the soil moisture condition of plastic limit, the best condition for the multiplication of *T. ferrooxidans* (Ueno *et al.,* 2003), aggregated.

3) Matric potential −3000 kPa, water content 0.30 kg kg^{-1}, near the shrinkage limit, allows low multiplication of the bacteria (Ueno *et al.,* 2003), aggregated.

Methods for drying, treatments of the soil after adjusting moisture condition, and the way of converting water content to matric potential were the same as in Ueno *et al.,* (2003).

2.2.2 Incubating Conditions

Twenty grams of moisture conditioned soil sample was put in a sterilized petri dish, 90 mm in diameter and 20 mm in height, and the dish was wrapped with Parafilm. Samples were incubated at 30℃ for six weeks, and bacterial count, soil pH (H_2O), and electric conductivity ($EC_{1:5}$) were checked every week from the start of the incubation. We opened the dishes every three days to exchange air.

2.2.3 Bacterial Count

We took four grams of wet soils in a centrifuge tube (50 mL), added 40 mL of sterilized distilled water, shook one minute by hand, left it still for five minutes, and then centrifuged (× 750 g, Nioh and Furusaka, 1972) it for 15 minutes. The supernatant were collected to count the "free form of *T. ferrooxidans* (FFT)". We again added 40mL of sterilized distilled water to the residue, shook by hand for one minute, and treated with supersonic vibration for three minutes. All the soil solution was collected to count the "adsorbed form of *T. ferrooxidans* (AFT)". FFT and AFT counts were obtained by Most-Probable-Number method using Silverman 9k liquid culture and 1.5mL tubes (Ueno *et al.,* 2003).

2.2.4 Soil pH (H_2O) and $EC_{1:5}$

Dishes for measuring soil pH (H_2O) and $EC_{1:5}$ were prepared and incubated as in the bacterial counting. Ten grams of wet soil were suspended in distilled water and the pH (H_2O, 1 : 5) of the suspension was checked with electro probe (HORIBA F-23). The soil solution was

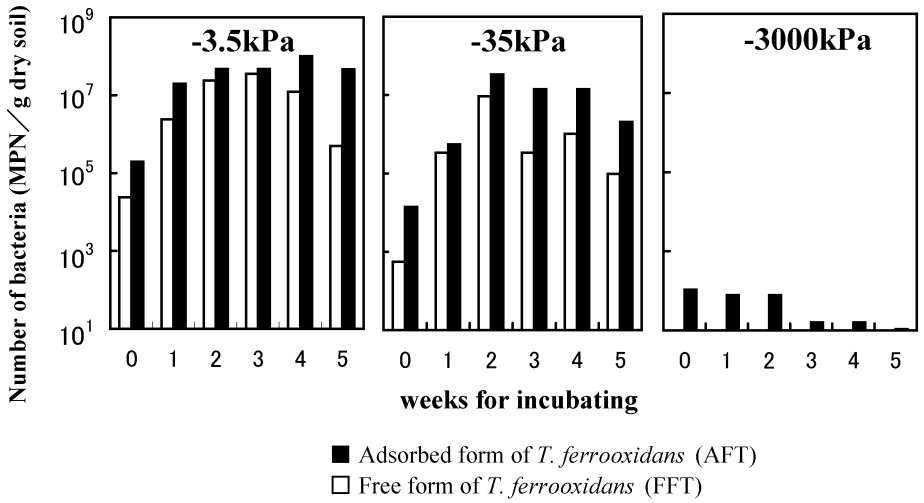

Fig. 2 The number of adsorbed and free form of *T. ferrooxidans* under three soil moisture conditions.

filtrated using 5B filter paper and $EC_{1:5}$ was measured using electro probe (HORIBA DS-14).

3. Results

3.1 Number of adsorbed and free form of *T. ferrooxidans*

Changes in the number of *T. ferrooxidans* are shown in Fig. 2. In every soil moisture condition, a larger number of bacteria were counted in AFT than FFT at 0 week of the incubation. The largest number was counted under the soil moisture condition of −3.5 kPa in both forms. Under the condition of −3.5 kPa, the difference in the population size between the forms was small at 0 week. After a logarithmic phase (from the second to fifth week), the population size decreased in FFT, but remained constant in AFT. Under the condition of −35 kPa, from 0 week to the second week the counts were less than those of −3.5 kPa, and the difference between the two forms of bacteria was larger. The multiplication rates at the logarithmic phase were higher than those of −3.5 kPa in both forms, especially in FFT. After the logarithmic phase, the number declined in both forms, and the declining rates were higher than those of −3.5 kPa, especially in FFT. Under the condition of −3000 kPa, *T. ferrooxidans* was not detectable in FFT, and the number did not increase throughout the incubation period in AFT.

3.2 Soil pH (H_2O) and $EC_{1:5}$

Changes in the soil pH (H_2O) are shown in Fig. 3. Under the condition of −3.5 kPa, the value dropped to 2.78 after a week, gradually declined to 2.11 from the second to fourth week, and then slightly increased in the fifth week. Under the condition of −35 kPa, the value dropped linearly from the start to the second week, gradually declined until the third week and marked a minimum of 2.19, and then slightly increased. Under the condition of −3000 kPa, the value declined in the first week, reached a minimum of 3.00, and slightly increased thereafter.

Changes in the soil $EC_{1:5}$ are shown in Fig. 4. The EC tended to increase while pH value decreased. The result was the same as previous reports and this phenomenon is assumed to cause production of sulfate ion and soluble iron (Ueno *et al.,* 2002 a b, 2003).

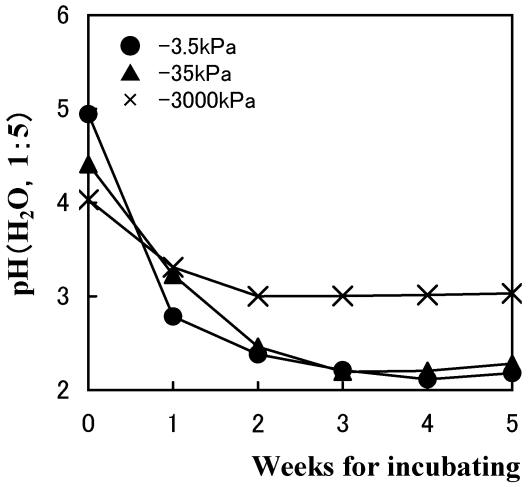

Fig. 3 Changes in soil pH in the incubating period.

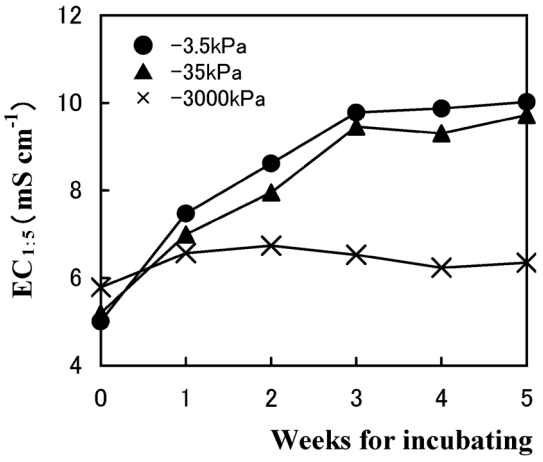

Fig. 4 Changes in soil EC in the incubating period.

4. Discussion

4.1 Microhabitat of *T. ferrooxidans*

In order to analyze the relation between physical condition of soil matrix and microhabitat of *T. ferrooxidans* in soil matrix, the microhabitats were modeled considering the number of FFT and AFT as shown in Fig. 5. The model is simplified to indicate only 2 : 1 clay mineral, H_2O, and bacteria. Although the components are drawn nearly to scale, except for the distance between clay minerals and macropore size, the model may not reflect the exact microhabitat because it has not been confirmed by a direct way such as by electric microscope. A more precise state should be researched by some direct method. In the model, however, at least the number of FFT represents that of weakly adsorbed bacteria to clay minerals, and the number of AFT represents that of bacteria adsorbed more tightly than FFT. From the difference in the adsorption rate and their number, we can easily imagine where in the soil matrix the bacteria conceivably stay and multiply in each soil moisture condition.

Under the soil moisture condition of −3.5 kPa, a large number of *T. ferrooxidans* was observed at the first stage of incubation. They increased during the logarithmic phase regardless of the adsorbed amount. These multiplication rates were lower throughout the soil matrix than the condition of −35 kPa, but the survival rate was higher in both forms of bacteria. Under the condition of −35 kPa, the multiplication rates of both bacterial forms were the highest of all conditions and the number of FFT declined at highest ratio. Under the condition of −3000 kPa, bacterial activity was very low.

Although factors causing these statuses should be studied by other experiments, we may assume that ; under the condition of −3.5 kPa, there is low stress for bacterial survival because of the wide space to move around between the clay minerals ; and under the condition of −35 kPa, soil forms aggregates with a lot more macro pores, generating larger specific surface area than the soil which had no soil structure as in the condition of −3.5 kPa (Fig. 5). These physical conditions are supposedly favorable for *T. ferrooxidans* to acquire CO_2, O_2, and nutrients, and to move and spread in the soil matrix. The significant decrease in FFT under the condition of −35 kPa may be explained by the lack of nutrient and space to

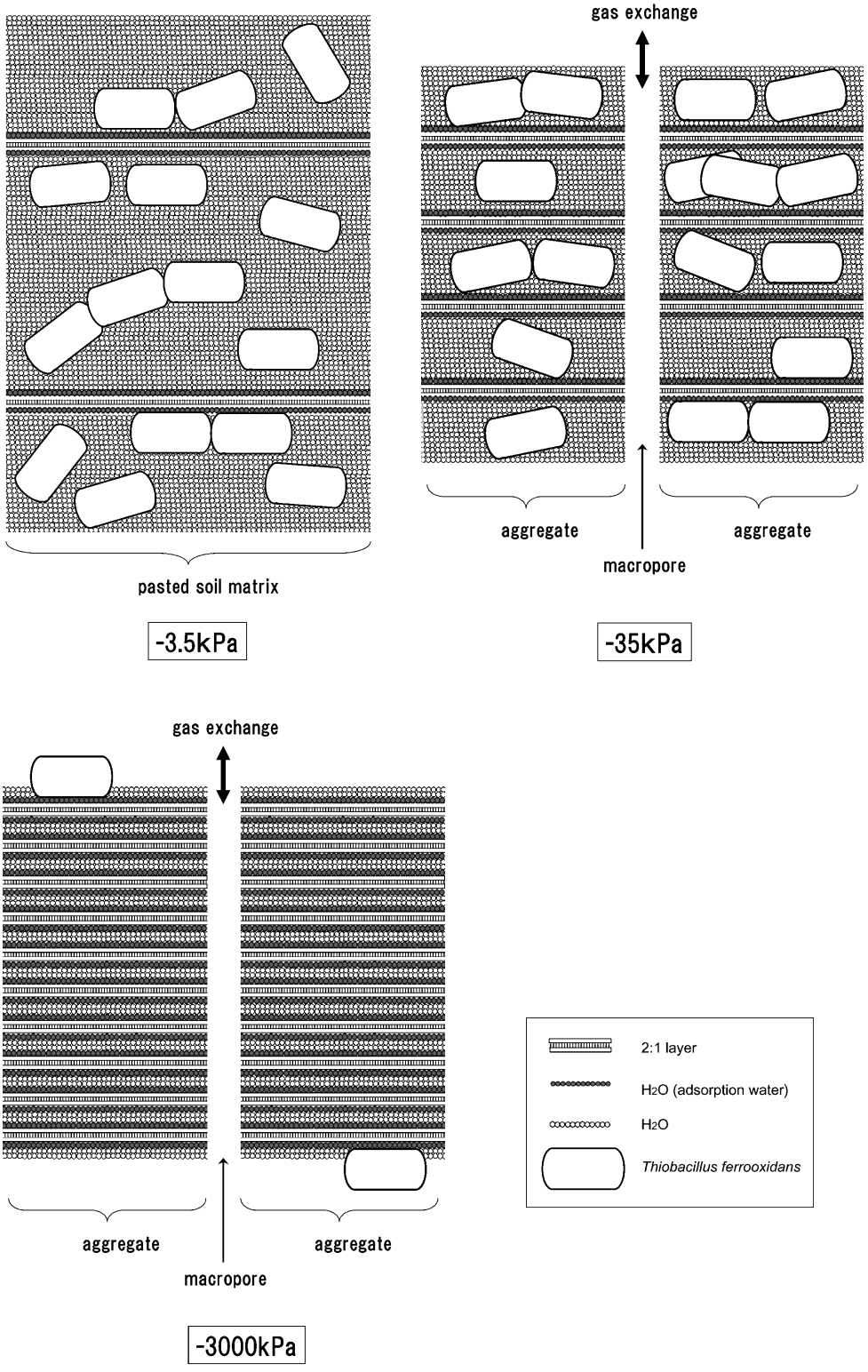

Fig. 5 Schematic model of microhabitat of *T. ferrooxidans* in different soil moisture conditions.

live after rapid increase in bacterial population. Under the condition of −3000 kPa, the soil moisture condition was nearly at the shrinkage limit, and distances between clay minerals should be extremely small (Fig. 5). In this condition, it is assumed that *T. ferrooxidans* is completely adsorbed to clay minerals and is not able to move against the molecular force and the van der Waals force. In addition, molecular diffusion of nutrients, water, and gasses may be extremely low, and material circulation in this dry and high-pressured condition is almost impossible that bacteria cannot survive.

4.2 Soil Acidification and Microhabitat of *T. ferrooxidans*

Soil acidification nearly stopped with the termination of the logarithmic phase under the condition of −3.5 kPa and −35 kPa. The minimum pH value was 2.11 and 2.19, respectively. These pH values were nearly the level of H_2O_2 (Table 1). On the other hand, the minimum pH value in −3000 kPa was 3.00. This difference in the minimum pH value was presumed to be caused by the difference in the bacterial activity. Adachi *et al.* (1992) indicate that over the drying conditin of pF 3.0 (−98.1 kPa) soil acidification is caused mainly by chemical reaction. Ueno *et al.* (2002 a) shows that under the condition of −31.0 kPa bacterial oxidation is superior to the chemical oxidation. The present study revealed that, under the condition of −3000 kPa, chemical oxidation seems to be the main factor for the soil acidification, and that *T. ferrooxidans* largely contributes to soil acidification under the condition of −3.5 kPa and −35 kPa.

5. Conclusion

In this study, we obtained following findings.

1) At the beginning of soil incubation, most *T. ferrooxidans* lived in the adsorbed form.

2) Under the condition of −3.5 kPa and −35 kPa, the bacteria multiplied both in the adsorbed and free form, and the survival rate was higher in the adsorbed form.

3) Multiplication rate of both forms were high under the condition of −35 kPa, and therefore soil acidification seemed to take place in the whole soil matrix under this condition.

4) Under the condition of −3000 kPa, bacteria were adsorbed tightly to the soil and could not live long, and the entire bacterial activity was very low.

These data indicate that population dynamics of *T. ferrooxidans* in soil matrix is seriously influenced by soil moisture conditions.

Acknowledgments

Authors sincerely thank Shuji Hamazaki in Shimane Prefecture for collecting soil samples, and students in the Lithosphere Management Lab. in Okayama University for lending their help in the experiments.

References

Adachi, T., Matsumoto, Y. and Hara, T. (1992) : Process of Acid Sulfate Soil Formation from the Viewpoint of Moisture Conditions on Coastal Muddy Soil. Translations of the Japanese Society of Irrigation, Drainage and Reclamation Engineering, **162** : 89–96 (in Japanese).

Attanandana, T., Vacharotayan, S. and Kyuma, K. (1981) : Chemical characteristics and Fertility Status of Acid Sulfate Soils of Thailand, pp. 137–156, Proc. of the Bangkok Symp. on Acid Sulfate Soils.

Dixson, J.B., Hossner, L.R., Senkayi, A.L. and Egashira, K. (1982) : Mineralogical Properties of Lignite Overburden as they Relate to Mine Spoil Reclamation, pp. 169–191, Acid Sulfate Weathering, SSSA Special Publ. NO.10, Soil Science Society of America, Wisconsin.

Fujii, N. and Yasuda, T. (1971) : Preliminary Study on the Clay Mineral Distribution in the Bottom Sediments of the Lake Nakaumi and Lake Shinji-ko West Japan, Bulletin of the Geological Survey of Japan, **22** : 593–602 (in Japanese).

Hattori, R. (1976) : Growth and Spore Formation of Bacillus subtilis Adsorbed on an Anion-Exchange Resin, J. Gen. Appl. Microbiol., **22** (4) : 215–226

Mehra, O.P. and Jackson, M.L. (1960) : Iron oxide removal from soils and clays by a dithionite-citrate system buffered with sodium bicarbonate, Clays and Clay Minerals, 7 : pp. 317–327, Pergamon Press.

Mielnich, P.C. and Dugas, W.A. (2000) : Soil CO_2 flux in a tall-grass prairie. Soil Biology and Biochemistry, **32** : 221–228.

Murakami, H. (1961) : A method for Semi-quantitative Analysis of Oxidizable Sulfur in Polder Soils Using Hydrogen Peroxide, Soil Science and Plant Nutrition, **32** : 277–279 (in Japanese).

Nioh, T. and Furusaka, C. (1972) : Studies on Glycine-percolated Soil IV Fractionation of Bacteria in Glycine-percolated Soil in "Two-layered Sucrose Solution System", Soil Science and Plant Nutrition, **18** (6) : 219–223.

Silverman, M.P. and H.L. Ehrlich. (1964) : Microbial formation and degradation of minerals, Adv. Appl. Microbiol., **6** : 181–183.

Singer, P.H. and Stumm, W. (1970) : Acidic Mine Drainage. The Rate-Determining Step, Science, **167** : 1121–1123.

Tanaka, H. and Sakamoto, M. (1972) : Studies on Soil Physical Conditions and the Ecology of Soil-borne Disease Bacteria (4), Bull. Inst. Agr. Res. Tohoku Univ., **23** : 141–158 (in Japanese).

Tamm, O. (1922) : Eine Methode zur Bestimmung der anorganischen Komponente des Gelkomplexes im Boden, Medd. Fr. Statens Skogsfoersoekasanstalt, **19** : 387–404.

Ueno, K., Adachi, T. and Narioka, H. (2002 a) : Influence of Temperature/Water Condition on the Acidification Process of Sediment in Lake Kojima. Journal of the Japanese Society of Soil Physics, **91** : 15–23 (in Japanese).

Ueno, K., Adachi, T. and Narioka, H. (2002 b) : Effects of Drying on Bacterial Activity and Iron Formation in Acid Sulfate Soils. Journal of the Japanese Society of Soil Physics, **91** : 49–57 (in Japanese).

Ueno, K., Adachi, T. and Narioka, H. (2003) : Influence of Moisture Content on Soil Respiration in Acid Sulfate Soils, Journal of the Japanese Society of Soil Physics, **93** : 35–45 (in Japanese).

Yoneda, S. (1958) : A Review on Soil and Nutrition in Reclamation Areas (II), Soil Science and Plant Nutrition, **28** : 455–459 (in Japanese).

異なる水分条件下のパイライト含有土壌における *Thiobacillus ferrooxidans* の個体群動態

上野　薫*・足立忠司**・成岡　市***

* 中部大学生物機能開発研究所，〒487–8501 愛知県春日井市松本町 1200

** 岡山大学環境理工学部，〒700–8530 岡山県岡山市津島中 3–1–1

*** 三重大学生物資源学部，〒514–8507 三重県津市栗真町屋町 1577

要　　旨

パイライト含有土壌の初期的酸化において触媒の働きを担う鉄酸化細菌 *Thiobacillus ferrooxidans* の微生息域と土壌水分との関係の把握を目的として，土壌水分条件を 3 段階に設定した潜在的酸性硫酸塩土壌の静置培養を行い，より強く土壌に吸着された *T. ferrooxidans*（AFT）と，より弱く吸着された *T. ferrooxidans*（FFT）を遠心法により分別し計数した。これら細菌数の経時的変化から，水分条件の異なる土壌における当該細菌の生息・増殖部位について推定した。その結果，どの水分条件下でも培養初期には AFT が多く存在すること，増殖率は，−35 kPa で吸着状態に関係なく最も高いこと，生残率は−3.5 kPa, とくに AFT で高いこと，−3000 kPa では FFT が検出されず，培養初期にわずかに生息していた AFT も長くは生存できないことが明らかとなった。これより，上記細菌の個体群動態は土壌水分条件により異なることが示されたが，微生息域の特定には直接的手法を用いた別途検証が必要と考えられた。

キーワード：パイライト，土壌水分，微生物的酸化，*Thiobacillus ferrooxidans,* 個体群動態

受稿年月日：2005 年 10 月 25 日

受理年月日：2006 年 1 月 4 日