Bull. Fac. Life Env. Sci. Shimane Univ., 12：3－6, September 30, 2007

Glucose-degrading activity in paddy soil under anaerobic and aerobic conditions

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Abstract Glucose degrading activity of gray low land paddy soil samples was measured anaerobically and aerobically by a mutarotase-glucose oxidase method after incubation under anaerobic and aerobic conditions. Cellulose degrading and protease activities were determined as well for comparison. Under anoxic conditions, the soil redox potential continuously decreased down to about －200 mV, and the concentration of Fe(II) increased up to about 3.0 mg g^{-1} dry soil at the end of the incubation, whereas the oxic conditions were maintained during the aerobic incubation. ATP contents of the anaerobic soil were almost half of the aerobic soil. In the soil incubated aerobically for 65 days, the glucose degrading activity was 0.37 μmol g^{-1} dry soil h^{-1} when measured aerobically, that is three times higher than when measured anaerobically. In the soil incubated anaerobically, the activity measured anaerobically constantly increased to 0.88 μmol g^{-1} dry soil h^{-1} during the same period, while ca. 0.1 μmol g^{-1} dry soil h^{-1} in the aerobic measurement. Cellulose degrading activity was also higher in the anoxic soil. In contrast, there was no significant difference in the protease activities between the oxic and anoxic soils.

Keywords: cellulose, degradation, glucose, paddy soil, protease

Introduction

Glucose is a main degradation intermediate of rice straw and the most abundant monosaccharide in rice paddy field (Murayama 1977, Chidthaisong et al. 1999), therefore, rate of degradation of glucose as well as rice straw is considered to be an important index of carbon cycle in the soil environment. Their degradation also influences the methane formation in rice paddy soil (Glissmann and Conrad 2002). To our knowledge, however, there have been a limited number of reports on the quantitative measurement of the degradation of glucose in paddy soils (Chidthaisong et al. 1999). In those studies, materials labeled with isotope were used to trace the fate of the degradation products in addition to the parent materials.

In this study, we measured glucose degrading activity of paddy soil samples anaerobically and aerobically by a conventional mutarotase-glucose oxidase method (Miwa et al. 1972). The activities of the soil samples incubated under anoxic and oxic conditions were examined and compared with cellulose degrading and protease activities.

Materials and Methods

Preparation of soil samples

Soil sample was collected from a gray low land paddy field (Paddy A) in Matsue, Shimane prefecture, Japan. The surface layer (0-2 cm) of the paddy soil was sieved through a 2 mm mesh, and supplemented with distilled water to achieve a final water content of about 55%. The physico-chemical properties of the soil sample were presented previously (Okamoto et al. 1999). About 500 g aliquots of the soil sample were set under aerobic and anaerobic conditions. For the aerobic conditions, each soil sample was put in a 0.03 mm-thickness polyethylene bag (30 cm x 20 cm x 1 cm), which was closed by a sealer. For the anaerobic conditions, it was put in a glass bottle (8 cm id x 13.5 cm), which closed with a cap. These soil samples were kept at 25℃ in darkness, and used for the further experiments after about 1, 13 (11), 22 (21), 32, and 65 (62) days of the aerobic and anaerobic incubations (Figures

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in parentheses indicate the date when the incubation period under the anaerobic conditions was different from that under the aerobic incubation). At appropriate sampling points, Fe (II) content determined by the colorimetric method using phenanthroline as a reactant (Motomura 1994), and Eh value of the soil samples were measured as an indicator of redox conditions. Aliquot (4 g) of the soil sample was taken in triplicate, and suspended with 40 ml of 1M acetate buffer (pH 3.0). After shaking for 5 min at 200 rpm, Fe(II) content of the filtrate was measured colorimetrically after addition of 0.25% phenanthrolin solution. The following experiments described below were performed taking three aliquots of the soil samples after mixing well.

Glucose degrading activity of soil

Aliquot (20 g) of the soil sample was taken into 50 ml glass vials. Twenty milliliter of distilled water saturated with air or nitrogen gas was added to the soil samples for the further aerobic and anaerobic incubation, and one milliliter of 150 mM glucose solution was added to achieve a final concentration of 5 mM. For the aerobic incubation, the vials were loosely capped, whereas the vials were sealed with PARAFILM (American National Can) as replaced the headspace with nitrogen gas, and capped tightly for the anaerobic incubation. These soil samples were kept at 25℃ with shaking at 120 rpm.

Immediately after the addition of glucose, and after 3, 6, 9, 12, and 20 h of the aerobic and anaerobic incubations, one milliliter aliquot of the soil suspension was taken in triplicate, and the concentration of glucose was determined by the mutarotase-glucose oxidase method using Glucose CII-Test Wako kit (Wako Pure Chemicals). After centrifugation of the soil suspension at 12000 rpm for 5 min, 0.5 ml of the supernatant and glucose standard solutions (0, 0.1, 0.5, 1.0 and 1.25 mM) were taken into the disposable glass test tube (16 x 100 mm, IWAKI), and one milliliter of the reaction reagent was added. The reaction mixture was kept at 37℃ for 5 min, and after dilution with 2 ml of distilled water, absorbance at 505 nm was determined.

Protease activity of soil

Aliquot (2 g) of the soil sample was taken for the assay of Z-FLase and caseinase activities. The procedure was described previously in detail (Okamoto et al. 1999). Briefly, the soil sample was suspended with 48 ml of 25 mM phosphate buffer (pH 7.0) containing 0.8 ml of toluene and 144 mg casein or 9.9 mg benzyloxycarbonyl-L -phenylalanyl-L -leucine (Z-FL, SIGMA), and incubated at 30℃ for 2 hours with shaking at 120 rpm. Amino residues produced were determined by ninhydrin reagent L8500 (Wako Pure Chemicals) using leucine as a standard.

ATP content in soil

ATP content was determined according to the procedure described previously (Suyama et al. 2001). Aliquot (1 g) of the soil sample was suspended with 10 ml ice-chilled TCA solution, and ATP was extracted by sonication (20 kHz, 200W) for 2 min. After centrifugation, ATP in supernatant was determined using ATP analyzer AF-100 (TOA Electronics Ltd.) by luciferin-luciferase system.

Cellulose degrading activity of soil

For the measurement of cellulose degrading activity, the soil samples were separately prepared in accordance with the procedures described above except that Benchkote sheets (a polyethylene backed filter paper, 4 cm x 4 cm, Whatman) were buried in the soil samples. After the incubation described above, three pieces of the Benchkote sheet were taken from each soil samples, and the weight loss was determined according to the procedures described previously (Tatsuyama et al. 1984).

Results and Discussion

Soils redox conditions and ATP contents

Under anaerobic conditions, the soil redox potential continu-

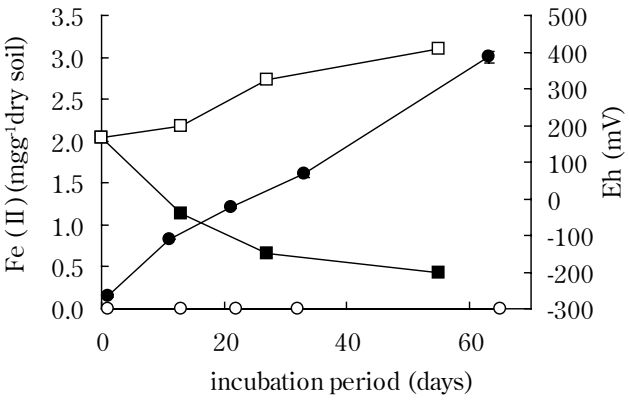

Fig. 1 Fe(II) contents (●, ○) and Eh values (■, □) in soil samples incubated under anaerobic (●, ■) and aerobic (○, □) conditions.

ously decreased down to about -200 mV, and the concentration of Fe(II) increased up to about 3.0 mg/g dry soil at the end of the incubation (Fig. 1). Whereas the oxic conditions were maintained during the aerobic incubation based on the increased redox potential and no detectable Fe(II) in the soil samples. These results indicate that containment of the soil sample in the 0.03 mm-thickness polyethylene bag did not inhibit permeability of oxygen to make the soil sample oxic.

ATP content decreased after 11 days of anaerobic incubation, and the amounts were almost half of those of aerobic conditions (Fig. 2). The difference was supposed to be attributed to the microbial different metabolic systems between aerobic and anaerobic conditions. The results are in good agreement with the previous observation in which Inubushi et al. (1989) reported decrease in ATP contents during anaerobic incubation of Japanese paddy soils.

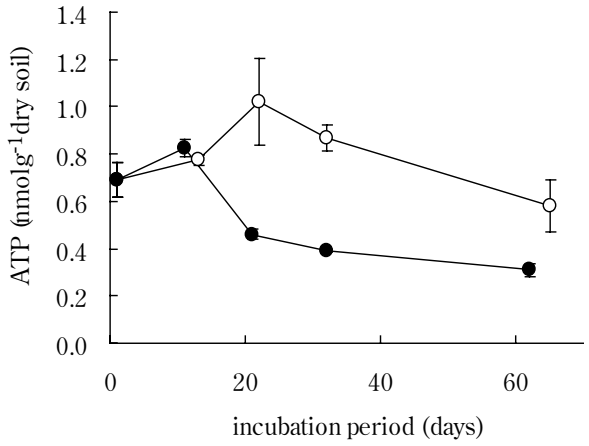

Fig. 2 ATP contents in soil samples incubated under anaerobic (●) and aerobic (○) conditions.

Glucose and cellulose degrading activities of soil

Change in the glucose degrading activity was shown in Fig. 3. In the soil incubated aerobically, the activity increased gradually from 0.15 to 0.37 μmol g^{-1} dry soil h^{-1} when measured aerobically after 65 days of incubation, while the activity measured anaerobically gradually decreased to 0.11 μmol g^{-1} dry soil h^{-1}.

In the soil incubated anaerobically, the activity constantly increased up to 0.88 μmol g^{-1} dry soil h^{-1} measured anaerobically during the same period, while the activity gradually decreased to 0.095 μmol g^{-1} dry soil h^{-1} when measured aerobically.

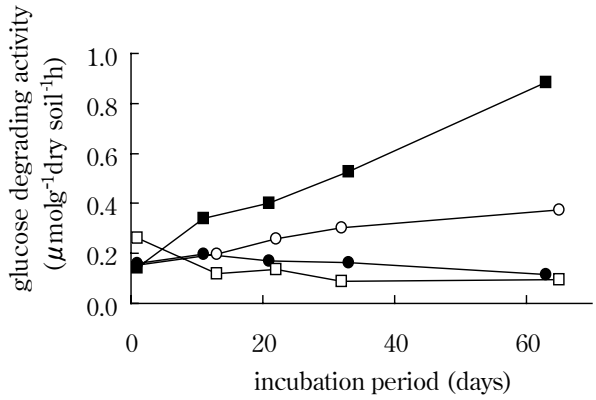

Fig. 3 Glucose degrading activity of soil samples incubated under anaerobic (□, ■) and aerobic (●, ○) conditions, measured anaerobically (●, ■) and aerobically (○, □).

The activity was influenced by the incubation conditions of soil. Facultative anaerobic microorganisms might proliferate under both aerobic and anaerobic conditions, but showed less potential in the degradation of glucose when the atmospheric conditions were changed. Generally, aerobic respiration is more efficient in producing ATP than fermentation and anaerobic respiration. Higher amount of ATP under aerobic conditions would reflect the efficiency, but higher activity in glucose degradation of the anaerobic soil suggests that the activity had no relationship with the ATP content. Cellulose degrading activity was also higher in the anaerobic soil as shown in Fig. 4. Flooding and drainage are rotated in paddy soils causing anaerobic and aerobic conditions, respectively. Degradation of cellulose and glucose seems to be more preferable during the flooded season, and higher temperature would also enhance the

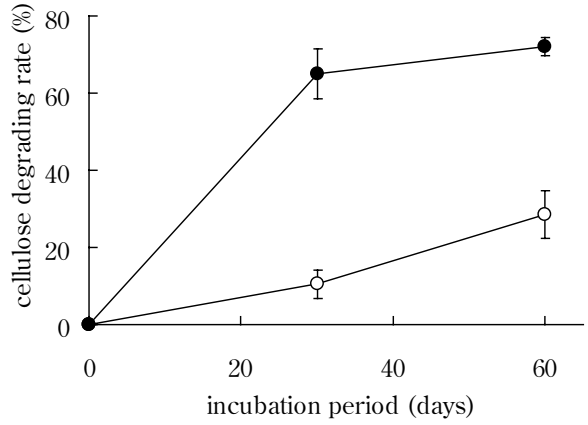

Fig. 4 Cellulose degrading rate in soil samples incubated under anaerobic (●) and aerobic (○) conditions.

degradation. Kanazawa and Yoneyama (1980) compared the degradation of ^{15}N-labelled rice straw in a paddy soil under flooded and upland conditions, and observed almost same rate of degradation. Murthy et al. (1991) examined mineralization of ^{14}C-labelled rice straw in anaerobic and aerobic soils, and reported higher rate of mineralization under aerobic conditions. Discrepancy of the results might be due to difference in materials used, as rice straw is composed of lignin and hemicellulose in addition to cellulose.

Protease activity of soil

There was no significant difference in Z-FLase and caseinase activities between aerobic and anaerobic soils (Fig. 5). These results indicate that the oxic conditions of soil have little influence on the protease activity. It is reasonably assumed by the hydrolytic nature of proteases. Compared the glucose and cellulose degrading activities with the protease activity from the standpoint of relative activities between the aerobic and anaerobic conditions, anaerobic condition seems to be suitable for the degradation of glucose and cellulose.

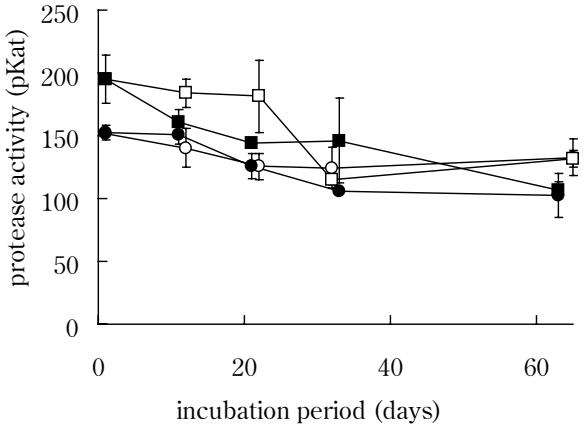

Fig. 5 Z-FLase (●, ○) and caseinase (■, □) activities in soil samples incubated under anaerobic (●, ■) and aerobic (○, □) conditions.

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