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The Formation of Insoluble Tc Depends on Bacterial Activity

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The formation of insoluble Tc (> 0.2 μm in size) in ponding water of paddy soil was investigated. The ponding water prepared by flooding with deionized water was collected at a one-week intervals during the 120 days of the experimental period. The collected sample was incubated with 3 kBq mL^{-1} TcO_4^- at 25 ˚C for 14 days. The mean value of relative amounts of insolubilized Tc was less than 0.6% of total Tc. This result suggested that flooding with deionized water was not effective for the formation of insoluble Tc. When the ponding water sample was incubated with TcO_4^- under anaerobic conditions, Tc was scarcely insolubilized. On the other hand, a significant fraction of the Tc was insolubilized in the ponding water sample supplemented with nutrients that promote bacterial activity. Also, the amount of insoluble Tc increased with time. From these results, it was concluded that the formation of insoluble Tc in the ponding water depends on enhanced bacterial activity rather than just anaerobic conditions.

1. Introduction

Technetium-99 (^{99}Tc) is produced by thermal fission of ^{235}U and ^{239}Pu with a yield of 6%, making ^{99}Tc relatively high in abundance among fission products. In addition, the half-life of ^{99}Tc is very long (2.1 × 10^5 y). Under aerobic conditions, Tc is present in the heptavalent form as pertechnetate (TcO_4^-). This chemical species is soluble and mobile in the environment and is readily taken up by plants.[1] The uptake of Tc by rice is an especially serious issue in the transfer of ^{99}Tc to humans. Therefore, it is important to predict the behavior of ^{99}Tc in agricultural environments.

Recently, the authors found that soluble TcO_4^- was changed to insoluble forms in ponding water in several types of paddy soil[2] and that the formation of insoluble Tc was mainly caused by bacteria.[3] The chemical species of the insoluble Tc was not identified, but it was probably TcO_2.[4] In the previous study, 0.3% glucose water was used for flooding paddy soil to demonstrate the possibility of forming insoluble Tc. Paddy fields in nature, however, are not flooded by glucose water even though there are other sources of organic carbon such as plant litter, added organic matter for fertilization, and remains from previous crops. Therefore, the aims in this study were to determine the amounts of insoluble Tc in ponding water without addition of glucose and to clarify the main factors causing Tc insolubilization.

2. Materials and Methods

Preparation of ponding water samples. Paddy soil containing Tc-insolubilizing bacteria[3] was collected from Koriyama City, Japan. Characteristics of the soil were described in a previous report.[2] The Tc-insolubilizing bacteria in the paddy soil have not been isolated yet. The soil was air-dried and passed through a 2-mm-mesh sieve. This procedure eliminated plant litter and previous crop residues from the soil. For flooding, 5 g of the air-dried soil were put in a polypropylene tube with a cap and the tube was filled with 7.5 mL of autoclaved deionized water. The flooded soil was statically incubated at 25 ˚C for 120 days under 12 h-12 h light-dark cycles. The initial conditions of the sample were aerobic because the sample had more than 40 mL of headspace air. Flooded soil samples were prepared in triplicate.

During the 120-day incubation period, 1.2 mL of the ponding water was collected once a week from each flooded sample. After collection, the flooded soil sample was supplemented with autoclaved deionized water to maintain the initial volume of flooding. Of the collected ponding water, 0.2 mL was used to determine the concentration of ferrous iron, and the rest was used for the following experiments.

Insolubilization of Tc in ponding water samples. The experimental procedure is shown in Figure 1. The stock solution of NH_4TcO_4 in H_2O was filter-sterilized by passing it through a 0.2 μm pore size membrane. In the aqueous solution, Tc is present as pertechnetate (TcO_4^-). The Tc solution was added to each of the collected ponding water samples at a final concentration of 3 kBq mL^{-1}. These samples were then statically incubated at 25 ˚C for 14 days in the dark. The initial conditions of these samples were aerobic because the samples had about 14 mL of headspace air. After incubating for 0, 4, 7, and 14 days, a part of the sample was passed through a 0.2-μm-pore size filter to separate insoluble Tc. The relative amount of insoluble Tc was calculated by dividing "(the ^{99}Tc radioactivity at day 0) – (the radioactivity of ^{99}Tc at each sampling time)" by "the ^{99}Tc radioactivity at day 0".

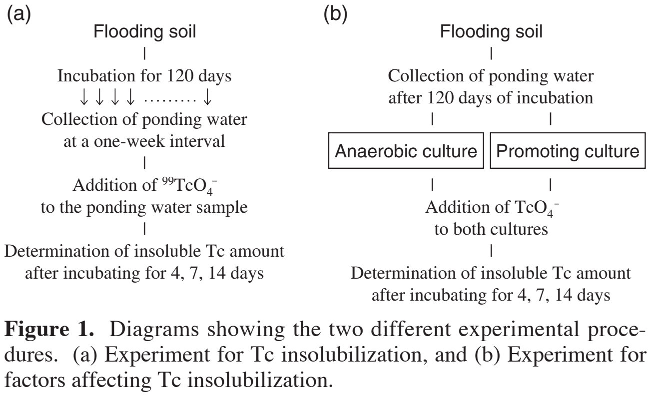

Figure 1. Diagrams showing the two different experimental procedures. (a) Experiment for Tc insolubilization, and (b) Experiment for factors affecting Tc insolubilization.

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Determination of Factors affecting Tc insolubilization. The experimental procedure is shown in Figure 1. To clarify the factors affecting Tc insolubilization, two subsamples were prepared using the ponding water from the 120-day flooding: 1) a sample incubated under anaerobic conditions (anaerobic culture), and 2) a sample with organic substrates added to promote bacterial metabolic activity (promoting culture). For the anaerobic culture, the ponding water sample was incubated in the AnaeroPack^{TM} System (MGC Co., Inc. Tokyo, Japan) for 14 days after addition of TcO_4^- at a final concentration of 3 kBq mL^{-1}. For the promoting culture, the following reagents were added to the ponding water sample (in milligrams per milliliter of culture): tryptone, 1.0; yeast extract, 0.5; and NaCl, 1.0. This subsample was also incubated for 14 days after addition of $^{99}TcO_4^-$ at a final concentration of 3 kBq mL^{-1}. Relative amounts of insoluble Tc were determined after incubating for 4, 7, and 14 days in the same way as mentioned above.

Analytical methods. The concentration of Fe(II) ions in ponding water samples was colorimetrically determined by the 1, 10-phenanthroline monohydrate method[5] at every time of sample collection.

The radioactivity of ^{99}Tc was measured by liquid scintillation counting of 0.1 mL filtrate samples mixed with 4 mL of liquid scintillation cocktail, Ultima gold LLT (PerKinElmer, Inc., Massachusetts, USA).

3. Results and Discussion

Formation of insoluble Tc. Variations in the relative amounts of insoluble Tc were small for 120 days of flooding (Figure 2). For 4-day incubation samples with different flooding periods, the mean value of the relative amounts of the insoluble Tc was 0.2 ± 1.7% of the total ^{99}Tc. Similar results were observed for the samples after incubating for 7 and 14 days (Figure 2), and mean values of the relative amounts of the insoluble Tc for both samples were less than 0.6% of the total ^{99}Tc. These data indicated that Tc was hardly insolubilized in the ponding water samples. However, the presence of Tc-insolubilizing bacteria in the paddy soil studied was certain because, as reported previously, a significant fraction of Tc was insolubilized in the ponding water samples prepared by flooding the same paddy soil with glucose water.[3] The addition of glucose as a carbon source promotes microbial metabolic activity, and the activated microbes rapidly exhaust molecular oxygen to decompose the organic carbon source. Both microbial activity[6] and anaerobic conditions[7] are responsible for removal of Tc from solution. The limited formation of insoluble Tc under the present conditions, therefore, was probably due to the low metabolic activity of the bacteria or the aerobic conditions in the ponding water samples. Among microorganisms, bacteria are key for Tc insolubilization in ponding water.[3] In the following experiment, the effects of the metabolic activity of bacteria and the aerobic conditions on insolubilization of Tc were investigated.

Factors affecting Tc insolubilization. Anaerobic conditions in the ponding water samples were evaluated by analysis of the ferrous ion because Fe(II) is generally released to ponding water from paddy soil under anaerobic conditions. The concentrations of Fe(II) ion in the ponding water samples were less than 0.45 μg mL^{-1} during the experimental period, while the addition of glucose to flooding water resulted in 143 μg of Fe(II) ion per milliliter after 7 days of flooding.[8] Therefore, the ponding water samples without addition of glucose were under aerobic conditions.

To clarify the effect of anaerobic conditions on Tc insolubilization, the ponding water sample spiked with ^{99}Tc was forcibly incubated under anaerobic conditions (anaerobic culture). Tc was hardly insolubilized at 14 days (Figure 3), suggesting that anaerobic conditions were a not key factor for Tc insolubilization in the ponding water.

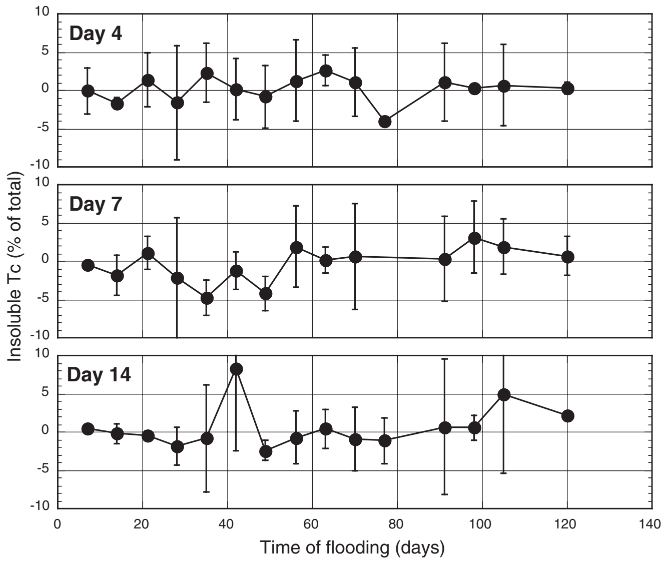

Figure 2. Formation of insoluble Tc in ponding water samples. The number of days means the time after the addition of ^{99}Tc to the ponding water. The means ± standard deviations (error bars) from three experiments are shown.

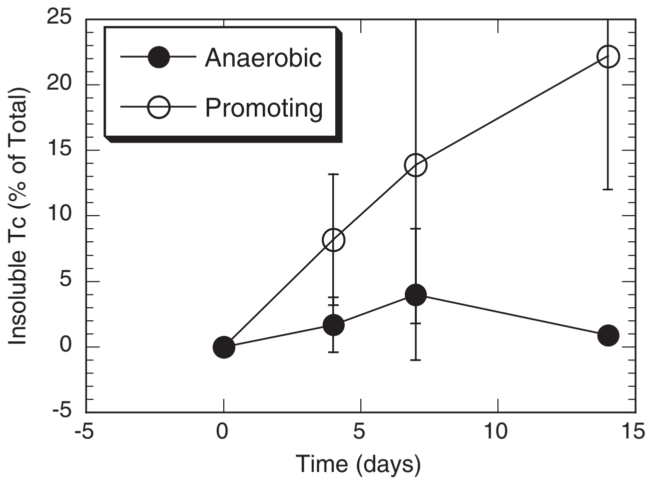

Figure 3. Formation of insoluble Tc in the anaerobic culture and the promoting culture. The means ± standard deviations (error bars) from three experiments are shown.

The effect of bacterial activity on Tc insolubilization was also determined by the addition of organic substances to the ponding water sample (promoting culture). Insoluble Tc was formed and its relative amount increased with time (Figure 3), while Tc was hardly insolubilized without addition of organic substances (Figure 2). These results suggest that Tc-insolubilizing bacteria were present in the ponding water sample and their metabolic activity resulted in formation of insoluble Tc in this sample. Organic substrates added to the cultures may function as electron donors because the previously known Tc-insolubilizing bacterium, *Escherichia coli*, requires organic substrates such as formate, pyruvate, glucose, or glycerol as electron donors.[9]

4. Conclusion

Insoluble Tc was scarcely formed in ponding water samples prepared by flooding of the air-dried paddy soil with deionized water. The amount of insoluble Tc was increased by the addition of organic substrates to the ponding water samples, but not by incubation under anaerobic conditions without addition of organic substrates. The formation of insoluble Tc in ponding water is therefore due to bacterial activity.

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