Research article

Mitigating Splash Erosion with Applying *Bacillus subtilis* Natto

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Abstract Soil erosion from upland fields affects not only soil productivity but also water environment. From the viewpoints of soil and water conservation, many strategies for mitigation of soil erosion were discussed. Especially, conservation strategy for mitigating splash erosion which is the first process of soil erosion should be considered. Moreover, biological crust mixing with microorganisms has been focused as one of the treatments for splash erosion. Therefore, two objectives were determined in this study. The first is to evaluate the effect of *Bacillus subtilis* Natto adding on mitigating splash erosion, and the second is to investigate the kinetic energy of raindrop to break the binding force of soil particles with hyphae of *Bacillus subtilis* Natto. Raindrop experiment was carried out with stainless cans and Mariotte bottle of splash erosion apparatus. Stainless cans were filled with soil, and then *Bacillus subtilis* Natto was applied. In addition, raindrop energy was changed through controlling the height of Mariotte bottle. Based on the experimental results, there was a tendency that soil loss from the cans applied *Bacillus subtilis* Natto was significantly lower than controlled cans. In addition, it was observed that *Bacillus subtilis* Natto mitigated splash erosion until kinetic energy of raindrop at 4.86×10^{-5} J/drop. Therefore, it was concluded that *Bacillus subtilis* Natto is applicable for mitigation of splash erosion.

Keywords splash erosion, *Bacillus subtilis* Natto, biological crust

INTRODUCTION

Large amounts of soils were released from upland fields by rainfall and surface runoff. Soil erosion is a cause of decreasing soil productivity. Moreover, soil erosion causes water pollution such as eutrophication in watersheds. Therefore, some conservation strategies for soil erosion such as buffer strips installation (Kawai et al., 2007; Gopal and Mihara, 2008; Siriwattananon et al., 2009, Torillo and Mihara, 2011, 2014) have been discussed.

On the other hand, conservation strategies for mitigating splash erosion which is the first process of soil erosion should been considered. In this connection, making soil crust has been considered as a proper treatment for mitigating splash erosion. Soil crust formation was affected by microorganism activity, root growing, soil animal activity or mineral interaction (Six et al., 2002). Therefore, microorganism growing is important for mitigating soil loss. There was a report by Shimomura et al. (2007) that growing microorganisms in soil is able to reduce soil loss. However, it was not discussed about specific species of microorganism for mitigate raindrop erosion.

So, this study focused on *Bacillus subtilis* Natto which is a kind of Gram-positive bacteria. *Bacillus subtilis* Natto can make spore which has tolerance for acid, ultraviolet or heat (Hosoi, 2003). In addition, *Bacillus subtilis* Natto creates hyphae which contain viscous material as γ-poly-glutamic acid (Hara, 1990).

In this study, two objectives were determined. One is to evaluate the effect of *Bacillus subtilis* Natto adding on mitigating splash erosion and the other is to investigate the kinetic energy of raindrop to break the binding force of soil particles with hyphae of *Bacillus subtilis* Natto.

METHODOLOGY

In this study two experiments were conducted, the evaluation of *Bacillus subtilis* Natto addition on mitigating splash erosion, and the investigation of the kinetic energy of raindrop to break the binding force of soil particles with hyphae of *Bacillus subtilis* Natto.

Evaluation of *Bacillus subtilis* Natto Addition on Mitigating Splash Erosion

Bacillus subtilis Natto used in this experiment was laboratory made (Fig. 1). It consists of a dry powder which contained 1.08×10^7 colonies formed units per gram (cfu/g) of *Bacillus subtilis* Natto.

Soil properties are shown in Table 1. Stainless cans of 1.1 cm diameter and 1.0 cm deep were filled up with Andosol soil. Then *Bacillus subtilis* Natto powder at 1 g/cm^2 was applied to soil. After applying *Bacillus subtilis* Natto, stainless cans were put in incubator set at 37 ºC for 2 weeks. Every day, physiological saline solution at 0.8 ml was applied to each can.

Fig. 1 *Bacillus subtilis* Natto powder

Fig. 2 Stainless can

Table 1 Characteristics of the soil (volcanic ash soil)

Specific gravity	Particle size distribution %					Soil texture	pH	EC µS/cm
	Gravel	Coarse sand	Fine sand	Silt	Clay			
2.63	1.97	12.3	27.8	0	39.3	CL	5.74	155

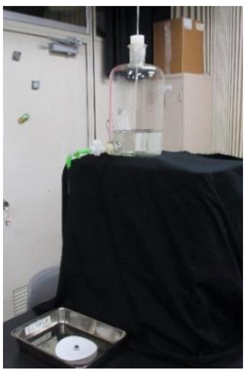

Fig. 3 Apparatus for raindrop experiment

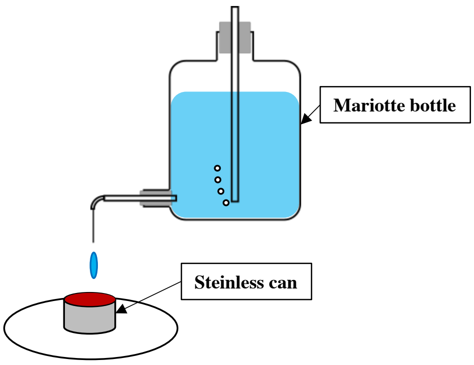

Fig. 4 Outline of raindrop experiment

Raindrop experiments were carried out with a Mariotte bottle as shown in Figs. 3-4. A needle of DIK-6000 artificial rainfall simulator was attached with Mariotte bottle. Water was dripped from a height of 18.0 cm. Based on the observation and calculation, kinetic energy of raindrops was 2.36×10^{-5} J. For an experiment, 50 raindrops were applied to soil sample of each can.

Kinetic Energy of Raindrop to Break Binding Force of Soil Particles with Hyphae

Bacillus subtilis Natto powder and soil sample used in this experiment were the same as the first experiment. *Bacillus subtilis* Natto at 1.01×10^{7} colonies were observed in the powder.

Raindrop experiments were conducted employing Mariotte bottle as well as the first experiment. Raindrop was applied at the heights of 20, 40, 60 and 80 cm. Kinetic energies of raindrop in each height were observed and calculated based on the Eq. (1). Raindrop mass was measured with dried filter paper and raindrop velocities of each height was measured by photographs shuttered at 1/100 second. The results of observed and calculated kinetic energy of raindrops are indicated in Table 2.

$$\text{Kinetic energies of raindrop, Ek (J)} = \frac{1}{2} \times M \times v^2 \qquad (1)$$

Where M is raindrop mass (kg), v is raindrop velocities of each height measured by photographs at 1/100 second (m/s).

Table 2 Raindrop energies in each height

Height of raindrop cm	Kinetic energy $\times10^{-5}$ J
20	3.26
40	4.86
60	9.66
80	13.8

Analysis of Soil Loss

After raindrop experiments, the mass of soil particles splashed out from the samples was measured. Then soil loss rate was calculated with Eq. (2).

$$\text{Soil loss rate (\%)} = \left(1 - \frac{\text{Remained soil mass in the stainless can (g)}}{\text{Initial soil mass in the stainless can (g)}}\right) \times 100 \qquad (2)$$

RESULTS AND DISCUSSION

Bacillus subtilis Natto Addition on Mitigating Splash Erosion

Fig. 5 shows the rate of soil loss in stainless cans. In control cans, about 20% of soil was released from cans. On the other hand, in case of stainless cans with *Bacillus subtilis* Natto, the rate of soil loss was reduced to 13%. Moreover, based on the results of statistical analysis, significant difference at $p \leqq 0.01$ was observed between the rate of soil loss of control and that of *Bacillus subtilis* Natto added.

In addition, as a result of microscope observation, hyphae of *Bacillus subtilis* Natto was observed in soil applied with *Bacillus subtilis* Natto as shown in Fig. 6. So, it was considered that *Bacillus subtilis* Natto formed biological crust with hyphae growing.

Therefore, it was considered that *Bacillus subtilis* Natto is applicable to mitigate splash erosion.

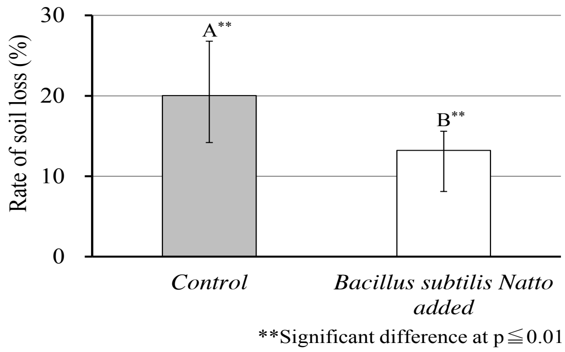

Fig. 5 Rate of soil loss

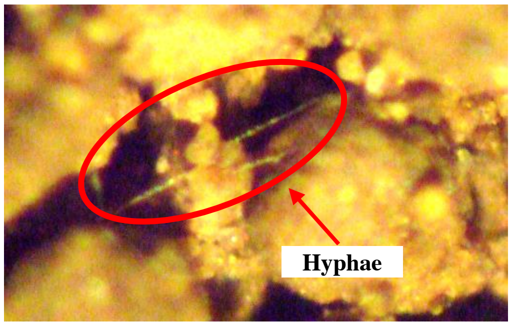

Fig. 6 Hyphae in soil

Kinetic Energy of Raindrop to Break Binding Force of Soil Particles with Hyphae

Figure 7 shows the relationship between the rate of soil loss and the kinetic energy of raindrop. According to the results, the rate of soil loss increased with the kinetic energy of raindrop in both cans of control and *Bacillus subtilis* Natto added. As a result of statistical analysis, confidence interval at 99% was observed for both regression lines of the rate of soil loss of control and that of *Bacillus subtilis* Natto added.

The changes in soil loss rate were shown in Fig. 8. *Bacillus subtilis* Natto reduced the soil loss rate at kinetic energy of raindrop from 3.46 to 4.86×10^{-5} J. In addition, significant difference was observed between the rate of soil loss of control and *Bacillus subtilis* Natto added. However, when the kinetic energy of raindrop was larger than 9.66×10^{-5} J, significant difference was not observed.

Therefore, it was considered that biological crust formed by *Bacillus subtilis* Natto was not broken by splash erosion until 4.86×10^{-5} J of kinetic energy.

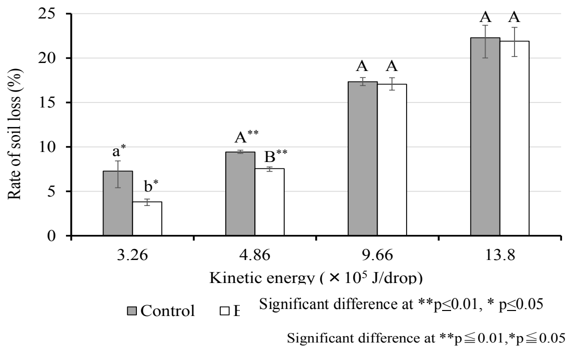

Fig. 7 Relationship between rate of soil loss and kinetic energy of raindrop

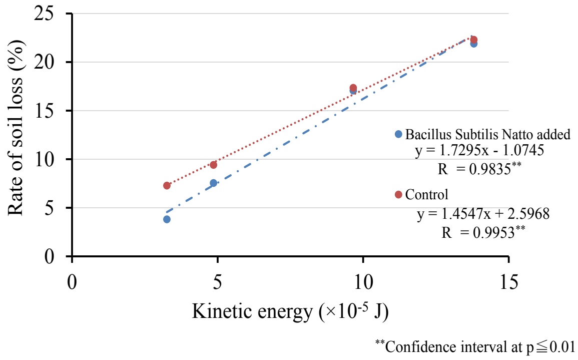

Fig. 8 Changes in soil loss rate

CONCLUSION

In this study, two experiments were carried out to investigate the effect of *Bacillus subtilis* Natto addition for mitigating splash erosion. Based on the experimental results, the rate of soil loss was decreased by *Bacillus subtilis* Natto addition. Moreover, hyphae of *Bacillus subtilis* Natto were observed in the soil which *Bacillus subtilis* Natto was applied. So, it was considered that *Bacillus subtilis Natto* is able to form biological crust with growing hyphae.

In addition, there was a tendency that *Bacillus subtilis* Natto mitigated splash erosion until kinetic energy of raindrop at 4.86×10^{-5} J/drop.

Therefore, it was concluded that *Bacillus subtilis* Natto is applicable to mitigate splash erosion. In addition, it was found out that biological crust formed with *Bacillus subtilis* Natto started to break at kinetic energy of raindrop 4.86×10^{-5} J/drop.

The results of this study showed that microorganisms which create viscid hyphae such as *Bacillus* sp. may be applicable to mitigate splash erosion.

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