Effects of microorganisms on hydraulic conductivity decrease in infiltration

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Summary
Microorganisms can clog pores in universal soil and decrease hydraulic conductivity and infiltration. We did three types of column experiments to clarify the effects. In all columns, glucose solution of 50 μg cm^{-3} was percolated for 120 days, and both the saturated hydraulic conductivity, K_s, and the volume ratio of gas phase, a, were measured continuously. The K_s decreased rapidly in the initial 10 days, and after while it slowly decreased for 110 days. By adding chloramphenicol in the second column as bactericide and cycloheximide in the third column as fungicide to the glucose solutions, we observed biological clogging by bacteria and fungi respectively, bacterial clogging proceeding more rapidly than the fungal clogging. The volume of gas phase increased and reached the maximum value of 30.6% after 103 days from the beginning of percolation. This large amount of gas was retained in the soil pores as bubbles and occluded the pathway of water, resulting in the decrease in K_s. When percolating solution was altered to sodium azide, a strong biocide, after 120 days the volume of gas phase decreased rapidly, and K_s increased simultaneously.

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Introduction

When soil is continuously submerged with nutrient solution, saturated hydraulic conductivity, K_s, of soil decreases with time. Allison (1947) explained that one of the causes of this decrease is biological clogging of soil pores with microbial cells and their synthesized products, slimes, or polysaccharides.

Biological clogging can occur when wastewater is recycled by sprinkler irrigation (de Vries, 1972), in septic tank filters (Kristiansen, 1981; Jones *et al.*, 1993), and in bioremediation practices (Lee *et al.*, 1988; Jennings *et al.*. 1995). On the other hand, biological clogging can be advantageous. Preul (1968) reported that infiltration rate of domestic wastewater stabilization ponds decreased in three years of operation and prevented excess infiltration. Chang *et al.*. (1974) showed that the growth of microorganisms that produces polysaccharides sealed animal wastewater ponds. Biological clogging can also control seepage in irrigation channels (Ragusa *et al.*, 1994) and small dams (Rengasamy *et al.,* 1996) at little cost.

Microbial cells which are responsible for biological clogging are those of the bacteria (Gupta & Swartzendruber, 1962; Frankenberger & Troeh, 1982), fungi (Seki *et al.*, 1996), and algae (Ragusa *et al.*, 1994). Cunningham *et al.* (1991) showed that K_s of glass spheres and quartz sand decreases with the development of biofilm of *Pseudomonas aeruginosa*. Biofilm forms when solution rich in nutrients is percolated in coarse-textured porous media (Rittman, 1993; Vandevivere *et al.*, 1995), while in natural soils microcolonies predominate (Harvey *et al*., 1984). Microbial synthesized polysaccharides (Fermann & Weaver, 1978; Whitefield, 1988) and polyuronide (Thomas *et al.*, 1966) also cause decrease in K_s (Avnimelch & Nevo, 1964; Vandevivere & Baveye, 1992a).

Bacterial synthesized gas such as methane can also cause K_s to decrease by occluding pore necks as bubbles with sand (Sanchez *et al.*, 1994), peat (Reynolds *et al.*, 1992), and Andisols (Seki *et al.*, 1996). Faybishenko (1995) showed that entrapped air exists in soil pores as either mobile air or immobile air, and it reduces hydraulic conductivity of soil.

Miyazaki (1993) showed that biological clogging is severer in the hardpan layer of paddy field soils, whereas in upland and forest Andisols it is not. One possible reason is that there are few microbes in paddy field soil than in upland and forest soil, and therefore increase in microbial number is large when nutrient solution is supplied. Marshall (1988) showed that in oligotrophic habitats, starved bacteria survive at solid surfaces, and when the substrate is continually replenished, rapid biofilm development is expected.

It is difficult to distinguish the contributions of each of the above-mentioned factors on the decrease in K_s. Here we show results of a series of column experiments and discuss the effects of microbial activities on decrease in K_s.

Materials and methods

Soil

The hardpan layer (25-30 cm depth) from paddy field of Andisols in Tokyo was used for column experiments. Characteristics of this soil are shown in Table 1. The soil was sieved to pass 2 mm and dried to the water content of 800 g kg^{-1}.

Table 1 Soil sample and experimental setup.

	Variable	Value
Soil characteristic	% clay	35
	% silt	15
	% sand	50
	Particle density /g cm^{-3}	2,64
	Total C /g kg^{-1}	31,2
	Total N /g kg^{-1}	2,51
	Number of bacteria /g[1)]	2.0×10^7
	Number of fungi /g[1)]	2.4×10^4
Experimental setup	Bulk density /g cm^{-3}	0,65
	% porosity	75,4

1) Dilute plate counting method (Seki *et al.*, 1996)

Flow system and procedures

We packed the sample with an acrylic plastic column of 5 cm diameter and 1 cm height (Figure 1) and saturated the sample over night from the bottom end of the column. The height of the column was set 1 cm because in most of the previous reports biological clogging proceeded within 1 cm from the inlet of nutrient solution (Thomas *et al.*, 1966; Rice, 1974; Seki *et al.*, 1996).

After packing, glucose solution was applied to the soil surface from a Mariotte bottle. The average hydraulic gradient in the column was set to 1 by keeping the height of Mariotte bottle and the drip tube constant. At the top and bottom ends of the column, glass filters of 4 mm thickness were set to support the sample.

Experimental conditions

We conducted three types of column experiments (Table 2). In the first column glucose

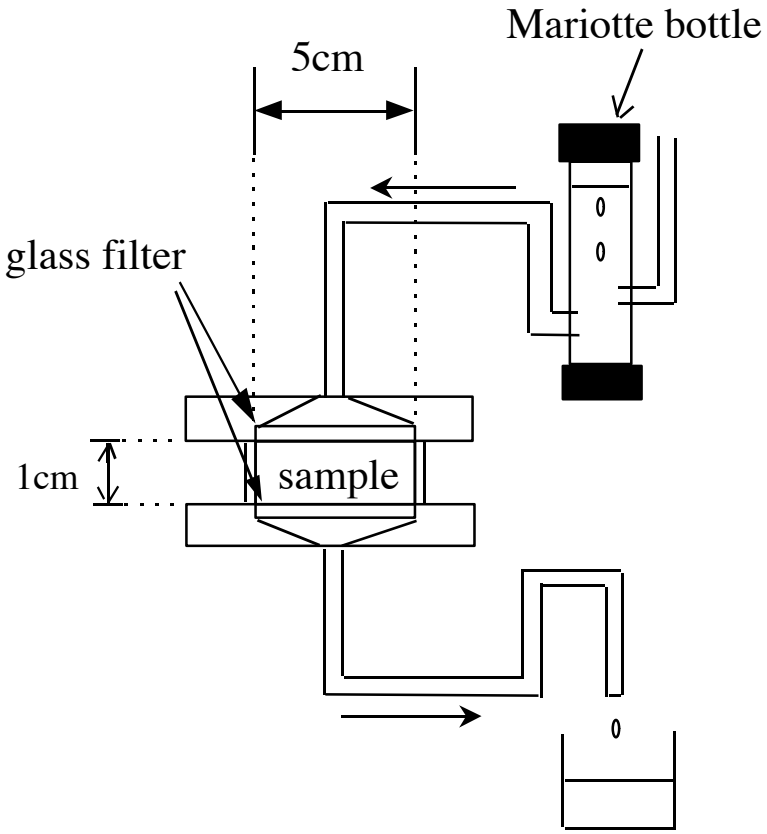

Fig.1 Schematic diagram of the flow system (not to scale).

solution of 50 μg cm^{-3} (GCO) was used as substrate for microbial growth. In the second column glucose solution with chloramphenicol (GCH) was used to kill bacteria. In the third column glucose solution with cycloheximide (GCY) was used to kill fungi. Bacterial clogging and fungal clogging can thus be identified separately. Seki *et al.* (1996) also did an experiment with sodium azide solution (SAZ) and we shall refer to this in the discussion.

Table 2 Solution applied to the flow system.

Abbreviation of run	Solution	Bacteria	Fungi
GCO	Glucose 50 μg cm^{-3} (Control)	+	+
GCH	Glucose 50 μg cm^{-3} + chl. 2 mg cm^{-3}	-	+
GCY	Glucose 50 μg cm^{-3} + cyc. 1 mg cm^{-3}	+	-
SAZ[1)]	Sodium azide 50 μg cm^{-3}	-	-

chl. chloramphenicol cyc. cycloheximide
+; Expected growth -; Expected decline
1) Seki et al. (1996)

In all three column experiments we altered flowing solution from glucose to sodium azide of 50 μg cm^{-3} to kill all the living microbes after percolating glucose solution for a given period. We covered three columns with black sheets to prevent photosynthesis. In this way algal growth was prevented, and there was no algal clogging (Ragusa *et al*., 1994). All the experiments were done under 30°C to enhance microbial activity. The average temperature of August in Tokyo in the last 30 years is 27.1°C.

Saturated hydraulic conductivity
We calculated the flux of solution from the measured value of the discharge rate. The hydraulic heads at the inlet and the outlet of the column were measured by piezometers. Then we calculated the saturated hydraulic conductivity, K_s, from Darcy's equation:

$$Q = -K_s \frac{\Delta H}{\Delta Z} \quad (1)$$

where Q is the flux of percolation, ΔH is the head difference between the inlet and outlet of the column, ΔZ is the difference in height between the inlet and outlet of the column. In this way, K_s of the column including the upper and lower filter was obtained. We assume that clogging at both the glass filters and soil proceeds at the same rate, because Seki *et al.* (1996) showed that the clogging of glass filters proceeds at nearly the same rate as the clogged region of the soil sample. By this assumption, the observed K_s is equal to the K_s of soil sample. Actually we use the term saturated hydraulic conductivity, K_s, even after providing evidence that the soil was not completely water-filled and the volume of gas phase increased to the maximum value of 30.6%. The term saturated means that the water was not retained in the soil by capillary action (Sanchez *et al.*, 1994).

Volume ratio of gas phase
During percolation we stopped the flow, removed the soil column from flow system, measured the mass of the column, and after that, put the column back in the system and

started percolation again. The volume ratio of the gas phase, a, was calculated by the following equation:

$$a = 1 - \frac{\rho_d}{\rho_p} - \frac{\rho_t - \rho_d}{\rho_w} \quad (2)$$

where ρ_d is the dry bulk density, ρ_p is the particle density, ρ_t is the wet soil density, ρ_w is the density of water. Wet soil density was calculated from the mass and the volume of the column, and other variables used in this calculation are shown in Table 1.

Results and Discussion

Saturated hydraulic conductivity

Figure 2 shows the changes in saturated hydraulic conductivities (above) and in the volume ratio of gas phase (below). In GCO run, K_s increased from the initial value of 2×10^{-3}cm s^{-1} to 4×10^{-3}cm s^{-1} at 0.65 day, and decreased rapidly until 4×10^{-5}cm s^{-1} at 10 day, followed by gradual decrease until 3×10^{-6} cm s^{-1} at 120 day. After we altered percolation solution from glucose to sodium azide at 120 day, K_s increased rapidly to 5×10^{-4}cm s^{-1} at 128 day, and decreased down to 2×10^{-5}cm s^{-1} at 137 day when it stabilized.

In GCH run, change in K_s was similar to GCO until 120 day, while after 120 day change in K_s was not similar to GCO. In GCY run, initial decrease of K_s was more rapid than in GCO run, and after 120 day K_s did not change as in GCO run.

In SAZ run, saturated hydraulic conductivity in the top 1-cm layer of 12-cm high column did not change much for 39 days.

Volume ratio of gas phase

Initial values of the volume ratio of gas phase, a, were 14.0%, 15.0%, 18.4%, for GCO, GCH, and GCY runs respectively. They decreased rapidly with percolation. In GCO run, a decreased initially and increased remarkably from 3.6% at 40 day to 30.6% at 103 day. In GCH run, a decreased gradually until 120 day. In GCY run, a fluctuated around 10% until 120 day. When sodium azide was percolated after 120 day, a decreased rapidly in GCO run and slightly in GCY run. The value of a was not measured in SAZ run.

K_s increase with air removal

Change in a as a function of cumulative discharge is shown in Figure 3. Linear regressions are also shown in the figure. Absolute values of the slope of the linear regressions are the rate of air removal by dissolution. We can calculate the amount of air dissolved in the unit volume of the percolating solution. These values are 0.74 μg m^{-3} for GCO, 1.11 μg m^{-3} for GCH, and 0.72 μg m^{-3} for GCY. For all the three columns, K_s increased with entrapped air dissolution. As an example, change in K_s as a function of a of GCO run is shown in Figure 4. As entrapped air is removed from the start of the percolation to 0.65 day, K_s increased from 2×10^{-3} cm s^{-1} to 4×10^{-3} cm s^{-1}.

In this experiment increase in K_s due to entrapped air dissolution started at the beginning of percolation and ended in one day, while in most of the previous percolating experiments it was followed by initial decrease in K_s and started from 10 to 20 day (Allison, 1947; Seki *et al.*, 1996). This difference arose from the fact that the column height of our experiments is much shorter than previous percolating experiments. The volume of percolating solution required for decreasing certain amount of a is inversely proportional to the column height.

Clogging by bacteria and fungi

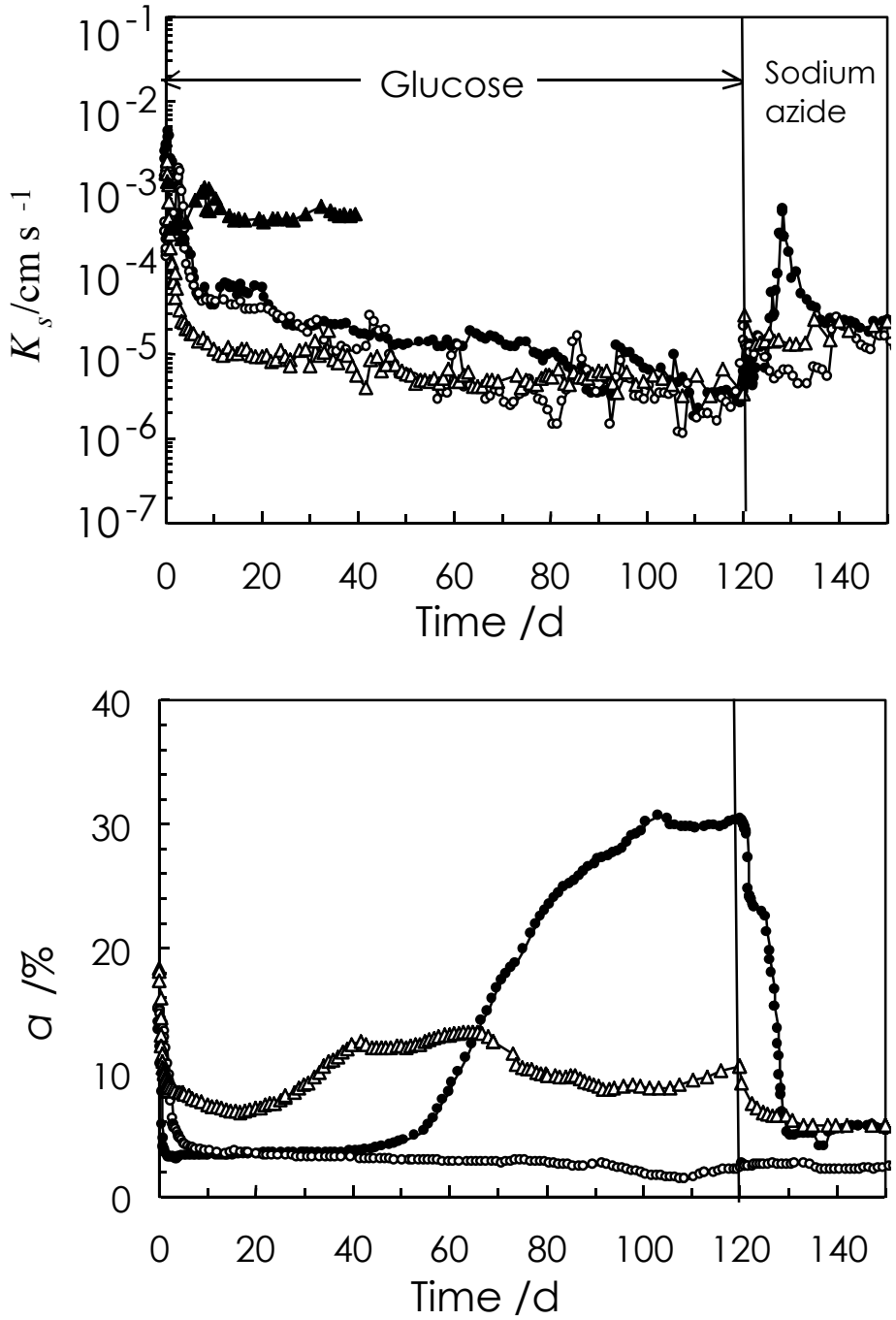

Fig. 2 Change in saturated hydraulic conductivity, K_s (above), and volume ratio of gas phase, a (below), of soil sample. The percolating solutions are GCO (●), GCH (○), and GCY (△). All solutions were altered from glucose to sodium azide at 120 day. Closed triangle (▲) at the top figure is a reference data (Seki *et al.*, 1996).

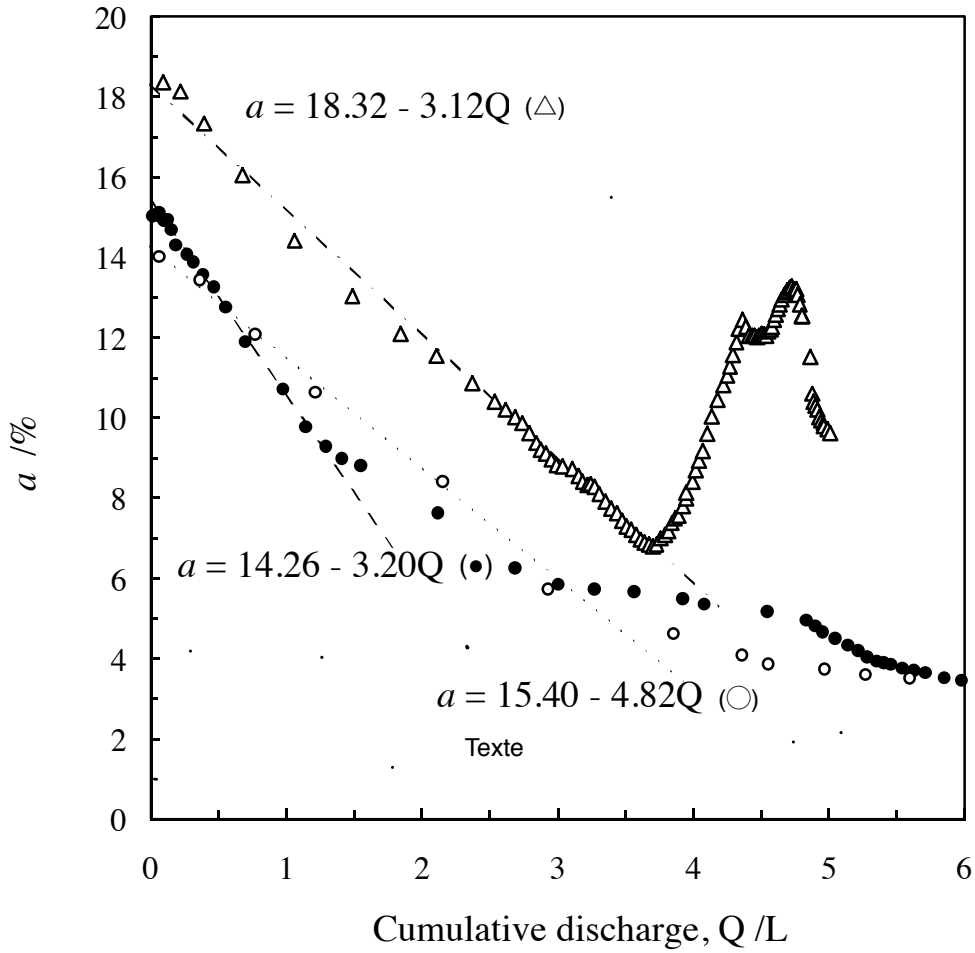

Fig. 3 Volume ratio of gas phase as a function of cumulative discharge. Percolating solutions are GCO (●), GCH (○), and GCY (△).

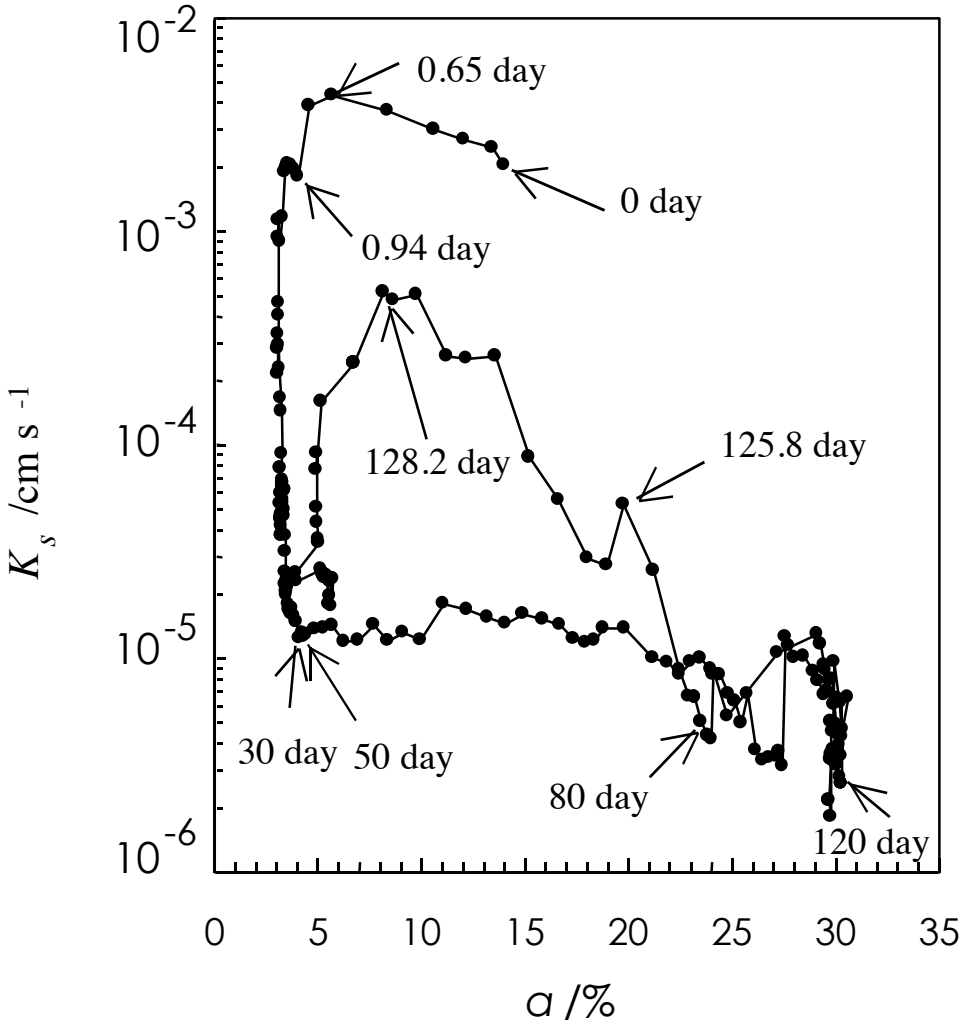

Fig. 4 Saturated hydraulic conductivity, K_s, plotted against volume ratio of gas phase, a, for GCO run.

For GCH and GCY runs, not so much increase of the volume ratio of gas phase was observed as GCO run (Figure 2). Therefore, we observed the single effect of biological clogging with microbial cells and their synthesized products on K_s. In the GCH run chloramphenicol worked as a bactericide, and fungi clogged the pores. In GCY run cycloheximide worked as fungicide, and the pores were clogged by bacteria. Figure 2 shows that bacterial clogging proceeded more rapidly than fungal clogging. It may be because bacteria grow faster on the soil surface than fungi grow. The rate of fungal clogging (the rate of decrease in K_s of GCH run) is almost the same as the rate of clogging in GCO run. It suggests that fungal clogging was predominant in GCO run, as Seki *et al.* (1996) showed by dilute plate counting method.

Possibility of dry bulk density increase

We calculated the volume ratio of the gas phase by assuming that the dry bulk density is constant during percolation. However, dry bulk density may change, because part of the increased microbial cells and their synthesized products accumulate in soil pores. We obtained the dry bulk density value of 0.65 g cm^{-3} gravimetrically after the end of the percolation, and therefore microbial cells and their synthesized products that accumulated in soil pores may have been destroyed during drying at 105EC. Dry bulk density measured in this way does not involve the mass of microbial cells and their synthesized products destroyed by drying. In this section we include the mass of microbial cells and their synthesized products in the dry bulk density, in other words, density of the entire solid phase.

Figure 2, the volume of gas phase decreased gradually from 15 to 108 day in the GCH run. The volume was calculated from the increase of the measured value of wet soil density during this period, but we cannot say whether this increase is the result of the decrease in volume of gas phase or the increase in dry bulk density or combined effect of these two phenomena. Since the calculation based on the first assumption was done in Figure 2, we now take the second assumption; we assume that volume of gas phase is constant from 15 day to 120 day and calculate dry bulk density change (Figure 5). Dry bulk density increased from 0.65 g cm^{-3} to 0.73 g cm^{-3} from 15 to 108 day. This is the largest estimate of the increase in dry bulk density, because we assumed that gas was not removed during 15 to 120 day. From mass balance estimates, the bulk density can not increase this much if only the applied glucose was the source of mass increase. Part of the mass increase may have been due to the accumulation of chloramphenicol.

Occlusion of pore necks by bubbles

In the GCO run the decrease in K_s was caused not only by biological clogging but also by occlusion of pore necks by bubbles of gas which occupied the volume of 30.6% at maximum. The bubbles were mainly of methane produced by bacteria (Seki *et al.*, 1996).

After percolation the solution was altered to sodium azide at 120 day in the GCO run, the volume ratio of gas phase decreased, and hydraulic conductivity increased simultaneously (Figure 4). It seems that the gas occlusion was one of the causes of decrease in K_s. By linear regression between cumulative discharge and the volume ratio of gas phase as in Figure 3, the amount of gas dissolved in the unit volume of solution was 4.2 μg m^{-3}. We could see bubbles of gas in the effluent discharging from the soil column. It indicates that part of the gas dissolved in the solution became bubbles.

In the GCH run methane-producing bacteria were killed, and no gas was produced. In the GCY run the volume ratio of the gas phase increased gradually from 6.8% at 17 day to 13.3% at 64 day, but it did not increase any more. The gas production was slower than that in GCO run because the K_s decreased more rapidly. The smaller value of K_s resulted in less solution percolating in any one period, which means that there was less substrate for methane production. This is why the volume of gas phase did not increase in GCY run as much as in

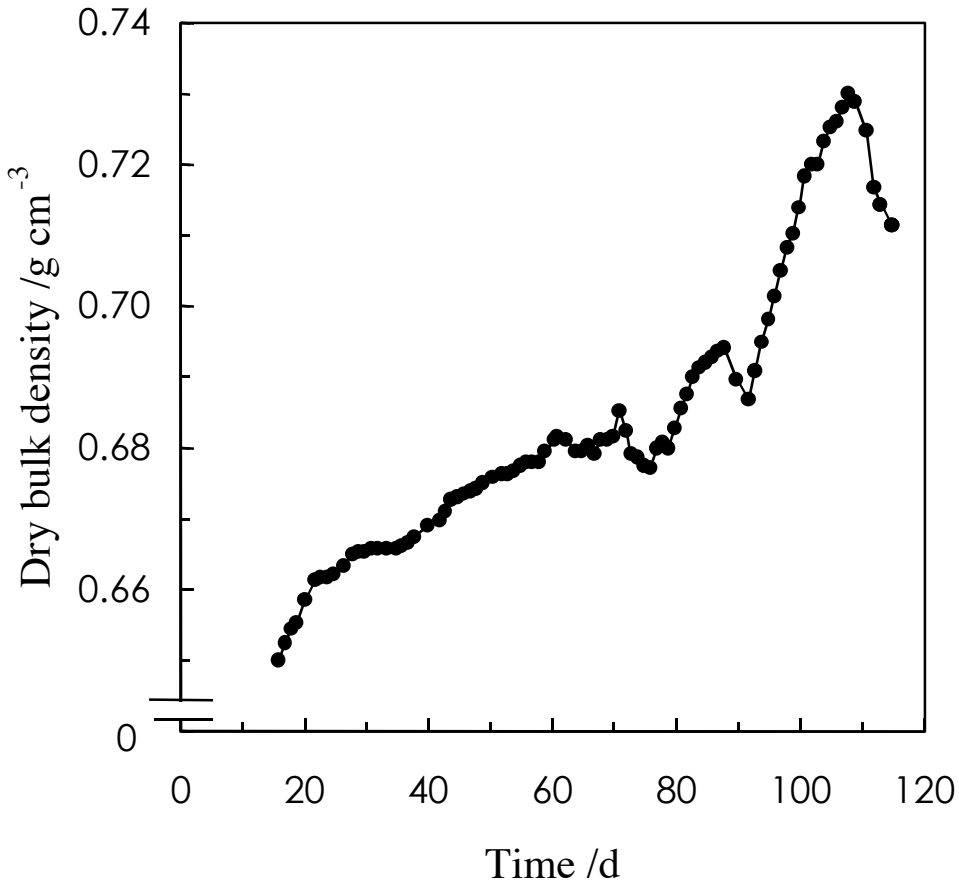

Fig.5 Change in dry bulk density for GCH run, calculated by assuming that volume ratio of gas phase is constant after 15 day.

GCO run.

Combined effect

The fact that in all columns K_s did not return to the initial value after the alternation of percolation to sodium azide shows that not all the dead microbial cells and their synthesized products were removed (Ripley & Saleem, 1973; Seki *et al.*, 1996). In GCO run, K_s increased from 120 to 128 day with gas removal and decreased from 128 to 137 day. Reduction of K_s in GCO run was caused by the combined effect of both biological clogging and gas occlusion. Therefore when gas was removed from 120 to 128 day, one of the causes of K_s reduction, that is gas occlusion, was eliminated and K_s approached to the initial value.

The reduction of K_s after 128 day is difficult to explain. The microbes was killed during the 120 to 128 day period, so the amount of living microbes did not change after 128 day. The volume ratio of gas phase did not change after 128 day, either. It means that the reduction of K_s during this period was not due to microbial clogging or gas occlusion, and may due to some kind of change in soil structure.

One explanation is as follows. As the volume of gas phase increased from 15 to 120 day, the microbially synthesized gas pushed apart soil particles. This gas was removed by dissolution or discharge during 120 to 128 day. At 128 day the pores previously occupied by the gas remained large, acting as the path for water, with the result that K_s increased. During 128 to 137 day, the pores previously occupied by the gas shrank and approached the initial size, and the K_s decreased again. However this explanation is not pursuasive, because the gas pressure may not be strong enough to push apart soil particles as postulated here.

The stabilized value of K_s at 150 day was approximately same as in the GCH and GCY runs. Biological clogging can decrease K_s to the order of 10^{-6} cm s^{-1} by reducing the cross sectional area of the pathway of percolating solution. Gas occlusion can also decrease K_s by blocking the pathway of water and it also change the arrangement of the soil particle. The combined effect of these two phenomena did not decrease K_s to much less than of 10^{-6} cm s^{-1}.

Conclusions

When glucose solution of 50 mg kg^{-1} is percolated through a soil column, K_s diminished as a result of biological clogging and gas occlusion. Fungal clogging was more important than bacterial clogging in our experiment. When fungi were killed by cycloheximide, pores were clogged by bacteria. Bacterial clogging was more rapid than fungal clogging, which suggests that bacteria grow on the soil surface more rapidly than fungi do.

The volume of gas phase increased with time and reached the maximum value of 30.6% after 103 days from the beginning of percolation. This large amount of gas was retained in the soil pores as bubbles and occluded the pathway of water, resulting in the decrease in K_s. Decrease in K_s was caused by the combined effect of both biological clogging and gas occlusion. We conclude that gas occlusion was one of the causes of decrease in K_s because when percolating solution was altered to sodium azide, a strong biocide, the volume of gas phase decreased rapidly and K_s increased simultaneously. We also suggested that occlusion of gas in soil pores altered the arrangement of soil particles.

In the past biological clogging and gas occlusion have been studied separately. However, they proceed simultaneously in natural conditions and affect K_s in a complex manner.

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