

## **Bioclogging of glass beads by bacteria and fungi**

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### **Abstract**

We analyzed biological clogging of glass beads in laboratory column experiments. Glucose solution of 50 g cm<sup>-3</sup> was percolated in columns packed with glass beads of 0.2-mm and 0.05-mm in diameters, and change in hydraulic conductivity was measured continuously for 30 days. The bioclogging at the inlet of solution proceeded rapidly in every column. When glass filter was placed on the top of the sample, the glass filter was clogged severely. To avoid filter clogging, we did the other column experiments without setting glass filter over the top of the sample. Hydraulic conductivity at the top layer of every column decreased in three orders of magnitude in 2 to 4 days, and stabilized with small tendency to decrease afterwards. The numbers of fungi as well as bacteria at clogged layers increased in every column. Therefore fungi are also important clogging material, although many researches so far have focused on bacterial clogging and neglected the effect of fungi. The thickness of the layer of clogged part depends on the particle size of the beads. In 0.2-mm beads, the thickness of the clogged layer was larger than 0.3 cm, while in 0.05-mm beads the thickness of the clogged layer was smaller than 0.3 cm. Shear stress of biomass at 0.2-mm beads is larger than the biomass at 0.05-mm beads because of larger flux at initial condition, and microbial cells moved downward by shear detachment force and the clogged layer became thicker. When deaerated distilled water was percolated, hydraulic conductivity changed in three stage, increase, decrease and increase, and finally two orders decrease in hydraulic conductivity was observed.

**Keywords:** clogging, bioclogging, bacteria, fungi, hydraulic conductivity, microbial effect

### **Introduction**

Clogging of soil pores by microbial cells and their exudate (bioclogging) causes decrease in hydraulic conductivity over a long period of time (Allison, 1947). Bioclogging phenomenon has been reported in wastewater sprinkler irrigation system (de Vries, 1972), septic tank filters (Kristiansen, 1981), and in bioremediation (Lee *et al.*, 1988). Baveye *et al.* (1998) made an exhaustive review of bioclogging.

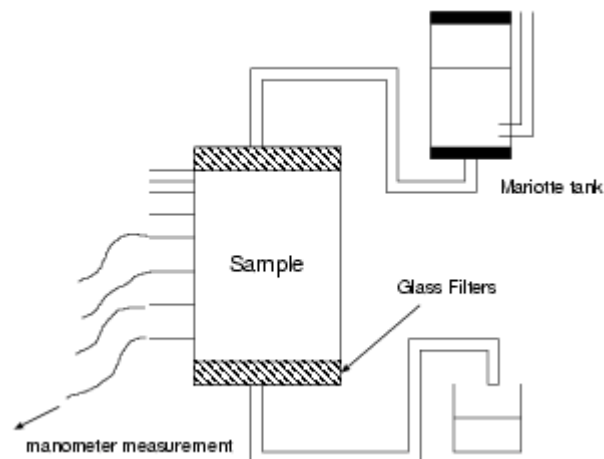
Attempt to make estimation of biological clogging by model is continued (MacDonald *et al.*, 1999; Seki and Miyazaki, 2001), but estimation of model parameters such as microbial growth and mass transfer is not easy. Field data and laboratory analysis is required to understand bioclogging mechanism. We conducted a series of experiments to see bioclogging of glass beads samples and analyze the mechanism.

### Materials and Methods

We conducted four types of column experiments by using glass beads of 0.2 and 0.05 mm under the constant temperature of 25 (Table 1). The permeameter is an acrylic plastic column of 5-cm diameter and 10-cm height (Figure 1). The top and bottom ends of the samples for Run 1 were supported by glass filters of 4-mm thick, and only bottom filter were used for Runs 2 to 4. Three piezometers were inserted equidistantly at different height of the column, 0, 3, 10, 17, 30, 50, 70, 90, 100-mm from the surface of sample.

**Table 1** Conditions of column experiments.

Run	Beads diameter [mm]	Water	Filter	Hydraulic gradient
1	0.2	Glucose 50 g m <sup>-3</sup>	Top and Bottom	0.22
2	0.2	Glucose 50 g m <sup>-3</sup>	Bottom	0.35
3	0.05	Glucose 50 g m <sup>-3</sup>	Bottom	1.10
4	0.2	Deaerated water	Bottom	0.12



**Figure 1** Schematic figure of sand and glass beads column.

We packed glass beads by hand in the water-filled column with bulk density of 1.60 Mg m<sup>-3</sup>. Glucose solution or deaerated water was supplied from the top of the column continuously for 30 days. Hydraulic gradient of the flow system was controlled by the height of a Mariotte tank and drip point at the end of effluent tube. We measured the pressure of water inside glass beads by reading the height of piezometers, and hydraulic head was calculated from average value of the three piezometer readings of each height. Saturated hydraulic conductivity,  $K_s$ , of each layer was calculated by Darcy's equation:

$$q = -K_s \frac{\Delta H}{\Delta z} \quad (1)$$

where  $q$  is the flux of flow (cm s<sup>-1</sup>),  $\Delta H$  (cm) is the hydraulic head difference across the layer, and  $\Delta z$  (cm) is the difference in height across the layer.

After percolation, we sliced the columns and measured the number of bacteria and fungi of different depth by dilute plate counting method. Bacteria were cultured in egg albumin agar culture and fungi were cultured in rose bengal agar culture.

## Results and Discussion

### Bioclogging at the top layer

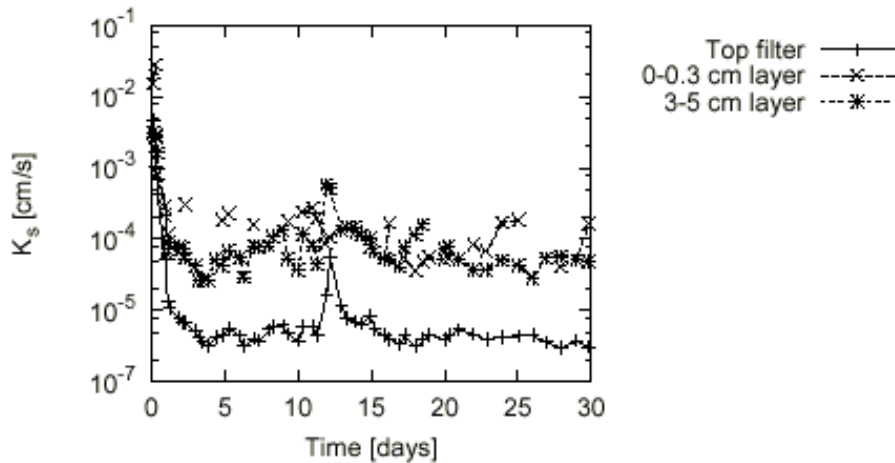
Figure 2 shows the change in saturated hydraulic conductivity of the top filter, 0-0.3-cm layer and 3-5-cm layer for Run 1. Hydraulic conductivity at the top filter suddenly decreased in two days from the initial value of  $5 \times 10^{-3} \text{ cm s}^{-1}$  to  $7 \times 10^{-5} \text{ cm s}^{-1}$  and stabilized during 30 days percolating period, with a small tendency to decrease and final value of  $3 \times 10^{-7} \text{ cm s}^{-1}$ . This drastic decrease in  $K_s$  at the top filter made it difficult to calculate  $K_s$  of other part of the sample, because hydraulic head difference of the unclogged layer became too small. In extreme case, hydraulic gradient indicated opposite direction of the flow, so that  $K_s$  value calculated from equation (1) became negative. It arose from the inaccuracy of the measurement of manometers and probably preferential flow in the column. For example, most of the plot of the 0-0.3 cm layer data in Figure 2 are missing because negative value was calculated. Accurate measurement of the  $K_s$  was not available, but at least we can say that  $K_s$  at the 0-0.3 cm layer did not decrease so much as the severely clogged layer. Hydraulic conductivity at the 3-5 cm layer also decreased but the decrease was not so severe compared with glass filter.

In order to eliminate the effect of filter clogging and clarify the mechanism of  $K_s$  change in the glass beads layer, rest of the experiments, Run 2 to 4, were conducted without top filters. Figure 3 shows the change in  $K_s$  of Run 2. In Figure 3,  $K_s$  decreased in three orders at the top 0.3-cm layer of the column from initial value of  $2 \times 10^{-3} \text{ cm s}^{-1}$  to the final value of  $3 \times 10^{-5} \text{ cm s}^{-1}$ . Decrease in hydraulic conductivity is similar to the top filter of Run 1. Hydraulic conductivity also decreased in the layer of  $0.3^{-1} \text{ cm}$ , but it is only two order decrease and not severe as the top layer of 0.3-cm.

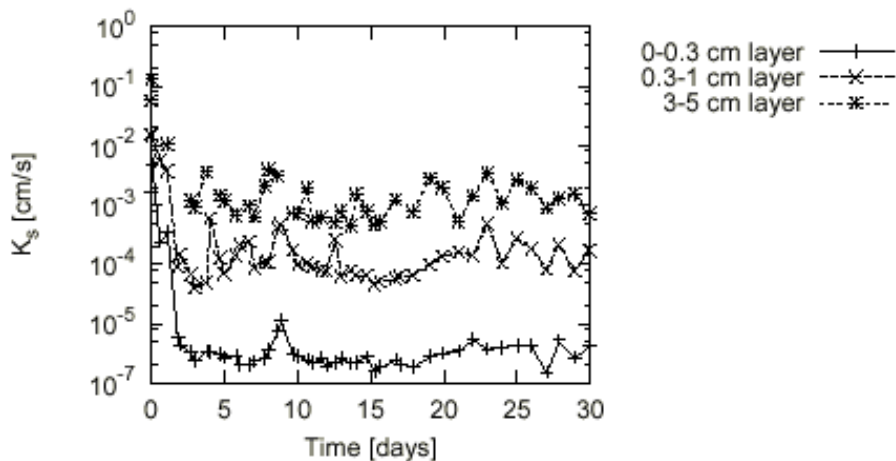
In both Runs 1 and 2, bioclogging occurred in the top few millimeters layer, which corresponds to top filter in Run 1 and glass beads layer in Run 2. The purpose of this experiment is to see the bioclogging of glass beads, not the supporting filter. In the experiments afterwards top filter was not used and effect of top filter was not involved.

### Effect of particle size

In Run 3, where glass beads of 0.5-mm diameter were used, saturated hydraulic conductivity changed as shown in Figure 4. The severest clogging was observed at the top layer of 0.3-cm. The rate of  $K_s$  decrease at the top layer was slower than the rate of  $K_s$  decrease in 0.2-mm beads column. In 0.2-mm beads column, (Figure 3), initial sudden  $K_s$  decrease occurred in two days, while in 0.05-mm beads column (Figure 4),  $K_s$  decrease at the top layer continued for 5 days. Final value of  $K_s$  at the clogged layer (top 3 mm layer) after 30 days, total experimental period, was  $4 \times 10^{-5} \text{ cm s}^{-1}$  for 0.2-mm beads and  $4 \times 10^{-7} \text{ cm s}^{-1}$  for 0.05-mm beads. In both columns, three orders decrease in  $K_s$  was observed. In 0.2-mm beads column,  $K_s$  of 0.3-1 cm layer also decreased, showing that the layer of the clogged part was thicker than 0.3 cm and thinner than 1 cm.

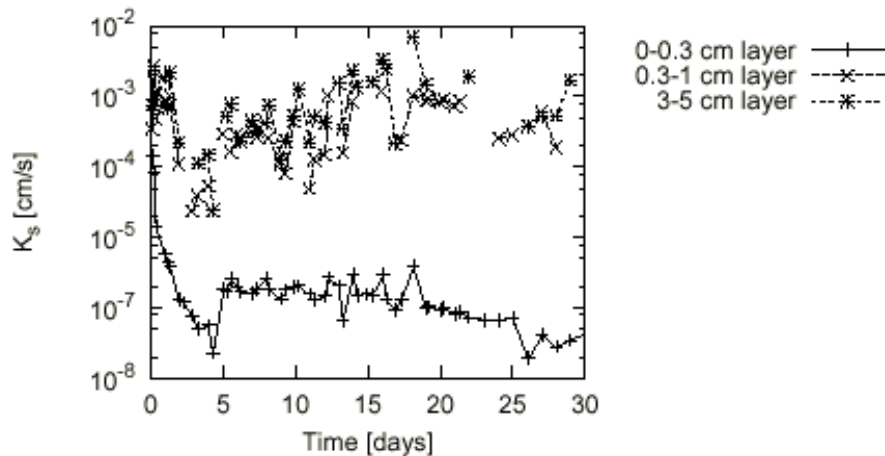


**Figure 2** Change in hydraulic conductivities of top filter and 0-0.3 and 3-5 cm layer of Run 1, where glass beads of 0.2-mm diameter was packed and glass filters are set at both top and bottom of the sample.



**Figure 3** Change in hydraulic conductivities of 3 layers of Run 2, where glass beads of 0.2-mm diameter were packed.

In 0.05-mm beads column,  $K_s$  of 0.3-1 cm layer did not decrease, and clogged layer of this column was thinner than 0.3 cm. MacDonald *et al.* (1999) analyzed shear detachment effect on biomass growth. Shear of biomass is larger for 0.2-mm beads than for 0.05-mm beads, because initial value of  $K_s$  was higher and flux was larger. Therefore microbial cells accumulated at the surface of glass beads had higher tendency to be detached by flow and to move downward, and the clogged layer for 0.2-mm beads may have become a little thicker than 0.05-mm beads column.



**Figure 4** Change in hydraulic conductivities of 3 layers of Run 3, where glass beads of 0.05-mm diameter were packed.

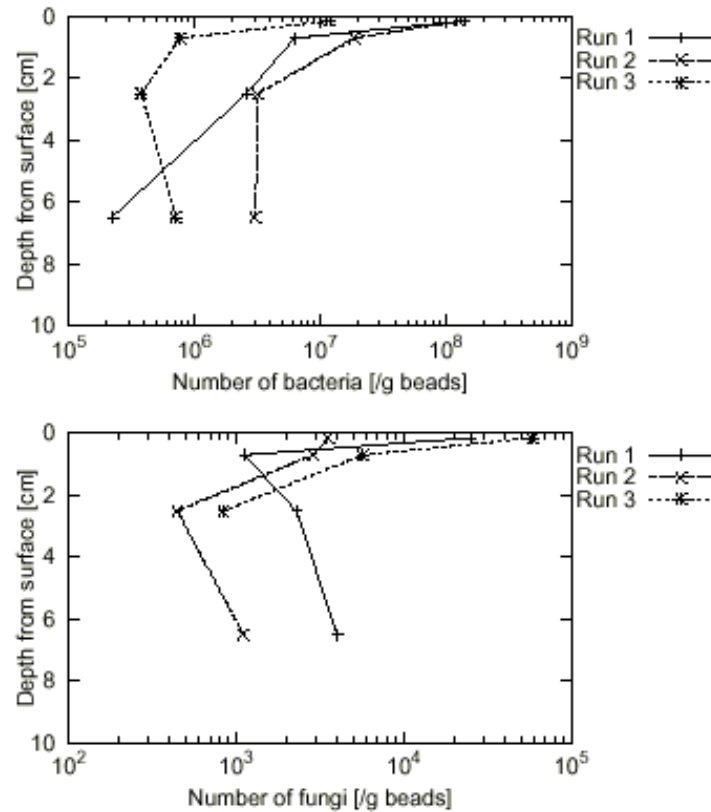
### Effect of bacteria and fungi

Profile of bacteria and fungi population was measured at the end of Runs 1 to 3 (Figure 5). Population of bacteria and fungi are about ten times higher at the top 0.15-cm layer in every Run. This matches the most clogged layer of Runs 2 and 3. It shows that increase in the numbers of bacteria and fungi caused decrease in  $K_s$ . Most researches on bioclogging so far focused on bacterial colonies, but Seki *et al.* (1996) pointed out that fungal clogging is more important than bacterial clogging in laboratory column experiment with Andisol. Seki *et al.* (1998) observed combined effect of bacteria and fungi on hydraulic conductivity decrease. The result of fungal count showed that fungi played an important role on the decrease in  $K_s$  in glass beads column.

Figure 6 shows the change in  $K_s$  for Run 4, where distilled and deaerated water without glucose was applied to 2-mm beads column. Hydraulic conductivity of the clogged 0.3-cm layer first decreased from  $2 \times 10^{-3} \text{ cm s}^{-1}$  at the start of experiment to  $2 \times 10^{-5} \text{ cm s}^{-1}$  at 6 days (first stage), and then increased to  $1 \times 10^{-3} \text{ cm s}^{-1}$  at 15 days (second stage), and after that it decreased to  $1 \times 10^{-5} \text{ cm s}^{-1}$  at 24 days (third stage).

This three stage of  $K_s$  change is similar to the three phase change of permeability proposed by Allison (1947). Allison (1947) explained the second stage of permeability increase by the effect of removal of gas occluded in the sample. However in our experiment, glass beads were packed in water by hand and the volume of entrapped air is not so large than in natural soil sample. Therefore the three stage change needs to be explained in different manner. This change may be explained as follows. At the first stage, aerobic bacteria and fungi grew with subtle nutrient still remained in the percolating water and gas dissolved in the column. They increased in number and caused bioclogging. At the second stage, anaerobic bacteria started to increase and aerobic bacteria and fungi decreased because no oxygen is supplied from the applied water. Some of the dead cells of microbes were washed away from the bottom of the columns, and therefore  $K_s$  increased. At the third stage, anaerobic bacteria increased and caused bioclogging. The rate of the growth of anaerobic bacteria is slower than that of aerobic bacteria, and anaerobic bioclogging took place after 15 days. This is one

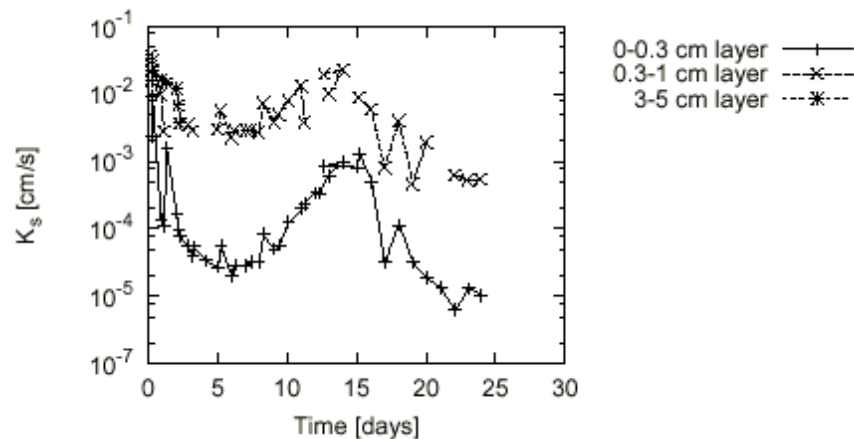
possible explanation but not confirmed yet. The most interesting point is that we observed bio-clogging in subtle nutrient and oxygen flow system.



**Figure 5** Profile of bacteria and fungi population measured by dilute plate counting method after each Run. Each plot represents the average of three replications.

### Conclusion

We observed bio-clogging in columns packed with glass beads having different diameters. The bio-clogging proceeds most severely at the inlet of solution, where nutrient and oxygen is supplied from the percolating water. Shear detachment of microbes may have influenced the thickness of the clogged layer. In 0.2-mm beads, effect of shear detachment was stronger than in 0.05-mm beads, resulting in stronger tendency for microbes to move downward and made clogging layer thicker. Bio-clogging was also observed in column with deaerated distilled percolating water, which shows that very subtle amount of nutrient and oxygen can cause bio-clogging. The behaviour of hydraulic conductivity change in the deaerated distilled water column was interesting, having three phase changes in time. These types of changes may be explained by the change of microcosm from aerobic to anaerobic, but fully understanding of these phenomena is left for future analysis.



**Figure 6** Change in hydraulic conductivities of 3 layers of Run 4, where glass beads of 0.2-mm diameter was packed and deaerated water was applied.

#### Acknowledgements

We thank Hiromi Imoto at the University of Tokyo for his help in experiments.

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