WATER RESOURCES RESEARCH, VOL. 37, NO. 12, PAGES 2995–2999, DECEMBER 2001

A mathematical model for biological clogging of uniform porous media

Katsutoshi Seki and Tsuyoshi Miyazaki

Department of Biological and Environmental Engineering, Graduate School of Agricultural and Life Sciences
University of Tokyo, Tokyo, Japan

Abstract. We develop a theory that explains the decrease in saturated hydraulic conductivity K_s due to biological clogging of porous media. Experiments show that discontinuous microcolonies in fine-textured soils decrease K_s more severely than biofilms do. However, most existing models for biological clogging assume that bacteria cells form biofilms which uniformly cover pore walls. We propose a mathematical model for biological clogging with a quantitative evaluation of the nonuniform microbial distribution of colonies. A series of equations describing the relation between the biological clogging and the saturated hydraulic conductivity are derived. The data of previous researchers are used to validate the model.

1. Introduction

An increase in the amount of biomass decreases the saturated hydraulic conductivity K_s of porous media by clogging pore spaces, i.e., blocking the pathway of water [*Allison*, 1947]. Biological clogging is observed in porous media ranging from glass beads [*Cunningham et al*., 1991] and sand [*Kristiansen*, 1981; *Vandevivere and Baveye*, 1992a, 1992b] to natural soils such as loamy sand [*Rice*, 1974], sandy soil, loamy soil and silty clay [*Chang et al*., 1974], Andisols [*Miyazaki*, 1993; *Seki et al*., 1996], podzol soil [*de Vries*, 1972], dairy waste pond soil [*Davis et al*., 1973], and irrigation channel soil [*Ragusa et al*., 1994]. The clogging materials can be bacteria [*Gupta and Swartzendruber*, 1962], fungi [*Seki et al*., 1996, 1998], algae [*Ragusa et al*., 1994], or microbial by-products such as polysaccharides [*Avnimelch and Nevo*, 1964; *Vandevivere and Baveye*, 1992a]. Gas, such as methane produced by bacteria, also causes K_s reduction by obstructing pore throats [*Sanchez de Lozada et al*., 1994; *Seki et al*., 1998].

Cunningham et al. [1991] measured the biofilm thickness and K_s of quartz sand and glass beads of different particle diameters. They analyzed their data using the Kozeny-Carman equation [*Kozeny*, 1927; *Carman*, 1937]. *Jennings et al*. [1995] also used this equation for the estimation of the relation between biofilm thickness and K_s.

Taylor et al. [1990] measured K_s reduction with biofilm growth and calculated biofilm thickness using the Kozeny-Carman equation combined with a cut-and-random-rejoin-type model. *Vandevivere et al*. [1995] evaluated the Kozeny-Carman equation and other mathematical models using the data of *Cunningham et al*. [1991] and *Vandevivere and Baveye* [1992b]. A given bulk volume of biomass per unit pore volume caused a larger K_s decrease in fine-textured porous media than in coarse-textured media (Figure 1). As a result, Kozeny-Carman and other equations accurately predicted biological clogging in 1-mm glass beads but were not able to model clogging in the finer fractions. This discrepancy was associated with the tendency of biomass to "pile up," i.e. to form biofilms on the coarse-textured material, which has a larger specific surface area than finer-textured material. Experimental observations confirmed this suspicion. It has been observed that microcolonies are formed in fine-textured porous media, including many natural soils [*Harvey et al*., 1984]. *Vandevivere et al*. [1995] showed that the resulting decrease in K_s is larger for microcolonies than for biofilms. They examined several existing models assuming that bacteria form uniform biofilms and found that none could predict K_s reduction by biological clogging of fine-textured porous media, where microcolonies are likely predominant.

In a new model for estimating K_s reduction due to biological clogging we consider the type of microorganism attachment on the soil surface. In this paper, we describe the model and apply the model to sets of experimental data obtained by *Cunningham et al*. [1991] and *Vandevivere and Baveye* [1992b].

Paper number 2001WR000395.
0043-1397/01/2001WR000395$09.00

2. Theory

In the first stage of colony formation, bacteria attach to the solid particle, first reversibly and then irreversibly with exopolysaccharide polymers [*Trulear and Characklis*, 1982] or with bacterial adhesion pili [*Bullitt and Makowski*, 1995]. Next, microcolonies begin to grow as bacteria adhere to the microcolony itself [*Costerton et al*., 1978]. As the colonized surface area increases, discrete microcolonies begin to converge, forming a biofilm. Microcolonies and biofilms are distinguished by the ratio of the colonized surface area to total surface area of the solid particle.

We define "colony-enveloping space" as the hypothetical space over which a film of uniform thickness L_b would cover the soil particle. L_b is equal to the maximum colony thickness (Figure 2). The colony-enveloping space is determined in the same way for biofilms, microcolonies, and colonies with other configurations [e.g., *Vandevivere and Baveye*, 1992b], meaning no assumptions about colony shape need be made in order to apply the model presented here.

According to the nonsimilar media concept [*Miyazaki*, 1996] the characteristic width of the pore d (L) is obtained from the transformation of (1) into (2);

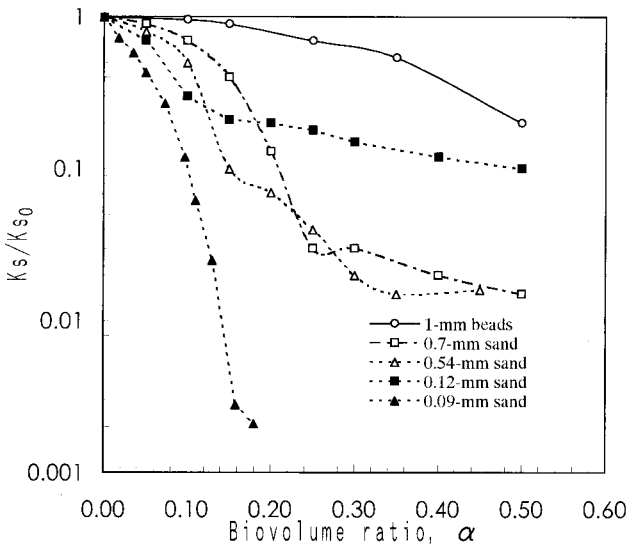

Figure 1. Relationship between the saturated hydraulic conductivity ratio and the biovolume ratio for *Pseudomonas aeruginosa* in 1-mm glass beads and in 0.70-, 0.54-, and 0.12-mm sand (data from *Cunningham et al*. [1991]) and for Arthrobacter sp. strain AK19 in 0.09-mm sand (data from *Vandevivere and Baveye* [1992b]) as summarized by *Vandevivere et al*. [1995].

$$\rho_b = \tau\rho_s\left(\frac{S}{S+d}\right)^3, \tag{1}$$

where S is the characteristic length of solid phase (L), τ is the shape factor of solid phase and is defined as the ratio of the volume of solid phase to the volume S^3, ρ_s is particle density ($M\,L^{-3}$), and ρ_b is bulk density ($M\,L^{-3}$) (Figure 3). According to *Miyazaki* [1996] the characteristic length of solid phase is

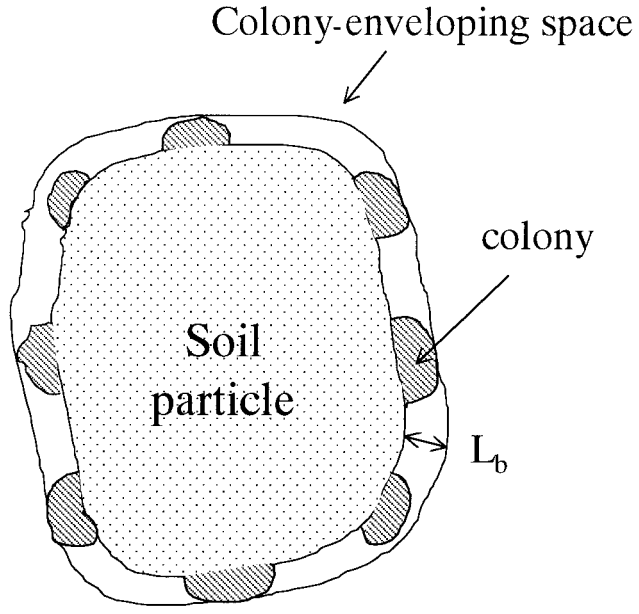

Figure 2. Conceptual illustration of colony-enveloping space around a soil particle. The thickness L_b is the maximum colony thickness. The colony-enveloping space concept is applicable to any kind of colony shape, including biofilm and microcolony.

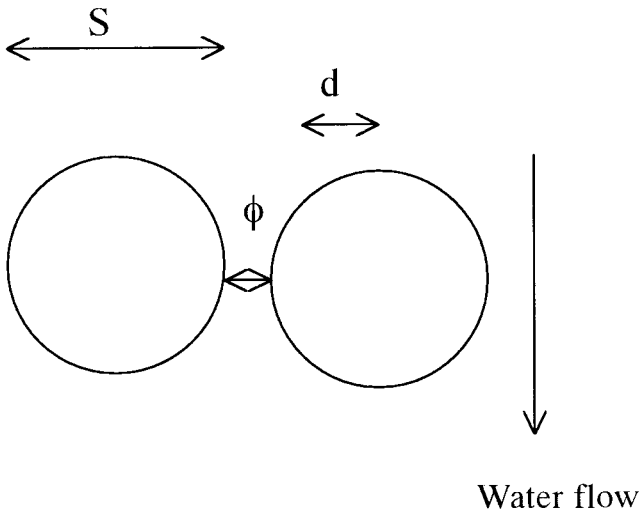

Figure 3. Sketch of the characteristic length of solid phase (S), the characteristic width of the pore (d), and the diameter of pore throat (ϕ). In this model the S value is set equal to the mean particle diameter, and d is assumed to be close to ϕ.

not directly measurable, but it is representative of length. In this model, where we deal with uniform porous media, we use the mean particle diameter as the characteristic length of solid phase. This is reasonable for uniform porous media but is not reasonable for nonuniform porous media. By arranging (1), we obtain

$$d = \left\{\left(\frac{\tau}{1-e}\right)^{1/3} - 1\right\}S, \tag{2}$$

where e is porosity.

The value of τ is defined for a "clean" porous medium, that is, a porous medium without any clogging materials on its surface. Therefore (2) is valid only for clean porous media before biomass accumulates.

After clogging by colonies the diameter of the pore throat ϕ decreases to ϕ' because the maximum colony thickness increases. *Dykaar and Kitanidis* [1996] developed a physically based model that suggests that the biomass nearest the pore throat is more effective at consuming the solute than biomass in the pore chamber. *Deleo and Baveye* [1997] showed that bacteria grow more profusely in crevices between the sand particles. Figure 2 suggests the following relationship

$$\phi' = \phi - 2L_b. \tag{3}$$

According to *Ewing and Gupta* [1994] the flux of water through a circular pore throat is proportional to the third power of the tube diameter under the same pressure gradient. Therefore we obtain

$$\phi'/\phi = (K_s/K_{s0})^{1/3}, \tag{4}$$

where ϕ is the pore throat diameter of a clean porous medium (L), ϕ' is the pore throat diameter of clogged porous medium (L), K_s is the saturated hydraulic conductivity of the clogged porous medium ($L\,T^{-1}$), and K_{s0} is the saturated hydraulic conductivity of the clean porous medium ($L\,T^{-1}$). We now assume that the characteristic length of the pore phase is close to the diameter of pore throat as $d \fallingdotseq \phi$. This assumption will

Table 1. Parameters Used for Calculation of Experimental Data Points in Figures 4 and 5

Sample	Mean Particle Diameter S, mm	Porosity e	Particle Density ρ_s,[a] g cm^{-3}	Bulk Density ρ_b,[b] g cm^{-3}	Shape Factor τ[c]
Glass beads	1[d]	0.48[d]	2.60	1.35	1
Glass beads	0.7[d]	0.48[d]	2.60	1.35	1
Glass beads	0.54[d]	0.35[d]	2.60	1.35	1
Glass beads	0.12[d]	0.48[d]	2.60	1.35	1
Sand	0.095[e]	0.39[e]	2.65	1.62	1

[a]Assumed.
[b]Calculated from porosity and particle density.
[c]From *Miyazaki* [1996].
[d]From *Cunningham et al.* [1991].
[e]From *Vandevivere and Baveye* [1992b].

be addressed in section 3. Using this assumption, (3) and (4) yield the following equation:

$$L_b = \frac{d}{2}\{1 - (K_s/K_{s0})^{1/3}\}. \tag{5}$$

From (2) and (5) we can calculate the maximum biofilm thickness L_b, using S, e, τ, and K_s/K_{s0}. The characteristic length of the solid phase of the clogged porous medium S' (L) is

$$S' = S + 2L_b. \tag{6}$$

Here we define the enveloping factor β, where $0 < \beta \leqq 1$, as the bulk volume of biomass per unit volume of colony-enveloping space. When the value of β is much smaller than 1, the biomass is known as a "microcolony." When β is close to 1, it is called a "biofilm." The process of colony growth includes the gradual increase of both L_b and β. The distinction between microcolony and biofilm is loose because the critical value of β separating biofilms from microcolonies is not defined.

The outline of the colony-enveloping space parallels the surface of the clean particle. Therefore the following relation is obtained:

$$\frac{V_s + V_f}{V_s} = \frac{S'^3}{S^3}, \tag{7}$$

where V_s is the volume of a clean particle (L^3) and V_f is the volume of a colony-enveloping space (L^3). From the definition of β and (6) and (7) we obtain

$$\frac{V_b}{V_s} = \frac{\beta V_f}{V_s} = \beta\left\{\left(1 + \frac{2L_b}{S}\right)^3 - 1\right\}, \tag{8}$$

where V_b is the volume of the biomass (L^3).

An alternative expression for the biomass volume to clean particle ratio is

$$\frac{V_b}{V_s} = \frac{\alpha e}{1 - e}, \tag{9}$$

where α is biovolume ratio, the bulk volume of biomass per unit pore volume of clean porous medium. Combining (8) and (9), β can be calculated by

$$\beta = \frac{\alpha e}{1 - e}\left\{\left(1 + \frac{2L_b}{S}\right)^3 - 1\right\}^{-1}. \tag{10}$$

We can calculate d, L_b, and β by using (2), (5), and (10).

3. Results and Discussion

Vandevivere et al. [1995] compared three types of mathematical models based on Ives [*Ives*, 1965], Maulem [*Maulem*, 1976], and Kozeny-Carman equations proposed by *Taylor et al.* [1990] with the data of *Cunningham et al.* [1991] and *Vandevivere and Baveye* [1992b]. The three models assume uniform biofilm formation, which corresponds to $\beta = 1$ in the current study. Substituting $\beta = 1$ into (10), we obtain

$$\alpha = \frac{1 - e}{e}\left\{\left(1 + \frac{2L_b}{S}\right)^3 - 1\right\}. \tag{11}$$

By rearranging (5) and (11), substituting expressions for d (equation (2)) and L_b (equation (11)) into (5), and solving for K_s/K_{s0}, we find

$$\frac{K_s}{K_{s0}} = \left\{1 - \frac{2L_b}{S}\left[\left(\frac{\tau}{1 - e}\right)^{1/3} - 1\right]^{-1}\right\}^3 = \left\{1 - \left[\left(\frac{\alpha e}{1 - e} + 1\right)^{1/3} - 1\right]\left[\left(\frac{\tau}{1 - e}\right)^{1/3} - 1\right]^{-1}\right\}^3. \tag{12}$$

Equation (12) was used to calculate the relationship between α and K_s/K_{s0} when $\beta = 1$, $\tau = 1$, and $e = 0.48$. (Other parameter values are listed in Table 1.) The resulting curve fits well with that of Mualem (Figure 4). Like the models of Kozeny-Carman, Ives, and Mualem, when $\beta = 1$, (12) overestimates the extent of clogging of 1-mm beads and underestimates the extent of clogging of 0.12-mm sand. *Vandevivere et al.* [1995] attributed underestimation of the extent of clogging of fine-textured porous media to the continuous biofilm assumption.

For each pair of α and K_s/K_{s0} values in Figure 1 we used (2), (5), and (10), with appropriate parameter values from Table 1, to calculate d, L_b, and β. This procedure was used to estimate the relationship between L_b and β (Figure 5). The values of β are especially small for the finest material (0.09-mm sand). *Vandevivere and Baveye* [1992b] observed a three-dimensional loose assemblage of colonies by scanning electron microscopy (SEM). According to their SEM photograph, values of β around 0.2 are plausible. Although the value of β is limited to the range of $0 < \beta \leqq 1$ by definition, some of the calculated values of β are >1. In particular, the values of β for 1-mm beads exceed 1 in the entire range of L_b. This may be a result of the inaccuracy of the assumption that the colony is either thickest at the pore throat or of uniform thickness throughout

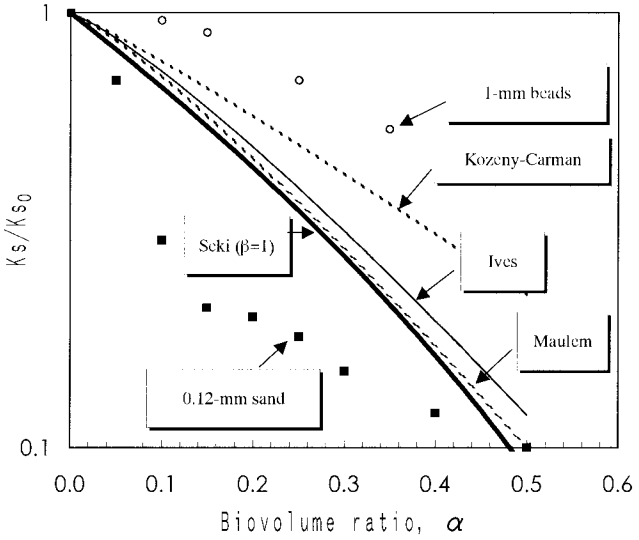

Figure 4. Comparison of K_s/K_{s0} versus α predicted by this study (Seki), *Ives*, *Mualem*, and *Kozeny-Carman* with the experimental data of *Cunningham et al*. [1991] (after *Vandevivere et al*. [1995]).

the pore. *Wanner and Gujer* [1986] observed that microbial colonies at the pore throat tend to be dislodged from the pore throats by moving water in 1-mm glass beads.

The empirical relationships between L_b and β listed in Table 2 for each size fraction were obtained by regressing the data in Figure 5. Figure 6 demonstrates the relationship between α and K_s/K_{s0}, given various values of L_b, calculated using (11) and (12). The model results match closely the data for all five samples. The curve for 0.09-mm sand is especially important, as the mathematical models of Ives, Maulem, and Kozeny-Carman were not able to explain this sharply decreasing curve.

In order to estimate the value of K_s from the value of α the

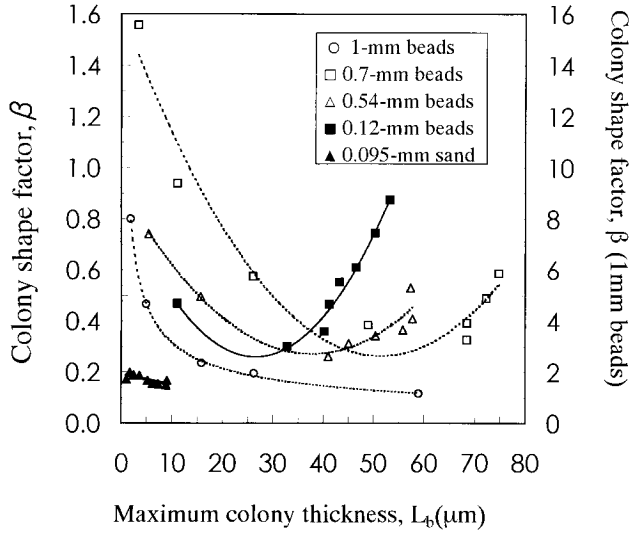

Figure 5. Change in L_b and β with bacterial growth, calculated from biovolume ratios and saturated hydraulic conductivities of Figure 1 (data from *Cunningham et al*. [1991] and *Vandevivere and Baveye* [1992b]). Note that the right axis applies to 1-mm beads. Equations for fitted curves in Figure 5 are shown in Table 2.

Table 2. Equations Used to Fit the Experimental Data in Figure 5

Sample	Fitted Equation
1-mm beads	$\beta = 11.383L_b^{-0.5566}$
0.7-mm beads	$\beta = 0.0004L_b^2 - 0.0377L_b + 1.3160$
0.54-mm beads	$\beta = 0.0005L_b^2 - 0.0343L_b + 0.9190$
0.12-mm beads	$\beta = 0.0009L_b^2 - 0.0462L_b + 0.8748$
0.09-mm sand	$\beta = -0.0044L_b + 0.1945$

relationship between L_b and β must be known or estimated. There are two ways to estimate this relationship. The first is to use calculations described in section 2. The second is to directly observe the shape of colonies. The kinetics of biofilm growth have been studied extensively [*Wanner and Gujer*, 1986; *Csikor et al*., 1995]. However, these studies are based on the assumption that a uniform biofilm is formed, that is, that $\beta = 1$ throughout the biofilm growth process. This study demonstrated that the $\beta = 1$ assumption is inadequate for fine-textured porous media. *Massol-deya et al*. [1995] demonstrated that the shape of thick biofilms is also nonuniform. For such materials, colony growth must be studied as changes both theoretically and experimentally for varying L_b and β. Estimation of β by direct observation is left for future research.

The merits of this model are as follows: (1) There is no assumption that a biofilm of uniform thickness is formed on the soil surface. (2) Assuming uniform colony distribution in the colony-enveloping space (i.e., $\beta = 1$), the curve of saturated hydraulic conductivity versus biovolume ratio resembles existing mathematical models on microbial clogging.

Limitations of this model include the following: (1) Measurement of colony thickness and colony shape factor are not presented. (2) Our model could not predict growth on 1-mm glass beads, yielding an unrealistic value of β.

In deriving (5), we assumed that the characteristic pore width d was equal the throat diameter ϕ. In the calculation of (2) the value of d is 0.154–0.243 times the value of S. Assuming cubic packing of circular grains, the diameter of the circle

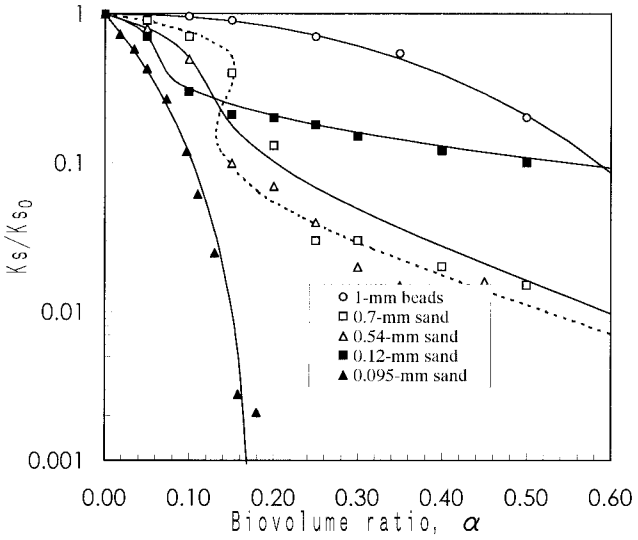

Figure 6. Relationship between the saturated hydraulic conductivity ratio and the biovolume ratio calculated by this model to the experimental data of Figure 1.

internally touching the four grains is 0.41 times the grain diameter. Assuming rhombohedral packing, the diameter of the internally touching circle is 0.077 times the diameter of the grains. The value of ϕ is supposed to be some value between these two values, i.e., 0.077 and 0.41, and the values obtained from (2) are in this range.

4. Conclusions

A mathematical model was developed for estimating saturated hydraulic conductivity decrease due to biological clogging of porous media having uniform particle size. The extent of biological clogging depends on the distribution of the colony. We defined the concept of "colony-enveloping space" and expressed nonuniform colony shape mathematically. The mathematical expressions of the colony shape were combined with the nonsimilar media concept [*Miyazaki*, 1996], and a series of equations describing the relationship between soil texture, microbial distribution, and saturated hydraulic conductivity were derived.

The relationship between biovolume ratios and saturated hydraulic conductivities of glass beads and sand was applied to our model [*Cunningham et al*., 1991; *Vandevivere and Baveye*, 1992b]. When we assume the formation of colonies as a uniform biofilm, our model yields results similar to previous mathematical models, particularly to the Mualem model. The underestimation of the extent of clogging of fine-textured materials is attributed to the assumption of uniform biofilm formation. The relationship between the maximum colony thickness and the colonial shape factor was then calculated. Finally, empirical curves relating biovolume ratio and the saturated hydraulic conductivity were determined.

Further research for estimating the enveloping factor is required for utilizing this model for prediction of the extent of clogging. The role of this paper is to give a new concept of an enveloping factor, which serves as a step toward the understanding of the growing process of colonies and the ensuing decrease in saturated hydraulic conductivity.

Acknowledgments. We acknowledge Samuel Colbeck, editor of *Water Resources Research*, Thomas MacDonald, and other anonymous reviewers, who gave us many helpful comments and encouragement. English language editor's comments were also helpful.

References

Allison, L. E., Effect of microorganisms on permeability of soil under prolonged submergence, *Soil Sci*., *63*, 439–450, 1947.

Avnimelch, Y., and Z. Nevo, Biological clogging of sands, *Soil Sci*., *98*, 222–226, 1964.

Bullitt, E., and L. Makowski, Structural polymorphism of bacterial adhesion pili, *Nature*, *373*, 164–167, 1995.

Carman, P. C., Fluid flow through a granular bed, *Trans. Inst. Chem. Eng*., *15*, 150–156, 1937.

Chang, A. C., W. R. Olmstead, J. B. Johansen, and G. Yamashita, The sealing mechanism of wastewater ponds, *J. Water Pollut. Control Fed*., *46*, 1715–1721, 1974.

Costerton, J. W., G. G. Geesey, and K. J. Cheng, How bacteria stick, *Sci. Am*., *238*(1), 86–95, 1978.

Csikor, Z., P. Miháltz, L. Czakó, and J. Holló, A pressure test method monitors biofilm coated particle fluidization, *Appl. Microbiol. Biotechnol*., *43*, 383–386, 1995.

Cunningham, A. B., W. G. Characlis, F. Abedeen, and D. Crawford, Influence of biofilm accumulation on porous media hydrodynamics, *Environ. Sci. Technol*., *25*, 1305–1311, 1991.

Davis, S., W. Fairbank, and H. Weisheit, Dairy waste ponds effectively self-sealing, *Trans. ASAE*, *16*, 69–71, 1973.

Deleo, P. C., and P. Baveye, Factors affecting protozoan predation of bacteria clogging laboratory aquifier microcosms, *Geomicrobiol. J*., *14*, 127–149, 1997.

de Vries, J., Soil filtration of wastewater effluent and the mechanism of pore clogging, *J. Water Pollut. Control Fed*., *44*, 565–573, 1972.

Dykaar, B. B., and P. K. Kitanidis, Macrotransport of a biologically reacting solute through porous media, *Water Resour. Res*., *32*, 307–320, 1996.

Ewing, R. P., and S. C. Gupta, Pore-scale network modeling of compaction and filtration during surface sealing, *Soil Sci. Soc. Am. J*., *58*, 712–720, 1994.

Gupta, R. P., and D. Swartzendruber, Flow-associated reduction in the hydraulic conductivity of quartz sand, *Soil Sci. Soc. Am. Proc*., *26*, 6–10, 1962.

Harvey, R. W., R. L. Smith, and L. George, Effect of organic contamination upon microbial distributions and heterotrophic uptake in a Cape Cod, Mass., aquifer, *Appl. Environ. Microbiol*., *48*, 1197–1202, 1984.

Ives, K. J., and V. Pienvichitr, Kinetics of the filtration of dilute suspensions, *Chem. Eng. Sci*., *20*, 965–973, 1965.

Jennings, D. A., J. N. Petersen, R. S. Skeen, B. S. Hooker, B. M. Peyton, D. L. Johnstone, and D. R. Yonge, Effects of slight variations in nutrient loadings on pore plugging in soil columns, *Appl. Biochem. Biotechnol*., *51-52*, 727–734, 1995.

Kozeny, J., Über kapillare leitung des wassers im boden, *Sitzungsber. Akad. Wiss. Wien*, *136*, 271–306, 1927.

Kristiansen, R., Sand-filter trenches for purification of septic tank effluent, 1, The clogging mechanism and soil physical environment, *J. Environ. Qual*., *10*, 353–357, 1981.

Massol-deya, A. A., J. Whallon, R. F. Hickey, and J. M. Tiedje, Channel structures in aerobic biofilms of fixed-film reactors treating contaminated groundwater, *Appl. Environ. Microbiol*., *61*, 769–777, 1995.

Miyazaki, T., Effects of microbiological factors on water flow in soils, in *Water Flow in Soils*, edited by T. Miyazaki, S. Hasegawa, and T. Kasubuchi, pp. 197–220, Marcel Dekker, New York, 1993.

Miyazaki, T., Bulk density dependence of air entry suctions and saturated hydraulic conductivities of soils, *Soil Sci*., *161*, 484–490, 1996.

Mualem, Y., A new model for predicting the hydraulic conductivity of unsaturated porous media, *Water Resour. Res*., *12*, 513–522, 1976.

Ragusa, S. R., D. S. de Zoysa, and P. Rengasamy, The effect of microorganisms, salinity and turbidity on hydraulic conductivity of irrigation channel soil, *Irrig. Sci*., *15*, 159–166, 1994.

Rice, R. C., Soil clogging during infiltration of secondary effluent, *J. Water Pollut. Control Fed*., *46*, 708–716, 1974.

Sanchez de Lozada, D., P. Vandevivere, P. Baveye, and S. Zinder, Decrease of the hydraulic conductivity of sand columns by *Methanosarcina barkeri*, *World J. Microbiol. Biotechnol*., *10*, 325–333, 1994.

Seki, K., T. Miyazaki, and M. Nakano, Reduction of hydraulic conductivity due to microbial effects, *Trans. Jpn. Soc. Irrig. Drain. Reclam. Eng*., *181*, 137–144, 1996.

Seki, K., T. Miyazaki, and M. Nakano, Effects of microorganisms on hydraulic conductivity decrease in infiltration, *Eur. J. Soil Sci*., *49*, 231–236, 1998.

Taylor, S. W., P. C. D. Milly, and P. R. Jaffé, Biofilm growth and the related changes in the physical properties of a porous medium, 2, Permeability, *Water Resour. Res*., *26*, 2161–2169, 1990.

Trulear, M. G., and W. G. Characklis, Dynamics of biofilm processes, *J. Water Pollut. Control Fed*., *54*, 1288–1301, 1982.

Vandevivere, P., and P. Baveye, Effect of bacterial extracellular polymers on the saturated hydraulic conductivity of sand columns, *Appl. Environ. Microbiol*., *58*, 1690–1698, 1992a.

Vandevivere, P., and P. Baveye, Relationship between transport of bacteria and their clogging efficiency in sand columns, *Appl. Environ. Microbiol*., *58*, 2523–2530, 1992b.

Vandevivere, P., P. Baveye, D. Sanchez de Lozada, and P. DeLeo, Microbial clogging of saturated soils and aquifer materials: Evaluation of mathematical models, *Water Resour. Res*., *31*, 2173–2180, 1995.

Wanner, O., and W. Gujer, A multispecies biofilm model, *Biotechnol. Bioeng*., *28*, 314–328, 1986.

T. Miyazaki and K. Seki, Department of Biological and Environmental Engineering, Graduate School of Agricultural and Life Sciences, University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-8657, Japan. (amiyat@soil.en.a.u-tokyo.ac.jp; seki@soil.en.a.u-tokyo.ac.jp)

(Received October 12, 1999; revised January 5, 2001;
accepted June 23, 2001.)