Biofilm morphology as related to the porous media clogging

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\section*{A B S T R A C T}

Aquifer recharge for the wastewater reuse has been considered and studied as a promising process to cope with the worldwide water scarcity. Soil clogging by an excessive growth of bacteria is often accompanied with the aquifer recharge. In this study, biofilm morphology and hydraulic conductivity were concurrently characterized at two flow rates and two levels of substrate concentrations. The experiments were conducted using a biofilm flow cell that was filled with glass beads. The biofilm images taken by confocal laser scanning microscopy (CLSM) were quantified by textural, areal, and fractal parameters. Hydraulic conductivity was monitored during the experiments. The flow velocity influenced the superficial morphology of biofilms and initial clogging time, while the substrate concentration affected biofilm density and clogging rate. Three different clogging mechanisms were suggested depending on the flow rate and substrate concentration: (1) clogging at a high flow rate can be accelerated by entrapped and accumulated biofilms, and can be easily eliminated by high shear force, (2) clogging at a low flow rate can be delayed for the time of local biofilm growths in the narrow pore necks, but the biofilm is rigid enough not to be sloughed, and (3) clogging in a solution with high substrate concentrations cannot be easily eliminated because of the growth of dense biofilms. The depicted biological clogging mechanisms will play a role in supporting studies about aquifer recharge.

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1. Introduction

Water scarcity or uneven distribution of freshwater has a significant impact in the world (Oki and Kanae, 2006). Many wastewater reuse projects have been conducted in Europe, and the necessity of integrated water management was presented (Bixio et al., 2006). The aquifer recharge for the wastewater reuse has been studied with promising results for coping with the worldwide water scarcity because of its cost effectiveness and sustainability. (Durham et al., 2003; Chu et al., 2004). The aquifer recharge has many advantages, such as groundwater resource preservation, safe water storage against evaporation and secondary contamination, water transportation, and water treatment (Aoki et al., 2005). The significance of specific criteria and guidelines was reported for the health and regulatory aspects of aquifer recharge (Asano and Cotruvo, 2004).

Soil aquifer treatment (Amy and Drewes, 2007), riverbank/riverbed filtration (Hiscock and Grischek, 2002), and constructed wetland (Baez-Cazull et al., 2008) represented examples of the aquifer recharge for water reuse. The contaminant removal accompanied with the aquifer recharge systems can be a result from natural physicochemical and biological reactions in the subsurface soil porous media. An
excessive growth of bacteria in soil brings about the biofilm-induced clogging of pores (Hill and Sleep, 2002). The clogged pores by a bioprocess will retard flow and significantly decrease the quantitative efficiency of the system (Schubert, 2002; Langergraber et al., 2003). On the other hand, the controlled soil clogging accompanied with bioprocesses improves water quality as retention time increases (Van Cuyk et al., 2001). Considering both positive and negative influence of pollutant removal (Bishop, 2007), biological clogging in porous soil must be depicted by examining a correlation between bacterial growth and hydraulic conductivity to control the aquifer recharge and improve its efficiency.

Biofilm-induced clogging in porous media has been researched in various experimental settings. An initial biofilm study in porous media was about the biofilm thickness as it related to porosity, permeability, and friction factor (Cunningham et al., 1991). Biofilms at the inlet of sand columns caused a severe reduction of the hydraulic conductivity (Vandevivere and Baveye, 1992). The authors demonstrated that the bacterial cells were sparsely and heterogeneously distributed on the solid surface. Wu et al. (1997) examined the influence of the biofilm growth on the hydraulic conductivity of soil columns and used destructive sampling at the end of the experiments to determine distributions of attached biomass on the soil. A two-dimensional random width network pore model was used to observe biofilm growth in porous media at different scales (Dupin and McCarty, 1999, 2000). Flow rerouting by heterogeneous colonization, pH effects on the biofilm morphology, sudden conductivity recoveries by sloughing events, and disinfection effects on the conductivity were observed in the work. Stoodley et al. (2005) revealed the effect of fluid flow on the biofilm transport in porous media suggesting periodic sloughing and regrowth events.

Considerable amount of study has been done on biofilm morphology on flat plates. Soil has heterogeneous and hierarchical porous media, and physical heterogeneity of soil affects bacterial transport and growth (Harvey et al., 1993). Biofilm growth in porous media induces anomalous fluid transport (Seymour et al., 2004). Characterization of biofilms in porous media should be carried out in experimental settings different from flat plates, especially when transport plays an important role. Because the physical structure of biofilms has high heterogeneity (Yang et al., 2000), heterogeneities of both porous media and biofilm structures should be considered at the same time.

The confocal laser scanning microscopy (CLSM) has made it more convenient to acquire and quantify images of undisturbed biofilm structures (Kuehn et al., 1998). Biofilm morphology quantification software has been developed to facilitate the quantification of the heterogeneity, the size, and the morphology of biofilms using textural, volumetric, and fractal parameters in two and three dimensions (Heydorn et al., 2000; Yang et al., 2000; Xavier et al., 2003; Beyenal et al., 2004; Rodriguez and Bishop, 2007).

The objectives of this study were (a) to observe and quantify the microscale biofilm morphology in the porous media made of glass beads as affected by the flow velocity and substrate concentrations, and (b) to interpret changes in the hydraulic conductivity based on the biofilm morphology. *Pseudomonas putida* (P. putida) was used as a single bacterial strain to develop biofilms in this study. This strain was chosen due to its effectiveness as a bioremediation of various organic contaminants in soil and groundwater systems (Prescott et al., 2002).

2. Materials and methods

2.1. Bacteria characterization

*P. putida* F1 was cultivated from frozen stock cultures and grown in a nutrient broth (NB) agar medium (Laboratories Conda, Spain: Gelatin Peptone 5.0 g, Beef Extract 3.0 g, and Agar 15.0 g in Distilled Water 1 L, and final pH 7.4) at 30 °C. In a preliminary experiment, the CLSM image of *P. putida* F1 taken on a glass plate without glass beads showed the presence of rod-shaped bacteria cell which was 2–3 μm in size similar to the report of Prescott et al. (2002). The microbial concentration was assessed by the optical density (OD) at a wavelength of 600 nm using a UV–VIS spectrophotometer (Shimadzu, Kyoto, Japan) (Won et al., 2007). In order to enumerate bacteria cells, the microbial concentration and OD were linearly correlated, and the following correlation equation was obtained with an R² of 0.99.

\[
x = 390.5 \cdot OD \quad (1)
\]

where \(x\) is the microbial concentration (mg/l).

The microbial growth of *P. putida* F1 was characterized in an isothermal batch system at 20, 25, and 30 °C with the microbial inoculum mass of 0.013, 0.025, and 0.126 mg. Substrate concentration (8000 ppm), volume (100 ml), and pH (7.4) were kept constant, and the isothermal shaker in which bacteria was grown was kept at 180 rpm. The Gompertz equation (Perni et al., 2005)

\[
\ln \left( \frac{X}{X_0} \right) = U \exp \left( - \exp \left( \frac{\mu_{\text{max}}}{U} (t_{\text{lag}} - t) + 1 \right) \right) \quad (2)
\]

was fitted to microbial growth curves to obtain the maximum growth rate, \(\mu_{\text{max}}\) (h⁻¹), and lag phase duration, \(t_{\text{lag}}\) (h), for each set of the experimental conditions. In Eq. (2), \(X_0\) equals the microbial concentration at the time, \(t\), equals 0, and \(U\) the limit of the value of \(\ln(OD/OD_0)\) as \(t\) approaches infinity. All model parameters were obtained from the nonlinear regression using curve-fitting software (DATAFIT 8.2, Oakdale Engineering, USA). Since the growth model, Eq. (2), covers the lag and exponential phases in the microbial growth curve, the nonlinear regression was conducted with the data until the maximum OD was reached.

2.2. Biofilm flow cell system

The biofilm flow cell system was set up for the monitoring of biofilm morphology and hydraulic conductivity in porous media, as shown in Fig. 1. The experimental system consisted of three parts: 1) the syringe pump supplying the NB solution with a constant flow rate, 2) a flow cell containing the porous media, and 3) effluent ports. The flow cell was constructed from aluminum plates measuring 60 × 25 × 15 mm³. The
upper section of the flow cell had inlet and outlet ports, with a rubber septum for bacteria injection near the inlet, and a glass plate for monitoring. The lower flow cell section was a container for the porous media measuring $40 \times 2 \times 2 \text{ mm}^3$ (total volume $= 160 \text{ ml}$). The void space was filled with acid-washed glass beads ranging between 425 and 600 $\mu$m (Sigma–Aldrich). The resulting porous media was classified as the medium sand in terms of the engineering classification of sediments defined by the American Society of Testing Materials (Fetter, 1994). The particle density ($\rho_p$) of the glass beads was 2.597 mg/mm$^3$ (Sigma–Aldrich). The bulk density ($\rho_b$) of the glass bead layer was measured for each experimental run. The porosity ($\phi$) of the porous media was computed from the following relationship (Fetter, 1994):

$$\phi = 1 - \frac{\rho_b}{\rho_p}$$

### 2.3. Experimental procedure

Experiments were run at two Darcy’s velocities ($q$), 1.25 and 7.50 mm/min, which were within the typical groundwater flow velocity range of 1–10 mm/min in sandy or gravelly aquifers (Harter, 2003), and at two substrate concentrations ($S$), 1000 and 8000 ppm. The values of $S$ were selected based on preliminary experiments in which the microbial growth was substantially attenuated as $S$ was changed from 8000 to 1000 ppm (data not shown). The combination of each velocity with each substrate concentration gave the total of four experimental conditions. Other parameters were kept constant (Table 1). The biofilm flow cell system was washed with 70% ethanol and autoclaved distilled water before every experiment. The NB solution was autoclaved and aerated by injecting the 0.2 $\mu$m filtered air inside the sterilized chamber at least 6 h before the experiment to ensure aerobic conditions. Before each experimental run, the bacterial strain was cultivated and transferred into 1 ml of phosphate buffered saline (PBS; pH 7.4, Sigma–Aldrich) solution after 12 h, when the bacterial growth reached the late exponential phase (OD $= \sim 1.5$). The transferred bacterial cells were vortexed for 1 min, harvested by centrifugation (9300 $\times g$ for 2 min), washed with the PBS solution, and vortexed again. In order to introduce the same biomass into the porous media in each experimental run, the bacterial suspension after the second vortexing was diluted with the PBS solution until the OD reached 1.0. The porous media was inoculated with 100 $\mu$l of the diluted bacteria suspension through the rubber septum using an autoclaved syringe. The microbial mass in the inoculum was 0.039 mg. After the inoculation, the flow was stopped for 30 min. Flow experiments were started in the

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**Table 1 – Parameters of experiments in the flow cell to observe biofilm morphology and hydraulic conductivity change in porous media as dependent on Darcy’s velocity ($q$) and the Substrate concentration ($S$).**

<table>
<thead>
<tr>
<th>Experimental setup</th>
<th>Bulk density ($\rho_b$) [mg/mm$^3$]</th>
<th>Porosity ($\phi$) [-]</th>
<th>Darcy’s velocity ($q$) [mm/min]</th>
<th>Substrate concentration ($S$) [ppm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.269</td>
<td>0.511</td>
<td>1.25</td>
<td>1000</td>
</tr>
<tr>
<td>2</td>
<td>1.323</td>
<td>0.491</td>
<td>7.50</td>
<td>1000</td>
</tr>
<tr>
<td>3</td>
<td>1.265</td>
<td>0.513</td>
<td>1.25</td>
<td>8000</td>
</tr>
<tr>
<td>4</td>
<td>1.306</td>
<td>0.497</td>
<td>7.50</td>
<td>8000</td>
</tr>
</tbody>
</table>
thermostatic room at 25 °C and continued for 60 h. Experimental conditions are summarized in Table 1.

2.4. Biofilm image acquisition and quantification

Bacteria in the porous media layer were stained with Syto 9 green fluorescent nucleic acid stain (5 mM solution in DMSO; Molecular Probes, USA). The Syto 9 was diluted to 2% with the autoclaved distilled water (100 μl), and injected directly into the porous media through the rubber septum of the flow cell. After 3 min, an autoclaved PBS solution was passed through the media at the same flow rate as in the experiment conditions for 1 h. This was done to remove any unbound stain and to decrease the background fluorescence.

Biofilm images were taken using a CLSM (LSM 5 PASCAL, Carl Zeiss, Germany). The 40 × 1.2 NA water immersion lens and the 20 × 0.5 NA lens were used to take images of bacteria in a pure culture and of biofilms in porous media, respectively. The 488 nm Ar laser and the 505–530 nm band-pass emission filter were used to detect the stained bacteria, and the differential interference contrast (DIC) images were then taken to confirm the morphology of the porous media. From the comparison of the fluorescence and DIC images, it was confirmed that cell staining was sufficient for observing bacteria cells with the CLSM.

The fluorescence and the DIC gray-scale images for the biofilm and porous media (glass beads), respectively, were sequentially taken by CLSM at the end of the operation time (60 h) for each experimental condition. More than five images (size: 483 × 483 pixels) were taken for each condition, with five images of biofilms on the solid surfaces for each condition (5 images × 4 conditions) tagged for further image analysis. Textural parameters, namely the textural entropy (E; the measure of the randomness of the image pixel distribution), the energy (E; the measure of the regularity in patterns of pixels), and the homogeneity (H; the measure of the similarity of the image structures located close to each other) were computed for the images. The textural parameters are used to measure the microscale heterogeneity of biofilms as a means of quantifying gray-scale intensity variations in biofilm images, and the computation methods for those parameters were described by Beyenal et al. (2004) and Yang et al. (2000).

The gray-scale images were then converted to binary images based on the optimal threshold (θ) computed by the image thresholding algorithm (iterative selection method) (Yang et al., 2001). These binary images were used to quantify the areal parameters, such as the specific biofilms (A*b); area occupied by biofilms in a unit of pore space), the specific biofilm contacts (N*b; biofilm contact length on a unit of solid surface), and the average biofilm depth (Z*b):

\[
A^* = A_p / A_p, \quad N^* = N_p / N_p, \quad Z^* = A_p / N_p
\]

where the total pore area (A_p) and the total biofilm area (A_b) were measured in pixel units using the porous media and the biofilm binary images, respectively. The biofilm contacts (N_p) and the porous media perimeter (N_p) were defined as the numbers of biofilm pixels neighboring the porous media and porous media pixels on the boundary of the porous media, respectively.

The binary images were also used to compute fractal parameters of the biofilm morphology, namely the fractal dimension, D_f to characterize the irregularity of the biofilm boundary (Yang et al., 2000). The higher D_f, the rougher is the biofilm boundary. Values of D_f were computed by the box counting method (Turner et al., 1998). Recently, it was reported that D_f was not sufficient for fully representing the fractal geometry (Pendleton et al., 2005), as fractals with identical D_f can have different appearances (Turcotte, 1997). In this context, the lacunarity, L, was computed to see how evenly biofilms are distributed (Mandelbrot, 1982). Large L implies large gaps (pores), and small L implies relatively well distributed pores inside biofilms. L was computed by the gliding-box algorithm introduced by Allain and Cloitre (1991). In this study, L was computed for the biofilm images at q = 7.50 mm/ min to compare biofilm coarseness, and the gliding-box size was fixed at 10 × 10 pixels, which was similar to a single biofilm pore size when S = 1000 ppm.

2.5. Hydraulic conductivity

The hydraulic conductivity (K) of the porous media was computed based on Darcy’s flow equation (Fetter, 1994),

\[
K = \frac{Q}{\Delta h / L / A}
\]

where Q equals flow rate (mm³/min), \(\Delta h\) represents hydraulic head loss (mm), and \(L = 40\) mm and \(A = 4 mm^2\) are the length and the cross-sectional area of the glass bead layer, respectively. Since the Darcy’s velocity \(q = Q / A\) was kept constant for each condition (Table 1), the hydraulic conductivity was computed from measurements of the hydraulic head difference between the piezometers in front of and behind the flow cell during the experimental runs (Fig. 1).

3. Results and discussion

3.1. Microbial growth

The microbial growth of P. putida F1 was characterized by varying the temperature and the inoculum mass in the isothermal batch tests. Growth curves and \(\mu_{max}\) computed using Eq. (2) are shown in Fig. 2. Four distinct phases (lag, exponential, stationary, and death phases) were clearly observed in microbial growth curves during an 80-h incubation. Lag phase durations were less than 10 h, and the maximum growths were reached at 1.7 of OD within 20 h. When inoculum mass were 0.013 and 0.025 mg at 20 °C, the microbial growth was delayed with substantially longer lag phases and less steep growth slopes. The growth slope was correlated with \(\mu_{max}\) as shown in the insert in Fig. 2. For each temperature, the \(\mu_{max}\) was not sensitive on the inoculum mass. Temperature dependence of \(\mu_{max}\) was clearly observed. At small inoculum mass (about 0.02 mg), the \(\mu_{max}\) at 20 °C was significantly less than those at 25 and 30 °C. The \(\mu_{max}\) values depended on temperature rather than on the inoculum mass. These preliminary experimental results indicated that the lag phase duration and the time for the maximum growth of the bacteria would be less than 10 and 20 h, respectively, in
Further flow cell experiments with 0.039 mg of the inoculum mass at 25 °C.

3.2. Biofilm morphology in porous media

Representative CLSM images of the biofilm morphology in porous media for each condition are shown in Fig. 3. The flow direction was from left to right of the images, and the green color indicates stained bacteria. For both substrate concentrations, biofilms were shallow and uniformly distributed on the glass beads surfaces at \( q = 1.25 \text{ mm/min} \). At \( q = 7.50 \text{ mm/min} \) with \( S = 1000 \text{ ppm} \), the biofilm morphology became irregular and biased to the flow direction. When \( S = 8000 \text{ ppm} \) at \( q = 7.50 \text{ mm/min} \), denser biofilms were more biased to the flow direction. In published work with biofilms on flat plates (Characklis and Marshall, 1990), biofilms were denser and more uniformly distributed as flow rate increased. This was because of more efficient substrate transfer to the biofilm and higher shear force. In our study, biofilms at higher \( q \) were more pronounced compared with the effect of the substrate concentration. The large coefficients of variations of both textural and areal parameters implied that the biofilm formation at the pore scale was heterogeneous even in the relatively homogeneous porous media.

Fractal dimensions \( (D_f) \) were higher at \( q = 7.50 \text{ mm/min} \) implying rougher and more irregular biofilm surfaces compared with the \( q = 1.25 \text{ mm/min} \) conditions. \( D_f \) at \( q = 7.50 \text{ mm/min} \) and \( S = 8000 \text{ ppm} \) was comparable with the \( D_f \) about 1.4 in 2-dimension for 3 days’ biofilm obtained by Jackson et al. (2001). As \( S \) was decreased to 1000 ppm, the \( D_f \) was increased to 1.542 showing the highest irregularity in Fig. 3c. Images at \( q = 7.50 \text{ mm/min} \) (examples in Fig. 3c, d) were compared in terms of \( L \). The lower \( L \) at \( S = 1000 \text{ ppm} \) meant that more gaps with similar sizes were well distributed, and it implied that biofilms were coarse. The high coefficient of variation of \( L \) at \( S = 8000 \text{ ppm} \) implied that the degree of biofilm accumulation was varied depending on the location in the porous media.

Studies have shown that substrate diffusion and fluid advection play important roles in substrate supply, especially in thick biofilm systems (Characklis and Marshall, 1990). In this study, the substrates could be supplied more deeply into the biofilms and thus the biofilms became denser at \( S = 8000 \text{ ppm} \). The insufficient substrate supply made the biofilms coarser at \( S = 1000 \text{ ppm} \).
3.3. Temporal variation of hydraulic conductivity

The normalized hydraulic conductivity (\(K/K_0\)) was used to compare normalized clogging rates for each experimental run (Fig. 4). The ratio \(K/K_0\) in all experiments eventually decreased, and the decreases were stepwise or fluctuated which meant that the decreased hydraulic conductivity was intermittently partially recovered. The degree of fluctuations at \(q = 7.50\) mm/min with \(S = 8000\) ppm was larger than in other conditions implying more frequent recovery events, due to higher flow rate and more rapid clogging due to higher substrate concentration. The initial decrease of \(K/K_0\) at \(q = 7.50\) mm/min occurred in less than 5 h which was earlier than at \(q = 1.25\) mm/min (about 30 h). The value of \(S\) did not affect the initial \(K\) decreases (Fig. 4). Towards the end of the experiments, more significant decreases of \(K/K_0\) were observed at \(S = 8000\) ppm. The overall decrease in \(K/K_0\) was affected by nutrition while the initial decrease was affected by flow rate.

The perfect clogging with \(K/K_0\) less than 0.01 at \(q = 1.25\) mm/min with \(S = 8000\) ppm occurred between 50 and 60 h, and so all experimental runs were stopped after 60 h for the comparison.

3.4. Development of clogging for biofilms of different morphology

Although Fig. 3 showed that biofilm morphology in each experimental condition was different, the biofilm-induced clogging shown in Fig. 4 occurred in all of the experiments. The flow rate and the nutrition influenced the biofilm morphology in a different manner. The superficial biofilm morphology and the initial decrease of \(K/K_0\) were dependent on the flow velocity, while the biofilm coarseness and the clogging intensity depended on the nutrition level (Figs. 3 and 4). This implies that different clogging mechanisms may dominate as...
the result of the interplay between flow and substrate conditions.

The increased shear force at higher flow rates facilitates biofilm erosion and detachment (Characklis and Marshall, 1990). The sloughed biofilm colonies can be entrapped and accumulated in narrow pore necks in porous media like in Fig. 3c, d. The decrease of $K/K_0$ at $q = 7.50 \text{ mm/min}$ occurred in less than 10 h (Fig. 4) which was even earlier than the time for the maximum microbial growth of bacteria (about 20 h, Fig. 2). It can be suggested that entrapment and accumulation of sloughed biofilms rather than local growth of biofilms accelerated clogging of the porous media. On the other hand, clogging at $q = 1.25 \text{ mm/min}$ started after about 30 h in Fig. 4 which was longer than the time for the maximum microbial growth of bacteria. This result and the biofilm morphologies at $q = 1.25 \text{ mm/min}$ (Fig. 3a, b) showed that the porous media was clogged due to local biofilm growths in narrow pore

### Table 2 – Mean and coefficient of variation values of morphological parameters of biofilm images depending on Darcy’s velocity ($q$) and the substrate concentration ($S$)

<table>
<thead>
<tr>
<th>$q$ (mm/min)</th>
<th>$S$ (ppm)</th>
<th>$K/K_0$</th>
<th>$\theta$</th>
<th>$L$</th>
<th>$E$</th>
<th>$TE$</th>
<th>$H$</th>
<th>$N_b^*$</th>
<th>$A_b^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.25</td>
<td>1000</td>
<td>0.128 (0.10)</td>
<td>0.006 (0.09)</td>
<td>0.381 (0.13)</td>
<td>0.008 (0.09)</td>
<td>0.018 (0.10)</td>
<td>0.001 (0.11)</td>
<td>0.002 (0.09)</td>
<td>0.001 (0.10)</td>
</tr>
<tr>
<td>1.25</td>
<td>8000</td>
<td>0.132 (0.05)</td>
<td>0.009 (0.09)</td>
<td>0.653 (0.13)</td>
<td>0.006 (0.09)</td>
<td>0.020 (0.11)</td>
<td>0.001 (0.11)</td>
<td>0.002 (0.09)</td>
<td>0.001 (0.10)</td>
</tr>
<tr>
<td>7.50</td>
<td>1000</td>
<td>0.182 (0.14)</td>
<td>0.003 (0.08)</td>
<td>0.965 (0.13)</td>
<td>0.005 (0.09)</td>
<td>0.030 (0.11)</td>
<td>0.001 (0.11)</td>
<td>0.002 (0.09)</td>
<td>0.001 (0.10)</td>
</tr>
<tr>
<td>7.50</td>
<td>8000</td>
<td>0.262 (0.08)</td>
<td>0.004 (0.08)</td>
<td>1.868 (0.13)</td>
<td>0.005 (0.09)</td>
<td>0.040 (0.11)</td>
<td>0.001 (0.11)</td>
<td>0.002 (0.09)</td>
<td>0.001 (0.10)</td>
</tr>
</tbody>
</table>

### Fig. 4 – Temporal variations of the normalized hydraulic conductivity ($K/K_0$) in log scale based on Darcy’s velocity ($q$) and the Substrate concentration ($S$).

### Fig. 5 – Temporal variation of optical density at 600 nm wavelength (OD) of effluents from the biofilm flow cell with Darcy’s velocity ($q$) = 1.25 mm/min and the Substrate concentration ($S$) = 8000 ppm.
necks rather than due to entrapment and accumulation that happened at \( q = 7.50 \text{ mm/min} \) (Fig. 3c, d).

The temporal variation of OD of effluents at \( q = 1.25 \text{ mm/min} \) and \( S = 8000 \text{ ppm} \) is shown in Fig. 5. This data showed how much bacteria and/or biofilms were flushed out from the porous media. The stepwise decrease of \( K/K_0 \) (Fig. 4) and the repeated peaks of OD in the effluents (Fig. 5) implied that both biofilm clogging and flushing occurred repeatedly in the porous media similar to the results of Stoodley et al. (2005). Immediately after inoculation, suspended bacteria was flushed out and detected, and then intermittent minor peaks were detected although hydraulic conductivity was not decreased (Fig. 4) within 30 h. It implies that suspended and/or sloughed bacteria at \( q = 1.25 \text{ mm/min} \) was not entrapped but constantly flushed out. The amount of flushed-out bacteria began to increase constantly after 30 h. This coincided with another steady state of hydraulic conductivity after a sudden decrease at 30 h (Fig. 4). The sudden decrease of flushed-out bacteria at 45 h indicated that the porous media was clogged by biofilm, and it was confirmed by sudden decrease of hydraulic conductivity at that time in Fig. 4.

4. Conclusions

The biofilm morphology and the hydraulic conductivity decrease in porous media were concurrently characterized at different conditions of the flow rate and the substrate concentration. Results showed that Darcy’s velocity influenced the superficial morphology of biofilm and initial time of clogging, whereas substrate concentration affected the biofilm density and the rate of clogging. Given the distinct differences in biofilm morphologies and the hydraulic conductivity decrease during the experiments, different clogging mechanisms were dependent on flow rate and substrate concentration.

(1) Clogging at high flow rates can be accelerated by entrapped and accumulated biofilms, but can be easily eliminated by high shear force.

(2) Clogging at low flow rates can be delayed for the time of local biofilm growths in the narrow pore necks, but the biofilm is rigid enough not to be sloughed.

(3) Clogging in a solution with high substrate concentration cannot be easily eliminated because of the high density of biofilms.

The biological clogging mechanisms in porous media which were depicted in this study will play a role in support of controlling aquifer recharge and improving its efficiency.

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