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REMOVAL OF MS2 BACTERIOPHAGES IN THE SLOW SAND FILTRATION PROCESS

USUWANIE BAKTERIOFAGÓW MS2 W PROCESIE FILTRACJI POWOLNEJ

Slow sand filtration is old sustainable water treatment technology used worldwide to treat surface waters by biological, physicochemical and physical removal mechanisms. One of the numerous advantages of slow sand filtration is elimination or limitation of disinfectant chemicals. However, it can pose a serious health threat if viruses or bacteria pass through to the slow sand filter effluent. Thus, a precise and reliable virus enumeration method is of great concern to scientists. Real time PCR assay is one of the most promising virus enumeration methods. The experiments of slow sand filtration were carried out in the laboratory at Gifu University in Japan. RRT-PCR, as well as traditional plaque methods were applied for enumeration of model virus (F-specific RNA bacteriophage MS2) reduction during the runs. Model virus reduction through the four identical columns operated under different conditions, characterized by velocity, light illumination and quantity of bacteria in raw water, were investigated. In the end, based on the experimental results, mathematical model of virus reduction, as well as a reduction of bacteria during the slow sand filtration was proposed. The predicted curves and measured points correlated quite well with each other.

1. Introduction

Slow sand filtration is a 200 - year - old sustainable water treatment technology used worldwide to treat surface waters by biological, physicochemical and physical removal mechanisms. Slow sand filtration is ideal for small communities, because it is simple to operate, does not require chemical pre-treatment, functions over a wide range of influent water quality without the need for process adjustment, requires minimal maintenance and is relatively cost-effective to build and operate. This is why slow sand filtration is not only used in rural regions of developing countries, but also in developed countries such as Japan. The treatment performance of slow sand filtration has been reported for a variety of water quality parameters, including the removal of bacteria, turbidity, organics and microbial pathogens. Of particular importance is the need to quantify the removal

and inactivation of enteric viruses that would likely be present in sewage-contaminated surface water. Viruses are typically difficult to remove by filtration, because their small size precludes straining, and their negative surface charge impairs attachment to granular media. Since one of the advantages of slow sand filtration is elimination or limitation of disinfectant chemicals, viruses can pose a serious health threat if they pass through to the slow sand filter effluent. There is a strong need for research on the slow sand filtration process to develop knowledge on how to control virus removal. Despite this need, there are very few studies in literature that report on virus removal by slow sand filtration [4]. Cryptosporidium or Giardia are most often relatively efficiently removed during slow sand filtration (>4 log), whereas removal of viruses or bacteria is much weaker (1-4 log). There is a limited number of viral removal reports due to lack of a simple and precise direct virus enumeration method. Apart from the traditional plaque method, the new genetic method was applied for virus enumeration during experiments.

There is also a lack of mathematical models describing virus removal during slow sand filtration reported in literature.

2. Material and methods

2.1. Laboratory set-up

The experiments were carried out in the laboratory at Gifu University in Japan. The set-up included two raw water tanks and four filtration columns, presented in figure 1. The tanks were filled with water collected from the river in the Gifu prefecture in Japan, guaranteeing biofilm growth in the filter media. Both of the tanks were equipped with mixing system protecting against settling of the particles scattered in raw water. One of the tanks was filled with river water after passing through a 0.5 micrometer mesh membrane, hoping to remove more of the bacteria. MS2 bacteriophages were dosed periodically to the raw water in both tanks, keeping the MS2 concentration constant during the entire run.

Afterwards, water from the mixing tank was pumped by means of a peristaltic pump to four identical filtration columns. The filtration columns were filled with sand media received from the full-scale slow sand filter at one of the Japanese water treatment plants. All of the columns were similar to each other. The inner diameter of the columns was equal to 25 mm, and the height of the sand media was 22 cm. The dried sand media was characterized by an effective diameter d_{10} equal to 0.15 mm, d_{60} = 0.38 mm. Uniformity d_{60}/d_{10} of the sand media was equal to 2.53.

Three columns (A,B,C) were operated at 4m/d, and one column (D) at 8 m/d for three months. As it was mentioned before, raw water inflowing to the columns was collected from one of the rivers located at the Gifu prefecture guaranteeing biofilm growth in the filter media. However, one of the four columns was supplied by the river water after passing through a 0.5 micrometer mesh membrane that should guarantee significant reduction of bacteria in the inflow. Another column was artificially illuminated during the day to simulated uncovered slow sand filter conditions.

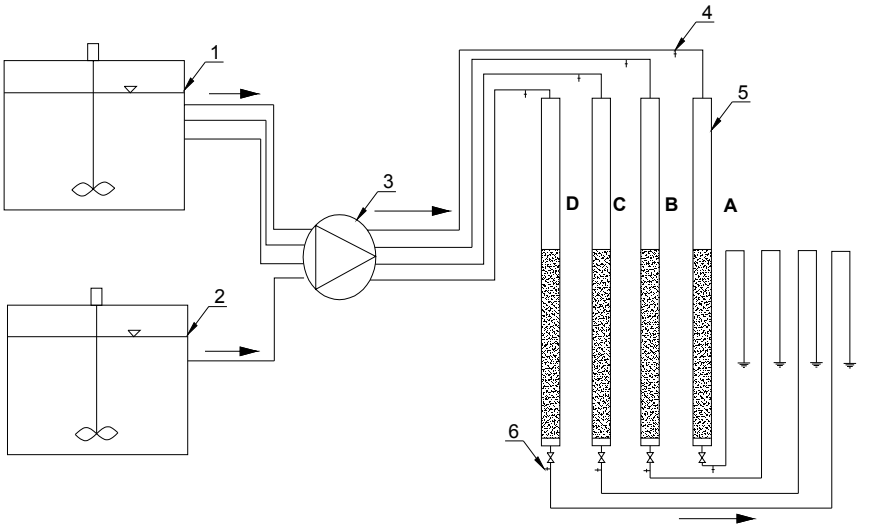

Fig. 1. Laboratory set-up (1 – raw water mixing tank, 2 – raw water mixing tank after 0.5 µm mesh membrane, 3 – peristaltic pump, 4 – inflow sampling points, 5 – filtration columns, 6 – outflow sampling points)

Rys. 1. Stanowisko laboratoryjne (1- zbiornik wody surowej 2. – zbiornik wody surowej po przefiltrowaniu przez 0,5 µm filtr mechaniczny 3. Pompa perystaltyczna 4 – punkty poboru na dopływie, 5 – kolumny filtracyjne, 6 – punkty poboru na odpływie)

2.2. Measurements

Removal of water quality indices, such as turbidity, particle counts, suspended solid, dissolved oxygen (DO), oxidation-reduction potential (ORP), pH, UV absorbance, MS2 bacteriophages and total bacteria, for different conditions of each of the columns were investigated. MS2 bacteriophages were used during experiments as surrogates for human enteric viruses. MS2 Bacteriophages are typically removed less than human enteric viruses during passage through granular media. Therefore, MS2 bacteriophages are good conservative model viruses. MS2 bacteriophages are characterized by a strong negative surface charge, low isoelectric point (pH =3.9) and very small size (26 nm) [4].

The suitability of coliphages as indicators of virus and fecal contamination of water and food has long been studied, and there is an abundant scientific literature addressing this topic compiled in several reviews [7,8,9,10,12].

For detection of the nano-sized coliphages, most of the previous studies employed the conventional plaque assay method. However, it is well recognized that the plaque method has the drawbacks of being time-consuming and being largely dependent on culture conditions or nutrient levels. Moreover, the plaque method can only detect infectious viruses. During the experiments, MS2 bacteriophages were measured by two

methods, the traditional plaque method and a new method based on the real-time reverse transcription polymerase chain reaction (RRT-PCR). The RRT-PCR method has the merit of being rapid and highly sensitive. This method not only allows investigators to discriminate between microorganisms from different sources, but it also allows detection of extremely small quantities of nucleic acid, hence drawing the attention of water treatment specialists for precise investigation of microbial behavior during water treatment [2]. With the RRT-PCR method, the total concentration of microorganisms, both infectious and non-infectious, can be quantified, showing to be very attractive for grasping information on the entire behavior of microorganisms and viruses. The successful development of a qPCR (quantitative PCR)-based method suitable for quantification of virus removal by nano-filtration was reported [13] in literature. There are also some examples of successful application [3] of the qPCR-based method for quantification of protozoa and bacteria remaining in the filtrate.

The quantity of male specific MS2 bacteriophage in the samples was determined following the conventional plaque method described in ISO 10705–2 [5], using Escherichia coli WG5 as the host strain.

The sand was cleaned, dried and put into the columns, keeping exactly the same composition in each of them. The experiment was carried out for 3 months.

Apart from virus experimental analysis, viable bacteria number and other water quality indices were also investigated. The standard plate count method was used for measurement of the viable bacterial number [1].

3. Experiments

For the whole run, the temperature was more less constant and equal to 20 degrees Celsius, pH varied between 7 and 8 and conductivity between 6 and 10 mS/m. The raw water was characterized by a quite high quality, and the concentration of dissolved oxygen didn't drop below 70%. Water quality parameters such as DO, ORP, pH, UV260 and DOC were not significantly reduced for the entire filtration run. The average values of the raw water quality indices during the experiments are presented in table 1.

Tab. 1. Average values of the raw water quality indices during the experiment

Tab. 1. Średnie wartości wskaźników jakości wody surowej podczas eksperymentów

H	EC [mS/m]	DO [mg/L]	DO [%]	ORP	Temp [°C]	UV260	DOC [mg/L]	Turbidity [NTU]	Suspension concentration [vol/vol]
7.43	7.52	6.24	71.93	229.07	20.77	0.12	1.21	0.9	0.000001

3.1. RRT-PCR and plaque assay methods for bacteriophage enumeration during slow sand filtration

Figures 2 - 4 present MS2 bacteriophage reduction during slow sand filtration as measured by the plaque and RRT-PCR methods. The graphs let us compare the results received based on both methods.

The dominant physical processes influencing the fate and transport of viruses in the filter media include advection, dispersion, diffusion, and adsorption and biological such as growth/decay. However, the replication and inactivation processes regard only active viruses. Since the plaque method can only detect active viruses, whereas the RRT-PCR method can detect both: active as well as non-active viruses, a higher virus removal as measured by the plaque rather than the RRT-PCR method would mean better removal of active than non-active viruses or more intensive virus replication than inactivation.

Slightly better virus removal was monitored by plaque method only in the beginning of slow filtration through columns A, B and D (fig. 2-4). However, for the rest of the filtration time, the RRT-PCR method gave higher virus reduction results than the plaque method, but the observed differences were not significant.

Higher virus removal measured by the genetic rather than the plaque method can suggest faster replication than the inactivation of viruses during slow filtration. It also explains the opposite relation at the beginning of the filtration run. Since MS2 coliphage replication takes place inside the bacteria cell, the low number of bacteria inside the sand media during the maturation stage causes virus replication to decrease.

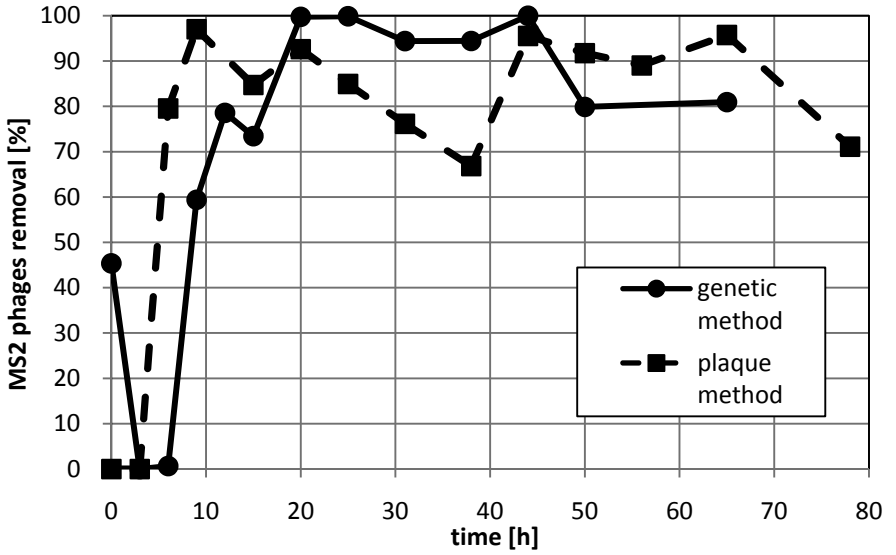

Fig. 2. Removal of MS2 phages flowing through column A measured by the RRT-PCR and plaque methods.

Rys. 2 Usuwanie fagów MS2 przepływających przez kolumnę A zmierzone metodami: RRT-PCR i łysinkową

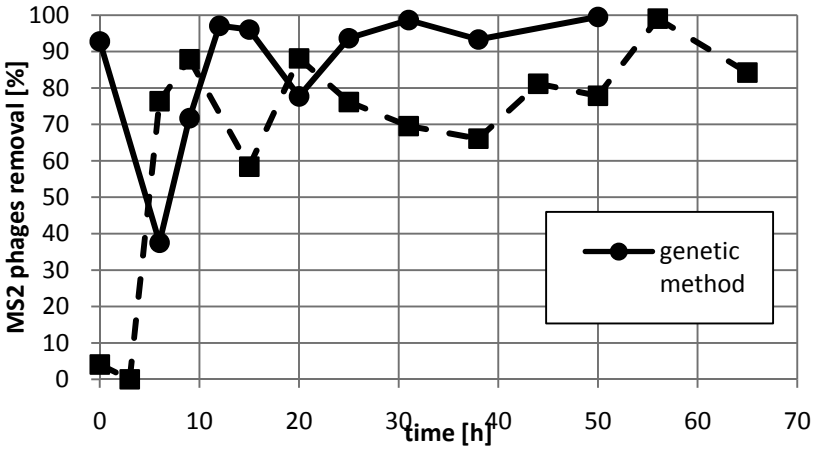

Fig. 3. Removal of MS2 phages flowing through column B measured by the RRT-PCR and plaque methods.

Rys. 3 Usuwanie fagów MS2 przepływających przez kolumnę B zmierzone metodami: RRT-PCR i łysinkową

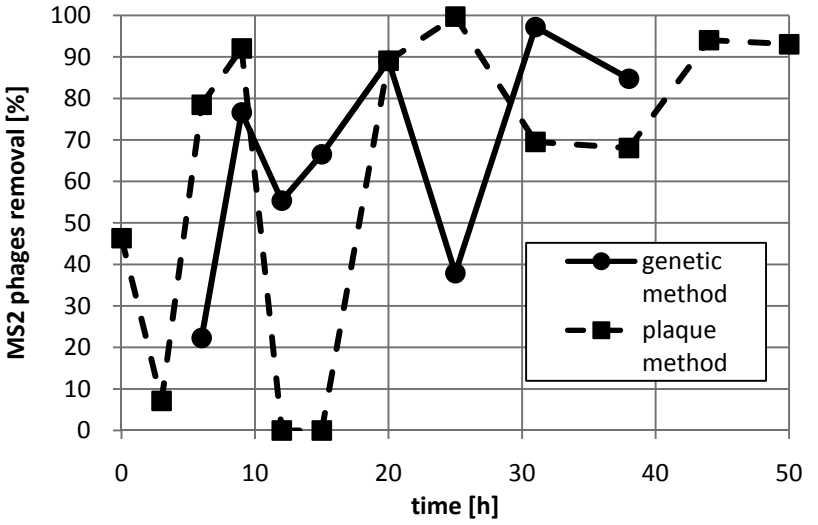

Fig. 4. Removal of MS2 phages flowing through column D measured by the RRT-PCR and plaque methods.

Rys. 4 Usuwanie fagów MS2 przepływających przez kolumnę D zmierzone metodami: RRT-PCR i łysinkową

3.2. Microbes removal during slow sand filtration

Generally speaking, the MS2 removal graphs (figure 5) for all of the columns are relatively similar to each other. Slightly poorer removal through column B was observed than through column A, which was a consequence of the two time higher filtration velocity and shorter contact time in column B than in column A. The most stable results were observed for filtration through column C. The virus removal curve for column C presents the quickest increase during the maturation period and later the highest removal during the stable stage. The experiment was carrying out for column C simulating sunlight illumination. A visibly intensive growth of the schmutzdecke layer and algae in the supernatant water inside column C caused a more effective removal of viruses flowing through column C than flowing through the remaining columns.

As it can be seen in figure 5, the maturing period lasted around 10 days for all of the filters. After this time, very little improvement of removal was observed. In accordance to other reports, a deterioration stage was almost unnoticed, due to very low filtration velocity. However, after 80 days of the filtration run, some decrease of virus removal occurred. However, this was difficult to prove, because of a sudden increase of filter head-loss and finish the run at the same time.

Column D was supplied by river water after the mesh membrane. Since one of the virus removal mechanisms is attachment to the cell of bacteria and predation by filter feeders, a lack of microorganisms larger than 0.5 micrometers in the raw water should initially reduce the removal of viruses. On the other hand, a lack of bacteria should have lowered replication of bacteriophages and improved their reduction during filtration. Removal of viruses through column D was lower and more unstable than in the other columns during the maturation stage. During the stable stage, a similarly high MS2 reduction was observed as in the columns supplied by river water without membrane pre-filtration.

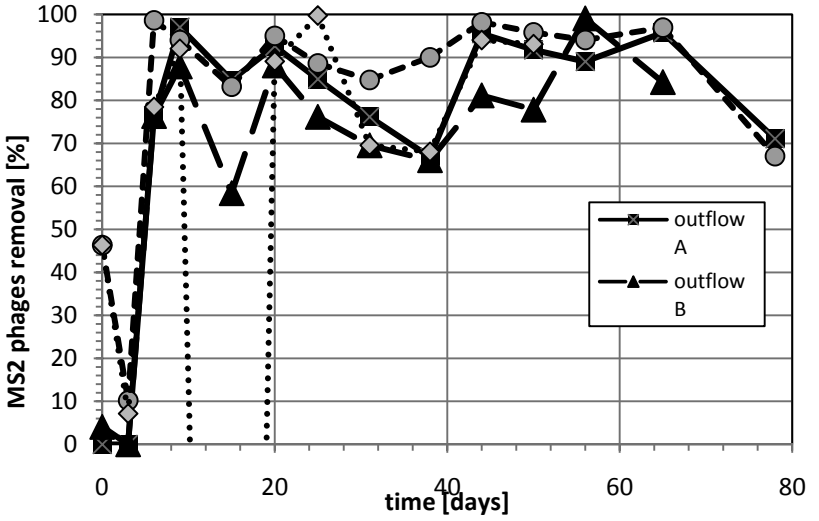

Fig. 5. Removal of MS2 phages flowing through column A,B,C,D measured by the plaque method.

Rys. 5 Usuwanie fagów MS2 przepływających przez kolumnę A,B,C,D zmierzone metodami łysinkową

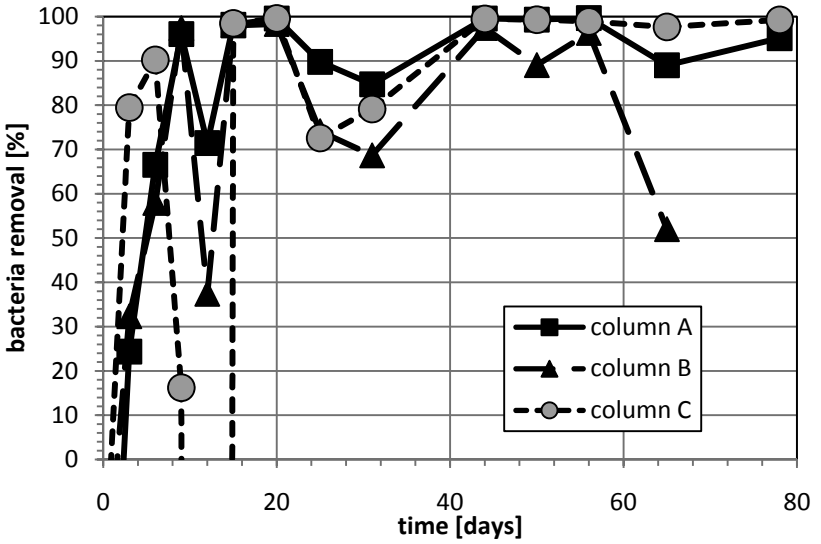

Fig. 6. Removal of bacteria flowing through column A,B,C,D measured by the plaque method

Rys. 5 Usuwanie ogólnej liczby bakterii przepływających przez kolumnę A,B,C,D zmierzone metodą łysinkową

Mathematical model of MS2 bacteriophage removal as well as viable bacteria during slow sand filtration were proposed based on experimental results. The phenomenological model contained mass balance (1) and kinetic equations (2) (3) was used to predict absolute deposit in slow filter:

$$\frac{\partial \sigma}{\partial t} = -\frac{\partial C}{\partial x} w \tag{1}$$

$$\frac{\partial \sigma}{\partial t} = \lambda C w \tag{2}$$

$$\lambda = \lambda_o + \left(\lambda_1 - \lambda_o\right)\left[1 - exp\left(\frac{\sigma^2}{\sigma_1^2}\right)\right] \tag{3}$$

Where: C – suspension concentration,
w – superficial velocity for the slow filter,
x – depth in slow filter,
t – filtration time,
σ – absolute deposit concentration,
λ – filtration coefficient,
λ_o – initial filter coefficient, for clean sand media (σ=0),
σ_1, λ_1 – parameters in expression (3).

$$\frac{\partial S_{microbs}}{\partial t} = D_d \frac{\partial^2 C_{microbs}}{\partial x^2} - \frac{\partial C_{microbs}}{\partial x} w \tag{4}$$

$$\frac{\partial S_{microbes}}{\partial t} = -\lambda_{microbes} w C_{microbes} \tag{5}$$

where: $\lambda_{microbes}$ – microbes removal rate coefficient
$C_{microbes}$ – microbes concentration.
$S_{microbs}$ – retained microbes concentration
D_d – hydrodynamic dispersion coefficient,

$$\lambda_{microbs} = \lambda_{o,microbs} + \left(\lambda_{1,microbs} - \lambda_{o,microbs}\right)\left[1 - exp\left(\frac{\sigma^2}{\sigma^2_{1,microbs}}\right)\right] \tag{6}$$

Kinetic equations (2) and (3) as well as (5) and (6) described two filtration stages: maturation and stable. The first one presents significant removal improvement with the maturation of the biofilm; the second one showed very slight improvement and stabilization. The deterioration of particle removal rarely observed during slow sand filtration was not taken into account. Equations (3) and (6) were originally proposed by Mackie [14] for the first two stages of rapid filtration. The equations (3) and (6) represent a curve that is monotonically increasing and levels off. Once σ has reached the value $2\sigma_1$ there is very little or lack of change in the value of λ.

Transport of biocolloids in granular media was described by equations (4) and (5).

Differential partial equations were solved based on finite difference method. Three coefficients, λ_o, λ_1, σ_1, included in equation (3) and three coefficients $\lambda_{microbes}$, $\lambda_{microbes}$, $\sigma_{1,microbes}$ in equation (6) were predicted based on the least square fitting. The biological activity of the biofilm was omitted in the model for experiments without light, since the mixed liquor volatile suspended solids (MLVSS) of the biofilm were quite small compared to the total specific deposit of the biofilm. Laboratory measurement of the ratio of MLVSS to the total specific deposit of the biofilm showed results that changed from 15% at the upper layer of the sand media to a much lower percentage at the bottom before regeneration.

The specific deposit at 4 m/d filtration velocity was calculated based on mass balance (1) and kinetic equation (2) as well as experimental results measured using a particle counter. Removal of MS2 bacteriophages as well as total bacteria for the same filtration run was calculated based on equations (4) and (5).

Removal of MS2 bacteriophages and total bacteria at 8 m/d filtration velocity were calculated based on equations (4) and (5) and new $\lambda_{o,microbs}$, $\lambda_{1,microbs}$, parameters. Viruses as well as most of the bacteria are small in size and their transport in the immediate vicinity of the collector surface is dominated by Brownian diffusion, whereas effects of interception and gravitation are negligible. In this case the collision efficiency, using spherical Happel's flow model, is given by the Smoluchowski-Levich approximation [11]

$$\eta = 4 A_S^{1/3} \left(1 - \varepsilon_o\right)^{2/3} N_{Pe}^{-2/3} \tag{7}$$

Where: ε_o – initial media porosity,

η – collision efficiency,

N_{Pe} – Peclet number,

$$N_{Pe} = \frac{d_p \cdot w}{D},$$

$$D = \frac{k_B T}{3\pi\mu d_p},$$

μ – water viscosity,

d_p – particle diameter,

D – mass diffusion coefficient,

k_B – Boltzman constant,

T – temperature,

$$A_s = 2\left(1 - p^2\right) / \omega,$$

$$\omega = 2 - 3p + 3p^5 - 2p^6,$$

$$p = (1 - \varepsilon_o)^{\frac{1}{3}}.$$

The single collector removal efficiency is related to the removal rate coefficient, by the expression:

$$\lambda = \frac{1.5(1-\varepsilon_0)^{1/3}}{d}\eta\alpha \tag{8}$$

where: d – grain (collector) diameter,

α – sticking (attachment) efficiency (represents the fraction of the particles colliding with collector that remain attached.

From equations (7) and (8) it follows

$$\lambda = \frac{1.5(1-\varepsilon_0)}{d} 4 A_S^{1/3} N_{Pe}^{-2/3} \alpha \tag{9}$$

Conditions of experiments carrying out for column A and B differed only in velocity. Since, even sticking efficiency is independent of hydrodynamic effects (velocity and dispersion) Peclet number is the only parameter dependent on velocity in expression (9). In consequence, removal rate coefficient, which is linearly dependent on Peclet number to the power of (-2/3), at different flow velocities is equal to the ratio of the two flow velocities to the power of (-2/3). From this it follows that

$$\frac{\lambda_{0,microbes(B)}}{\lambda_{0,microbes(A)}} = \frac{\lambda_{0,microbes(B),c}}{\lambda_{0,microbes(A),c}} = \left(\frac{w_{(B)}}{w_{(A)}}\right)^{-2/3} = \left(\frac{8}{4}\right)^{-2/3} = 0.63, \tag{10}$$

$\lambda_{o,microbes(A)}$ – initial value of microbe removal coefficient for column A (4m/d) based on best fitting,

$\lambda_{o,microbes(A),c}$ – initial value of microbe removal coefficient for column A (4 m/d) calculated from equation (9),

$\lambda_{o,microbes(B)}$ – initial value of microbe removal coefficient for column B (8 m/d)calculated from equation (9).

From equation (10) it follows that $\lambda_{o,microbes(B)}$, parameters for column B were counted from the equation:

$$\lambda_{0,microbes(B)} = \lambda_{0,microbes(A)} \left(\frac{w_{(B)}}{w_{(A)}} \right)^{-2/3} = \lambda_{0,microbes(A)} \left(\frac{8}{4} \right)^{-2/3} = 0.63\lambda_{0,microbes(A)}, \tag{11}$$

Since $\lambda_{1,microbes(B)}$ is microbe removal coefficient at relatively low deposit concentration, similar expression to (11) is used for calculation:

$$\lambda_{1,microbes(B)} = \lambda_{1,microbes(A)} \left(\frac{w_{(B)}}{w_{(A)}} \right)^{-2/3} = \lambda_{1,microbes(A)} \left(\frac{8}{4} \right)^{-2/3} = 0.63\lambda_{1,microbes(A)}, \tag{12}$$

Parameter σ_1 was kept for column B the same as predicted based on best fitting for column A.

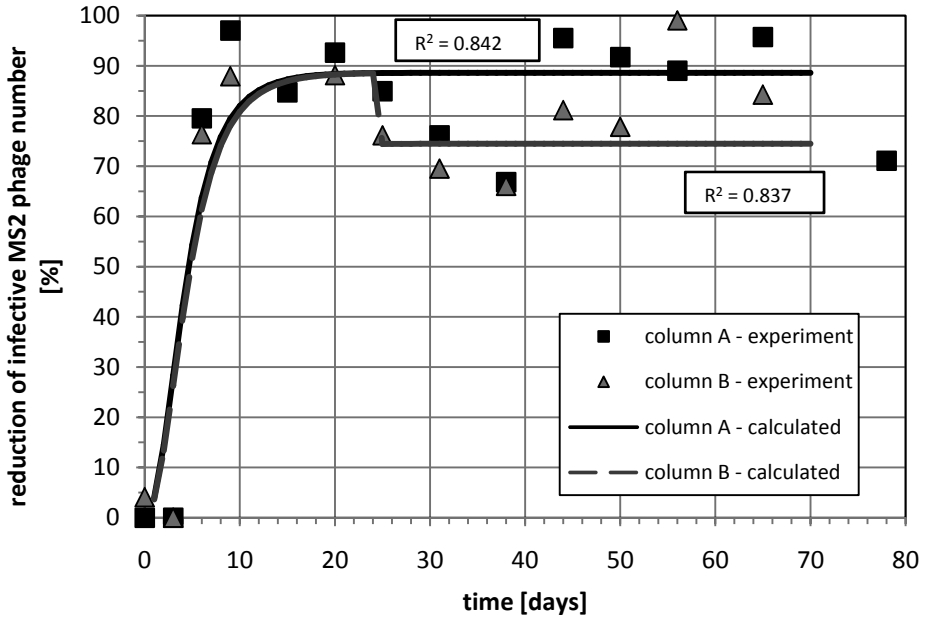

Fig. 7. Removal of MS2 bacteriophages flowing through column A,B measured by the plaque method.

Rys. 7 Usuwanie MS2 bakteriofagów przepływających przez kolumnę A,B zmierzone metodą łysinkową

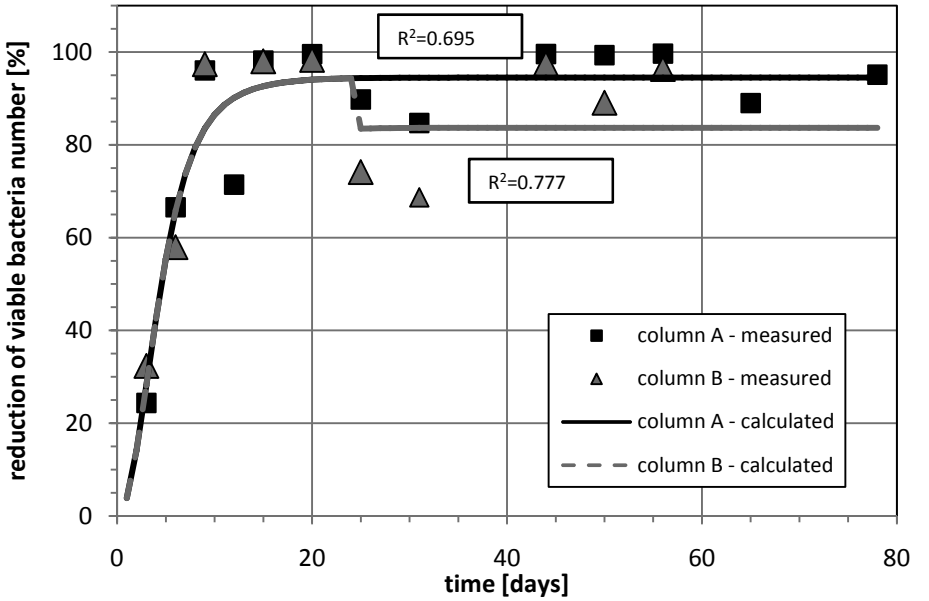

Fig. 8. Reduction of viable bacteria in column A,B by the plate count method

Rys. 8 Redukcja żywych bakterii przepływających przez kolumnę A,B, zmierzone na płytce Petriego

The coefficients of determination R^2 were counted for the sets of data presented in figures 6 and 7. The coefficient of determination R^2 is the proportion of variability in a data set that is accounted for by the mathematical model. R^2 is used to provide a measure of how well experimental data is likely to be predicted by the mathematical model. The values of R^2 were calculated based on the mathematical definition:

$$R^2 = 1 - \frac{SS_{err}}{SS_{tot}} = 1 - \frac{\sum_{t=1}^{n} \left(y_i - f_i \right)^2}{\sum_{t=1}^{n} \left(y_i - \overline{y} \right)^2} \tag{13}$$

Where: SS_{err} – the residual sum of squares,
SS_{tot} – the total sum of squares,
y_i – measured values,
f_i – calculated values,
$\overline{y}$ – average of the measured values.

The coefficients of determination were calculated for the sets of data and presented in figures 6 and 7. The values of R^2, higher than 0.8 for modeled virus reduction and not lower than 0.7 for modeled bacteria reduction, suggest a quite good fit of all of the curves to the experimental data. However, a better fitting of the curves for infective MS2 bacteriophage reduction was noticed than for viable bacteria.

Quite high correlation between calculated and experimentally predicted set of data (figures 6 and 7) suggests suitable description of virus deposition kinetic by equations (5) and (6).

4. Conclusions

The experiments of MS2 bacteriophage reduction during slow sand filtration have been carried out in one of the Japanese laboratories. Generally, MS2 enteric virus reduction measured by the RT-PCR and plaque methods have shown quite similar results for all of the runs. However, in the ripening stage, a slightly higher reduction of MS2 phages was detected by the plaque method, whereas during the stable stage, a higher reduction was measured by the RT-PCR method. The higher reduction measured by RT-PCR during the stable period suggests a more intensive replication than inactivation of viruses.

The experiments carried out on four columns operating under different conditions showed a very low, unstable reduction of viruses during the maturation period and a near lack of reduction for the initial condition. The highest and most stable virus reduction was observed for the column simulating the uncovered filter with artificial light illumination. The virus reduction in this filter varied between 1 log to 2 log. The biofilm inside the illuminated column matured faster than in the other columns, attaining a much earlier stable period and higher virus reduction. As was supposed, a higher velocity caused a shorter contact time and lower virus reduction. However, no significant influence of velocity was observed. The reduction of viruses in raw water filtered through the 0.5 micrometer mesh membrane eliminating more of the coliform bacteria was characterized by lack of stability and lower efficiency than through the remaining columns. A much higher viable bacteria reduction than virus was observed. This comes as no surprise, since very low MS2 phage reductions were reported by many scientists. Curves predicted based on the mathematical model for infectious MS2 phage reduction and viable bacteria reduction for different velocities fit quite well to the experimentally measured set of data. The coefficients of determination for MS2 reduction data were higher than 0.8 and not lower than 0.7 for viable bacteria reduction.

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