ENGINEERING JOURNAL

Article

Bacteriophage Removal Efficiency of In-line Coagulation with Ceramic Membrane Filtration

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Abstract. The main objectives of this study are to evaluate the removal efficiency of bacteriophage Qβ using in-line coagulation with ceramic membrane filtration at different coagulant dosages, ceramic membrane pore sizes and initial bacteriophage Qβ concentrations. Raw water was collected from the Ping River, Chiang Mai, Thailand, and spiked with bacteriophage Qβ to prepare an initial concentration of 8×10^6 PFU/ml. According to the resulted, it was found that the smaller pore sizes membrane yield higher bacteriophage Qβ log removal. However, the use of a ceramic membrane alone could not remove bacteriophage Qβ completely. In-line coagulation combined with ceramic membrane filtration was conducted. The optimal polyaluminum chloride (PACl) doses for the 1.0, 0.5 and 0.1 μm pore size membranes were 2.5, 2.0 and 1.5 mg-Al/L, respectively. Bacteriophage Qβ removal at the optimal PACl dose was more than 6.7 log in all cases. The results of effect of different initial bacteriophage Qβ concentration on the removal efficiency showed that in-line coagulation with ceramic membrane at all pore sizes can completely remove bacteriophage Qβ when the initial concentration was low (5×10^5 PFU/ml). However, the higher initial bacteriophage Qβ concentrations (4×10^6 and 8×10^7 PFU/ml) required a higher PACl dose to obtain effective bacteriophage Qβ removal.

Keywords: Bacteriophage, ceramic membrane, in-line coagulation, PACl coagulation.

1. Introduction

Human enteric viruses have been recognized as major causes of waterborne out-break which have been reported worldwide in both developed and developing countries [1, 2]. Human virus analysis requires advanced technologies and specialists. The F-specific RNA bacteriophage is an enterobacteriaceae virus of the Leviviridae family that is physically and genomically analogous to human enteric viral pathogens found in sewage. It is abundant in sewage and easy to enumerate using standardized ISO methods (ISO10705-1), making them a good prospective indicator of viral contamination in a marine environment. It has been proposed as indicators of viral contamination in the environment and used to model the behavior of human pathogen enteric viruses. F-specific RNA bacteriophage Qβ (diameter 0.023 μm, pI 5.3) has been used extensively as a surrogate virus for waterborne viruses because of its morphological and structural resemblance to human enteric viruses, which need to be removed during the treatment of drinking water [3-7]. In addition, its survival characteristics in aquatic environments are similar to those of human enteric viruses. The average concentrations of F-specific RNA bacteriophage that commonly found in wastewater are ranging from 1 to 5 log10 plaque-forming units (PFU)/ml [8-10] and 1 to 3 log10 PFU/ml in river water [9, 11-12]

The one of the most important factor for human survival is access to the high quality of potable water [13]. Membrane filtration is the promising technology which has been used extensively all over the world for potable water production and wastewater treatment. The installation of microfiltration (MF) and ultrafiltration (UF) facilities has increased considerably over the past decade [14]. MF and UF are considered capable processes for providing better drinking water quality. The solid-liquid separation process in water treatment using ceramic membranes has garnered greater interest over the last decade [15]due to its several advantages such as its strength, resistance to acidity, thermal superiority, chemical stability and low environmental impact [16]. The membranes are used to remove particulate matter such as inorganic particles as well as microorganisms including bacteria and virus. Nevertheless, MF devices whose pore sizes are not small enough to reject smaller particles as viruses, causing health concerns [4].

The addition of a coagulant before ceramic membrane filtration is one of the possible pretreatment processes to increase treated water quality. It has been reported that some microorganisms have a tendency to be adsorbed onto the coagulated floc particles, which are retained by ceramic membranes. In-line coagulation is an application applied to small membrane systems. It is referred to the use of coagulation before membrane filtration without a sedimentation step [17, 18] and can enhance microorganism removal onto the membrane surface.

Thus, the objective of this study is to investigating the bacteriophage Qβ removal efficiency of in-line PACl coagulation with ceramic membrane filtration along with the effects of the initial bacteriophage Qβ concentration and PACl dosage.

2. Materials and Methods

2.1. Raw Water

Raw water was collected from the Ping River in Chiang Mai, Thailand, in December during the winter season. It contained a turbidity about 40-80 NTU. Raw water was transported in polyethylene tanks and stored in a refrigerator at 4°C before analysis. pH of the raw water was directly measured by a Model F-21 Horibra pH-meter. A HACH Turbidity meter Model 2100 was used to measure the turbidity of the raw water in nephelometric turbidity units (NTU). The parameters were analyzed in triplicate.

2.2. In-line Coagulation Combined with Ceramic Membrane Filtration

The ceramic membrane modules used in this study of nominal pore sizes of 1.0 μm, 0.5 μm and 0.1 μm were provided by Metawater Co., Ltd., Japan. The ceramic membrane modules, which were installed in a stainless steel vessel vertically, were operated in dead-end mode. The purged pressure was controlled by adjusting the nitrogen gas pressure regulator. Raw water was spiked with bacteriophage Qβ and mixed by using jar test apparatus at a mixing speed of 150 r/min for 60 second to prepare an initial concentration of 8.00×10^6 PFU/ml. The spiked-bacteriophage Qβ raw water was poured into a pressurized tank. Polyaluminum chloride (PACl) was used as the coagulant for in-line coagulation combined with ceramic

membrane filtration experiment. PACl dosage of 1.5, 2.0, 2.5 and 3.0 mg-Al/L were added to the spiked-bacteriophage Qβ raw water and immediately mixed. After that, the coagulated water in the pressurized tank was allowed to flow through an 8-meter-nylon tube prior flowing to the bottom end of the ceramic membrane module. The feeding control was regulated by nitrogen gas at a controlled pressure of 0.2 MPa as shown in Fig. 1. The spiked- bacteriophage Qβ raw water and permeate water were collected to evaluate the bacteriophage Qβ removal efficiencies.

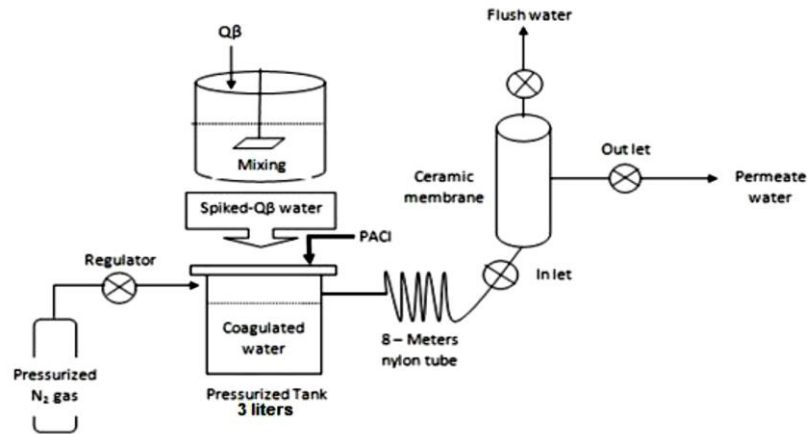Fig. 1.   Schematic diagram of in-line coagulation combined with a ceramic membrane.

2.3. F-specific RNA Bacteriophage Qβ and Bacterial Host Preparation

The F-specific RNA bacteriophage Qβ was used as a spiked model virus. Bacteriophage Qβ was analyzed by an overlay plaque assay using Salmonella typhimurium WG 49 as the host strain. A WG49 host strain working culture was prepared by using the pour plate technique that uses MacConkey agar as the medium. The culture plates were incubated overnight at 37°C. Then it was kept in the refrigerator at 4°C for 3 weeks. Inoculums cultures were prepared by picking up 3-6 colonies with agar from the working culture plate and adding them into the 10 ml of the Tryptone-yeast extract-glucose-broth (TYGB). The culture was incubated with shaking for 24 hours at 37°C. About 10 ml of the inoculum culture could be used with 500 ml of the tryptone-yeast extract-glucose agar (TYGA). The concentration of the microbe was reported as PFU/ml. In addition, E. coli K12 A/λ (F+) was used as the host strain for an enumeration of the F-specific RNA bacteriophage. The procedure for enumerating E. coli K12 A/λ (F+) is described the same as above. bacteriophage Qβ stock solution at a concentration of about 10^8 PFU/ml was sub-cultured in the LB broth feed with E. coli K12 A/λ (F+) every 2 weeks.

2.4. Overlay Plaque Essay

The procedures used were in accordance with the standardized protocol (ISO, 1997). The WG49 host strain was incubated in TYGB for 18±2 h at 37°C with shaking at 150 rpm. About 1 milliliter of the dilution sample and 1 milliliter of the exponentially growing WG 49 host culture were added to molten Semi-solid TYGA. This was mixed and poured into a petri dish. When higher bacterial background flora may have interfered with the growth of the host and replication of phages, nalidixic acid and kanamycin was added to suppress contaminant growth. The overlays were incubated over-night at 37°C. The bacteriophage Qβ concentrations reported in the plaque forming unit (PFU) were average from the triple plate count that illustrated a standard deviation of less than 30%. The detection limit of the plaque assay, which corresponds to the smallest amount of phages that could be detected, was 1 PFU/ml.

3.   Results and Discussion

3.1. Raw Water Characteristics

The characteristics of the raw water from the Ping River were analyzed. The average pH and alkalinity values of the Ping River water were 7.69 and 108.87 mg/l CaCO3, respectively. It can be noticed that the pH of the raw water was nearly neutral. In order to prevent pH drops during the coagulation/flocculation process, conventional water coagulation uses alum (aluminum sulfate) as the coagulant was generally required the additional alkalinity in case of low alkalinity raw water. PACl are synthetic polymers dissolved in water that react to form insoluble aluminium poly-hydroxides. PACl solutions are not as acidic as alum. Therefore, PACl was induced as the coagulant without pH adjustments in this study. The turbidity of the raw water was 41.77 NTU, which is considered low. This is because the raw water in this study was collected in the winter.

The concentration of bacteriophage Qβ in the raw water samples was observed to be under the detection limit. As a result, it was necessary to apply a larger water sample volume in the plaque assay and/or apply the virus concentration method in order to increase the bacteriophage Qβ concentration in the water samples prior to the plaque assay. Thus, bacteriophage Qβ was spiked in all experiments according to the recommendation of U.S. Environmental Protection Agency, regarding the control of the quality of treated surface water by membrane filtration. The virus feed concentration has to be sufficiently high to allow the demonstration of up to 6.5 log removal when the surrogate is removed to the detection limit.

3.2. Removal of Bacteriophage Qβ by In-line Coagulation with Ceramic Membrane

The efficiency of the bacteriophage Qβ removal process can be expressed by the log removal, which is defined as log C_i/C_f, where C_i and C_f represent the bacteriophage initial concentration and the bacteriophage in the filtrate water, respectively. Figure 2 illustrates the log removal of bacteriophage Qβ by the ceramic membrane with and without PACl coagulation.
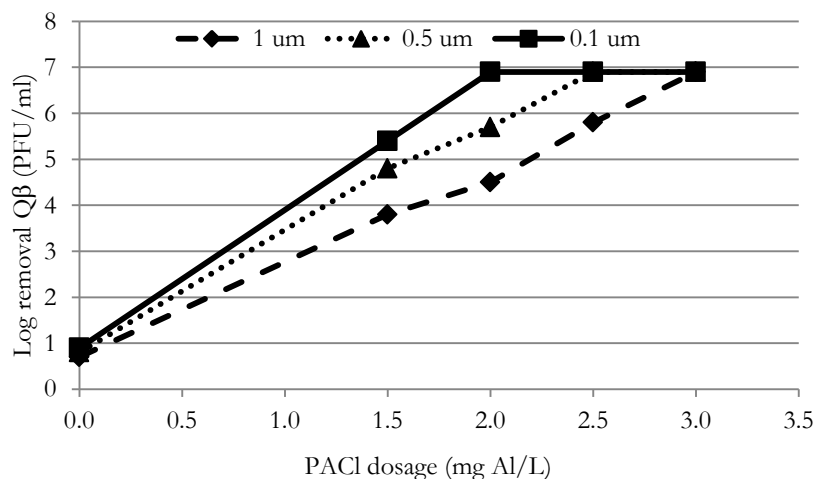

Fig. 2.   The log removal of bacteriophage Qβ by the ceramic membrane with and w/o coagulation.

In this study, raw water was spiked with bacteriophage Qβ at 8.00×10^6 PFU/ml. The effect of the pore size of the ceramic microfiltration membrane on bacteriophage Qβ removal was studied. The results show that the bacteriophage Qβ concentrations were reduced from 8.00×10^6 PFU/ml in the spiked raw water to 1.78×10^6 PFU/ml, 1.18×10^6 PFU/ml and 9.33×10^5 PFU/ml by ceramic membrane with pore sizes of 1.0 μm, 0.5 and 0.1μm, respectively. The log removal of the three pore sizes of ceramic membranes without PACl coagulants were lower than 1.

Since the diameter of the bacteriophage Qβ (0.023 μm) was smaller than the ceramic membrane pore sizes [19], a bacteriophage Qβ removal of lower than 1 log removal was observed in a previous study.

According to several studies, ceramic membrane cannot be expected to be an effective barrier for virus sized particles based on their nominal pore sizes. Two mechanisms, however, are recognized: (a) removal by the filtration effect of the fouling layer on the membrane surface, and (b) the aggregation of virus particles into larger particles and thus improved rejection [20].

In order to enhance the efficiency of bacteriophage Qβ removal, the in-line coagulation with ceramic membrane filtration was conducted. The results of in-line coagulation at PACl dosages 1.5, 2.0, 3.5 and 3.0 mg-Al/L with the 1.0 μm pore-sized ceramic membrane show that it can remove bacteriophage Qβ at an initial concentration of 8.00×10^6 PFU/ml to 1.23×10^3, 2.56×10^2, 1.20×10^1 and 1.00×10^0 PFU/ml, respectively. Similarly, in-line coagulation with the 0.5 μm pore-sized ceramic membranes could remove bacteriophage Qβ from an initial concentration of 8.00×10^6 PFU/ml to 1.16×10^2, 1.60×10^1, 1.00×10^0 and 1.00×10^0 PFU/ml, respectively. Meanwhile, the 0.1 μm pore sized ceramic membrane showed that it could remove bacteriophage Qβ from an initial concentration of 8.00×10^6 PFU/ml to 3.20×10^1, 1.00×10^0, 1.00×10^0 and 1.00×10^0 PFU/ml, respectively.

The resulted shows that the addition of the PACl coagulant could enhance the removal of bacteriophage Qβ. From the log removal results in Fig. 2, it can be seen that increasing the PACl dosage can enhance the log removal of bacteriophage Qβ to the highest log removal as 6.9. The results show that the removal of the bacteriophage Qβ concentration increased from 0.7 log removal by the 1.0 μm pore-sized ceramic microfiltration membrane to 3.8, 4.5, 5.8 and 6.9 log removal at the PACl dosages of 1.5, 2.0, 2.5 and 3.0 mg-Al/L, respectively. Similarly, the removal of the bacteriophage Qβ concentration by the 0.5 μm pore-sized ceramic microfiltration membrane also increased from 0.8 to 4.8, 5.7, 6.9 and 6.9 log removal at the PACl dosages of 1.5, 2.0, 2.5 and 3.0 mg-Al/L, respectively. The smallest pore size (0.1 μm) presented the highest log removal. The log removal increased from 0.9 to 5.4, 6.9, 6.9 and 6.9 at the PACl dosages of 1.5, 2.0, 2.5 and 3.0 mg-Al/L, respectively. High bacteriophage Qβ log removal was obtained from smallest pore size membrane even at the low PACl dosage.

The ceramic membrane microfiltration at all pore sizes with the lowest PACl dose (1.5 mg-Al/L) could not reduce bacteriophage Qβ completely. Bacteriophage Qβ weakly absorbed to aggregates and passed through the membrane. The ceramic membrane with a pore size of 0.1 μm achieved a log removal of 6.9 with a PACl dose of 2.0 mg-Al/L. Meanwhile, the ceramic membrane with a pore size of 1.0 μm showed lower performances (4.5 log). These results corresponded to those of [4] which reported that the coagulant dosage strongly affected virus removal with the coagulation-MF hybrid system: the larger the coagulant dose, the greater the proportion of virus was removed.

From a review of literature, removal mechanisms for enteric viruses by membrane filtration have taken into account size exclusion, the electrostatic repulsion between a charged membrane and a charged virus, and the adsorption of viruses to the membrane material. It can be described that PACl coagulation with a ceramic membrane might contribute to bacteriophage Qβ removal by adsorption or attraction on suspended PACl with enough adsorption potential. Bacteriophage Qβ capture in the PACl cake layer and are clogging in the constricted membrane pore, including membrane physical sieving. In addition, clay particles naturally contain a negative charge, when the amino acid-RNA composition of bacteriophage Qβ is also negatively charged due to the pH of the surface water (pH 7-8) [21]. Therefore, bacteriophage Qβ could not be absorbed with clay particles without a coagulant. PACl could reduce the negative charge of the amino acid so that it could reach its isoelectric point (no charge amino acid). Then the amino acid-RNA composition could become non-charged and adsorbed onto negatively charged clay particles to form larger aggregates.

Coagulation effectively aggregated bacteriophage Qβ to form larger sized aggregates and also increased the coagulation time. Therefore, the aggregated bacteriophage Qβ was large enough to be retained on the ceramic membrane surface. Thus, coagulation at the PACl doses of 3.0 mg-Al/L, 2.5 mg-Al/L and 2.0 mg-Al/L combined with ceramic membrane microfiltration offered the best conditions for bacteriophage Qβ reduction with the three membranes (1.0 μm, 0.5 μm, and 0.1 μm) when the initial concentration of bacteriophage Qβ was 8.00×10^6 PFU/ml.
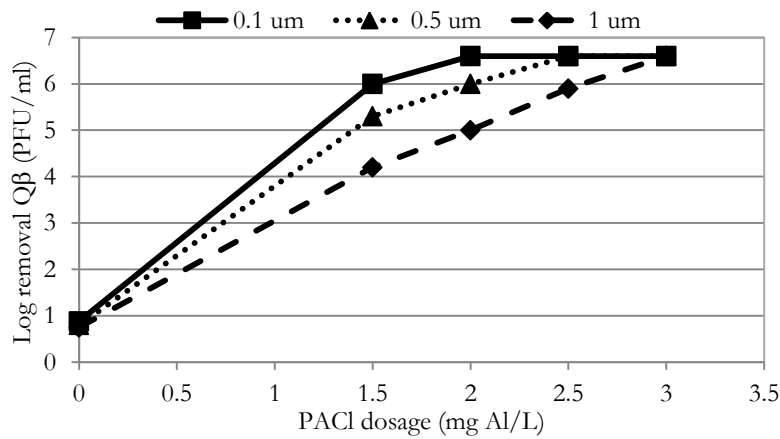
3.3. Removal of Bacteriophage Qβ with Different an Initial Concentration by In-line Coagulation with Ceramic Membrane Microfiltration

This part investigates the effect of different an initial concentrations of bacteriophage Qβ in the feed water on the bacteriophage Qβ removal. The different initial concentrations were varied at 5.00×10^5 PFU/ml, 4.00×10^6 PFU/ml and 8.00×10^7 PFU/ml. Three pore sizes of ceramic membranes (1.0 μm, 0.5 μm and 1
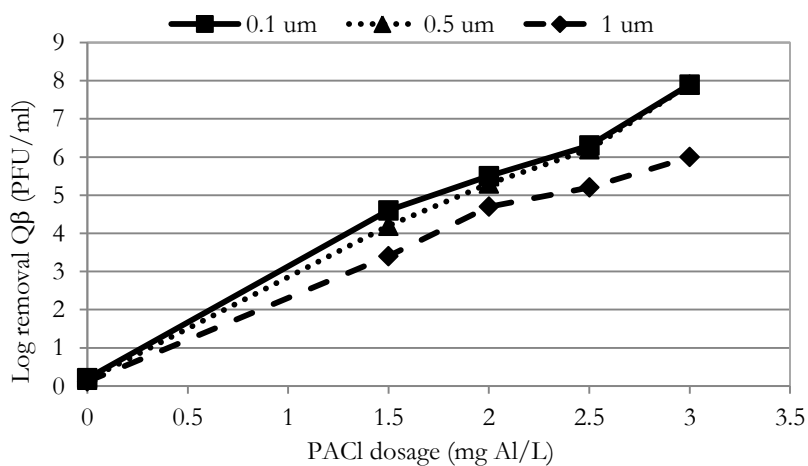
μm) were used and the PACl concentrations were varied at 1.5, 2.0, 2.5 and 3.0 mg-Al/L. The results of the bacteriophage Qβ reduction at different an initial bacteriophage Qβ concentrations are shown in Fig. 3.
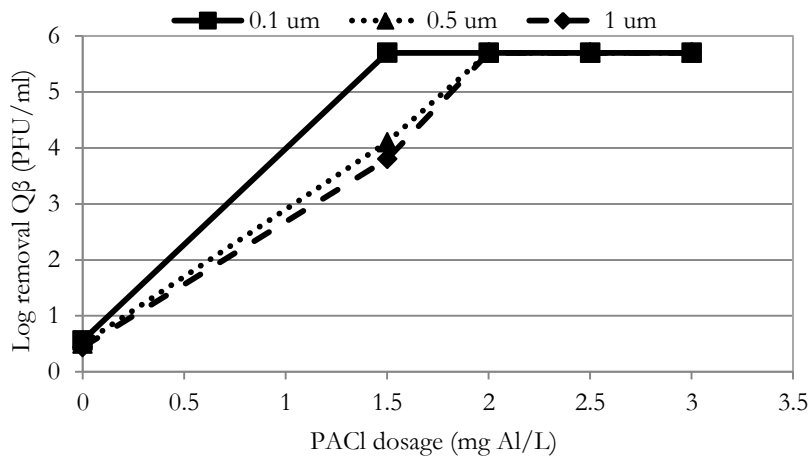
(a) initial bacteriophage Qβ concentration of 5.00×10^5 PFU/ml

(b) initial bacteriophage Qβ concentration of 4.00×10^6 PFU/ml

(C) initial bacteriophage Qβ concentration of 8.00×10^7 PFU/ml

Fig. 3   The log removal of bacteriophage Qβ by in-line coagulation with ceramic membrane filtration at different an initial bacteriophage Qβ concentrations.

The results from Fig. 3 show that the bacteriophage Qβ in the spiked raw water at 5.00×10^5 PFU/mL was completely retain by the 0.1 μm ceramic membrane at all PACl dosages. It also achieved the highest log removal efficiency (5.7 log). On the other hand, bacteriophage Qβ remained present in the filtrate of the larger pore-sized ceramic membranes at the lowest PACl dose (1.5 mg-Al/L). To remove all bacteriophage Qβ in the spiked raw water, the 0.5 μm and 1.0 μm pore-sized ceramic membranes required a PACl dosage of 2.0 mg-Al/L. However, when the initial Qβ concentrationwas increased to 4.00×10^6 PFU/mL, the ceramic membrane pore size 0.1 μm with low PACl dose (1.5 mg-Al/L) cannot remove bacteriophage Qβ completely. The combination of the 0.1 μm pore-sized ceramic membrane with 2.0 mg-Al/L allowed for the highest log removal (6.6 log), which was similar when the higher PACl doses (2.5 and 3 mg-Al/L) were employed. On the other hand, the ceramic membranes with larger pores (0.5 μm and 1.0 μm) required more PACl to totally remove the bacteriophage Qβ. The most effective PACl dose for removing bacteriophage Qβ completely with the 0.5 μm and 1.0 μm pore-sized membranes were 2.5 and 3.0 mg-Al/L, respectively.

For the highest initial bacteriophage Qβ concentration at 8.00×10^7 PFU/mL, the obtained results showed that the highest log removal (7.9 log) was observed by the highest coagulation (3.0 mg-Al/L) combined with 0.5 and 0.1 μm pore-sized ceramic membrane. On the other hand, a log removal (4.6 log) was achieved by coagulation with the 0.1 μm pore-size ceramic membrane at the low PACl dose (1.5 mg-Al/L). This indicated that the growth of aggregates was not sufficiently large enough to remove bacteriophage Qβ with low PACl doses with all the evaluated ceramic membranes. The high initial bacteriophage Qβ concentration required a higher PACl dosage to improve aggregation and its removal efficiency. Increasing the coagulant dosage could make the aggregates adequately large enough to be rejected by the ceramic membrane. Incidentally, the performance of the 1.0 μm pore-sized ceramic membrane was lower than those of the smaller pore sizes as bacteriophage Qβ was not retained by ceramic membrane microfiltration. The highest PACl dosage in this study was not able to sufficiently form large enough aggregates of bacteriophage Qβ that could be rejected by ceramic membrane microfiltration. A higher PACl dosage was required to remove bacteriophage Qβ in the spiked raw water completely.

Increasing the initial bacteriophage Qβ concentration affected the removal of the bacteriophage Qβ concentration by ceramic membrane microfiltration. The highest log removal was different following the initial bacteriophage Qβ concentration. Increasing the concentration of bacteriophage Qβ in the raw water from 10^5 to 10^7 PFU/ml affected a more than 1 log drop in removal using coagulation with ceramic membrane microfiltration. The most effective PACl dosage from a previous study did not suitably remove the concentration of bacteriophage Qβ. These results relate to those of [22] who noticed that increasing the concentration of bacteriophage in the feed solution from 10^6 to 10^9 PFU/ml caused more than a 1 log drop in removal using ultrafiltration. Thus, the bacteriophage Qβ in the feed water can have an effect on the removal of bacteriophage Qβ of ceramic membrane microfiltration with in-line coagulation.

4. Conclusion

Based on the obtained results from this study on bacteriophage Qβ removal by in-line PACl coagulation with ceramic membrane filtration, it can be concluded that ceramic membranes with pore sizes larger than 0.1 μm cannot act as a physical barrier to bacteriophage Qβ. Thus, ceramic membrane microfiltration alone cannot remove this virus in water. PACl coagulations have a strong effect on bacteriophage Qβ removal. The most effective PACl dosages with ceramic membranes with 0.1, 0.5 and 1.0 μm pore sizes for bacteriophage Qβ (8.00×10^6 PFU/mL) removal were 2.0, 2.5 and 3.0 mg-Al/L, respectively. When an initial bacteriophage Qβ concentration was 5.00×10^5 PFU/ml, in-line coagulation with ceramic membrane filtration can retained all bacteriophage Qβ from raw water. However, the removal efficiency were decreased with the increasing of an initial bacteriophage Qβ to 4.00×10^6 and 8.00×10^7, respectively. The higher initial bacteriophage Qβ required a larger PACl dosage to improve aggregation and the removal efficiency when the larger pore-sized membrane was utilized.

Acknowledgements

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