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REMOVAL OF VIRUSES BY MICROFILTRATION MEMBRANES AT DIFFERENT SOLUTION ENVIRONMENTS

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ABSTRACT

Rejection change by *nuclepore* microfiltration membranes with solution environment was investigated by using the RNA coliphages Qβ, MS2, *fr* and DNA coliphage T4. The obtained rejection results showed a higher rejection at lower pH than at higher pH for all viruses. The highest rejection for all viruses were obtained at pH closer to their isoelectric points and also the rejection variation indicates a similar pattern of behavior. This phenomenon of higher virus rejection at lower pH is explained with a possible viruses aggregation with each other due to their own electrostatic charge and isoelectric points. Also it was observed that the virus rejection was enhanced when they are in mixed environment. Finally the effect of protein was studied where, the virus rejection below pH 5.0 showed protein influence. © 1999 IAWQ Published by Elsevier Science Ltd. All rights reserved

KEYWORDS

Isoelectric point; membrane; microfiltration; pH; rejection; virus.

INTRODUCTION

Control of pathogenic microorganisms in reclaimed water has recently attracted a lot of attention and deeper recognition because of the present strict regulatory requirements and the anticipation of more stringent future water quality regulations (Gupta and Chaudhuri, 1995; Naranjo *et al.*, 1993; Sakoda *et al.*, 1997). Among the present water borne microorganisms, virus removal needs more emphasis because of their low infection dose, long term survival in the environment, low removal efficiency in conventional wastewater treatment etc. Aggravating the situation, in recent times the chemical disinfection process (chlorination) is proved to inherit some major drawbacks such as its ineffectiveness against microorganisms associated with suspended solids, dependence on environmental conditions, the formation of disinfection by-products etc. Hence in recent times the membrane technology is becoming a popular treatment process for efficient removal of microorganisms such as bacteria, virus and *Cryptosporidium*. In this regard, the low-pressure driven membrane processes such as the microfiltration and ultrafiltration has a high potential to be used as a primary disinfection process, enabling use of less disinfectant in treatment processes.

According to the studies conducted by Kolega *et al.* (1991) their hollow fiber microfiltration systems have demonstrated removals of 5-6 orders of magnitude of enteroviruses from treated sewage despite the pore size of the filter being much larger than the virus size. Further, there are a number of examples confirming

the pore size of a membrane alone is inadequate to describe the filtration characteristics of a membrane specially in varying environmental conditions (Cliver, 1965; Scutt, 1971). Thus in using the membrane process for disinfection, the information about the effect of solution environments on virus removal by membrane filtration is essential.

Considering the above, the main objective of this study is to investigate the effect of pH on rejection of virus in a microfiltration process using RNA coliphages Qβ, MS2, *fr* and DNA coliphage T4. The obtained rejection values are further examined with respect to the virus isoelectric point (pI) pH values, where a close relationship between pI (isoelectric point pH value) value and rejection is suggested. Also the factors such as the effect of protein concentration, membrane adsorption qualities and ionic strength on rejection characteristics of virus too is examined.

EXPERIMENTAL PROCEDURE

Membrane setup and materials

Filter apparatus. All the experiments for rejection analysis were conducted on a 25mm diameter ultra-holder unit, (*Advantec* model no. UPH-25K, shown in Fig. 1) having a volume of 12ml. This unit could be pressurized to a level more than 500kPa. Here V_1, V_2 and V_3 are N_2 control valves.

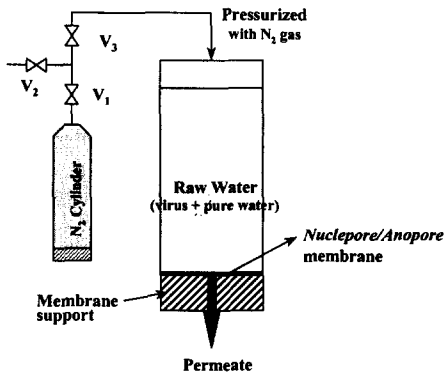

Figure 1. The dead end (*Advantec*) filter apparatus.

Virus. In this study the RNA coliphages were used to represent the pathogenic viruses since their size and structure very closely resembles that of the pathogenic enteroviruses (Kamiko and Ohgaki, 1989, Sakoda, *et al.*, 1997). Four strains of *E. coli* phages T4, Qβ, MS2, and *fr* of which T4 virus has an irregular shape and RNA coliphages Qβ, MS2 and *fr* have an icosahedron shape (which is very much similar to a sphere) were used as model viruses. The detailed characteristics of the above RNA coliphages and T4 phage are given in Table 1.

Table 1. Characteristic details of used model viruses

E.coli phage	Size* (nm)	pI value
Qβ	25##	5.3**
MS2	26##	3.9**
T4	80##	4.2**
fr	19**	8.9~9.0**

* Sizes are given in equivalent diameter (equivalent diameter can be said to be the diameter of a sphere equivalent to that virus volume), ## details from Tortora *et al.* (1992), ** details from Sakoda *et al.* (1997).

Virus culture media. The detailed compositions of the culture media (broth) used for cultivation and storage of the above virus are as given below. The media was prepared by mixing Polyposphate 10.0g, Yeast extract 5.0g, NaCl 5.0g, Glucose 1.5g, $MgSO_4.7H_2O$ 0.2g and $MnSO_4.4H_2O$ 0.05g in 1L of water. Always the media was autoclaved at 121°C for 20 minutes before use.

Experimental procedure and rejection evaluation

Sample preparation and experimental procedure. All experimental investigations were conducted in dead end filtration mode without stirring. The membranes were mounted on the *Advantec* ultrafiltration membrane unit at least 1 hour before each experiment and then this membrane unit was kept under UV radiation for sterilization. The unit was pressurized using N_2 gas maintaining a constant pressure level throughout the experiment. The applied pressure was 20kPa and 100kPa respectively for 0.2 and 0.05μm pore size *nuclepore* membranes.

Concentrated virus solutions (about 10^9-10^{12}PFU/ml) were diluted in pure water at appropriate levels to prepare the feed samples. The virus concentration in feed samples ranged between 10^3-10^6PFU/ml. Sample pH was adjusted using dilute HCl and NaOH solutions while the additional conductivity was adjusted with NaCl solution. All experiments were conducted at approx. 25°C temperature. The initial samples during filtration were taken for rejection measurements to avoid a possible concentration at the membrane surface. Concentration of virus were measured with the double layer agar plate counting method using host cells of *E coli* B, *E coli* K12 C15, *E. coli* K12 A/λ for the detection of T4, Qβ, MS2 (Ketratanakul *et al.*, 1989) and *E. coli* K12 strain 3300 (*ATCC(R)* 15767-B1) for *fr* virus (Knolle, 1964) respectively.

The protein concentration in the raw water samples was measured according to the Bicinchoninic acid (BCA) protein assay (Pierce, Chicago) method. Here the absorbency of the samples at 562nm light intensity was compared against the standard curve to obtain the protein level. The BSA used in this study was free of protease and was manufactured by *Acros Organics.*

RESULTS

Qβ, MS2, T4 and *fr* filtration at mono culture environments

Virus Qβ and MS2 rejection. In order to investigate the behavior of Qβ and MS2 rejection when they are in monoculture environment, a set of experiments was conducted in the following manner. Concentrated monoculture (pure culture) samples of Qβ and MS2 diluted in clean water were filtered through 50nm *nuclepore* membranes at various pH levels between 4.0 to 10.2. The transmembrane pressure was maintained at 100kPa throughout the experiments. The obtained rejection results of Qβ and MS2 are shown in Fig. 2a and b respectively.

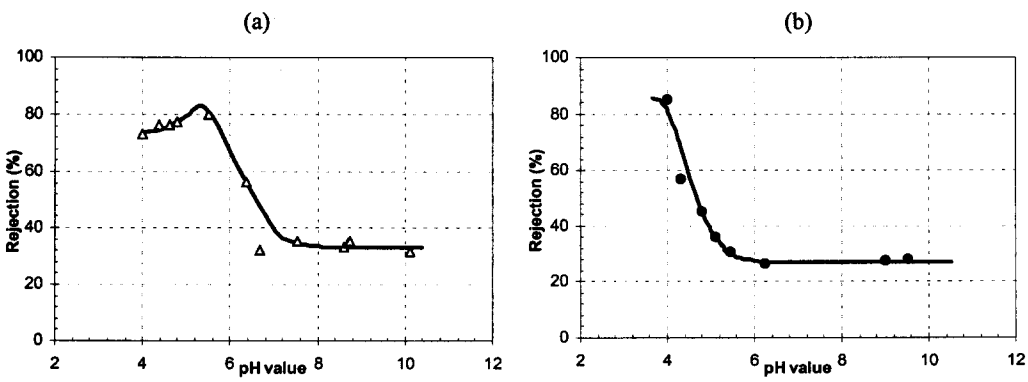

Figure 2 (a) Rejection of virus Qβ in monoculture at different pH levels; (b) Rejection of virus MS2 in monoculture at different pH levels

According to the obtained results, the rejection level of both Qβ and MS2 is not the same within the studied pH range. Both the viruses showed higher rejections at lower pH and maximum rejections were obtained closer to their individual isoelectric points (5.3 pH for Qβ and 3.9 pH for MS2).

Virus T4 and fr rejection. Experiments with monocultures of T4 and *fr* were conducted in a similar way to that of Qβ and MS2. Here the T4 and *fr* viruses were filtered using 200nm and 50nm *nuclepore* membranes at 30kPa and 100kPa pressure levels respectively. The obtained rejection curves of T4 (pI point 4.2) and *fr* (pI point 8.9-9.0) are shown in Figs 3 and 4 respectively. These rejection results show a very close resemblance to what was obtained for Qβ and MS2. Here the rejection of T4 and *fr* virus too shows a rapid decrease with increasing pH close to their individual pI values. In addition a constant high level of rejection below their pI values was obtained for Qβ and *fr*.

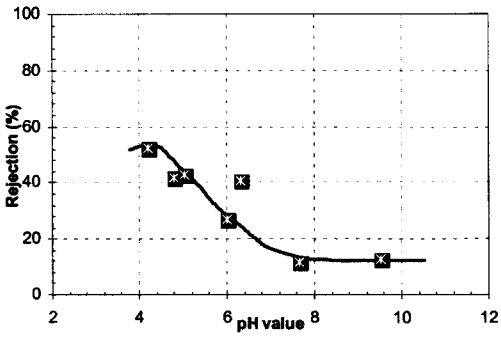

Figure 3. Rejection of T4 virus with pH change, 200nm (0.2μm) *nuclepore* membranes at 0.3 kg/cm^2 pressure.

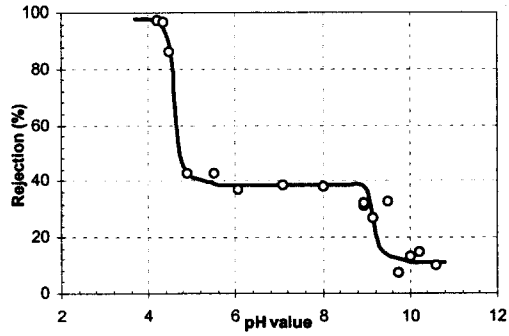

Figure 4. Rejection of *fr* virus with pH change, 50nm *nuclepore* membranes at 1.0 kg/cm^2 pressure.

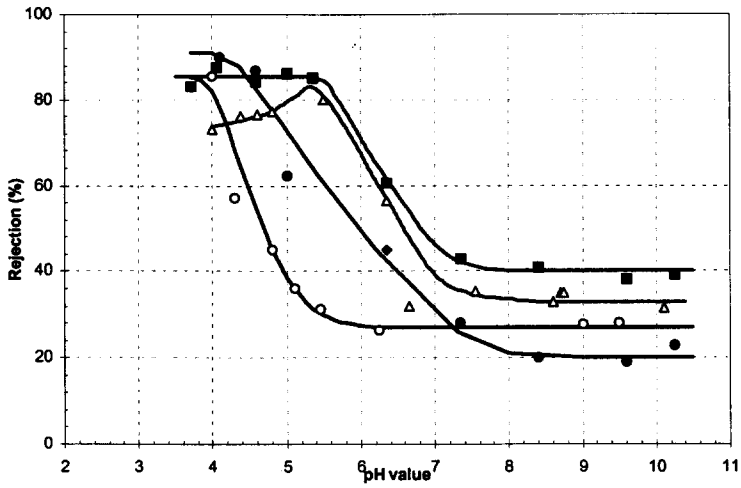

Figure 5. Rejection of virus Qβ and MS2 in mixed culture at different pH, Δ Qβ monoculture, ■ Qβ mixed culture, O MS2 monoculture and ◆ MS2 mixed culture.

Qβ and MS2 filtration in mixed virus environment

In order to investigate the behavior of virus Qβ and MS2 rejection, when they are in mixed culture environment, another set of experiments was conducted in the following manner. Mono-cultures of Qβ, MS2 and T4 were diluted in clean (pure) water and experiments for rejection at various pH levels were conducted similar to that of mono culture experiments. The pH was varied within 3.7 and 10.5. The obtained

virus rejection results are shown in Fig. 5 for both Qβ and MS2. Also indicated in the figure are the respective monoculture rejection curves.

The results obtained here show a similar variation to that of monoculture results. But a closer examination shows that the virus rejection at lower pH has decreased for any particular pH value in the monoculture virus samples compared to the mixed virus samples. This is more prominent in the case of MS2 than in Qβ in neutral and lower pH range. But at higher pH the rejection is almost the same.

Protein and conductivity interference

Protein influence on virus rejection. As indicated, the culture broth media contains various impurities like protein, NaCl etc. The approximate protein concentration in the raw virus samples was approximately about 0.8 mg/l. The dilution of concentrated virus (stored in culture broth) contributes this protein to the raw solutions. Since certain proteins are known to have influence on particle rejection, the effect of culture broth concentration on rejection was examined. The broth concentration as protein in the raw virus samples was adjusted to 0.8mg/l, 5.0mg/l and 50.0mg/l levels. At these broth levels the rejection of Qβ was investigated within the pH range of 4.25 and 9.0. The obtained results are shown in Fig. 6. The results indicate that Qβ rejection at its isoelectric point is increased with broth concentration.

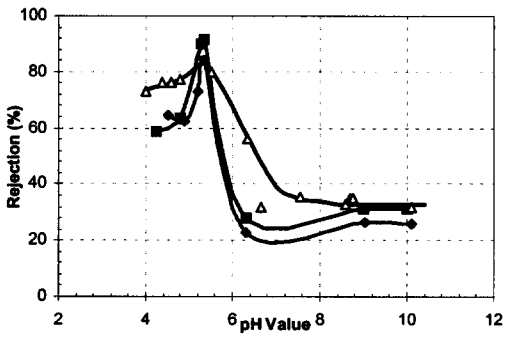

Figure 6. Rejection of Qβ virus at different broth concentrations 50nm *nuclepore* membranes at 100kPa pressure. Δ 0.8 mg/l, ◆ 5mg/l and ■ 50 mg/l.

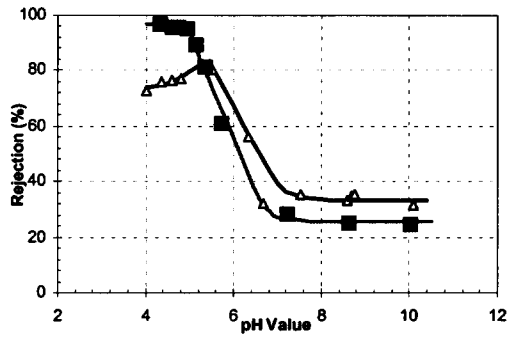

Figure 7. Rejection of Qβ virus with a concentration of 5mg/l BSA protein, 50nm *nuclepore* membranes at 100kPa pressure. ■BSA 5mg/l, Δ without BSA.

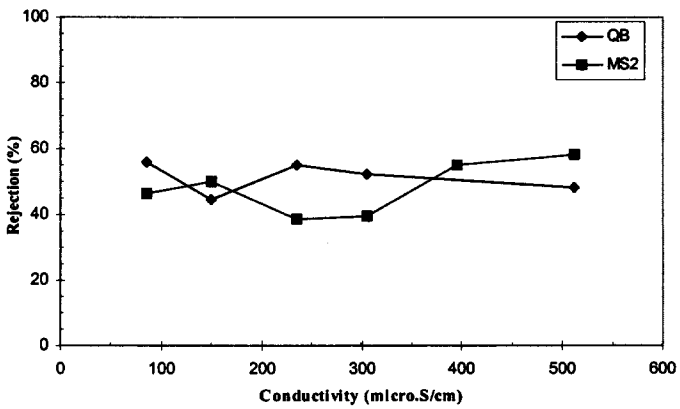

Figure 8. The effect of conductivity on Qβ and MS2 rejection for 50nm *nuclepore* membrane.

Further analysis was done with Bovine Serum Albumin (BSA). These experiments were done to obtain the influence of protein on virus rejection characteristics with respect to protein pI point. It is known that the

BSA protein has a pI point of 4.6-5.0 pH (Fane, A. G., *et al.*, 1983, Richard Bowen W., and Quan Gan, 1990). The rejection results obtained for Qβ rejection at a BSA concentration of 5mg/l are shown in Fig. 7. Here the obtained rejection values show that the protein influence is more prominent at its isoelectric point.

Effect of conductivity on virus rejection. In order to study the effect of conductivity on virus rejection, another separate set of experiments were conducted at constant pH level (6.25 to 6.35) but with a variable ionic strength. The ionic strengths of the samples were adjusted using a NaCl solution. The obtained results for both Qβ and MS2 are shown in Fig. 8. The obtained results show an almost constant level of rejection for both Qβ and MS2 in the studied conductivity range.

Adsorption of virus. According to the manufacturer specifications, the *nuclepore* membrane carries a slight negative charge. Hence, three adsorption tests were carried out with Qβ virus, at pH levels 4.5, 6.5 and 9.0 to determine the adsorption characteristics. The obtained results showed that the Qβ adsorption to *nuclepore* membranes was negligible within the above pH range.

DISCUSSION

Analyzing the Qβ, MS2, T4 and *fr* rejection for mono virus environments shown in Figures 2a, b, 3 and 4, indicates that the rejection for all phages shows variation with pH change. This change in rejection can be due to the possible difference in coagulation of virus with each other when they are in different environments. The obtained results for all viruses, shows a higher rejection at lower pH followed by a decrease in rejection at alkaline pH. That is, if the solution pH is less than the pI point, the rejection of virus is small and at pI points highest rejection is observed. Also away from the pI points, the virus in alkaline pH range rejection is the lowest but remains constant. Higher rejection results at lower pH values were observed by Roger Floyd and Sharp, (1977) with polio virus and reovirus aggregation at a very high virus concentration of about 10^9-10^{12}PFU/ml. Our results show that this phenomenon can be seen even with much lower concentrations of 10^3 to 10^6 PFU/ml for all viruses Qβ, MS2, T4 and *fr*.

This phenomenon of virus rejection change with pH could be associated with the mechanism of virus coagulation. The viruses Qβ, MS2, T4 and *fr* have different isoelectric points as shown in Table 1. Isoelectric point pH is such that for Qβ since its pI point is 5.3, it will have no net charge at pH 5.3 but, above pH 5.3 it will have a net negative charge and below pH 5.3 it will carry a net positive charge. When considering the common behavior obtained for virus rejection, at pH values above their pI points, the virus particles are in dispersed form because of the repulsive nature of viruses with each other due to their high negative charge. Closer to their isoelectric points, the viruses are in a state where their positive and negative charged sites are equally distributed. This equal distribution of positive and negative sites at virus isoelectric point leads to a maximum virus-virus coagulation with each other thus obtaining maximum rejection at pI points. For RNA phages Qβ and *fr*, the high rejection values below their isoelectric points are believed to be related to interaction of the impurities such as protein in the solution. Since the protein isoelectric points are in the range of pH 4.5 to 5.0, the protein and virus Qβ and *fr* exist in opposite polarities, values below these virus isoelectric points tending to make for rapid aggregation. This aggregation would cause the high rejection even below their pI values to continue.

Another interesting point in these results is the variable peak rejection values. The approximate peak rejection values for Qβ, MS2, T4 and *fr* at their individual pI points are 84%, 85%, 53% and 40% respectively. One major reason for this variable peak rejection is the different virus size to membrane pore size ratios (Herath *et al.*, 1998). Since the virus size to pore size ratio of Qβ and MS2 are (0.5, 0.52) higher than that of T4 and *fr* (0.4, 0.38), both Qβ and MS2, have higher peak rejection values than T4 and *fr* respectively.

Compared to the rejection values obtained for the mono virus culture case, the mixed virus case shows a slight improvement in rejection. The explanation for this increase in rejection is possibly higher rate of coagulation in mixed environment than in mono environment because the viruses would carry different charges in a certain pH range since the three viruses in the mixture Qβ, MS2 and T4 have different isoelectric points. Hence as obtained in Fig. 5 when viruses are in mixed environments, they tend to coagulate not only with the same virus but also with different viruses.

In order to clarify the above explanation for the changes in virus rejection, other possible factors affecting the results should also be analyzed. The other possibilities may be the effect of conductivity change, protein in raw water and possible adsorption of virus onto the membrane.

Generally as mentioned before, the protein molecule itself has its own isoelectric point and it is in the range of pH 5.0 and its size compared to the virus size is very small. Hence the 0.8mg/l of broth protein which is in the raw water can have a significant influence on the rejection of the above viruses. Inspecting the obtained results in this connection with increased broth concentration and with the addition of BSA protein, which are given in Figures 6 and 7 respectively suggests that, at lower pH, protein does influence the virus rejection. Considering the results in Fig. 7 with BSA, shows that the Qβ rejection increases until the BSA pI point is reached. This may be because, the virus and BSA molecules having an opposite charge in this pH range coagulate with each other. This may be the reason for obtaining higher rejection values for Qβ and *fr* below their pI points. Also, comparison of results in Figs 6 and 7 suggests the overall isoelectric point of broth solution should be in the range of pH 5.0, where a maximum enhancement of virus rejection at higher broth concentrations was observed.

At very low pH (near pH 4.0) *fr* virus shows very high rejection characteristics. This behavior can be related with the phenomenon of reversible structural changes in protein which, is believed to occur at very low pH range. The protein molecule is said to undergo striking reversible structural transitions at low pH values (generally around and below pH 4.0) which leads to changes in certain of their physicochemical properties such as size, adsorption characteristics etc. (Richard and Quan Gan, 1990; Bloomfield, 1966; Gregorio and Young, 1964). It is believed that these changes would enhance the coagulation process up to a very high level so that a high degree of rejection is achieved. In case of *fr* virus, it acquires a larger net positive charge at pH round 4.0 than Qβ, hence this makes the effects of this structural change more significant for *fr* virus Qβ as obtained in experimental results.

The experiments conducted for conductivity and for adsorption showed that neither conductivity nor adsorption plays any significant role in changing virus rejection in the studied pH range.

CONCLUSIONS

The rejection change was explained by the virus-virus and virus-impurity coagulation depending on individual isoelectric points. This could be achieved at even very low concentration of viruses of about $10^{3\text{-}5}$PFU/ml. The obtained rejection results show an identical variation with pH change (over pI values) and a maximum rejection at isoelectric points for all model viruses prompting that the rejection characteristics of viruses are very similar with each other. The rejection of viruses was closely related to their isoelectric point.

Also the results showed an enhanced virus rejection when they are in mixed environment possibly due to the enhancement of virus-virus coagulation. The impurities like protein in solution are believed to increase virus rejection, since in our study we observed a considerably high virus rejection at their isoelectric point even with microfiltration membranes which have larger pores compared to the virus size.

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