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Removal of mixtures of viruses using microfiltration membrane

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ABSTRACT

Membranes are finding wide spread applications in disinfection processes for raw water and municipal effluent reuse. This article describes a fundamental study for transmission of mixtures of real viruses through 0.22 μm hydrophobic microfiltration (MF) membrane. Two non-human animal viruses Foot-and-mouth disease (FMD) and Infectious Bovine Respiratory (IBR) in dilute solution were selected as viruses and challenged with the membrane. An attempt was made to address the main questions for process performance in terms of transmission (or rejection) of virus and flux, under various operating conditions, including transmembrane pressure, stirring and time. The effect of the presence of large virus (IBR, 150 nm) on rejection of small virus (FMD, 25 nm) was elucidated. It was found that IBR virus was completely rejected by the membrane regardless of the operating conditions. The small FMD virus was significantly removed depending on the conditions being higher for lower transmembrane pressure. The presence of large IBR virus enhances the removal of small virus.

Although numerous studies have focused on phages (mainly viruses of bacteria), limited researchers investigated the MF capability for removal of human or animal viruses from water or wastewater. This study is an answer to the questions arising for removal of real viruses from water by MF membrane.

Keywords: Membrane; Microfiltration; Virus; FMD; IBR

1. Introduction

The demand for potable water is increasing and therefore investigation of alternative methods for production of high-quality water is a vital requirement for human life. Other issues such as reusing domestic effluents are of growing interest.

There are many approaches that are used for disinfection of water. Each method has some difficulties and disadvantages [1,2]. Membranes are capable for removing viruses completely (using ultrafiltration) or significantly (using microfiltration (MF)) under appropriate conditions. For removal of poliovirus suspensions from water using 0.22 μm hydrophobic MF membrane, retentions greater than 99% were achieved [3,4].

Viral removal depends on membrane characteristics including surface properties [5] and interfacial properties of viral particles [6]. An appropriate membrane structure, that is thick multi-layered with porous structure improves the virus-trapping capacity [7].

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Interactions of viruses with the surfaces of membranes improve the removal efficiency. This includes the electrostatic [8] and hydrophobic interactions [9]. High retention was obtained with a hydrophobic membrane compared with hydrophilic due to the hydrophobic interaction [9].

Pre-conditioning of the viral suspension influences the removal capability. Aggregation around the isoelectric point of a virus or in the presence of salt improves the removal of viruses [9]. The aggregation is significantly enhanced by the conditions of ionic strength and pH [8].

Manipulating the operating condition including tangential flow [10] or membrane structure such as introducing silver nano-particles [11] enhances the viral removal. Silver-impregnated polysulfone membranes showed a significant improvement in viral removal. Moreover, this type of modification reduces the biofouling problem [11].

MF may be combined with other processes to improve viral removal. Around 6-log reduction was achieved for removal of bacteriophages using coagulation–MF system [12]. The MF membrane in combination with coagulation retained the virus to a similar extent as the ultrafiltration membrane [13].

In this work a fundamental study was carried out for the elucidation of transmission of mixtures of animal viruses Foot-and-mouth disease (FMD) and Infectious Bovine Respiratory (IBR) through MF membrane. Obviously, the viral removal depends on the operating conditions. The rejection of viruses and permeate flux were obtained during time under various transmembrane pressures and stirring. Feed containing viruses only and mixtures of viruses with different concentrations were employed in this study. One of the aspects of the current study was using real viruses instead of bacterial viruses, that is, phages to eliminate any doubt about the MF capability for removal of real viruses from water and waste water.

2. Materials and methods

2.1. Membrane

Millipore hydrophobic MF membrane (GVHP) with a nominal pore size of 0.22 μm was used for all experiments. The characteristics of the membrane are presented in Table 1. This membrane possesses a slightly negative charge [3,4] under the conditions used (pH 6–8).

Table 1
Characteristics of the GVHP membrane

GVHP membrane	
Manufacture	Millipore
Polymer	PVDF[a]
Nominal pore size	0.22 μm
Hydrophobicity	Hydrophobic
Water flux (l/m^2h)[b]	7,000−7,700
Resistance (m^{-1})[c]	(4.7–5.1) × 1.0^{10}

[a]Polyvinylidenefluride.
[b]Measured at 100 kPa.
[c]Calculated from pure water flux.

2.2. Filtration procedure

All experiments were carried out in 110 ml capacity batch cell, with a membrane area of 15.2 cm^2. A diagram presentation of the filtration procedure is depicted in Scheme 1. The cell consisted of a cylindrical vessel containing the test solution; two circular end pieces all were made from Perspex and a porous medium to support the membrane. The top end piece of the cell contained a feed and a gas inlet and pressure relief valve. Stirring was achieved by an internal magnetic bar (25 mm long, 6.4 mm diameter) suspended 2 mm above the membrane. Nitrogen gas was used to pressurize the cell to operating pressures of 25, 50, 100 and 150 kPa. The experiments were started by quickly pressurizing the cell after 100 ml of the suspension was poured into it. The suspension was continually fed from a reservoir connected to the cell to replenish permeate. In one set of the experiment to determine the effect of transmembrane pressure (ΔP) on performance the filtration was carried out by passage of 1,000 ml of the feed.

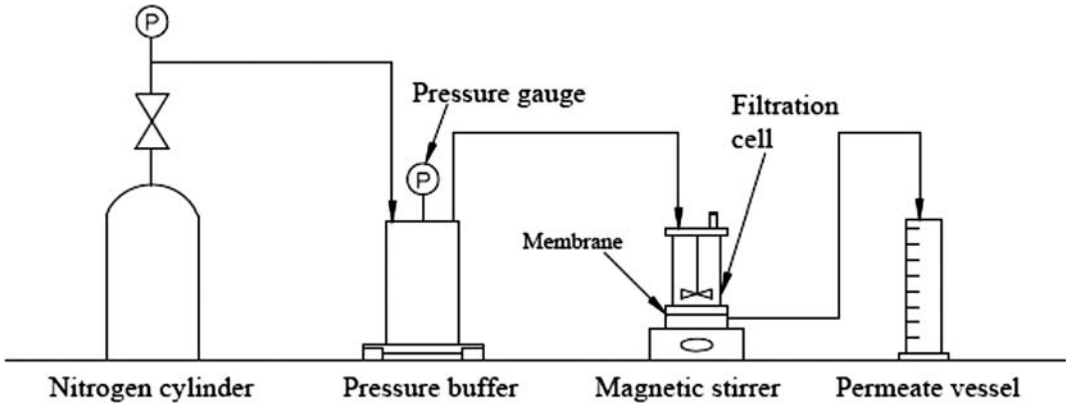
The concentration of virus in permeate was estimated on the basis of an average value from the total permeate. The concentration in the feed was also based on an average value for the feed obtained by assuming a constant rejection during the batch filtration. In another set of experiments aimed at assessing the variation of rejection with time the filtrate was collected and the viral concentration was assayed on samples from this permeate. The viral concentration in the feed was obtained by mass balance and the average values were used when calculating rejection.

For stirred experiments, the magnetic stirrer was started with a stirring speed of 400 rpm prior to the filtration. The experiments were carried out at ambient temperature (25 ± 1 °C). The flux was measured gravimetrically by continuously weighting permeate.

2.3. Viruses

2.3.1. Viral specifications

Two animal viruses that are not zoonosis, that is, FMD and IBR viruses were used in this study. FMD is



Scheme 1. Schematic of dead end cell.

an extremely contagious viral disease of colvan-hoofed animals. Its hosts are extremely wide being capable of infecting nearly 70 species within 20 families of mammals. The hosts of FMD are bovidae (cattle, zebus, domestic buffaloes, and yaks), sheep, goats, swine and all wild ruminants. Camelidae (camels, dromedaries, llamas, and vicunas) have low susceptibility. In cattle, the disease is characterized by fever and vesicular lesions on the mouth, tongue, muzzle, hooves, and udder. Myocarditis may occur in young animals sometimes resulting in death. There are seven distinct serotypes namely: A, O, C, SAT1, SAT2, SAT3, and Asia1. Infection with one serotype does not confer immunity against another. In this research, FMD virus type "O" was used.

FMD virus is preserved by refrigeration and freezing and progressively inactivated by temperatures above 50˚C. The virus is inactivated by pH < 6.0 or >9.0 and can be disinfected by sodium hydroxide (2%), sodium carbonate (4%), and citric acid (0.2%). The virus is resistant to iodophores, quaternary ammonium compounds, hypochlorite and phenol, especially in the presence of organic matter. It can be destroyed by heat, sunlight, low humidity, or certain disinfectants. However, it may remain active for a varying time in a suitable medium such as the frozen or chilled carcass of an infected animal or on contaminated objects.

FMD virus is a non-enveloped, 23–27 nm in diameter and $6–9 \times 10^6$ D molecular weight virus with icosahedra symmetry. The genome is a single linear molecule of RNA and is about 7.2–8.4 kb in size. Viral replication is restricted to the cytoplasm of the cell [14–17].

IBR, caused by bovine herpesvirus is a disease of domestic and wild cattle. This virus is a member of the genus Varicellovirus in the subfamily alphaherpesvirinae, which belongs to the Herpesviridae family. The viral genome consists of double stranded DNA that code for about 70 proteins.

IBR virus plays a prominent role among causes of undifferentiated bovine respiratory disease and abortion. It also causes conjunctivitis, infectious pustular vulvovaginitis, balanoposthitis, and rarely, encephalitis. The virus is readily transmitted and has worldwide distribution. Some cattle develop a latent infection, which can be reactivated. The disease can be diagnosed by clinical signs and lesions and by a variety of virology and immunologic techniques.

IBR virus belongs to herpes viridae with a lipoprotein envelope, 120–200 nm in diameter and $65–60 \times 10^6$ D molecular weight. The genome of IBR virus is double-stranded DNA [18–20].

2.3.2. Viral preparation

FMD virus. Baby Hamster Kidney (BHK) cells are commonly used for the production of bulk amounts of FMD viral. For FMD virus preparation, firstly, BHK cells were cultured as a monolayer by stocker medium with 3–4% bovine serum and incubated at 37˚C. After 48 h, media of flasks were changed by Earl medium containing 0.5% bovine serum and 1–2 ml FMD virus type "O" (Tissue culture infective dose [TCID] 50%, 10^7/ml). The flasks were incubated at 37˚C for 12 h. When Cytopathic Effects (CPE) were observed (about 90–100%) on BHK 21 cells, the media were harvested. Viral suspension should be clarified by 0.2 μm membrane for separation of cell debris. Viral titration was performed by Reed and Menuch method. The result was achieved at (TCID 50% = 10^7/ml). Finally, viral pool was dispersed into 200 ml flasks and store at −20˚C until usage.

IBR virus. For viral preparation, Bovine Kidney cell line was cultured as a monolayer by DMEM medium

with 10% bovine serum. This was incubated for 48–72 h at 37˚C. When the cells were grown with a good density, the media were changed by DMEM with 2% serum. This was inoculated with local isolate of IBR virus at a multiplicity of 0.1 plaque forming unit/cell and was incubated at 37˚C for 48–72 h. After CPE were observed between 90 and 100%, the media were harvested as a IBR suspension virus. The cell debris was separated using centrifuge at 5,000–6,000 rpm for 15 min at 4˚C. Finally, the supernatant containing clarified virus was titrated by Reed and Menuch method (TCID 50% = 10^7/ml) and stored at −70˚C.

3. Results and discussion

3.1. Rejection

The efficiency of the membrane for viral removal can be expressed either by rejection or log reduction value (LRV) which are defined as:

$$R = (1 - C_p/C_f) \times 100$$

$$\mathrm{LRV} = \log \frac{C_f}{C_p}$$

where C_f is the feed concentration and C_p is the permeate concentration. The IBR virus was totally rejected by the MF membrane. Considering that the separation of viruses by MF may be based on virus size, complete rejection of IBR (200 nm) by GVHP membranes (220 nm) is expected. The scanning electron microscope (SEM) micrographs (Fig. 1 for 50 kPa and Fig. 2 for 100 kPa operating pressure) clearly show the aggregate of IRB virus (highlighted in the figure) on the surface of the membrane. Similar results were obtained for other operating conditions. The filtration of Earl medium (without virus) remains no sign of any deposition on the membrane surface (Fig. 3). This image provides a basis for recognition of viruses on the remaining on the membrane surface (compare Fig. 3 versus Figs. 1 and 2).

FMD virus with smaller diameter (23–27 nm) has more chance to pass through the membrane with

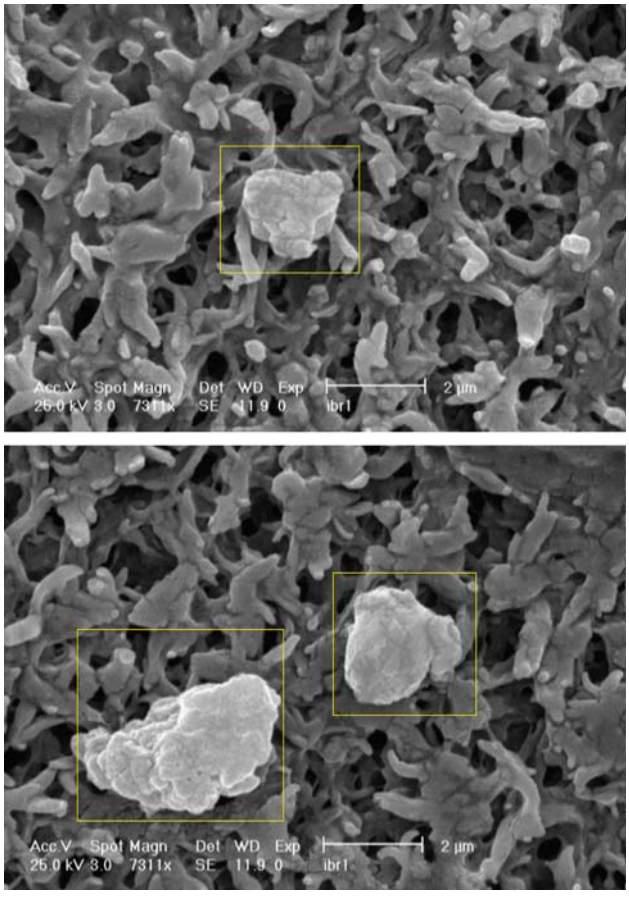


Fig. 1. SEM micrographs of GVHP membrane surface after filtering IBR virus (10^4/ml, 50 kPa, unstirred, various locations of the same membrane).

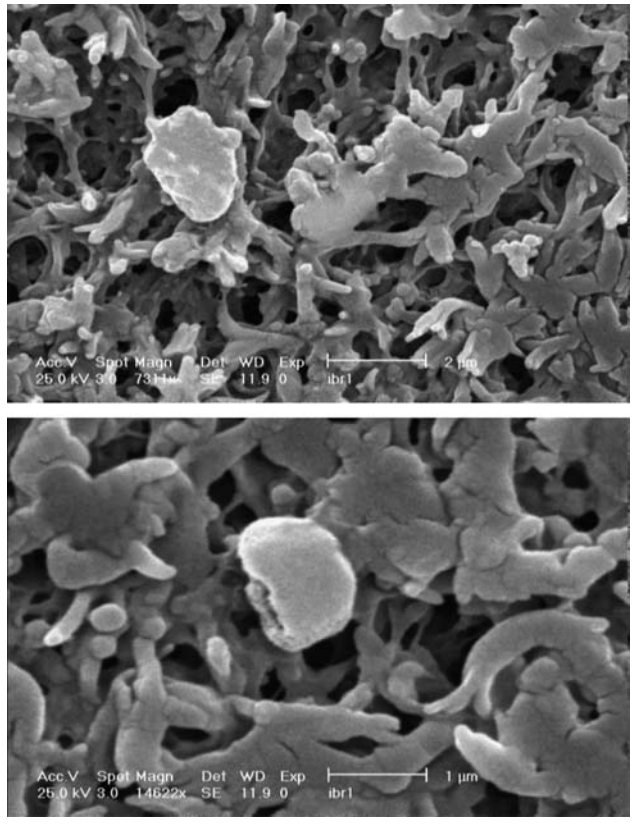


Fig. 2. SEM micrographs of GVHP membrane surface after filtering IBR virus (10^4/ml, 100 kPa, unstirred, magnification top 7,000 and down 14,000).

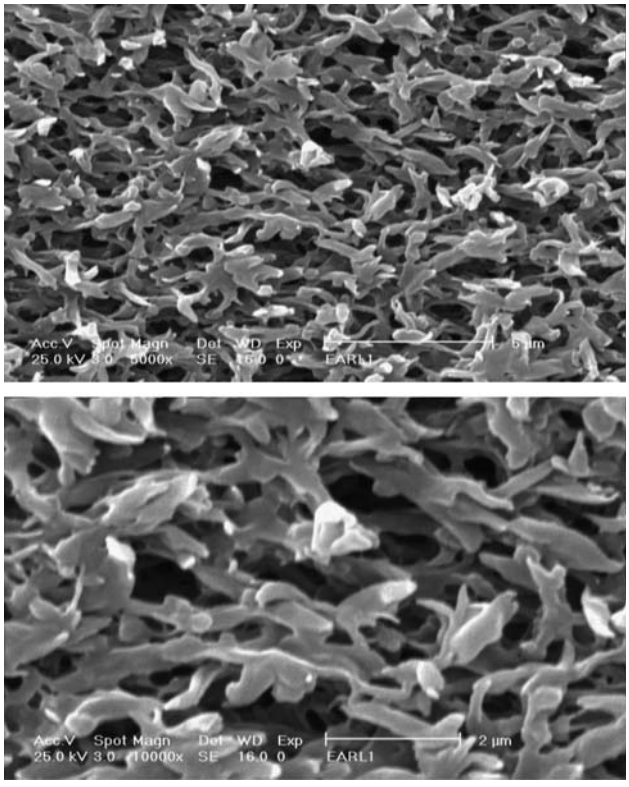


Fig. 3. SEM micrographs of GVHP membrane surface after filtering Earl medium (100 kPa, unstirred, magnification top 5,000 and down 10,000).

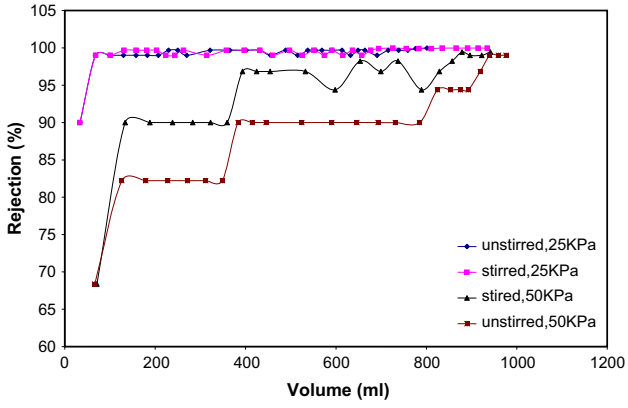


Fig. 4. Profiles of FMD viral rejection using GVHP membranes (initial concentration 10^6/ml).

large pore size (220 nm). However a pronounced part of the FMD viruses is retained by the membrane (Fig. 4). The highest viral rejection (99.96% or 3.5 LRV) was obtained for lowest transmembrane pressure (25 kPa) and stirred condition for 10^6/ml FMD viral concentration. This trend is expected. Increasing the applied pressure or reducing the turbulency facilitate the leakage of viruses through the membrane into the permeate. The practical implications are that improved viral removal can be achieved by operating with stirring and low rather than high applied pressure.

The GVHP membrane and FMD virus have opposite charges (negative for membrane vs. positive for virus) [21–24] that can enhance the viral rejection due to adsorption of FMD into the membrane pores. Further deposition within membrane pores, results in reduction of membrane pore size which enhances viral rejection. Pressure increment at constant concentration leads to faster permeation of feed through the membrane with lower stoppage of viruses within the pores and less chance for establishment of deposition. Favorite conditions lead to cake deposition which can be viewed using SEM (Figs. 5 and 6). The cake assists

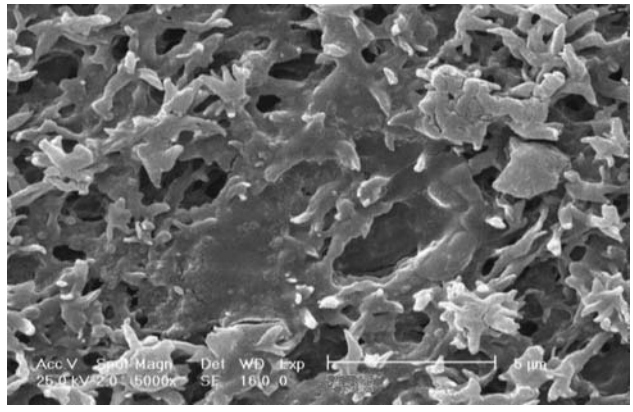


Fig. 5. SEM micrograph of GVHP membrane surface after filtering 300 ml of FMD virus suspension (10^6/ml, 25 kPa, unstirred).

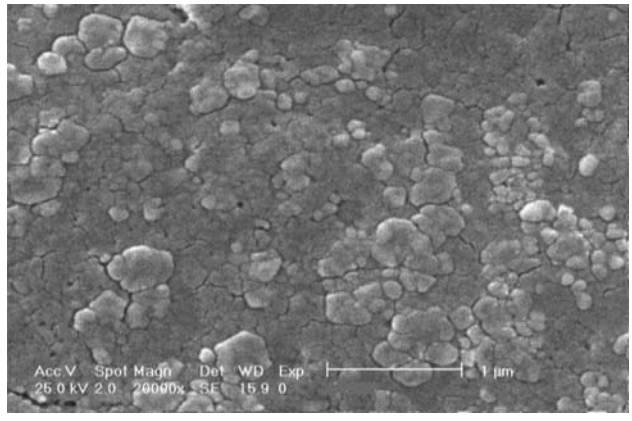


Fig. 6. SEM micrograph of GVHP membrane surface after filtering 950 ml FMD viral suspension (10^6/ml, 25 kPa, unstirred).

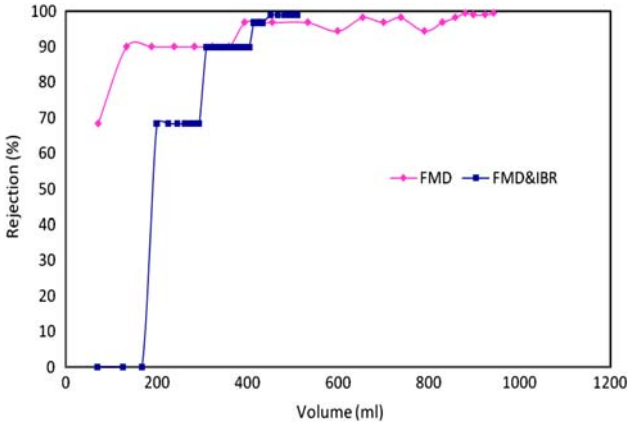


Fig. 7. Rejection profiles of FMD virus (initial concentration 10^6/ml) with and without IBR virus (initial concentration 10^6/ml) using GVHP membranes (50 kPa, 400 rpm).

the removal of viruses. In other words, the viruses are removed by other viruses as a secondary membrane.

In another set of experiments both IBR and FMD viruses were mixed and the capability of viral removal by MF membrane was measured. The effect of large IBR virus, with complete viral removal, on rejection of small FMD virus, with pronounced but not complete viral removal, is presented in Fig. 7. Eventually, nearly, complete viral removal was obtained for both operations, that is, with or without the presence of IBR virus. However IBR virus facilitates reaching to almost complete removal in shorter time with less passage of feed through the membrane. This is due to deposition of IBR virus on the membrane surface and faster establishment of the secondary membrane. Faster formation of cake layer is demonstrated in SEM micrographs (compare Fig. 8 for FMD virus without IBR virus and Fig. 9 for mixture of FMD and IBR viruses).

The establishment of cake layer which acts as a secondary membrane is responsible for complete or elevated viral removal. The parameters affecting the deposition of cake layer, including transmembrane pressure, viral concentration, stirring etc., influence the viral removal. Understanding the rationale for

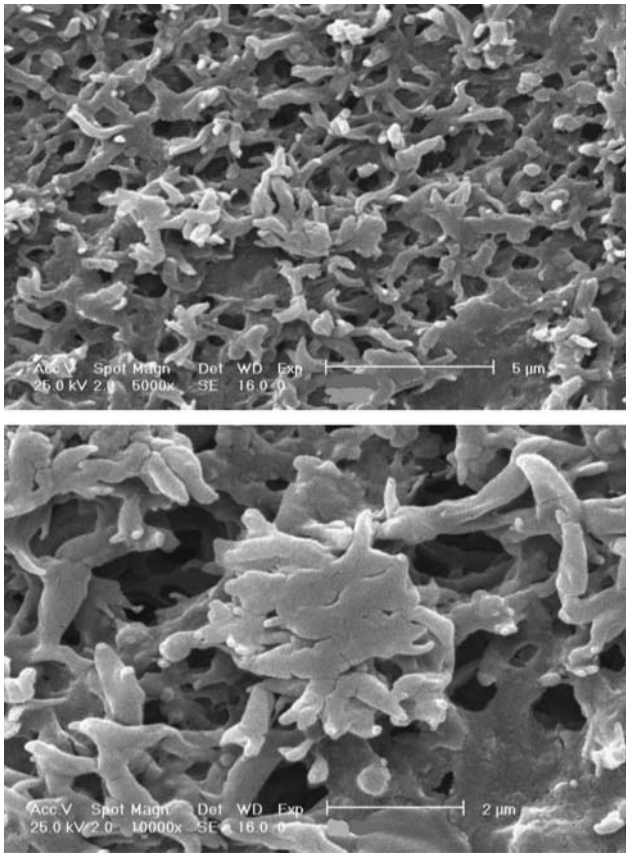


Fig. 8. SEM micrographs of GVHP membrane surface after filtering FMD virus (10^6/ml, 50 kPa, unstirred, magnification top 5,000 and down 10,000).

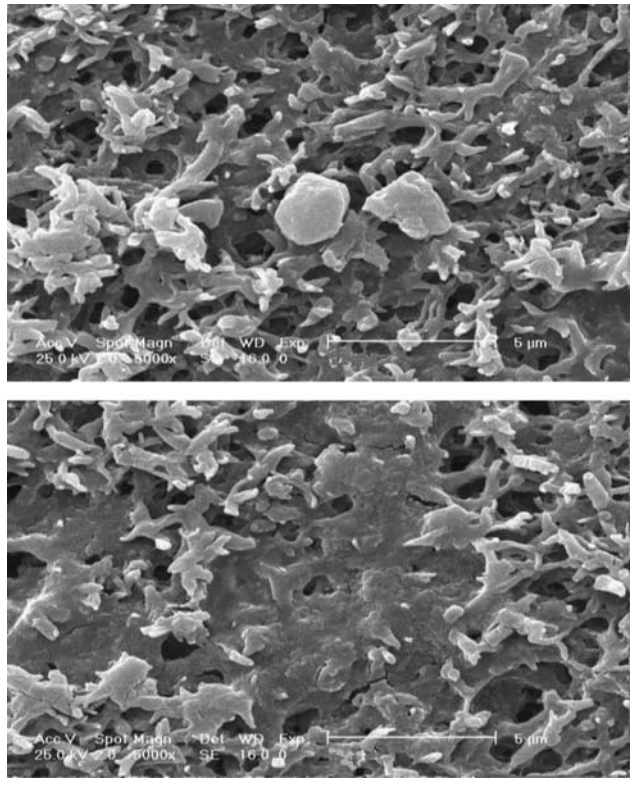


Fig. 9. SEM micrographs of GVHP membrane surface after filtering FMD virus (initial concentration 10^6/ml, 50 kPa, unstirred, IBR concentration 10^6/ml, various locations of the same membrane).

cake formation leads to manipulation of operating conditions for highest viral removal. Apparently higher concentration, lower applied pressure, and unstirred condition facilitate cake layer formation. An appropriate combination of the mentioned parameters leads to establishment of cake layer (see Figs. 10 and 11) and nearly complete removal of virus from water (Fig. 12). The latter demonstrates that viral rejection is initially nil due to favorite conditions for virus passage through the membrane. By cake formation during time, the viral retention is improved leading to almost complete removal.

3.2. Flux

Another key factor for estimation membrane efficiency is flux which indicates the process productivity. The parameters influencing the rejection may affect the flux. The obtained results indicate that stirring did not significantly change the flux (Fig. 13). This means that flux was not controlled by a hydrodynamically responsive polarization layer, presumably due to the dilute nature of the feed. In general, flux is controlled by the material deposited on the membrane surface or within the membrane pores.

Introducing IBR virus to the suspension containing FMD virus significantly reduces the flux (Fig. 14) due to greater deposition of cake layer on the membrane surface. However, increasing the driving force, that is, transmembrane pressure may overcome the low flux for mixed suspensions (Fig. 15).

The characteristic of cake layer is vital for manipulating the process productivity. Flux (J) may be obtained as a function of transmembrane pressure (ΔP) and viscosity (μ) from the following expression:

$$J = \Delta P/\mu(R_m + R_d) \Rightarrow R_d = \frac{\Delta P}{\mu J} - Rm$$

where R_m and R_d are resistances of virgin membrane and deposited layer.

Assuming a constant membrane resistance, during the filtration process, indicates that the deposit resistance depends on transmembrane pressure and flux. Prior to complete cake layer formation, increasing the applied pressure results in flux enhancement. Further

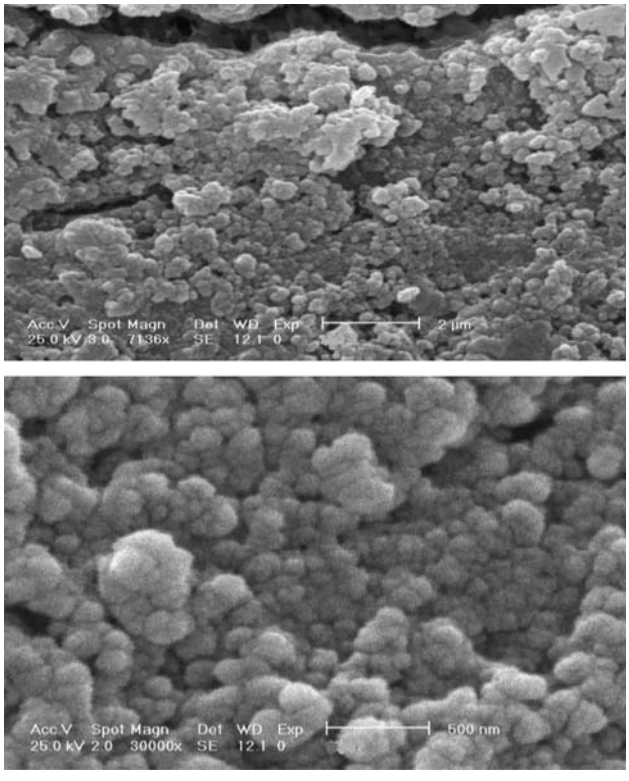


Fig. 10. SEM micrographs of GVHP membrane surface after filtering FMD viral (initial concentration 10^7/ml, 100 kPa, unstirred, IBR concentration 10^6/ml, magnification top 7,000 and down 30,000).

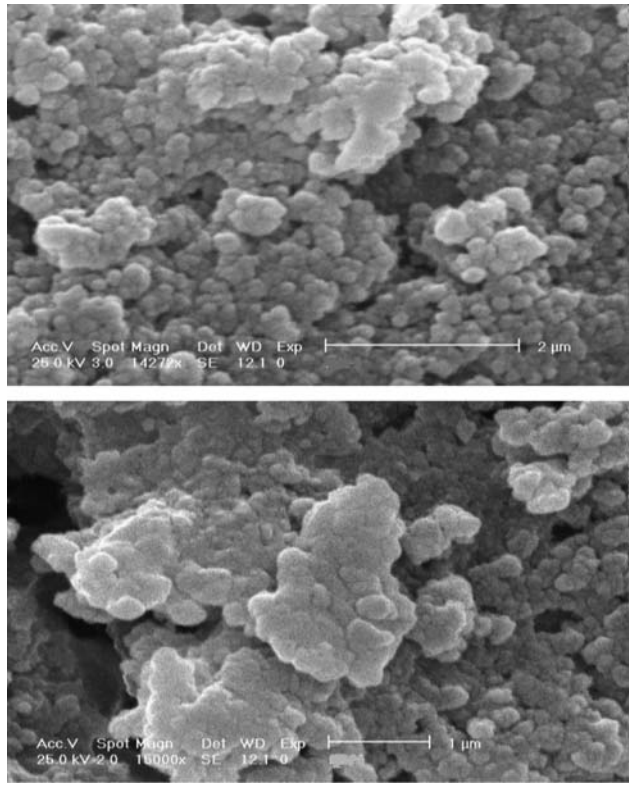


Fig. 11. SEM micrographs of GVHP membrane surface after filtering FMD viral (initial concentration 10^7/ml, 150 kPa, unstirred, IBR viral concentration 10^4/ml, magnification top 14,000 and down 15,000).

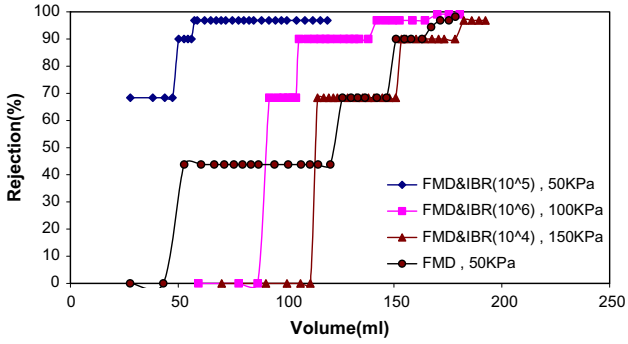


Fig. 12. Rejection profiles of FMD virus (initial concentration 10^7/ml) with and without IBR virus using GVHP membranes as a function of volume (unstirred).

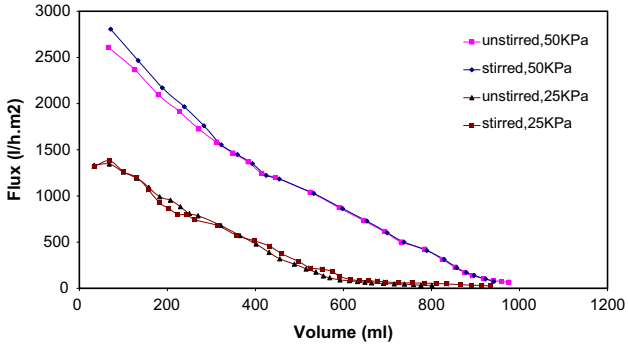


Fig. 13. Flux profiles of FMD virus (initial concentration 10^6/ml) using GVHP membranes as a function of volume.

pressure increment, after the establishment of cake layer, results in an enhancement in deposit resistance leading to the sharp flux decline.

The obtained results showing flux losses and deposit resistances for FMD viral, with 10^6/ml virus concentration, are presented in Table 2. Increasing the pressure reduces the flux loss and deposit resistance for suspensions containing FMD virus only. However, a contrary result was obtained for mixtures of FMD an IBR viruses (Table 3). For mixtures of viruses, greater flux loss and elevated deposit resistance were obtained for higher applied pressures. These contradictory results may be explained by non-formation of cake layer in the case of FMD virus and establishment of cake layer for mixtures of FMD and IBR viruses. For the former case, there is no cake to be compressed with increasing pressure. Accordingly, enhancement of driving force leads to further flux improvement with no pronounced effect deposit resistance. For mixtures of viruses, by cake layer formation, increasing the pressure leads to compression of cake layer leading to higher deposit resistance.

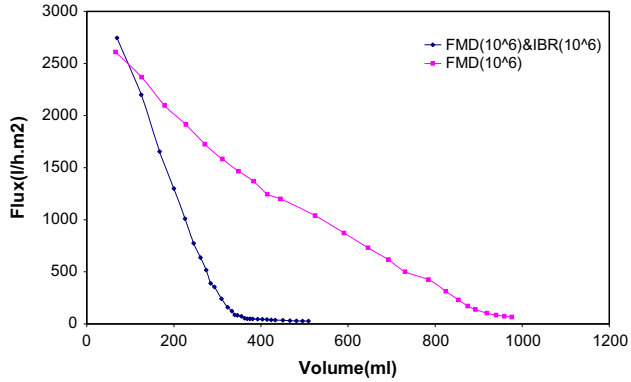


Fig. 14. Flux profiles of FMD virus (initial concentration 10^6/ml) with and without IBR virus (initial concentration 10^6/ml) using GVHP membranes as a function of volume (50 kPa, 400 rpm).

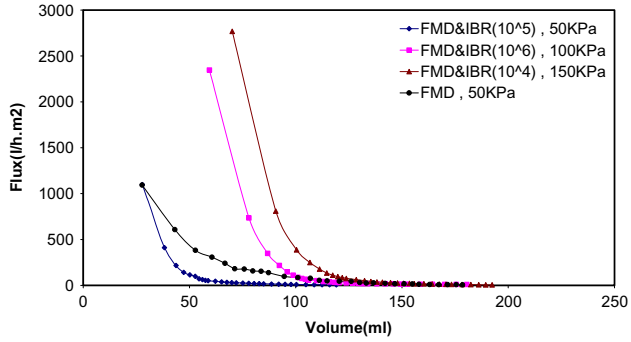


Fig. 15. Flux profiles of FMD virus (initial concentration 10^7/ml) with and without IBR virus using GVHP membranes as a function of volume (unstirred).

Table 2
Effect of transmembrane pressure on deposit resistance (R_d)[a] and flux loss (J_{loss})[b] of FMD virus (initial value 10^6/ml) using GVHP membrane under stirred conditions (400 rpm) after filtering 1,000 ml suspension

ΔP (kPa)	J_{loss} (%)	R_d (10^{10} m^{-1})
25	98.0	0.38
50	97.4	0.30
100	66.9	0.03
150	42.0	0.02

[a]Obtained from $J = \Delta P/\mu(R_m + R_d)$.
[b]Flux loss (%) = (ΔJ (initial − final)/initial flux) × 100.

The two affecting parameters, that is, concentration and pressure compete for affecting the cake layer formation and its characteristics. Table 4 demonstrates the influence of pressure and concentration on R_d and J_{loss}. Increasing the pressure from 50 to 100 kPa and

Table 3
Effect of transmembrane pressure on deposit resistance (R_d) and flux loss (J_{loss}) of mixtures of FMD virus (initial value 10^7/ml) and IBR virus (concentrationof IBR virus is negligible vs. concentration of FMD virus) using GVHP membrane under stirred conditions (400 rpm) after filtering 1,000 ml suspension

ΔP (kPa)	J_{loss} (%)	R_d (10^{10} m^{-1})
50	98.6	1.1
100	99.2	2.9
150	99.3	3.6

Table 4
Effect of transmembrane pressure and concentration on deposit resistance (R_d) and flux loss (J_{loss}) of mixtures of FMD (C_1) and IBR viruses (C_2) using GVHP membrane under stirred conditions (400 rpm) after filtering 1,000 ml suspension

ΔP (kPa)	C_1 (mL^{-1})	C_2 (mL^{-1})	J_{loss} (%)	R_d (10^{10} m^{-1})
50	10^6	10^6	99.0	0.7
100	10^6	10^4	98.0	0.3
150	10^{10}	10^6	99.5	1.9

reducing the IBR viral concentration from 10^6/ml to 10^4/ml, results in a reduction in flux loss and deposit resistance. This can be explained by non-formation of cake layer due to dilute nature of the feed with lower IBR viral concentration. However, the effect is pronounced by jointly increasing concentration and pressure and cake deposition.

4. Conclusions

In MF of FMD viral suspensions using 0.22 μm hydrophobic GVHP membrane, higher rejection of virus was obtained for stirred conditions and lower transmembrane pressure. Rejections greater than 99% were achievable. Evidence from electron microscopy indicates that the viruses were retained by adsorption and formation of a deposit layer on and within the membrane. IBR virus with appropriate concentration provides further sites for adsorption as well as blocking and obstructing the pores and forming a secondary barrier against viral transmission. The ability of the membrane to retain FMD virus is gradually increased during time. Superior viral removal may be achieved by stirring, low transmembrane pressure and in the presence of IBR virus with appropriate concentration.

In MF of FMD virus, flux was unaffected by stirring due to the dilute nature of the feed. For mixture of FMD and IBR viruses, the flux was improved at stirring condition. The completely retentive membrane for IBR virus results in concentration polarization in the vicinity of the membrane surface which can be disturbed by stirring to produce higher flux. The transmembrane pressure and concentration influence the resistance and flux loss.

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