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Chapter 21

Nanofiltration technology for removal of pathogens present in drinking water

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1. Introduction

Water is the essence of life present on earth. Various biological processes in living organisms are solely dependent on the presence of water. Shortage of potable water has emerged as an important challenge to current world generation. Furthermore, the contamination of fresh drinking water by pathogenic microbes as well as their presence in treated water poses a serious challenge to human health globally. The drinking water, under natural condition, is very often susceptible to contamination by nuisance microbes such as bacteria, viruses, and protozoa. To date, more than 100 waterborne pathogenic microorganisms belonging to bacteria, viruses, and protozoa have been identified in contaminated drinking water. Because the contamination happens during withdrawal, collection, storage, and transportation of drinking water, the microbial contamination at source is minimal as compared to places of storage. Importantly, these small infectious entities pose significant limitations in their assessment because of smaller size. The inherent dynamic behavior of conventional water treatment systems leading to process inefficiency for elimination of different organic and inorganic contaminants (Upadhyayula et al., 2008) as well as pathogenic microbes is well recognized. Slight changes in the feedwater quality in terms of pH, temperature, and

a. Equally contributed to this chapter.
transparency in addition to nature and concentration of microbial pathogens significantly affect the treatment efficiency of traditional water purification practices (Kfir et al., 1995). Furthermore, changes in water characteristics may modify the equilibrium between the absolute elimination and nonabsolute elimination of a treatment system (Assavasilavasukul et al., 2008; Lechevallier and Au Keung, 2004). The treatment in conventional process may also allow the luxuriant growth of noxious cyanobacterial strains followed by secretion of health hazardous toxins culminating into poor water quality. Also, the generation of disinfection by-products (DBPs) after application of chlorine and other disinfectant results into water with compromised features (Gopal et al., 2007; Dong et al., 2019). In this context, many of pathogenic microbes generally encountered in drinking water are resistant to chemical treatment processes (Mohammed, 2019) that might pose serious hazards to human health. Most importantly, the deliberate introduction of pathogenic microbes and their toxins (biothreat agents) in water treatment units or water supply systems with an aim to disturb mass public health as reported in developed countries such as the United States is also a major issue (Horman, 2005; Nuzzo, 2006). These limitations of conventional treatment units have necessitated the research on membrane processes for the removal of contaminants of either organic or inorganic origin. Among different membrane-based contaminant removal practices, microfiltration (MF), ultrafiltration (UF), reverse osmosis (RO), and nanofiltration (NF) have gained global popularity for water purification as compared to conventional treatment practices. This chapter has been framed to encompass the different waterborne pathogens, important diseases caused by them, and membrane-based processes for their effective removal with particular emphasis on NF technology. The advantages, limitations, and future perspectives of NF process in this connection have also been added.

2. Waterborne diseases

According to an estimate, around 1.5 million deaths of children have been reported to result from diarrhea (Fenwick, 2006). Astonishingly, diarrhea has been ranked as the second important disease responsible for heavy mortality in children globally. Worldwide, 90% cases of diarrheal diseases are the outcomes of improper sanitary practices and consumption of contaminated drinking water (UNICEF, 2012). The water-transmitted diseases are also one of the important factors for global economic loss. Apart from the expenses made by human, the World Bank has calculated annual global expenses equivalent to US$260 billion caused by unavailability of safe drinking water and cleanliness (WHO, 2012).

The World Health Organization (WHO) has documented the annual death occurrences reaching up to 5 million caused by waterborne diseases. Children from Asian and African nations below the age of 5 years are the most frequent
victims of disease transmitted through microbially contaminated water (Seas et al., 2000). The population from developed and rural areas is most commonly affected by diseases caused by microbially contaminated drinking water. However, the life-threatening impact of microbially contaminated water has also been observed for human population in developed countries. As per an estimate, in the United States of America, 5.6 lacs people are affected annually to a greater extent from diseases occurring from microbially contaminated water, while 7.1 million are expected to be affected moderately by waterborne microbial diseases leading to an average 12,000 deaths per year (Medema et al., 2003). Moreover, a minimum of 33 outbreaks of waterborne illness during the year 2009–10 in the United States of America is described (Center for Disease Control and Prevention, 2013). Such disease incidences indicate the development of easy and low-cost technology for detection of pathogens even at very low density as well as introduction of suitable measures to control the severe human diseases resulting from the utilization of microbially contaminated drinking water. Some of the important waterborne diseases and their causal organism are presented in Table 21.1.

3. Sources of pathogen contamination in drinking water

Generally, most of the waterborne pathogens gets their entry into water through humans and animals excreta (Swaffer et al., 2018) and urine. Application of livestock manures for enhancing plant productivity may also lead to microbial contamination of water systems (Bergion et al., 2018). Some of the pathogenic bacterial species such as *Legionella* has been suggested to transmit through aerosol formation followed by inhalation (Percival et al., 2004). Different species of *Cryptosporidium* are known to contaminate the water system via surface runoff from livestock farm houses destined for milk or meat production (Ruecker et al., 2007). In addition, for distribution of water, in many countries, the drinking water is supplied through long networks of pipes. The pipe-based translocation system serves as the substrata for the attachment of bacterial species with the tendency to form biofilm. The developed biofilms, on the other hand, may also support the existence of pathogenic microbes either for short time or longer durations. The hygienically important pathogenic organisms may include fecal indicator coliforms (*Escherichia coli*), some species of *Campylobacter*, *Mycobacterium* (Lehtola et al., 2007), opportunistic bacterial species such as *Legionella* and *Pseudomonas aeruginosa*, and viruses responsible for intestinal disease such as adenoviruses, caliciviruses, small round viruses, rotaviruses, etc. In addition, the originated biofilms also promote the suitable environmental habitats for helminths and nuisance protozoa including *Entamoeba histolytica*, *Giardia lamblia*, and *Cryptosporidium parvum* (Wingender and Flemming, 2011). The survivability of attached pathogenic organisms is largely decided by their physiological and biological characteristics apart from surrounding ecological conditions.
| Pathogen type | Pathogen          | Disease      | Remarks                                                                                                                                 |
|---------------|-------------------|--------------|------------------------------------------------------------------------------------------------------------------------------------------|
| Bacteria      | *Salmonella* spp. | Typhoid fever| Human excreta, sewage, and agricultural runoff as sources of contamination; one of the major reason for intestinal diseases; survivability under range of nonhost environmental conditions |
|               | *Shigella* spp.   | Shigellosis  | Careless handling of feces, poor water quality as the source of bacteria; mostly recorded in developing nations                              |
|               | Pathogenic *Escherichia coli* | Gastroenteritis | Responsible for different diarrheal diseases; may be hemorrhagic and invasive in nature; the pathogen is the useful indicator for fecal matter contamination |
|               | *Campylobacter* spp. | Gastroenteritis | One of the most common causes of gastroenteritis; sewage and fecal matter are suspected sources of contamination of drinking water |
|               | *Vibrio cholerae* | Cholera      | Human excreta as the contaminant source; the disease burden is much prevalent in sub-Saharan Africa                                         |
|               | *Yersinia enterocolitica* | Gastroenteritis | Human and animal excreta as the source of contamination                                                                                     |
| Virus         | Rotaviruses       | Diarrhea     | Common pathogen for diarrhea in children; the pathogen is resistant to changes in environmental conditions; transmitted through water contaminated with human feces; the victims excrete around $10^{11}$ particle per gram of excreta |
### TABLE 21.1 Some important waterborne pathogens and diseases caused by them.—cont’d

| Pathogen type                     | Pathogen                     | Disease                        | Remarks                                                                                                                                 |
|-----------------------------------|------------------------------|--------------------------------|----------------------------------------------------------------------------------------------------------------------------------------|
| Enteric adenoviruses              | Gastroenteritis              |                                | Second major reason for children gastroenteritis; the pathogen is resistant to conventional treatment processes; may be used as viral indicator for water quality |
| Caliciviruses                     | Gastrointestinal illness     |                                | The viruses are resistant to harsh environmental conditions of stomach; human excreta as the source of contamination                   |
| Astroviruses                      | Gastroenteritis              |                                | The pathogen is transmitted through sewage contaminated water; important factor for nonbacterial diarrhea in different age group humans |
| Small round viruses               | Gastroenteritis              |                                | The viruses are resistant to extreme environmental conditions; transmission through human feces contaminated water                    |
| Enteroviruses                     | Aseptic meningitis          |                                | Transferred through human excreta; these pathogens are indicator of viral contamination in drinking water; responsible for more than 85% cases of aseptic meningitis |
| Coronaviruses                     | Gastroenteritis; respiratory diseases |                                | Transferred through fecal wastes finding their ways in water; virus survival up to 96 h in human excreta                             |
| Hepatitis A virus                 | Hepatitis A                  |                                | Transferred through human feces; low density in drinking water could have major disease outbreak                                     |
| Hepatitis E virus                 | Hepatitis E                  |                                | Highly fatal for pregnant women; transmitted through sewage contaminated water                                                      |

*Continued*
Therefore, biofilms present in water transportation system may act like the active reservoir for pathogenic microorganisms and pose serious health hazards to human health if not managed properly.

### 4. Currently available techniques for pathogen removal

The contamination of drinking water with pathogenic microorganism (Amin et al., 2019; Sanganyado and Gwenzi, 2019) is a severe threat to human health. Disease outbreaks in both the developing and developed countries caused by drinking water contamination have lead to millions of death worldwide (Ashbolt, 2004). Many of the waterborne disease can be controlled through the application of physicochemical methods. Most of the modern water purification techniques are relied on the application of physical and chemical treatment technologies. The traditional methods for managing the pathogenic microbes in drinking water systems involve the heating (Zaman et al., 2019), treatment with ozone, chlorine and UV irradiation (Antony et al., 2012),

| Pathogen type | Pathogen                  | Disease       | Remarks                                                                                                                                 |
|---------------|---------------------------|---------------|----------------------------------------------------------------------------------------------------------------------------------------|
| Helminth      | *Ascaris lumbricoides*    | Ascariasis    | Transferred through human excreta                                                                                                                                                             |
| Protozoa      | *Entamoeba histolytica*   | Amebiasis     | Transferred through human excreta contaminated water; children are much affected; the cysts are generally resistant to destruction caused by chemical disinfectants |
|               | *Giardia lamblia*         | Giardiasis    | Transmitted through water contaminated with human excreta; children are much affected                                                                                                        |
|               | *Cryptosporidium parvum* | Cryptosporidosis | Disease is spread through consumption of drinking water polluted with human and animal excreta harboring cysts; much prevalent in developing countries; the cyst of pathogen in suspension may survive for 1 year at 4°C |
or filtration through membrane system (Ostarcevic et al., 2018). The conventional treatment technologies for selected pathogenic microbes are effective but are costly and may generate excessive secondary pollutants with toxicity greater than parent molecule necessitating the improvement in currently employed methods. The formation of trihalomethanes as a DBP after treatment with chlorine may be detected up to a level equivalent to 160 ppb causing significant human health hazard (Bellar et al., 1974; Costet et al., 2011). On the other hand, the added chemicals itself may be hazardous beyond the recommended dose. The chemical treatment techniques for managing the microbial contamination in drinking water are primarily based upon the oxidation of organic component of living cells. The application of a particular disinfection process is, however, not equally effective for eliminating every kind of pathogenic microbes present in drinking water, thus indicating the process limitation. For instance, viruses are generally less susceptible to commonly exploited chemical disinfection processes.

Application of membrane-based techniques for microbial decontamination (Torii et al., 2019; Németh et al., 2019) has gained considerable momentum in water purification industries. The superiority of membrane-based techniques over conventional treatment methods can be expressed in terms of (i) produced water with consistent characteristics, (ii) rapid and effective elimination of chemically resistant pathogens, (iii) inhibition of bacterial remultiplication, (iv) negligible residual chemical hazard, and (v) promising efficacy to decontaminate the polluted water with the quality reaching at standard levels.

The size-exclusion property of membrane processes is important in view of pathogen removal efficiency (Bennett, 2008). Membrane with a specified pore size could not be considered fit for removal of every pathogenic microorganisms. Under certain conditions, with the passage of time, the membrane may suffer from low pathogen removal efficiency because of development of breaches, salt deposition, and biofilm formation in applied filter (Stoica et al., 2018), thereby limiting the overall performance of the filtration technology. On the other hand, the removal of pathogenic microbes via membrane filtration is also affected by different factors including the surface characteristics (Langlet et al., 2008) as well as the time of pathogen’s interaction with membrane (Langlet et al., 2009).

5. Membrane-based technologies for pathogenic contaminant removal

Worldwide increasing demands for synthetic membranes have been expected due to scarcity as well as the microbiological contamination of fresh drinking water. Water purification practices based on pressure-driven membrane filtration can be broadly categorized as (i) MF and UF involving convective separation through membrane with characteristic porous structure and (ii) RO and NF involving diffusion-aided separation through semipermeable
membrane displaying dense structure (Antony et al., 2012; Betancourt and Rose, 2004). The membrane-dependent water treatment technologies have contributed significantly in generation of potable water especially in the United States and Europe. The techniques have been successfully employed to manage not only the pathogenic microbes but also for the elimination of secondary by-products and inorganic as well as organic contaminants of hazardous nature commonly encountered in drinking water.

The low pressure operated MF and UF proved as potential alternatives to conventional treatment methodologies. The aforementioned process may involve the removal of pathogens by size exclusion, association with charged membrane to produce a layer, interaction with membrane developed biofilm. Separation of a particular pathogenic microbe is governed by its natural characteristics, features of applied membrane, and physicochemical characteristics of aqueous medium (Jacangelo et al., 1995). Some of the important information about MF, UF, and RO are presented below.

### 5.1 Microfiltration

The process is based on the low pressure guided separation through membrane having pore size in the range of 0.1–10 μm allowing efficient water flow rate. The technique is useful in removing the particle supposed to interdict the further water purification steps. Apart from the removal of pathogenic waterborne microorganisms, the technique could be applied successfully for clarification, pretreatment, and removal of coarse particles (Jacangelo et al., 1997; Van der Bruggen et al., 2003).

### 5.2 Ultrafiltration

In contrast to MF, the porosity of membrane employed here for separation process ranges from 0.002 to 0.1 μm, leading to low water filtration rate and thus requirement of high pressure. Currently the process is being practiced for the elimination of pathogens and particulate materials. The pore size of membranes used for UF in water treatment process generally varies from 0.01 to 0.5 μm and is quite effective in removal of most of the pathogenic protozoan cysts having the size in the range of 4–15 μm (Jacangelo et al., 1997). In general, the membrane used for purification processes are consisted of polysulfone or polyethersulfones. The membranes can be regenerated by suitable treatment methods and can be utilized multiple times for removal of pathogens present in drinking water. One of the experimental investigations has reported the complete removal of poliovirus with the UF membranes having pore size 0.2 nm (Madaeni et al., 1995). Another study conducted by Jacangelo et al. (1995) has reported the elimination of MS2 virus higher than 6 logs utilizing the UF membrane with pore size as reported by Madaeni et al. (1995).
5.3 Reverse osmosis

The process of RO utilizes the denser membrane system without any definite porosity (Van der Bruggen et al., 2003). The transfer through membrane is hence slow and infiltration is not the outcome of pores present but the high pressure—based removal of targeted contaminant (waterborne pathogen). The broad range of water purification process is effective in simultaneously eliminating both microbial and organic contaminants (Wintgens et al., 2005; Malaeb and Ayoub, 2011). The high pressure is more desirable for decontamination of feedwater having high osmotic pressure rendered by increased concentration of soluble substances. The RO process is high energy demanding to maintain the pressure required for permeation of molecules. The separation of pathogens is chiefly attributed to the size-exclusion phenomena in addition to other factors including membrane physicochemical characteristics, functional groups associated with pathogens, and the characteristics of feedwater (Antony et al., 2012). The commonly available module designs for RO devices include spiral wound and hollow fibers. The hollow fibers module with greater density is advantageous in the sense that it allows the fast generation of contaminant free water but has limitations because of rapid membrane fouling (Malaeb and Ayoub, 2011).

6. Nanofiltration technology for removal of pathogenic microbes

The NF has emerged as an innovative technology to solve the problem of drinking water contamination (Emamjomeh et al., 2019). Since its introduction during late 80s, the NF membrane—based separation process so far has achieved multiple improvements. There was continuous rise in numbers of publication starting from 1995 to 2018 (Fig. 21.1). The bibliometric analysis
based on web of science data revealed the “Journal of Membrane Science,” “Desalination,” “Desalination and Water Treatment,” “Separation and Purification Technology,” and “Water Research” as some of the important research journals having significant research publications (Fig. 21.2). Most of the NF-based laboratory and field studies have been conducted in the area of engineering, water resources, polymer science, and chemistry (Fig. 21.3), indicating the interest of researchers from wide disciplines in NF technology. The pore size of membrane employed in NF process is generally less than 10 nm (Bohonak and Zydney, 2005). Based on size-exclusion phenomenon, the NF technology could be utilized for the removal of pathogens present in drinking water together with the organic and inorganic contaminants generally encountered in drinking water and wastewater. However, its expensive nature, high energy requirement, loss in membrane properties after fouling as a
prevalent issue, and disposal problems are the major limitations (Snyder et al., 2007; Luo et al., 2014) restricting its widespread dissemination.

6.1 Mechanism of separation by nanofiltration membranes

NF is a complicated phenomenon and is considerably regulated by fluid flow, its characteristics, and interfacial episodes occurring on the outer membrane surface and within porous structures of membrane. The separation through NF membranes is the integrated outcome of Donnan, transport, dielectric, and steric effects. The Donnan effect refers to the equilibrium and interaction between a soluble charged molecule and charge existing on membrane (Donnan, 1995). The charge existing on membrane has been assumed to result from dissociation of different ionic groups present on membrane as well as from porous conformation of membrane (Hall et al., 1997; Hagmeyer and Gimbel, 1998; Ernst et al., 2000). The ions may be of acidic, basic, and amphoteric nature depending on the type of materials used for membrane fabrication. The dissociation of surface available ionizable groups is significantly governed by pH of the solution intended for its purification. Note-worthy, the membranes with amphoteric behavior may display isoelectric point at a particular pH value (Childress and Elimelech, 1996). Apart from the presence of surface ionizable groups, under some conditions, the mild ion exchange property of NF membrane could also lead to adsorption of ions present in solution to be treated and hence may affect the membrane characteristics (Afonso et al., 2001; Schaep and Vandecasteele, 2001). According to the prevailing conditions accounted by charges on solute molecule and NF membrane, there may be attraction or repulsion. The process of dielectric extrusion as a possible mechanism of NF is very little known and the so-called two important hypotheses “image forces” and “salvation energy barrier” have been proposed to explain the mechanistic details of interaction (Yaroshchuk, 1998; Bowen and Welfoot, 2002). Both phenomena are attributed to the intense spatial internment, nanoscale dimension existing in the NF membrane, and charge. Solutes mobilizing in the solution phase are affected by drag forces imposed by solvent passing through pores with different dimensions. Thus, the transport of solute molecule through different confined pores is considerably affected by characteristics of solvent, and the free movement of solute is hindered. The overall transport of solute is thus determined by convection and diffusion process. Nevertheless, the real mechanistic details of NF membrane separation process are still unresolved, and interestingly the contribution of dielectric exclusion in membrane separation needs further work in this direction (Oatley et al., 2012). In general, uncharged solute molecules are rejected because of their size differences (size exclusion). The application of NF technique for separation of different pathogens generally encountered in drinking water is discussed briefly and provided in Table 21.2.
| S. No. | Bacteria | Virus | Protozoa | Remarks | References |
|-------|----------|-------|----------|---------|------------|
| 1     | *Escherichia coli* | —     | —        | Silver-based nanofilter and nanomembrane as efficient system for pathogen removal as compared to polysulfone membrane; the silver-based nanomembrane exhibited antimicrobial activity | Mangayarkarasi et al. (2012) |
| 2     | —        | HIV, bovine diarrhea virus, porcine pseudorabies virus, reovirus type 3, simian virus 40, bovine parvovirus | —        | Cellulose-based nanofilter membrane (planova 15N and planova 35 N); around 8 log removal of test virus; could be opted for virus removal from blood plasma | Burnouf-Radosевич et al. (1994) |
| 3     | —        | MS2   | —        | Carbon nanotube filter for elimination of model virus MS2; electric current—assisted rejection of virus particle was determined as 5.8—7.4 log removal; membrane fouling was very less after four time runs | Rahaman et al. (2012) |
| 4     | *Clostridium perfringens* | PRD-1 phage, MS-2 phage, *Giardia lamblia* cysts, *Cryptosporidium parvum* oocysts | —        | Efficient removal of the pathogens through NF membrane; higher log removal as compared to conventional techniques | Taylor et al. (2002) |
| 5     | *Bacillus subtilis*, *E. coli* | —     | —        | 4 log removal of *B. subtilis* and 3 log removal of *E. coli*; membrane filter coated with graphene-derived nanomaterials possessed antibacterial properties and could be applied for water purification; among different coating materials poly(N-vinylcarbazole)-graphene oxide (PVK-GO) displayed best performance in elimination of selected test bacterial species | Musico et al. (2014) |
|   | – | Hepatitis A virus (HAV) and B19V | – | Antibody assisted effective removal of nonenveloped virus HAV and B19V through NF membrane | Kreil et al. (2006) |
|---|---|-----------------------------------|---|---------------------------------------------------------------------------------|------------------|
| 7 | – | Model virus (pseudorabies virus, herpes simplex virus, poliovirus) and plasma-borne viruses (hepatitis virus and parvovirus B19) | – | Around 4–6 log removal; applicable for biopharmaceutical products | Burnouf and Radosevich (2003) |
| 8 | – | XMuLV and MMV | – | The designed hollow fiber membrane was able to remove virus by 3–4 log removal irrespective of antibody concentration; regenerated cellulose hollow fiber displayed better performance as compared to flat sheet membrane system | Marques et al. (2009) |
| 9 | – | Bacteriophage PP7 | – | More than 4 log removal of enveloped virus was observed indicating effective virus retention | Tarrach et al. (2007) |
| 10 | – | Retrovirus, simian virus 40, pseudorabies virus, poliovirus | – | The NF membrane with size 15 and 35 nm were reported to eliminate 6–7 log removal of retrovirus, simian virus 40, pseudorabies virus; the membrane with 15 nm pore size was efficacious in restricting the passage of poliovirus; the | O’Grady et al. (1996) |

Continued
| S. No. | Bacteria/Pathogen | Virus | Protozoa | Remarks                                                                                                                                                                                                 | References                  |
|-------|-------------------|-------|----------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------|
| 11    |                   | 3      |          | process was also efficient for purification of immunoglobulins                                                                                                                                               |                             |
| 11    |                   | Xenotropic murine leukemia virus (XMuLVs) and murine minute virus (MMVs) |          | Nanofilters were able to achieve approximately 6 log removal of test virus                                                                                                                                  | Zhao et al. (2007)            |
| 12    | *Staphylococcus aureus, E. coli* | Poliovirus sabin 1 |          | Carbon nanotube–based NF membrane was quite effective in removal of bacterial and viral contaminants; could outcompete the ceramic and polymer-derived membrane filters; easy to clean the nanofilter by autoclaving or ultrasonication and could be used repeatedly | Srivastava et al. (2004)     |
| 13    |                   | MS2 bacteriophage |          | The yttrium oxide–coated filter showed around 100% elimination of MS2 virus; the virus elimination was effective at pH range 5–9                                                                               | Wegmann et al. (2008)        |
| 14    |                   | MS2 bacteriophage |          | Carbon nanotube–based membrane loaded with oxides of copper, titanium, and iron was analyzed for elimination of virus; differences in virus removal at different pH ranges were noticed; copper oxide–coated carbon nanotube displayed best virus removal efficiency at varying pH ranges | Németh et al. (2019)         |
|   |   |   |   |
|---|---|---|---|
| 15 | T4 and f2 | The membrane (100 nm) bioreactor showed 5.8 log removal of T4; log removal for f2 was determined as 0.5; effective over conventional virus removal techniques | Zheng and Liu (2007) |
| 16 | Hepatitis C virus | Cellulose nanocomposite membrane filter exhibited nearly 100% removal of hepatitis C virus; 2CC-SE-L5 membranes had superior performance over 2SC-L5 | Huang et al. (2016) |
| 17 | *E. coli* | More than 6 log removal of *E. coli* was recorded for cellulose acetate–based composite NF membrane containing silver nanoparticle and silver ions exchanged with zeolite; membrane harboring silver ion exchanged with zeolite represented excellent bactericidal activity | Beisl et al. (2019) |
| 18 | *E. coli* | Higher than 4 log removal was found for membrane bioreactor integrated with NF; could be applied for treatment of hospital wastewater | Tran et al. (2019) |
| 19 | Chikungunya virus (CHIKVs) and Mayaro virus (MAYVs) | NF membrane with pore size equivalent to 35 nm was effective in removing Chikungunya virus (CHIKVs) and Mayaro virus (MAYVs); the process can be employed to filter the viruses from medicinal products | Yue et al. (2019) |

Continued
| S. No. | Bacteria      | Virus                  | Protozoa | Remarks                                                                                                                                 | References               |
|-------|---------------|------------------------|----------|-----------------------------------------------------------------------------------------------------------------------------------------|--------------------------|
| 20    | —             | Porcine circovirus-1   | —        | The filter with pore size equivalent to 19 nm was effective in reducing the level of Porcine circovirus-1 either in presence of antibody IgG or in absence of antibody; the filter with 35 nm pore size was unable to completely restrict the passage of test virus; higher log removal of virus was observed for membrane with smaller pore sizes | Bao et al. (2018)        |
| 21    | —             | Bacteriophage Qβ       | —        | The membrane was generated via photopolymerization of diol molecule and imidazolium; nanostructured membrane displayed nearly 100% rejection of bacteriophage Qβ (log removal value >4); the rate of water flow varied between 19 and 61 L m²/h at a pressure 0.3 MPa; removal of virus was regulated by content of ionic liquid | Hamaguchi et al. (2018)  |
6.2 Bacteria

Bacterial pathogens transmitted through supplied drinking water have resulted into several disease outbreaks and mortality in both developed and developing countries. The NF technology has been described to eliminate the bacteria and viruses from both surface and groundwater. The laboratory and field condition evaluation of NF membrane (Fyne process) of lake water was reported by Patterson et al. (2012). The NF experiments were conducted with varied tubular membrane system having different molecular weight cut-off (MWCO). The filtration process was effective in restricting the passage of bacterial pathogen *Bacillus subtilis*. The NF process evaluation for removal of test bacteria (*B. subtilis*) in recycle and dead end mode was based on filters ES404 and CA2PF. The effectiveness of ES404 (MWCO = 4000 Da) in elimination of test bacteria was slightly higher than the CA2PF membrane (MWCO = 2000 Da). Experimental investigations with NF membranes have represented the promising results (Lovins et al., 2002). The nanofilter NF1 was found to restrict the passage of *Clostridium perfringens* (∼1–5 µM) in four challenges out of seven independent membrane-based separation tests. Higher than 6 log rejection of *B. subtilis* as test pathogen from surface water (lake water) under field and laboratory condition is illustrated in tubular NF membrane (ES404 and AFC30) evaluated in recycle and dead end mode without the introduction of coagulating substances (Sinha et al., 2010). Field test—based studies on ES404 and AFC30 membrane NF demonstrated average log removal value as 5.5 and 4.7, respectively, for *B. subtilis*. The AFC30 membrane with low molecular cut-off (MCO), however, exhibited comparatively weaker removal of test pathogen. Kim et al. (2016) have demonstrated the potential of carbon nanotube and silver composite—based nanofilter in removal of waterborne bacterial pathogen *Staphylococcus aureus*. The composite material loaded onto the nanofilter leading to creation of nanopores was shown to be responsible for elimination of bacterial pathogen absolutely. Furthermore, the membrane loaded with composite material displayed considerable antibacterial activity against the *S. aureus* and *E. coli* rendered by silver nanoparticles. Most interestingly, the antibacterial effects of silver nanoparticle would be helpful in designing the membrane with negligible biofouling, an important limitation of the most of the currently used membrane-based filtration techniques.

6.3 Virus

Viruses are the important pathogens often detected in drinking water leading to numbers of severe human diseases. Because of resistance, application of chemical processes such as chlorination has been found to have no profound effect on most of the viruses as compared to pathogenic bacteria, calling for the need of membrane-based technologies. The development of
membrane-based filtration technology has gained considerable success to eliminate their presence in potable water. Studies conducted on removal of virus based on NF technology have demonstrated promising results. Because the smaller sized virus particles of coliphage Qβ as model virus may traverse the NF membrane, the elimination of particular viruses could not be expected to be the only outcome of pore size. The passage of smaller virus particles was expected to result from exceptionally larger membrane pores, not considered for porosity evaluation. However, retention value reaching up to 100% could be achieved for small viruses through NF technology (Urase et al., 1996). Most importantly, the NF membrane used in the study was not able to act as absolute barrier against the Qβ virus having diameter equivalent to 23 nm. Mostafavi et al. (2009) have described the potential of carbon nanotube—based nanofilter for the separation of MS2 virus for purification of contaminated water. The hollow nanofilter as synthesized through spray pyrolysis displayed efficient permeation and was very much effective in virus removal at pressure ranging from 8 to 11 bar. Studies have been conducted for removal of two bacteriophages MS-2 and PRD-1 (size ranging from 28 to 65 nm) from surface water through sand filtration coupled with NF (Yahya et al., 1993). The removal efficiency of slow sand filtration was recorded as 99% and 99.9% for bacteriophage MS-2 and PRD-1, respectively. On the other hand, NF process was able to remove the test viruses by 4-6 log units. Otaki et al. (1998) have described 6 log removal of coliphage Qβ and 7 log removal of poliomyelitis virus vaccine from river water by using NTR-729HFS4 NF membrane. In this sequence, studies performed by Urase et al. (1996) have reported the efficacy of NF process for rejection of model viruses Qβ and T4. The elimination of test virus ranged from 2 to 6 log units.

The effectiveness of NF technique for elimination of MS2 virus under field and laboratory condition is demonstrated (Patterson et al., 2012) and suggested the process utilization for small-scale drinking water systems. The two NF membrane systems ES404 (MWCO = 4000 Da) and AFC30 (MWCO = 350 Da) applied for MS2 virus elimination indicated the better performance of ES404. The designed membrane system ES404 achieved approximately complete elimination (around 6 log removal) during three runs performed in recycle mode. On the other hand, the average log removal of test MS2 virus under similar conditions was observed to be 4.8 for AFC30 membrane. Studies with NF membranes have displayed the outstanding performance (Lovins et al., 2002). Out of seven tests performed for membrane separation of virus MS2 (0.025 µM), in five cases there was complete absence of model virus in permeate after passing through nanofilters comprising of composite thin film (NF1). In two of the runs, there was presence of test organism indicating the fact that the log removal value is not absolutely regulated by microbe size and concentration. For cellulose acetate NF membrane (NF2), log removal value was very weak for PRD-1 and MS2 phage probably because of constraints in maintaining the pH of medium. For composite thin film membrane (NF3), the MS2 virus passed the membrane barrier
at three challenges out of four runs conducted. The PRD-1 phage was able to traverse the membrane at two occasions out of four runs performed. Overall, the composite thin film—based nanofilter was able to achieve 4.5−5.5 log removal value for test microorganism and in more than 50% challenges there was complete removal of test microbes. The study pertaining to elimination of MS2 virus from lake water has shown the effectiveness of membrane NF under field condition (Sinha et al., 2010). The NF membrane ES404 and AFC30 under recycle mode achieved the mean log removal value 4.6 and 4.3, respectively, for the MS2 bacteriophage and fulfilled the guidelines of USEPA. The tighter membrane AFC30 could not exhibit better log removal performance for MS2 virus. The membrane filter could also be loaded with composite of metal nanoparticle exhibiting antibacterial and carbon nanotube to improve the separation performance of membrane separation process. Kim et al. (2016) have reported the potential of carbon nanotube and silver composite—based nanofilter in removal of waterborne viruses such as polio-, noro-, and coxsackieviruses. At ambient temperature, the membrane filter loaded with >0.8 mg/cm² of composite material was remarkably effective in completely restricting the passage of viruses at a pressure equivalent to 5 kgf/cm². The composite material loaded membrane was found effective in inhibiting the passage of a mixture of poliovirus and norovirus as revealed through real-time reverse transcription quantitative polymerase chain reaction (RT-QPCR). The elimination of virus was ascribed to the binding of the viruses on comprehensive mesh consisting of carbon nanotube and silver present on membrane surface; therefore, increase in the density of composite material was associated with the increased removal of test virus.

Development of NF devices working on the principles of tangential and dead end flow has been evaluated for different plasma-borne viruses and model viruses including HIV, hepatitis virus, parvovirus, reovirus, poliovirus, herpes simplex virus, etc. Some of the filtration devices available in the market are Viresolve, Omega VR, Planova, Viresolve NFP and NFR, and Ultipor.

6.4 Protozoa

The elimination of pathogenic protozoans including *Giardia* and *Cryptosporidium* from contaminated drinking water is a worldwide challenging problem for various government organizations as well as water purification companies. Conventional chlorination is practiced for the inactivation and killing of such pathogens present in drinking water; however, generation of DBPs and resistance to chemicals necessitate the development of membrane-based separation techniques. The combination of RO with NF may improve the removal of targeted pathogen of concern. The integrated approach involving NF has shown 8−10 log removal of *Giardia* and *Cryptosporidium* for Lake Arrowhead, California (Madireddi et al., 1997). Studies on NF membrane—assisted elimination of *C. parvum* oocysts (4−6 μm) and *G. lamblia* cysts (8−14 μm) are described (Lovins et al., 2002). Out of 22 runs carried out for
separation of protozoan oocysts and cysts, 16 challenge tests displayed the complete rejection in case of composite thin film--based membrane filtration. Microbial elimination was attributed to the membrane module configuration and structure.

7. Advantages of nanofiltration technology over other virus elimination processes

There are numerous advantages associated with the application of NF for the efficient removal of contaminants present in drinking water. Some important benefits of NF technology are presented below.

7.1 Specificity

NF membrane devices are specifically designed to eliminate the viruses from contaminated sources. In most of the cases, the working parameters such as flow rate, temperature, nature of membrane, load of virus, and filter area are optimized for efficient and reproducible elimination of virus of concern. In contrast to the NF membrane--based virus elimination technique, other separation process such as centrifugation, chromatographic devices destined for purification of a protein molecule could removes viruses as an accidental phenomenon (Burnouf, 1993). Furthermore, the virus exclusion from contaminated water based on other existing techniques as compared to NF membrane techniques may be expensive and time-consuming.

7.2 Expectedness of virus removal

The virus removal through NF membranes occurs via sieving process and hence could be predicted for its efficiency and reproducibility if the process conditions are well-defined. The sieving is very much effective in restricting the passage of virus particles having size higher than mean pore size of NF membrane. Under the conditions where size of virus particles is equivalent to or lower than the membrane pore size, the other operating conditions could have considerable effects on virus removal. This includes congregation of virus particles and their adsorption on membrane filter rendered by surface charge. Examination of NF membrane integrity has demonstrated insignificant variations in the efficiency of virus removal during long-term run signifying the process consistency.

7.3 Process effectiveness and robustness

NF is efficient in removing high concentration of both enveloped and non-enveloped virus particles. The effectiveness of process is largely determined by the pore size of the membrane and the virus particle size. Most importantly, so far, no other membrane-based technique could have been established that
could remove both enveloped and nonenveloped virus particles to an equivalent level as displayed by NF membranes relying on small size pores.

7.4 Process elasticity and easiness

Very few separation techniques have represented the considerable flexibility as demonstrated by NF techniques. Nanofilters can be utilized at different phases of synthesis, generally not exhibited by other virus elimination processes. To date, NF membranes with different pore sizes and surface areas have been synthesized and commercialized for virus particles permitting the wide scale and straightforward application.

7.5 Viral markers

Opposed to the virus inactivation techniques resulting into killing of virus particles and leaving behind the markers such as antigens, coat proteins, and nucleic acids, the NF is potentially able to eliminate these viral signatures and could be utilized for process validation.

7.6 Toxicological assessment

The chemical processes generally used for the killing of viruses are health hazardous (mutagenic, carcinogenic, teratogenic) and pose negative impact to natural environment, requiring their precise detection and effective removal. Such toxicological assessments are not essential in case of NF-based virus elimination. However, the assessment of leaching of surface adhered virus particles in treated water should be taken into account as a priority during determination of NF process authentication.

In addition, the downstream contamination of treated water is rare in case of the NF techniques because of its application before the end of production process. Furthermore, the quality of production also remains unchanged while those based on heat or chemicals for elimination of virus particles may deteriorate the characteristics of treated water to an unacceptable level.

8. Future improvements in the technology

The combined application of different membrane-based technologies could be a promising approach over traditionally used methodologies to eliminate the pathogenic microbes resistant to chemical disinfection processes simultaneously with the effective inhibition of bacterial species remultiplication and removal of hazardous chemicals leading to water purification and meeting the standard criteria. Under real conditions, the NF technology may not absolutely serve as the strong barrier for human pathogens including bacteria and viruses (Adham et al., 1998). Furthermore, the functional efficiency may be reduced
with the passage of time because of formation of breaches in the membrane indicating the continuous monitoring of the membrane integrity. The breaches in membranes are generally produced through chemical or biological deterioration processes, aging, scratches caused by particle like substances, membrane fouling, detachment of membrane layers, improper fitting of connectors, O-ring, etc. Apart from abovementioned facts, the defects in membrane may also result during membrane manufacturing processes. Hence, testing the membrane integrity at certain interval of time would be helpful in maintaining the better efficiency of any membrane-based process for water purification targeting against the pathogenic microbes generally encountered in drinking water. The loading of membrane with silver nanoparticle could be a viable approach to stop the membrane biofouling, an important limiting factor diminishing the overall performance of separation process. However, there may be leaching of the loaded nanomaterials into the permeate at some extent. Further improvement in this direction would surely increase the separation efficiency of membrane and could be recommended for industrial scale application to disinfect the drinking water contaminated with different waterborne human pathogens.

9. Conclusions

The human pathogens transmitted through drinking water are one of the major causes of diseases and mortality at global scale. The conventional physicochemical techniques have limitations in separating many of the waterborne pathogens. NF technology on the other hand has shown promising application in purification of water contaminated with pathogenic bacteria, viruses, and protozoa. The discipline of NF technology has gained significant momentum in the academics and water processing industries. During the long course of laboratory studies, there was rapid rise in the number of research publications worldwide. Their ease in operation, high efficacy in removal of different pathogens, absence of DBPs, process flexibility, and robustness have promoted further research in separation technology to improve its overall performance in the area of membrane technology. However, the changes in membrane properties during the long run, clogging of filters, and leaching of adsorbed contaminants in permeate at some extent could limit its exploitation at industrial scale.

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Further reading

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