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Elimination of Pathogenic Bacteria from Milk Using Membrane Technology: A Review

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Abstract With technological advancements, membrane filtration is becoming increasingly popular in removing harmful microorganisms from milk and this article discusses many such efforts in a detailed manner. Retention of bacteria, vegetative spores and cells, even greater than 99% in most cases, is a clear indication of how efficient membrane technology is in removing these microorganisms from milk. It has been observed that in most of the cases, size exclusion acts as the dominant mechanism for achieving the desired separation. However, there are few studies that also reported about successful retention of bacterial cells from milk through the mechanism of electrostatic and hydrophilic/ hydrophobic interaction. An important aspect of implementing membrane for milk purification is that the presence of proteins and fat globules in milk causes severe membrane fouling and hence, this should be taken care of either by maintaining high cross flow velocity or generating back pulses during filtration. Besides, the microfiltration should also be carried out up to the critical microfiltration time so that the spores cannot germinate and contaminate the milk again. Moreover, an optimum pore size of the membrane is of the utmost importance for a proper balance between the membrane's rejection efficiency and the quantity of permeate produced, without compromising in milk's sensory and organoleptic attributes. It is worth mentioning that the use of third generation membranes with narrow pore size distribution is found to be fruitful in achieving good bacterial rejection from milk. Therefore, it can be inferred that with proper maintenance of all the above-mentioned factors, membrane filtration can definitely become a good alternative to conventional milk pasteurization process.

Keywords Membrane, Milk, Ceramic, Polymeric, Pathogen, Bacteria

1. Introduction

Milk can be considered as one of the most important fluids after water, upon which human lives are completely dependent. From an infant to an aged person, almost everyone except the lactose intolerant and vegan people use milk in their food in some way or the other. In fact, the demand for lactose free dairy products is also so high among the lactose intolerant people that lactose free dairy products are equally dominating the global dairy market. Milk, besides being a rich source of calcium, also provides other healthy nutrients such as protein, phosphorus, carotene, lactose, just to name a few. Drinking milk not only helps in the growth and development of the bones, but it can also be useful in preventing various undesired health consequences such as breast cancer, colon cancer, rickets, obesity in children, and so on [1]. It is worthy to mention that milk contains all the nine essential amino acids, which ultimately helps in maintenance of a good health through activities like blood glucose regulation, wound healing, nitrogen species scavenging, erythropoiesis, just to name a few [2,3]. The numerous advantages associated with drinking milk have resulted in an exponential increase in milk production worldwide and subsequent growth of the

dairy industry. It has been reported that the world has seen a significant increase in milk production from 530 million tonnes in 1988 to 843 million tonnes in 2018, which is more than 59%. India being the largest producer, contributes to almost 22% of world's production, followed by the European Union and United States of America (Figure 1) [4]. This huge quantity of milk production in different countries of the world as evident from Figure 1 is the clear indication of expansion of dairy industry in global market. It has been found in literature that the global market export value of milk has increased to 55.75 billion U.S. Dollars from 39.83 billion U.S. Dollars, thus signifying tremendous expansion of milk industry across the world [6]. Similarly, the lactose free dairy industry was also expected to earn an annual turnover of € 9 billion in 2022, as compared to € 6.33 billion in 2017 [7].

However, this huge quantity of milk produced needs to be purified before consumption as it may also be the shelter for various pathogens; some of them may cause serious health consequences to human being. Source of such pathogens can be multiple, starting from faeces of cattle that may eventually contaminate their teats and udder bladder to diseases like mastitis, which is a kind of bacterial infection affecting the cows [8]. Even milk can get contaminated during its storage as well as packaging. The presence of contaminants in milk reduces the shelf life of the product and can cause life hazards.

Pathogen such as *Clostridium tyrobutyricum* containing milk, when used for making cheese, can cause degradation of the quality of cheese produced due to the formation of fermentation by-products. Hence, it is of utmost importance to remove all the harmful bacterial spores from milk without the removal of necessary proteins and fats [9].

The conventional way of milk sterilization makes use of high temperature pasteurization. However, thermotolerant bacteria can withstand very high temperatures, making the pasteurization process an ineffective one. In some circumstances, the inactivated bacteria continue to release some enzymes, which may cause spoilage of the milk [10, 11]. Besides, the temperature rise may also cause change in phase, denaturation of protein as well as loss in sensory attributes of milk [12]. These disadvantages raised the need for an efficient alternative for bacteria removal from milk, bringing membrane filtration into the picture. This article, hence, describes many such efforts of milk purification through the use of membrane technology.

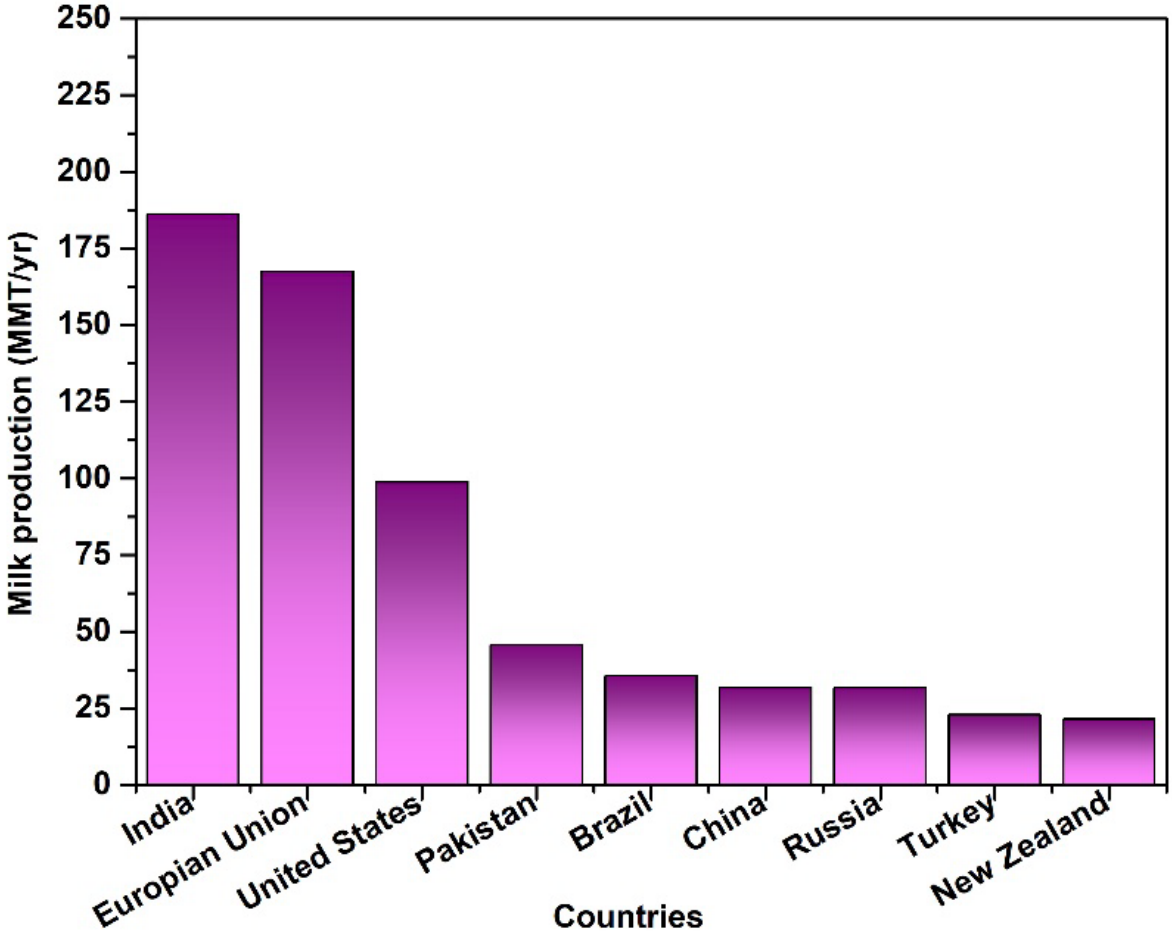

Figure 1. Country wise milk production in the year 2018 [5]

2. Microfiltration of Milk

As previously mentioned, contaminated milk can be the house of various bacteria such as *Pseudomonas aeruginosa, Bacillus anthracis, Bacillus cereus, Salmonella, Staphylococcus aureus, Clostridium botulinum,* and so on. These microorganisms, when entering into human body via food, can cause serious health consequences. Even the toxins released from some of these microorganisms can also lead to harmful consequences. For example, the toxins released by *Staphylococcus aureus* and *Clostridium botulinum* can lead to diseases like emesis and paralysis, respectively [13]. Table 1 demonstrates the detailed characteristics of various such bacterial cells, found predominantly in contaminated milk. As an efficient alternative to high temperature pasteurization due to the inherent disadvantages associated with the latter process, membrane microfiltration was adopted in the late-80s to sterilize milk before its use. It is to be mentioned that microfiltration uses the concept of passing the milk through porous membrane structures under a suitable pressure gradient for separating the microorganism present in milk [32]. Being an initiative in this area, the process adopted by Olesen et al. in late-80s was able to remove almost 99.99% of the total bacteria present in the feed milk [33]. Following the footsteps of Olesen, another group of scientists carried out microfiltration to separate *Salmonella* and *Listeria* cells from milk. These cells are representative of the pathogenic cells bearing the same nomenclature. *Salmonella* infection can induce symptoms like diarrhea, fever, etc., in human beings, while the infection from *Listeria* can be more fatal to human causing meningitis, abnormal birth consequences, miscarriages, etc. [34, 35]. A commercial membrane with 1.4 μm pore size was able to achieve log normal reductions (LRVs) up to 1.9 and 2.5 at 35 ˚C for *Listeria* and *Salmonella,* respectively. It was observed that with increasing temperature, retention of *Salmonella* increased while no significant variation was observed in the rejection of *Listeria,* the latter being more heat resistant. Moreover, it has also been mentioned that use of pre-microfiltered milk as feed resulted in lesser retention of the strains by the ceramic membrane. The probable reason for this is the reduction of milk components during pretreatment, which is responsible for membrane fouling. This reduction in thickness of fouling layer as a consequence of pretreatment results in reduced resistance for retention of strains on the membrane surface [36]. Though effect of milk temperature during milk microfiltration is known to have minimal effect on membrane's rejection performance, but it has pronounced effect on the quantity of permeate flux produced. It has been reported that an increase in feed temperature can significantly increase the quantity of permeate flux obtained. Literature mentioned about obtaining permeate flux of around 30 L/m^2h using cold microfiltration, whereas hot milk at 50°C, when microfiltered produces flux of approximately 200 L/m^2h [37]. This might be because of the pronounced reduction in the apparent viscosity of feed as well as permeate at higher temperatures [38]. An interesting fact to mention here is that use of high temperature milk as the feed for microfiltration can drastically increase the number of cleaning cycles and subsequent loss in the production step. This is because of the fact that at higher feed temperature, a sudden and steep decrease in the feed pH takes place. This sudden drop in pH leads to formation of a compact cake layer due to lesser repulsion amongst the deposited proteins and more intense protein-protein interactions amongst the casein micelles. However, the prolonged filtration time at lower feed temperature can never overcome the losses in the amount of permeate flux obtained at a comparatively higher feed temperature [39].

Table 1. Characteristics of bacteria found in milk

Bacteria	Sources	Size	Shape	Health consequences	References
Pseudomonas aeruginosa	Waste feed, contaminated teat dips, manure, and animal skin etc.	Diameter: 0.5-0.8 μm; Length: 1.5-3.0 μm	Rod	Infections in lung (pneumonia), blood etc.	[14, 15, 16]
Bacillus anthracis	Anthrax affected cattle	Diameter: 1.0-1.2 μm; Length: 3.0-5.0 μm	Rod	Anthrax	[17, 18]
Bacillus cereus	soil-contaminated udders and teats and the milking equipment	Diameter: 1.0 μm; Length: 3.0-4.0 μm	Rod	Diarrhea, Nausea, Vomiting	[19, 20, 21]
Salmonella	Faeces	Diameter: 0.5-1.5 μm; Length: 2.0-5.0 μm	Rod	Salmonellosis	[22, 23, 24]
Staphylococcus aureus	Mastitis affected cattle	1.0 μm	Spherical	Food intoxication	[25, 26, 27]
Clostridium tyrobutyricum	Faeces, colonized skin and hair of animals	Diameter: 1.1-1.6 μm; Length: 1.9-13.3 μm	Rod	-	[28, 29]
Clostridium botulinum	Feed, Water and other environmental factors	Diameter: 0.9-1.2 μm; Length: 4.0-6.0 μm	Rod	Paralysis, Death	[13, 30, 31]

Commercial Membralox ceramic membrane with a similar pore diameter was also used for this purpose. This membrane module, however, displayed significant reduction (four to five log cycles) in bacterial spores present in the feed. The results demonstrated that both the permeate and feed contain nearly the same concentration of other components (except for fat and casein protein) in the milk. Casein protein molecules and fat globules, having higher molecular weight could not pass through the small membrane pores and was retained on the membrane surface. Nevertheless, the permeate obtained showed the existence of the minute amount of fat globules in it, owing to the smaller size of some fat globules that had easy access through the membrane matrix to the permeate [40]. Other literature using the same pore sized membrane also reported similar pattern of bacterial retention [41]. Another study carried out by Holm et al. also showed promising results in bacteria retention by microfiltration membrane. In this study, milk was first separated into cream and skim milk portions, the latter being used for microfiltration. The microfiltered milk permeate could reduce the bacterial content by 99.7% and hence, it could be used for commercial purposes without further sterilization [42]. A similar approach was made by another group of researchers, where after separation of the cream from raw milk, the use of a 1.4 μm pore-sized ceramic microfiltration membrane could reduce the total bacterial count of skim milk by 2-3 LRV [43]. It is noteworthy to mention that the retentate obtained after microfiltration was not used for the final product development, unlike the Bactocatch process. In Bactocatch process, the retentate obtained is mixed with the separated cream, sterilized and then added to the permeate for final product development [42, 44]. As in the Bactocatch process, heat treatment is given to only a small portion of milk, the organoleptic and sensory properties of the milk are retained. This process, though is becoming popular due to no loss of sensory properties of milk, cannot guarantee complete removal of pathogenic bacteria from milk and hence, cannot be recommended as the safest process for getting pure milk free of any harmful pathogens [12, 45]. However, it should be kept in mind that while carrying out microfiltration of skim milk, it is always better to use higher cross flow velocities in conjunction with lower transmembrane pressure so as to reduce the membrane fouling, which can alter the selectivity of the membrane to a greater extent by retaining the important proteins present in milk. It has been reported that in case of multi-channel tubular membranes, velocity of fluid flowing through it follows a parabolic profile, with velocity being maximum at the center and gradually decreasing to zero in the boundary layer next to the membrane surface. Therefore, channels situated at the center of a multi-channel tubular membrane experiences higher fluid velocity as compared to the ones situated away from the center. This lower value of cross flow velocity, led to the formation of thicker cake layer on the outer channels, thus proving the importance of high velocity in reducing concentration polarization and subsequent membrane fouling (Figure 2) [46, 47]. Although higher cross flow velocity reduces the membrane fouling, however, it increases the operational cost of the process, making microfiltration an expensive process. In view of this, Jonsson and co-workers suggested using back shock with the reverse asymmetric membrane, which effectively reduces the intensity of concentration polarization of the membrane. In the case of the reverse asymmetric membrane, feed is allowed to pass through the support layer to skin layer. This causes deposition of contaminants inside the support layer, which can be removed by pulsations created by back shock. This method was proved to be very effective in obtaining higher LRVs at lower cross flow rates [9]. However, it should be kept in mind that there should be an optimum frequency for back pulsing, as too high or too low frequencies may not reduce the impact of fouling significantly. Experiments have revealed that a low value of back pulsing frequency may not be sufficient for scraping out the foulants from the membrane surface. Similarly, a very high value of back pulsing frequency may lead to abnormal increase in the quantity of permeate produced, thus forcing the foulants to move along with the permeate and get deposited on the membrane surface. Besides, it has also been reported that a pulse with shorter duration is more beneficial in removing the fouling layer from membrane surface as compared to a pulse with longer duration [48]. The significant effect of back pulsing on membrane's operating time can be understood from Figure 3. Therefore, it can be inferred that back pulsing can be opted for partial cleaning of membrane by periodic reversal of transmembrane pressure during microfiltration [49].

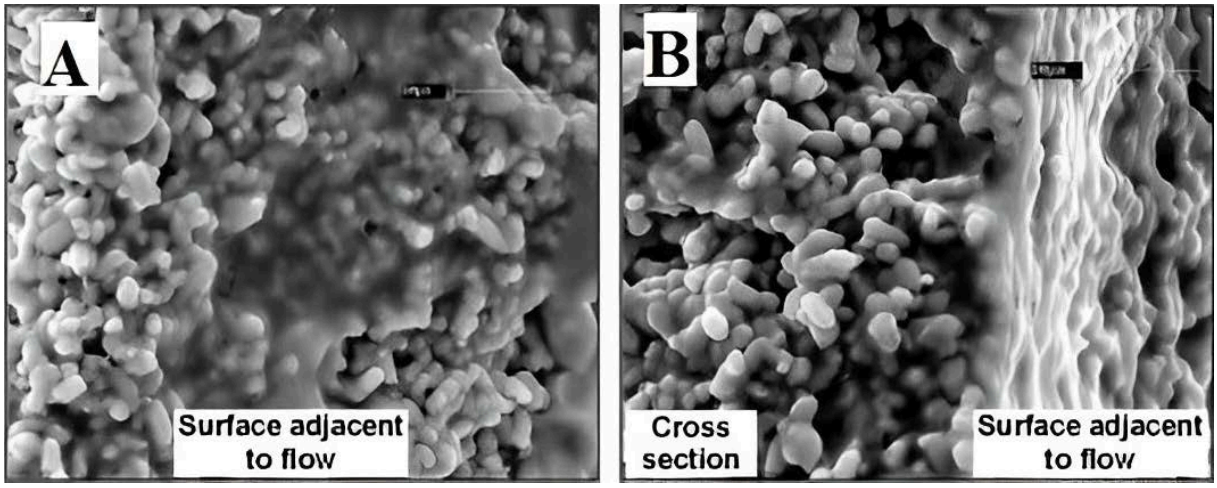

Figure 2. SEM images of ceramic membrane sections to show the effect of cross flow velocity [A – inner channel (high velocity); B – outer channel (low velocity)] [47]

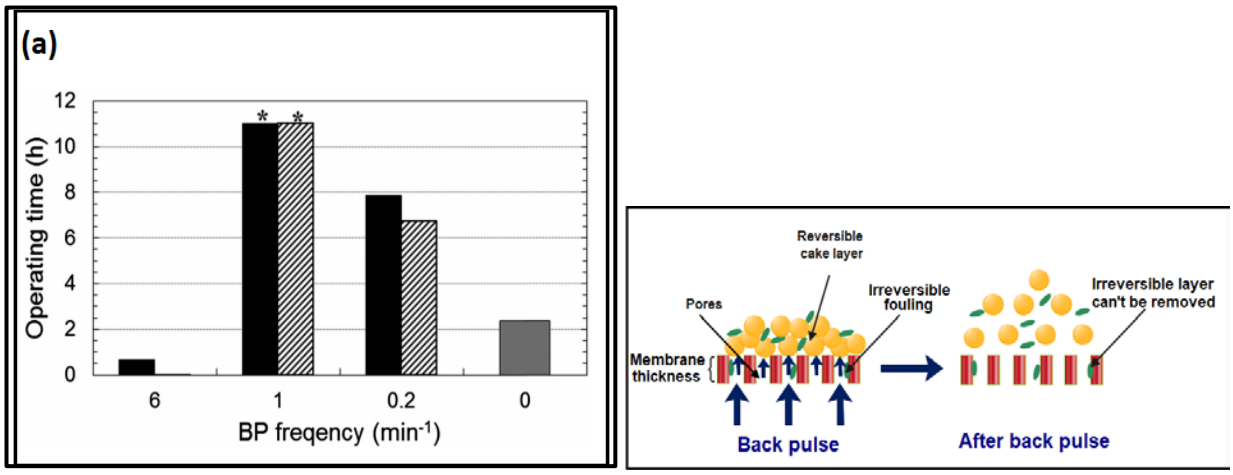

Figure 3. **(**a) Variation in membrane operating time with the use of back-pulse of duration 0.2 s (black bars) and 2.0 s (hatched bars) at different frequencies (Cross-flow velocity = 5.5 ms^{-1}). The grey bar represents result of the reference experiment without back-pulse (asterisk signifies that the experiments ran for 11 h without significant fouling [48] (b) Mechanism of back pulse (Schematic)

Among various third generation membranes with same mean pore diameter, the ones with narrower pore size distribution is found to show better microbial retention efficiency as compared to the ones with broader pore size distribution. This significant variation can be observed in case of Membralox and Sterilox membranes, where both the membranes possess similar mean pore size. But it is the much narrow pore size distribution of Sterilox membrane, which helped it to achieve 3-4 log removal of bacteria from milk as compared to Membralox membrane, which could achieve only 2-3 log removal [50].

Pasteurization after microfiltration is also considered as one of the effective ways to reduce the bacterial count in milk. Milk sterilized through this process is found to have higher pH and lower value of titratable acidity, signifying high stability of the milk. Nevertheless, with increasing time, pH of the microfiltered milk also starts decreasing as a result of either Maillard reaction or proteolysis of the milk proteins. The extent of proteolysis, however, depends on the content of the somatic cell in the milk. The particle size of milk was seen to increase with increasing time due to agglomeration and followed a monodisperse variation [51].

Elwell and co-researchers also drew similar inferences from a series of trials conducted to remove bacterial spores from the milk via microfiltration. Their investigation declared that the storage temperature of the milk is an important parameter for increasing its shelf life. It was observed that microfiltered milk, when stored at a lower temperature, delays the growth rate of bacteria, enhancing the shelf life [52]. A smaller pore-sized polyvinylidene fluoride membrane was also used to investigate its microfiltration ability in separating bacterial spores from skim milk. The membrane with 0.22 µm pore size retained almost 99% of the total bacteria present in the milk, probably through the principle of size exclusion, without even affecting its taste. Removal of bacterial spores also enhanced the shelf life of the milk [10]. Tomasula et al.

reported that microfiltration carried out with 0.8 µm membrane also portrayed promising results in attaining log normal reductions above five. However, lesser pore sized membranes have the disadvantage of retaining casein protein of milk as the molecular weight of casein protein is very high [53, 54]. Moreover, the flux obtained through such membranes is also very low in quantity. Observing these disadvantages, most of the research groups used a membrane having a mean pore size of 1.4 µm for the microfiltration of the milk, which can facilitate satisfactory removal of bacterial contaminants from milk without compromising on the flux or retention of healthy milk components [55, 56, 57]. Another inference obtained from the study carried out by Tomasula et al. points out that microfiltration should always be carried up to the critical microfiltration time; otherwise germination of spores may take place in the microfiltered milk. The high shear experienced during the membrane filtration process causes the spores to pass through the membrane pores causing damage to the spores itself. These spores, when remain in microfiltered milk for a long duration, may germinate and cause spoilage of milk. It has also been found that the permeate obtained after microfiltration should rather be stored at lower temperatures than pasteurizing at higher temperatures, because heat shock aids in germination of damaged spores in the milk permeate [55].

Another fact worth mentioning is that though in most of the cases the size exclusion mechanism plays the key role in efficient microbial separation from milk, but there are some other mechanisms too that may affect the bacterial retention performance of a ceramic membrane. Surface charge and its hydrophilicity are two such properties, which can play a pivotal role in achieving successful separation of bacteria from milk through membrane. Experiments conducted using *Bacillus licheniformis* and *Geobacillus sp.* revealed that both these microbial cells as well as the membrane surface became negatively charged at the ionic strength of milk, thus causing electrostatic repulsion to occur between the microbes and the membrane surface. This repulsive force between the two prevents the microbial cells to come near the membrane surface and penetrate through it. Another crucial factor determining the membrane's microbial retention performance is the hydrophilic property of the bacterial cells and the membrane surface. It has been well-reported in literature that the bacterial cells can either be hydrophobic or hydrophilic in nature. Again, the ceramic membranes are usually considered to be quite hydrophilic, though adsorption of various protein molecules on membrane surface during microfiltration makes it hydrophobic during later stages of microfiltration. In such a situation, when the properties of the membrane surface as well as the bacterial population becomes similar to each other, the repulsive force acting between the two restricts passage of cells through membrane pores, thereby enhancing bacterial rejection capacity of the membrane [58].

The schematic representation of various probable mechanisms of bacterial retention using membrane technology is illustrated in Figure 4. Moreover, the literature available regarding the retention of bacteria present in the milk using membrane filtration technology is presented in Table 2.

3. Conclusions and Future Prospects

Membrane filtration is observed to be an efficient alternative for the separation of bacteria and spores from milk. It has been found that most of the research groups have opted for membranes with a pore size of 1.4 µm, as very big pore sized membranes reduce bacterial retention and very low pore sized membranes accelerate membrane fouling along with increasing casein retention. It has also been observed that along with pore size and fouling, cross flow velocity and time of filtration also play a key role in determining membrane performance. However, all the membranes used in the literature are commercial ones, either ceramic or polymeric, thus increasing the cost of the process [59]. Moreover, the use of polymeric membranes cannot be appreciated as they are highly unstable in harsh environments [60, 61]. As in the milk industry, the use of high temperature may be required in many circumstances; hence the use of polymeric membranes cannot be appreciated. The above-mentioned issues, can however be addressed by the use of low-cost ceramic membranes made using naturally available materials such as clays, fly ash, just to name a few. Use of low-cost ceramic membranes has already been started in most of the separation processes and such processes are seen to have good separation efficiencies too [62, 63]. Besides that, use of naturally available clays in membrane manufacturing being a cost effective phenomenon, is an example of process intensification, which ultimately signifies a sustainable process. Membrane filtration, is itself a sustainable technology with higher selectivity, better yield, lesser operating cost as well as energy consumption associated with it [64]. Along with that, the use of naturally available low-cost minerals further drastically reduces the cost of the process. Moreover, use of materials like fly ash helps in mitigating the environmental issues caused by its improper disposal [65]. Moreover, utilizing such type of materials reduces the extraction of valuable compounds of earth crust such as bauxite, primarily used for producing alumina, which is widely used for ceramic membrane manufacturing [66, 67, 68]. The research works of Bouazizi et al., Vasanth et al., Singh and Bulasara are some examples of successful implementation of low-cost ceramic membranes in separation processes. These works mentioned about manufacturing membranes with average pore sizes in the range of 1.2-1.4 µm using naturally available materials such as Moroccan clay, kaolin, fly ash etc., which were quite successful when implemented in various separation

processes such as treatment of oil-in-water emulsion, bacteria and dye removal from water, and so on [69, 70, 71, 72]. The most important feature about these membranes is that the pore size of these membranes lies in the range of pore diameter preferred for carrying out microfiltration of milk for bacteria removal and hence, it can be thought that these membranes will be instrumental in carrying out successful microfiltration of milk, without even compromising with the quality as well as the quantity of permeate obtained. The kaolin based ceramic membrane made by Kumar et al. has a pore size of 0.309 µm and was successfully used for treating dairy wastewater [73]. Similarly, research works of Kaniganti et al. mentions about the use of 1.5 µm pore sized low-cost ceramic membrane in separation of bacteria from its solution and reported 100% rejection efficiency [74]. Vasanth et al. also implemented kaolin based ceramic membrane with an average pore size of 1.3 µm in separation of bacteria from water and achieved very good retention of bacterial spores [70]. Therefore, it can be concluded that use of these membranes made using naturally available raw materials should be strongly recommended for a sustainable milk microfiltration process.

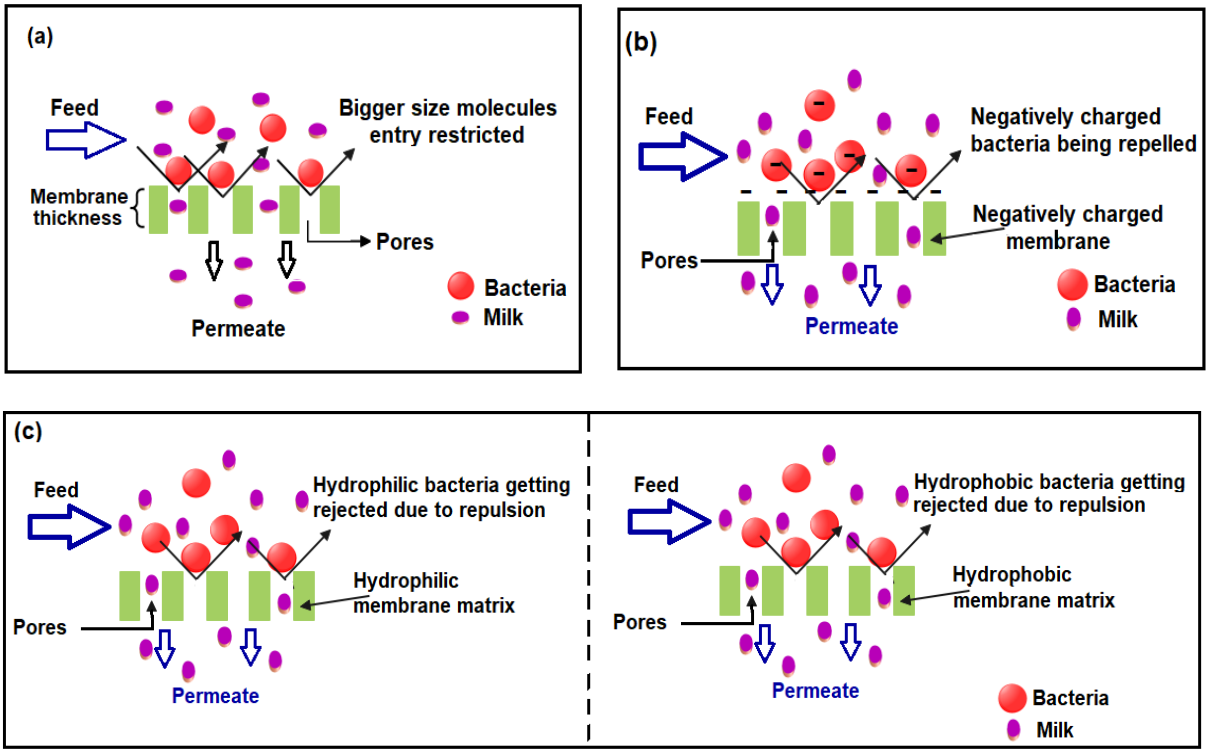

Figure 4. Different mechanisms for bacteria removal from milk using membrane technology (a) Size exclusion process (b) Electrostatic interaction (c) Hydrophobic/ hydrophilic interaction

Table 2. Summary of literature regarding microfiltration of milk for bacteria rejection

Membrane	Pore size	Bacteria	Conditions	Rejection	Reference
Membralox filter	1.4 µm	-	C_f: 10^8-10^9 CFU/mL; pH: 6.7±0.1; TMP: 1 bar	4-5 LRV	[40]
Membralox cartridge filter	1.4 µm	*Salmonella* and *Listeria* cells	T= 35 ˚C; C_f: 10^2-10^6 CFU/mL	*Salmonella*: 2.5 LRV *Listeria*: 1.9 LRV	[36]
Membralox multi-channel ceramic filter	1.4 µm; 0.8 µm	*Bacillus anthracis*	CFV: 6.2 m/s; TMP: 127.6 kPa	4.5±0.35 LRV; 5.91±0.05 LRV	[53]
Ceraflo α-alumina ceramic membrane (asymmetric); hollow fiber (polyethersulfone-polyvinylpyrrolidone) membrane (reverse asymmetric)	1 µm (asymmetric membrane); 0.54 and 0.87 µm (revere asymmetric membrane)	*Clostridium tyrobutyricum; Bacillus cereus*	-	4-5 LRV	[9]
Commercial Inside ceram (ZrO_2- TiO_2/TiO_2), Isoflux (TiO_2/TiO_2, Sterilox-GP (multi-layer α-alumina) membrane	1.4 µm	-	CFV: 6m/s and T = (21±2) ˚C; C_f: 50000-100000 CFU/mL	3.5-5 LRV	[58]
Commercial ceramic membrane	1.4 µm	-	T = 50 ˚C	High SCC[1]: TBC[2]: 3.56 LRV PBC[3]: 1.9 LRV Low SCC: TBC: 3.57 LRV PBC: 1.61 LRV	[51]
Membralox ceramic filter	1.4 µm	-	T = 50 ˚C; permeate pasteurized at 72 ˚C; C_f: 1475-3600 CFU/mL	3.79 LRV (without pasteurization); 5.63 LRV (with pasteurization)	[52]
Commercial Millipore polyvinyledenefluoride	0.22 µm	Vegetative cells and spores	-	99.1-99.5%	[10]
Commercial tubular membrane (TAMI)	1.4 µm	-	T = 6-7 ˚C; CFV: 5-7 m/s; TMP: 1.3 bar	>4 LRV	[46]

1 SCC: Somatic cells count;
2 TBC: Total bacteria count
3 PBC: Psychrotrophic bacterial count

Table 2 Continued

Membralox ceramic membrane	1.4 µm	-	T = 50 ˚C	2-3 LRV	[43]
Societe des Ceramiques Techniques α-Alumina membrane	1.4 µm	*E.Coli, B. Cereus*	T = 40 ˚C (Beginning); 60 ˚C (End)	99.7%	[42]
Commercial tubular membrane (TAMI)	1.4 µm	Vegetative cells	T = 6±1 ˚C; CFV: 7 m/s; TMP: 75.8 kPa; C_f: 4.11± 0.48 CFU/mL	3.4 LRV	[38]
Isoflux tubular membrane	1.2 µm	*Bacillus licheniformis; Geobacillus sp.*	T = 6 ˚C; CFV: 4.1 m/s; TMP: 69-74 kPa; C_f: 10^6 CFU/mL	*Bacillus licheniformis*:4.57 LRV; *Geobacillus sp:* > 6 LRV	[59]
-	1.4 µm	*Bacillus cereus*	-	> 3.5 LRV	[33]
Alumina Membralox membrane	1.4 µm	*Clostridium tyrobutyricum*	T = 50±1 ˚C	> 4.5 LRV	[41]
Membralox XLAB3 membrane	1.4 µm	-	T = 40 ˚C; CFV: 7 m/s	> 3LRV	[49]
Isoflux tubular membrane	0.8 µm, 1.4 µm	-	T = 50 ˚C; P: 75 kPa	3 LRV	[54]

Nomenclature:

LRV	Log reduction value
C_f	Feed concentration
T	Temperature
TMP	Transmembrane pressure
SCC	Somatic cells count
TBC	Total bacteria count
PBC	Psychrotrophic bacterial count
CFU	Colony forming unit

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