Nanocellulose-Based Membrane Filtration Material

Subjects: Microbiology
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Nanocellulose is among the emerging materials of this century and several studies have proven its use in filtering microbes. Its high specific surface area enables the adsorption of various microbial species, and its innate porosity can separate various molecules and retain microbial objects.

Keywords: nanocellulose ; membrane filter ; microbes ; surface functionalization

1. Introduction

Throughout the evolutionary process, among the significant issues faced by society today are the protection of natural resources and the implementation of eco-friendly approaches to sustaining a high quality of life. Environmental pollution is a worldwide concern and the majority of pollutants have long-term negative impacts on humans. Focusing on microbial pollution, the most common bulk transportation media for particulate contaminants are air and water. Microbes are microscopic living organisms that can be found everywhere, including in water, soil and air, but they are too small to be seen with the naked eye. These microbes are commonly viruses, bacteria, and fungi and may involve microscopic parasites. Certain microbes are harmful to our health, while others are beneficial. **Table 1** shows several types of infectious diseases caused by microbes.

Table 1. Several infectious diseases caused by microbes.

Infectious Disease	Microbe That Causes the Disease	Type of Microbe	Reference
Coronavirus (COVID-19)	**Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)**	**Virus**	[1]
Cold	**Rhinovirus**	**Virus**	[2]
Chickenpox	***Varicella zoster***	**Virus**	[3]
German measles	**Rubella**	**Virus**	[4]
Whooping cough	***Bordatella pertussis***	**Bacteria**	[5]
Bubonic plague	***Yersinia pestis***	**Bacteria**	[6]
TB (Tuberculosis)	***Mycobacterium tuberculosis***	**Bacteria**	[7]
Malaria	***Plasmodium falciparum***	**Protozoa**	[8]
Tinea barbae (dermatophyte infection)	***Trichophyton rubrum***	**Fungus**	[9]
Athletes' foot	***Trichophyton mentagrophytes***	**Fungus**	[10]

Microbes can also be transmitted through the air. According to the World Health Organization (WHO), airborne transmission differs from droplet transmission as it refers to the presence of microbes within droplet nuclei that are typically less than 5 μm in diameter and can circulate in the air for significant periods and be transmitted to others over distances more than 1 m [11]. Whereas droplet transmission occurs when a person is in close contact (within 1 m) with a symptomatic patient with respiratory symptoms such as coughing or sneezing and is thus at risk of exposure to potentially infective respiratory droplets (typically >5–10 μm in diameter). Nowadays, the threat of the newly discovered infectious coronavirus disease (COVID-19) is worrying, as this pandemic outbreak has already killed millions of people worldwide. The outbreak is exacerbated by the occurrence of frequent mutations, which makes it difficult to rapidly produce omnipotent vaccines [1]. Therefore, an effective, robust, and inexpensive air-borne virus removal membrane filter is an urgent need to provide a means to prevent virus spread in hospitals, transportation hubs, schools, and other venues with high social traffic turn-over in order to minimize the risks arising from the COVID-19 pandemic.

Microbe removal can be done through a variety of methods, such as, filtration (either depth filtration or surface screening), partitioning and fractionation (centrifugation), and chromatography (ion-exchange, affinity, gel permeation) [12]. Of these different techniques, filtration is a desirable choice, as it is non-destructive and non-interfering, implying that it will not threaten the quality of biological samples or induce immune reactions. Membrane filters have been made from a variety of synthetic and semi-synthetic polymers, designed to achieve a desired filtration pore size. The membrane filter is also an effective and widely used method for detecting microbiological pollution in collection samples. It requires less planning than certain other conventional methods and is one of the few methods that allows for microorganism separation and subsequent determination. Microbes cannot be retained by the normal membrane filter because the membrane pores are too large. Therefore, it is critical to have a more effective material for microbe filtration, and there are studies that have led to the discovery of new filtering media made from cellulose with efficient filtration capability. The ultimate objective would be to be able to effectively and securely filter microbes from the environment at an affordable cost.

2. Recent Developments on Nanocellulose as a Filtration Material against Microbes

Several developments concerning nanocellulose-based membrane filters capable of removing microbes will be reviewed. An important aspect of the modification of nanocellulose materials is to increase the binding affinity of the materials towards microbes. There are a number of studies that focused on the filtration removal of viruses and bacteria; however, very limited studies have been conducted, which concern other types of microbes, such as fungi, algae and protozoa.

2.1. Viruses

The ensnarement of viruses is one of the most crucial steps in biopharmaceutical and clinical processes and applications [13]. Of the various types of microbes, virus is among the smallest and most difficult to deal with, as compared to other microbes.

Exploration of nanocellulose as a filtration material against several types of viruses has received much research attention. As mentioned earlier, several viruses, including COVID-19, are airborne viruses that can be dispersed and spread through human nasal or saliva secretions from an infected person. Therefore, in order to minimize infection risks from viruses, an efficient, robust and affordable air-borne virus removal filter is an urgent requirement. Multiple research articles were recently published with regard to this type of air filter.

Several factors, such as filter thickness, pore size, number of layers, size of the virus, the charge on the filter surface, its ionic strength and surface chemistry are usually influenced by the efficiency of the air filtration process [12]. Generally, the use of size-exclusion type filtration has several benefits, such as flexibility and ease of use since it provides virus removal predictability through its physical properties, allows for the filtration of viral markers, enabling easy validation of the filtration process, and does not use toxic or mutagenic chemicals for viral inactivation [12][14][15].

Gustafsson et al. (2018) [16] evaluated membrane filter made from nanocellulose in a mille-feuille arrangement of varying thicknesses using a simulated wastewater matrix to explore its ability to remove viruses for drinking water purification applications. The filtrations of various samples of simulated wastewater with its total suspended solid content being 30 nm latex particles as surrogate waste material and 28 nm ΦX174 bacteriophages as the viral contamination. The authors examined the performance of these membrane filters at a pressure of 1 and 3 bar with varying thicknesses of 9 and 29 µm. The data they obtained demonstrate that a membrane filter made from 100% nanocellulose has the capacity to efficiently remove even the smallest of viruses, with up to 99.9980–99.9995%.

Manukyan et al. (2019) [17] fabricated nanocellulose-based mille-feuille type membrane filter for use in upstream applications for serum-free growth media filtration and it was designed to remove ΦX174 bacteriophages. The filter performance was evaluated based on its ability to filter small–medium-sized viruses using varying thicknesses of the fabricated membrane filter (i.e., 11 and 33 µm), as well as by varying the operating pressures (i.e., 1 and 3 bar). Based on their results, the 33 µm thick filter showed more stability and had better virus removal as compared to the 11 µm thick filter, although their flux was nominally lower. The findings of this study suggest that the nanocellulose membrane filter would be a viable alternative for the filtration of large volumes of cell culture media in upstream bioprocessing.

Asper et al. (2015) [18], in their study, used a membrane filter composed of 100% CNF to remove xenotropic murine leukaemia virus (xMuLV). It was found that the particle retention properties of the nanocellulose membrane filter were verified following the filtration of 100 nm latex beads, as shown in **Figure 1**. The results of this filtration of xMuLV suggested that the nanocellulose membrane filter was useful for removal of endogenous rodent retroviruses and retrovirus-like particles during the production of recombinant proteins.

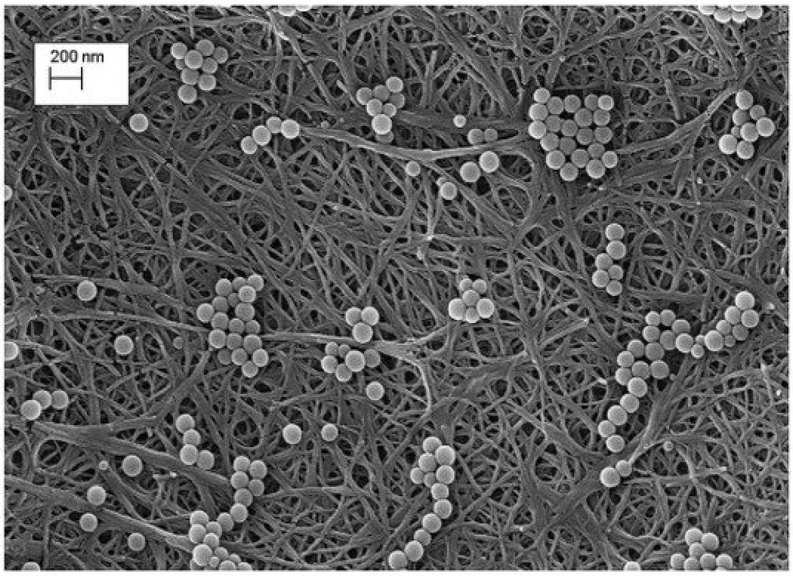

Figure 1. SEM images of 100 nm latex beads retained on the nanocellulose membrane filter. Reproduced with permission from [18].

Metreveli et al. (2014) [12] reported that one of the most challenging tasks for designing the virus removal membrane filter is tailoring the membrane upper pore size cut-off so that the filter retains viruses with a particle size of between 12 and 300 nm, while allowing for the unhindered passage of proteins which typically range between 4 and 12 nm in size. Therefore, high porosity of the nanocellulose-based filters is required to circumvent the problem of low permeance. In their study, the developed nanocellulose membrane filter, sized at 70 μm with a total porosity of 35%, was able to remove Swine Influenza A Virus (SIV), which had a particle size of 80–120 nm. The latex beads and SIV particles are observed as stacked structures on the surface of the porous membrane filter. They also found that the proteins pass unhindered through the membrane filter. Therefore, the pore size distribution presented in their work is promising for virus filtration applications, especially for large viruses ≥50 nm.

Besides this, Mautner et al. (2021) [19] also produced BNC membrane filters with high porosity for optimized permeance and rejection of nm-pollutants. The BNC was treated with organic liquids (alcohol, ketone, ether) before being further processed into the membrane filter. The treated BNC membrane filter has a porosity of 67%, which is higher than the untreated BNC membrane filter (33%). It also exhibits 40 times higher permeance, caused by a lower membrane density. Despite their higher porosity, the developed membrane filter also still has pore sizes of 15–20 nm, which is similar to the untreated BNC membrane filter. Thus, the developed membrane filter enables the removal of viruses by a size-exclusion mechanism at high permeance.

The strength of the nanocellulose is also important in designing a good membrane filter to remove viruses via a size exclusion mechanism. A study by Quellmalz and Mihranyan, (2015) [20] found that the citric acid cross-linked nanocellulose-based membrane filter has better mechanical performance than the untreated nanocellulose. It was observed that the untreated nanocellulose membrane filter was readily cracked at pressure gradients above 15 kPa, which could be limiting for its industrial application. The improved strength of the cross-linked nanocellulose membrane filter enables increasing the pressure gradient applied for filtration without compromising the integrity of the filter. It is concluded that citric acid cross-linking of nanocellulose is beneficial to be used in several industrial applications for removing viruses.

Previous studies on the surface modification of nanocellulose have led to the improvement of filtration efficiency against viruses. Electrostatic interaction between nanocellulose and viruses is improved dramatically with the incorporation of quaternary compounds as discussed. For instance, viruses such as coronavirus have a negatively charged surface and would interact with the cationic or anionic charge of nanocellulose-quaternary compounds [21]. **Figure 2** shows a schematic diagram of the coronavirus structure with proteins embedded in its bilayer membrane and negatively charged lipid head groups protruding to the outer side of the membrane.

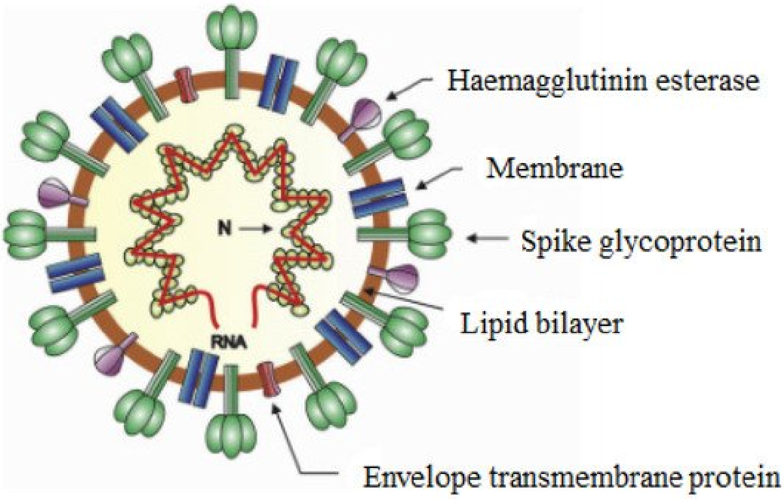

Figure 2. A structure design of coronavirus particle. Reproduced with permission from [22].

The entrapment of the virus onto nanocellulose matrix is due to the presence of electrostatic force attraction between the negatively charged virus particle and the positively charged nanocellulose membrane. Several studies have demonstrates successful results in filtering negatively charged viruses using cationic nanocellulose. For example, Mi et al. (2020) [23] developed a filtration setup using modified CNC with a positively charged guanidine group to adsorb porcine parvovirus and Sindbis virus and to completely filter out those viruses from water. It is interesting to point out that this filtration system has exceeded the Environment Protection Agency (EPA) virus removal standard requirement for portable water. In addition to the electrostatic interaction between the virus and guanidine group, Meingast and Heldt (2020) [24] outlined that the complete virus removal from water was also due to the protonated guanidine groups on the cationic CNC forming ionic and hydrogen bonds with the proteins and lipids on the virus surface.

Other than that, Rosilo et al. (2014) [25] in their study observed a very high affinity binding between the cationic CNC (known as CNC-g-P(QDMAEMA)s) mixture and cowpea chlorotic mottle virus (CCMV) and norovirus-like particles in water dispersions. Of note, this cationic CNC mixture was prepared by surface-initiated atom-transfer radical polymerization of poly(N,N-dimethylamino ethyl methacrylate) and its subsequent quaternization of the polymer pendant amino groups.

In addition, the anionic CCMVs could also be removed using functionalized lignin with a quaternary amine. In their study, they found that the CCMVs would form agglomerated complexes with cationic lignin [26]. Therefore, suggesting its potential use as material in the development of membrane filter for the removal of CCMVs.

Besides that, Sun et al. (2020) [27] reported that covalent modification on CNF (i.e., functionalization of nanocellulose) using polyglutamic acid (PGS) and mesoporous silica nanoparticles (MSNs) resulted in the successful filtration of EV71 virus and Sindbis virus. This is particularly due to the interaction between two exposed positively charged amino acids (His10 and Lys14) and the negatively charged MSNs on the modified CNF [27].

Table 2 summarizes the development of nanocellulose-based membrane filtration material for virus removal that have been discovered/explored so far. In addition to the guanidine groups, lignin, nanoparticles, and citric acid, nanocellulose could also be functionalized with several other compounds, such as small organic molecules, porphyrin dendrimers and others polymers in order to make it positively or negatively charged [28]. However, it is important to note that not all of these examples have been tested as a filter to remove viruses. It can be summarized that several present studies have shown the capability of nanocellulose as a filtration material for virus removal. Separation by size exclusion and adsorption mechanism are the most common approaches. Factors such as pore size distribution, porosity, thickness, strength and surface functionalization of nanocellulose can greatly influence the filtration efficiency.

Table 2. Nanocellulose developed filtration material for virus removal.

Microbes	Type of Nanocellulose	Functionalization	Findings	Reference
A/swine/Sweden/9706/2010 (H1N2)—Swine influenza	**BNC**	**Not applicable**	• The newly developed non-woven, μm thick membrane filter consisting of crystalline BNC able to remove virus particles solely based on the size-exclusion principle, with a log 10 reduction value ≥6.3, thereby matching the performance of industrial synthetic polymer virus removal filters currently in use.	[12]
Xenotropic murine	**BNC**	**Not applicable**	• The developed BNC membrane filter could remove the endogenous rodent retroviruses and retrovirus-like particles.	[18]
MS2 viruses	**BNC**	**Not applicable**	• This study highlights the efficiency of the nanocellulose-based membrane filter in removing/filtering out the ΦX174 bacteriophage with value of 5−6 log virus clearance (28 nm; pI 6.6).	[29]
ColiphagesΦX174	**BNC**	**Not applicable**	• The nanocellulose-based membrane filter exhibited 5−6 log virus clearance of MS2 viruses (27 nm; pI 3.9). This study also showed the possibility of producing cost-efficient viral removal filters (i.e., manufacturing process).	[29]
Parvoviruses	**BNC**	**Not applicable**	• The developed filter was the first non-woven, wet-laid membrane filter composed of 100% native cellulose. This study showed that the non-enveloped parvoviruses could be eliminated using this filter.	[30]
EV71	**CNF**	**Polyglutamic acid and mesoporous silica nanoparticles**	• This study showed that the modified microfibers could strongly adsorb the epitope of the EV71 capsid which is useful for virus removal.	[27]
Sindbis virus	**CNC**	**Guanidine**	• Functionalization of guanidine on CNC resulted in over 4 log removal value against the Sindbis virus.	[23]

Microbes	Type of Nanocellulose	Functionalization	Findings	Reference
Porcine parvo virus	**CNC**	**Guanidine**	• Authors also revealed that functionalization of guanidine on CNC managed to remove the Porcine parvo virus with over 4 log removal value.	[23]

2.2. Bacteria

The development of nanocellulose as a filtration material against bacteria also been widely discovered. Generally, the diameter of most waterborne bacteria is larger than 0.2 μm [31]. Thereby, it would be easy for nanocellulose-based membrane filters to entrap most bacteria species using the size-exclusion mechanism. Moreover, the modification of nanocellulose by surface functionalization can also be performed to increase the removal efficiency of bacteria. In this review, we highlight several findings concerning bacterial removal using nanocellulose based membrane filters.

Wang et al. (2013) [32] demonstrated that a multi-layered nanofibrous microfiltration system with high flux, low-pressure drops and high retention capability against bacteria (*Brevundimonas diminuta* and *Escherichia coli*) was possible by impregnating ultrafine CNF into an electrospun polyacrylonitrile (PAN) nanofibrous scaffold supported by a poly (ethylene terephthalate) (PET) non-woven substrate. The CNF was functionalized prior to impregnation with carboxylate and aldehyde groups using TEMPO oxidation. It was observed that this CNF-based microfiltration membrane exhibited full retention capability against those bacteria.

Otoni et al. (2019) [33] developed a cationic CNF compound using Girard’s reagent T (GRT) and shaped it into foam using several protocols, such as cryo-templating to remove the ubiquitous human pathogen *Escherichia coli*. The porosity of this foam, which is associated directly with its surface area and pore size plays a significant role in the removal of *Escherichia coli*. The cryogel foams produced by this method had porosities of *circa* 98% and were established to be able to achieve an approximately 85% higher anti *Escherichia coli* activity when compared to sample foams made up of unmodified CNF. The cationic CNF using GRT demonstrated good potential for both air and liquid filtration, with excellent absorbency through functional coating. Access to safe drinking water in high- and low-income countries has become one of the biggest challenges in the world as natural resources become scarcer.

Gouda et al. (2014) [34] invented a modified electrospun CNF containing silver nanoparticles (AgNPs) as a water disinfecting system for water purification systems. The AgNP content, physical characterization, surface morphology and antimicrobial efficacy of the developed membrane filter were then studied. AgNP, which belongs to the group of biocidal nanoparticles, has antimicrobial properties and is commonly used due to its size quantization effect. This can cause a shift in the reactivity of metals in the nanoscale. The developed membrane filter had an excellent ability to remove bacteria, including *Escherichia coli*, *Salmonella typhi*, and *Staphylococcus aureus* with a percentage filtration of more than 91% in contaminated water samples.

Ottenhall et al. (2018) [35] developed a CNF-based membrane filter, modified with polyelectrolyte multilayers to produce multilayer cationic polyvinyl amine (PVAm) and anionic polyacrylic acid (PAA). The authors successfully modified the CNF with cationic polyelectrolyte PVAm, together with the anionic polyelectrolyte PAA in either single layers or multilayers (3 or 5 layers) using a water-based process at room temperature. Based on filtration analysis, the functionalized CNF-based membrane filters with several layers were physically able to remove more than 99.9% of *Escherichia coli* from water. The three-layer membrane filter could remove more than 97% of cultivatable bacteria from natural water samples, which was a remarkable performance, as compared with the simple processing technique using plain nanocellulose filters.

Table 3 summarizes the effectiveness of nanocellulose-based membrane filters that have been functionalized with bioactive compounds for the removal of bacteria. It can be concluded that bacterial separation by size exclusion mechanism is easier as compared to the virus. This is because the size of bacteria is usually larger as compared to a virus. The surface functionalization on nanocellulose is capable of introducing anti-bacterial properties to the developed filtration material. However, limited studies were reported for the removal of other bacteria species using nanocellulose-based filtration material.

Table 3. Nanocellulose developed filtration material for bacterial removal.

Microbes	Type of Nanocellulose	Functionalization	Findings	Reference
Escherichia coli	**CNC**	**Silver nanoparticles**	• It possesses high adsorption capacity and is reusable. Beneficial in total removal of *Escherichia coli*.	[36]
Bacillus subtilis* and *Escherichia coli	**CNF**	**ZnO and CeO_2**	• It has high anti-bacterial activity, MIC_{50} against Bacillus *subtilis* (10.6 μg mL^{-1}) and *Escherichia coli* (10.3 μg mL^{-1}).	[37]
Escherichia coli	**BNC**	**Not applicable**	• The significance of Brownian motion caused by microorganisms captured with BNC-based membrane filter through theoretical modelling and filtration experiments was investigated by the authors. • It was found that the BNC-based filter was capable of filtering the bacteria.	[38]
Escherichia coli*, *Staphylococcus aureus	**CNF**	**Activated carbon**	• The two-layer AC/OCNF/CNF membrane able to remove *Escherichia coli* bacteria up to ~96–99% and inhibits the growth of *Escherichia coli* and *Staphylococcus aureus* on the upper CNF surface	[39]
Escherichia coli	**BNC**	**Silver nanoparticle**	• Higher amount of silver nanoparticles loaded onto the BNC membrane surface could increase the inhibition zone hence highlighting its good antimicrobial property against *Escherichia coli*.	[40]
Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa	**BNC**	**Silver nanoparticle**	• BNC-silver nanoparticle membrane showed strong antimicrobial activity against Gram positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria.	[41]
Escherichia coli, Staphylococcus aureus	**BNC**	**Silver nanoparticle**	• The developed Ag/BNC membrane exhibited good property as antimicrobial agent against *Escherichia coli* and *Staphylococcus aureus* as the antibacterial ratio against *Escherichia coli* and *Staphylococcus aureus* reached 99.4% and 98.4%, respectively.	[42]
Escherichia coli	**CNF**	**Polyethersulfone (PES) membranes**	• TEMPO oxidized-CNF coating is effective against *Escherichia coli*. The effectiveness was attributed to the pH reduction effect induced by carboxyl groups	[43]

2.3. Other Types of Microbes

Nanocellulose would also be able to act as a removal agent for other types of microbes which are larger in size than bacteria, such as fungi, algae and protozoa. However, it is noteworthy that there is still a lack of studies regarding this matter. To the best of our knowledge, there are no available reports on the development of a nanocellulose-based membrane filter for the removal of fungi.

Algae is also a major contributor to microbial contamination in water resources and their presence could change the taste or odour of water. For example, blue–green algae and coloured flagellates (especially the *Chrysophyta* and *Euglenophyta* genera of algae) are the best-known algae that cause contamination in water resources. Furthermore, green algae may also be a significant contamination factor as well [44]. Hence, the potential of nanocellulose should be explored by scientists to define their role as a membrane filtration material suitable for removing algae and protozoa from the contaminated water efficiently. Algae and protozoa are known to have a larger size than viruses and bacteria, thus the removal of these microbes could be effectively carried out using the size-exclusion mechanism.

However, similar to viruses and bacteria, the nanocellulose needs to be modified with other compounds such as metal nanoparticles, enzymes and proteins in order to increase its filtration efficiency [45]. Studies have shown that different charges between the cellular membrane of algae and protozoa do play a dominant role in the adsorption/retention of these microbes on a filtration membrane's surface (i.e., through the electrostatic interaction principle) [35][46].

A previous study carried out by Ge et al. (2016) [47] discovered the sustainability and the most efficient approach in harvesting algae using a modified CNC. The modification was made by introducing a 1-(3-aminopropyl)-imidazole (APIm) structure as a reversible coagulant. As shown in **Figure 3**, the coagulation process occurs when the positively charged CNC–APIm interacts with the negatively charged *Chlorella vulgaris* in the presence of carbon dioxide (carbonated water). Their findings are in agreement with the works of Qiu et al. (2019) [48], in which it was found that harvesting efficiency could reach up to 85% with only 0.2 g CNC–APIm mass ratio, 5 s of CO_2 sparging time, and a 50 mL/min flow rate. This signifies that the CNC–APIm complex could be an alternative to current conventional coagulants for harvesting algae in industrial applications.

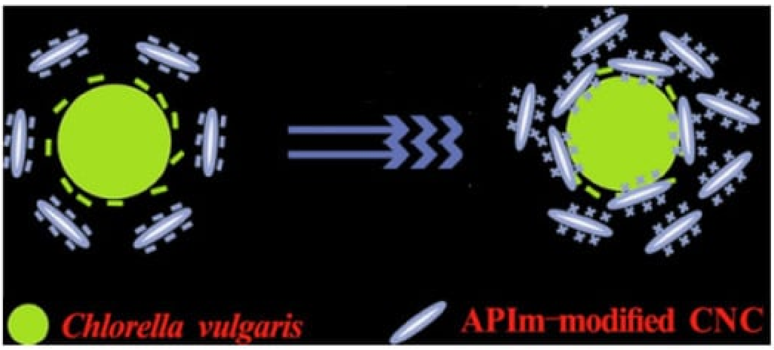

Figure 3. Illustration on the electrostatic attraction between *Chlorella vulgaris* and APIm-modified CNC. This figure was adapted from [47].

Algae harvesting is important for biodiesel industry and many studies have been carried out to increase its sustainability on a global scale. For example, the capability of CNF and CNC in harvesting algae was investigated by Yu et al. (2016) [49]. In their study, they discovered that the CNF did not require any surface modification to harvest the algae, as it played a role as an algae flocculant via its network geometry, something that the CNC (even cationic modified CNC) could not do. By referring to **Figure 4**, flocculation of algae did not happen when CNC was used, as the freely moving algae cannot be entrapped by the nanoparticle structure formation of CNC. However, this study only focuses on the flocculation capability of CNF and CNC, which could lead to a further study on the filtration efficiency of both materials for algae harvesting. This finding could indirectly point to the development of a nanocellulose-based membrane filter for algae removal in the future.

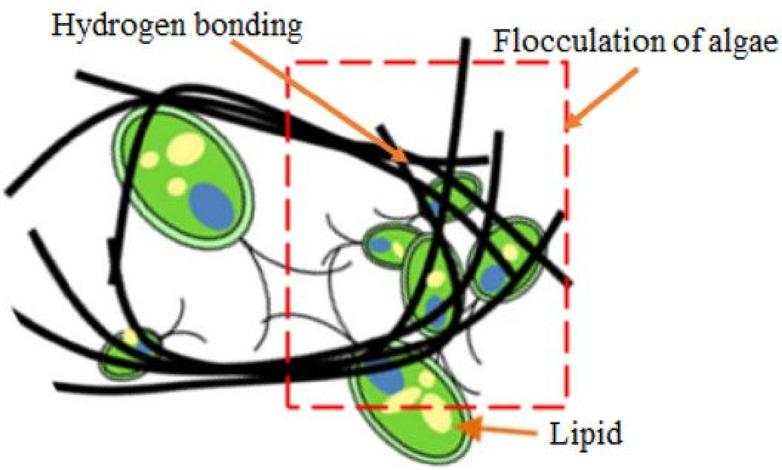

Figure 4. Schematic design of CNF induced microalgal flocculation. This figure was adapted from [49].

Overall, nanocellulose has shown its capability to filter algae. The functionalization is also important to improve filtration efficiency. However, the development of nanocellulose as a filtration material of fungus and protozoa is still limited. It is important to further investigate the capability of nanocellulose to filter these microbes. Moreover, several other factors which could influence the filtration efficiency, as discussed before, can also be considered for future studies.

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