Chapter

# Innovative Separation Technology Utilizing Marine Bioresources: Multifaceted Development of a Chitosan-Based System Leading to Environmentally-Friendly Processes

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## Abstract

Chitosan, known as a most typical marine biological polymer, has a fruitful capability of biocompatible gel formation. Attempts of chitosan have been made to develop it from the multifaceted viewpoint of separation technology. The physico-chemical properties of chitosan containing a lot of hydroxyl groups and reactive amino groups help to build the characteristic polymer networks. The deacetylation degree of chitosan is found as the most influential factor to regulate properties of chitosan hydrogels. The antibacterial activity of the chitosan membrane is one of its notable abilities because of its practical application. The chitosan, its derivatives, and the complex formation with other substances has been used for applications in filtration and membrane separation processes. Adsorption processes based on chitosan have been also developed widely. Moreover, complex of chitosan gel helps to immobilize adsorbent particles. The chitosan membrane immobilizing Prussian-Blue for cesium ion removal from the aqueous phase is one of the leading cases. To elaborate the adsorption behavior on the chitosan immobilizing adsorbent, the isothermal equilibrium and mass transfer characteristics can be discussed. The adsorption process using chitosan-based membranes in combination with filtration in a flow process is advantageous compared with the batch process. More advanced studies of chitosan aerogel and chitosan nanofibers have been proceeded recently, especially for adapting to water purification and air filtration.

**Keywords:** chitosan, membrane, deacetylation degree, filtration, adsorption, water treatment, aerogel, nanofiber

## 1. Introduction

A separation process, which is often called the “downstream process”, plays a key role of product manufacturing through chemical or biochemical reaction as well

as a synthesis process called the "upstream process" [1]. To ensure quality and cost of final products, the separation process is important and has been developed in line with the social demands [2]. For chemical and biochemical industries, the separation process aims to purify objective substances, eliminate undesirable substances, and fractionate each component from their mixture. As environmental awareness around the world increases recently, new separation technologies, such as wastewater treatment [3], advanced desalination [4], air cleaning [5] *etc.*, are in great demand. In addition, materials used in such separation processes are not only expected to be efficient, low cost, easy operation, but also required to be environmentally-friendly.

Chitin and chitosan obtained from crustaceans possess sufficient environmental adaptability as well as an attractive potential to build various types of functional media, e.g., membranes [6–10], micro/nanoparticles [11, 12], and nanofibers [13–16]. Many studies have devoted to develop the novel media adapting separation processes using chitosan. The separation performance of such chitosan media should be strongly influenced from chemical properties characterized by deacetylation degree (*DD*) at amino groups in the chitosan molecular chain. Nevertheless, it was less mentioned that the *DD* could steer not only the structure of the prepared chitosan gels but also the characteristics as separation media.

This chapter describes the preparation and physicochemical properties of novel chitosan-based media and demonstrates the promising ability of chitosan with focus on principal studies for environmentally-friendly separation processes. The essential factors which regulate the performance of separation media prepared from chitosan, such as *DD*, molecular weight, and options of cross-linker, are explained. In particular, the notable impacts of *DD* on the mass transfer mediated by chitosan membrane, the mechanical property, and the antibacterial activity, are introduced based on our previous research [17–19]. Separation media prepared from chitosan are often combined with various adsorbents [20, 21], carbon nanotubes [22, 23], or other functional materials [24, 25]. In such case, the behavior of mass transfer into the chitosan hydrogel is complicated to quantitatively evaluate. The present chapter shows the determination of effective diffusion coefficient of cesium ions in chitosan membrane immobilizing Prussian Blue particles [20]. Furthermore, the chitosan aerogels with macro-porous structure is proposed for selective separation for anionic dye from aqueous phase. Chitosan nanofibers incorporated with polyethylene terephthalate (PET) non–woven are also covered to describe in an application of air filtration.

## 2. Physicochemical properties and gelling characteristics

Chitin and chitosan are known as secondary abundant polymers obtained from the external skeleton of crustaceans such as crab and shrimp [26]. Apart from this, chitin is also found in and produced from the exoskeleton of insects or the cell walls of fungi and yeast [6, 27, 28]; however, this contribution is much less than that from the marine resources. In recent years, chitin and chitosan have gained attention instead of raw materials of petroleum origin owing to the inevitable depletion of fossil fuels and the prevention of climate change [29]. This section looks at the physicochemical properties of chitosan and focuses on the gelling characteristics to build environmentally-friendly separation media.

### 2.1 Chemical composition and gelling ability for separation media

**Figure 1** shows the chemical conversion between chitin and chitosan. The chemical composition of chitin can be described as a long-chain polymer,

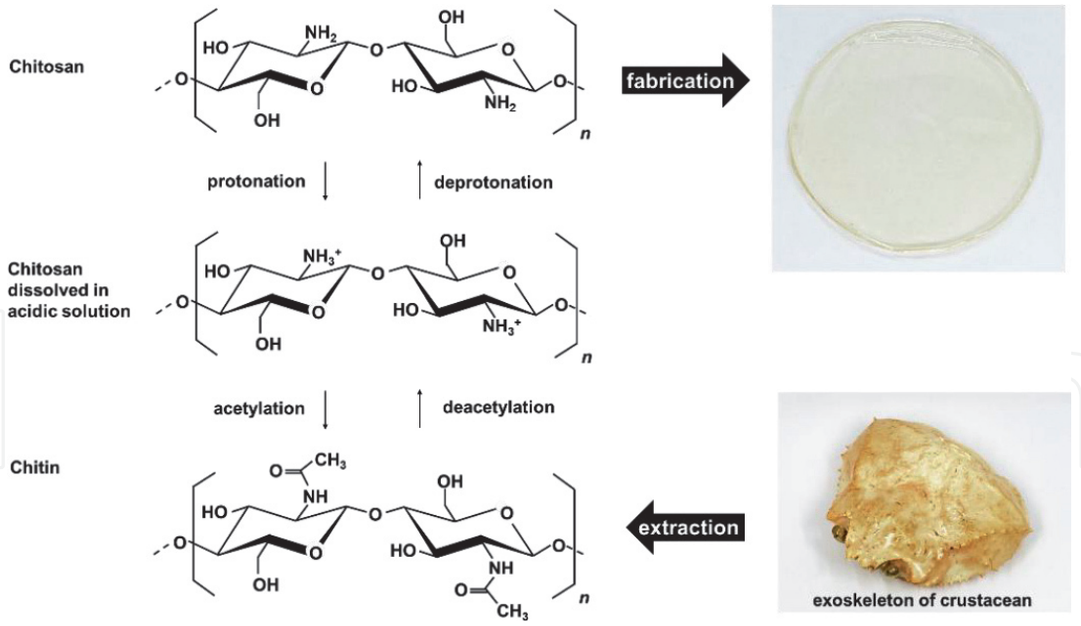


**Figure 1.**
*Chemical conversion among chitin, chitosan, and furthermore functional separation media.*

poly(β-(1 → 4)-*N*-Acetyl-D-glucosamine). Chitosan, poly(β-(1 → 4)-D-glucosamine), is obtained mainly by transforming partial deacetylation of chitin in an alkaline condition, such as using sodium hydroxide aqueous solution. It has been reported that chitosan and its oligosaccharides not only possess hydrophilicity, non-toxicity, biodegradability, and biocompatibility, but also possess antimicrobial activity, antioxidant properties, and an affinity for proteins [7, 26].

Chitosan is insoluble in water at neutral pH or in any organic solvent. Consequently, an acidic aqueous solution, such as acetate buffer solution, is usually employed to dissolve chitosan, whereby the acid dissociation constant of chitosan is found as pKa ≈ 6.5 [30]. Chitosan can be dissolved in acidic solutions by protonation of amino groups in glucosamine units.

Deprotonating a chitosan solution through an acid–base neutralization leads to formation of a water-insoluble gel structure without cross-linker due to intermolecular hydrogen bonding [8]. The salt (*e.g.* NaCl) coexisting with chitosan in an acidic solution acts as counter ions and disrupts intramolecular hydrogen bonding, and then the flexibility of chitosan molecular chains increases [31]. In addition, pH neutralization influences the formation of a polymeric network [9]. Therefore, the neutralization condition should be optimized. From the convenient gelling process, chitosan hydrogels have been developed widely as immobilizing matrices, with enzymes, carbon nanotubes, and electroconductive polymers as typical examples [10, 32, 33].

## 2.2 Deacetylation degree

Deacetylation degree (*DD*) is the most important factor to regulate physico-chemical properties. The deacetylation degree of chitosan samples was determined using the colloidal titration method-based experimental conditions in previous works [34, 35]. We dissolved chitosan powder (0.5 g) in 5% acetic acid solution, and then increased the total weight of chitosan–acetic acid solution to 100.0 g by adding acetic acid. We mixed a 1 g sample of this chitosan–acetic acid solution to 30 ml of deionized water. The titrant was 0.0025 N potassium polyvinyl sulfate (PVS-K), and the indicator was 1% toluidine blue. The terminal point of titration was clearly

indicated by the color changing from blue to claret. The deacetylation degree was calculated using the following equations.

$$X = f \times 0.0025 \times 10^{-3} \times v \times 161 \quad (1)$$

$$Y = 0.5 \times 10^{-2} - X \quad (2)$$

$$DD\ (\%) = \frac{X/161}{X/161 + Y/203} \times 100 \quad (3)$$

In these equations, $X$ is the equivalent mass of glucosamine contained in a 1 g sample of the chitosan–acetic acid solution, $v$ [ml] is the volume of 0.0025 N PVS-K solution, and $f$ is its concentration factor. Y is the mass of acetyl glucosamine contained in a 1 g sample, calculated as the difference between the mass of the sample and the value of $X$. We evaluated the deacetylation degree of the chitosan samples as a molar fraction of glucosamine [36].

### 2.2.1 Control of the deacetylation degree

A chitosan membrane was prepared by the casting method in combination with *N*-acetylation reaction [17]. The deacetylation degree (*DD*) decreased linearly with increasing added amounts of acetic anhydride (**Figure 2**). Stoichiometric control of the deacetylation degree to the desired level was successfully performed. However, gelation reaction due to excess addition of acetic anhydride inhibited formation of the chitosan membrane.

The gelation behavior of chitosan, which has various degrees of acetylation (*DA*) of amino groups, was investigated to ensure preparation of the designed membrane structure [37]. The gelation behavior was evaluated by the gelation time and the quantity of syneresis, and useful information not only for preparing a membrane but also for preparing an immobilized carrier or a chemical reaction system was obtained in this work.

### 2.2.2 Water permeation mechanism of an N-acetylated chitosan membrane

A novel model of the water permeation mechanism in an *N*-acetyl-chitosan membrane with a cellular structure was proposed [18]. Although the entire membrane structure has a hydrophilic character, the cellular structure incorporates junction zones that practically prevent water permeation.

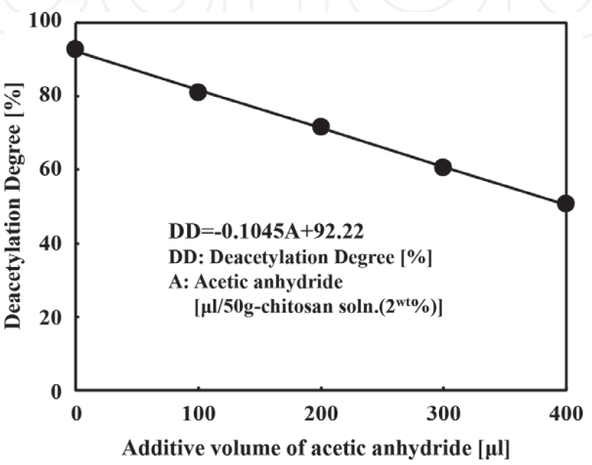


**Figure 2.**
*Effect of acetic anhydride on* N*-acetylation of chitosan. Acetic anhydride was added to 2 wt.% chitosan solution (50 g) [17] with permission from Elsevier.*

Chitosan membranes with a controlled degree of deacetylation (*DD*) were prepared using a casting method. Changes in the total water content and the pressure driven water flux of the membrane were observed with a change in *DD* (**Figure 3**). The membrane properties were analyzed and evaluated using water permeability measurements, scanning electron microscopy (SEM), X-ray diffraction (XRD), and differential scanning calorimetry (DSC). SEM observations indicated that the membrane structure was an individual cellular structure and that this cellular structure grew with decrease in *DD* (**Figure 4**). From XRD measurements, the intensity in the range from 10° to 20° were detected in the chitin (*DD* = 1.1%) and the chitosan membranes (71.3% < *DD* < 92.2%), which indicated that the crystal structure of the membrane was amorphous regardless of *DD*. The free water content ($W_f$), the freezable bound water ($W_{fb}$), and the bound water not able to freeze ($W_b$) were evaluated by DSC. The total water content and the sum of free water content ratios ($W_f$ + $W_{fb}$) decreased with increasing *DD* whereas $W_b$ gradually increased [18]. That suggests the membrane prepared from lower *DD* chitosan formed remarkable cellular structure. The free water was mainly contained inside of the cellular structure, and resulted in swelling the chitosan membrane (**Figure 5**).

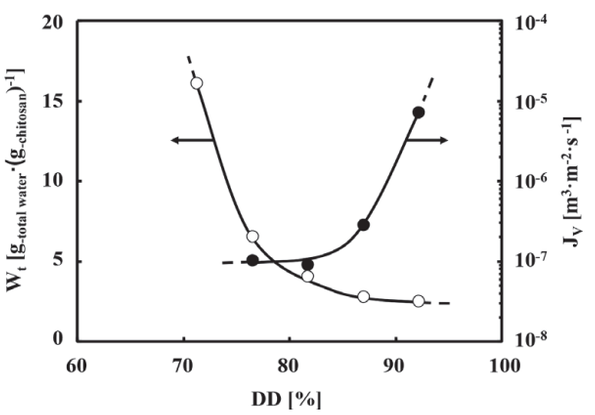


**Figure 3.**
*Change in water flux (50 kPa, 298 K, μL = 0.901 mPa s) and total water content of membrane with regulated* DD. *(●) water flux,* $J_v$ *and (○) total water content,* $W_t$ *[18] with permission from Elsevier.*

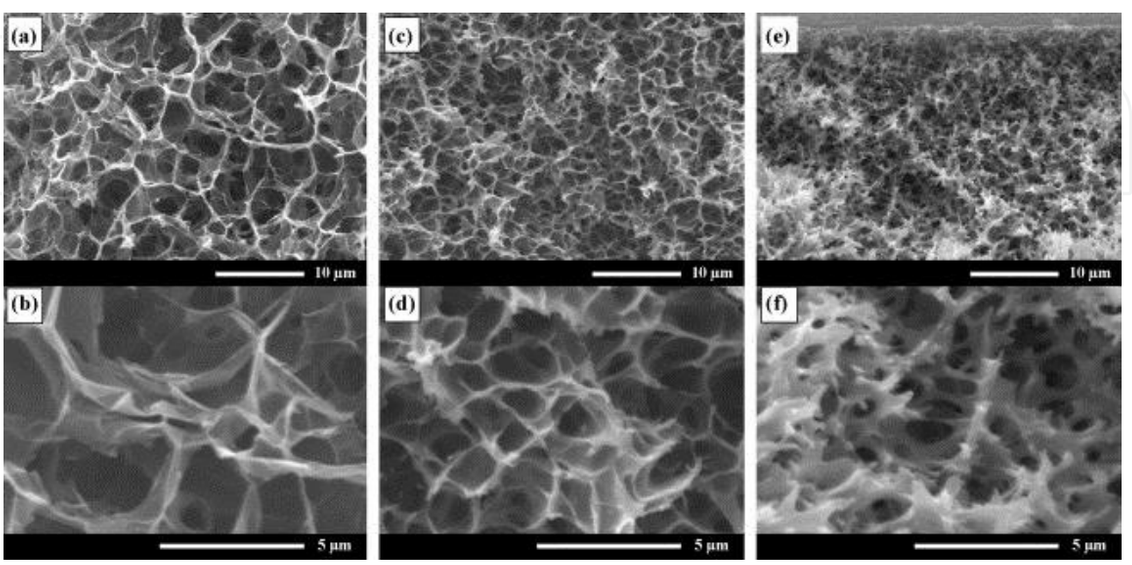


**Figure 4.**
*SEM images observed at cross-section of the membranes prepared from different DD chitosan [18] with permission from Elsevier. (a) and (b) DD 71.3%; (c) and (d) DD 81.8%; (e) and (f) DD 92.2%. The full length of reference scaling measure in (a), (c) and (e) indicates 10 μm. The full length of reference scaling measure in (b), (d) and (f) indicated 5 μm.*

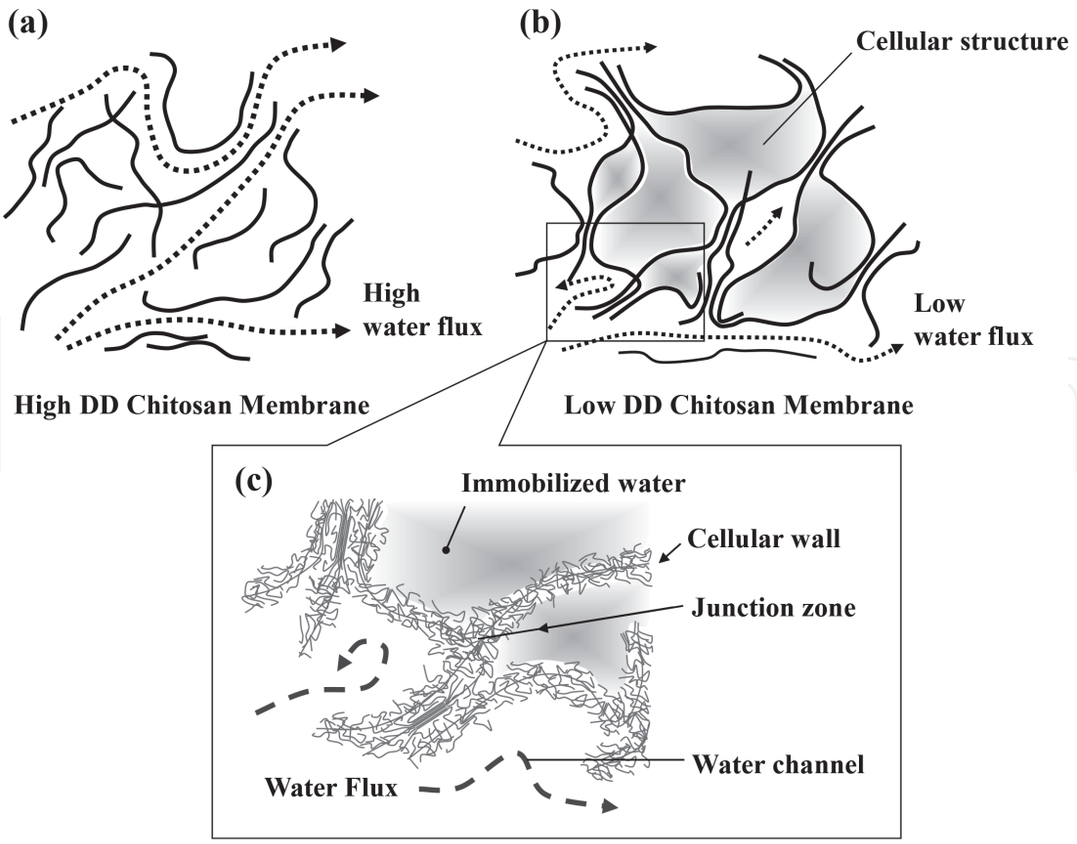


**Figure 5.**
*Schematic illustration of the water permeation mechanism in an* N*-acetyl-chitosan membrane with a cellular structure. (a) the structure of a high* DD *chitosan membrane, (b) the structure of a low* DD *chitosan membrane, and (c) the detailed image of a low DD chitosan membrane with its cellular structure and water channels. The cellular structure illustrated in (b) is composed of immobilized water, the cellular wall, and the junction zone. The structure prevents water flux [18] with permission from Elsevier.*

Pressure driven water flux was measured using the ultrafiltration apparatus; it was dependent on the operational pressure, membrane thickness, and the feed solution viscosity, and obeyed the Hagen-Poiseuille flow. At a higher *DD*, water permeation proceeded due to degradation of the cellular structure; the amount of water in permeation channels was greater than that for lower *DD* membranes even though the total water content in the membrane was less. The water flux of the chitosan membrane was determined by the water content constructing channels through the membrane and not by the total water content in the membrane.

### *2.2.3 Antibacterial activity*

The antibacterial activity is also explained, because of its long-time practical application. The antibacterial activity of chitosan membranes was investigated by a conductimetric assay using a bactometer [19]. The growth of the gram-positive sample (*S. aureus*) was more strongly inhibited by chitosan than the gram-negative sample (*E. coli*). This inhibitory effect was recognized as a bactericidal effect. Antibacterial activity was also observed and was dependent on the shape and specific surface area of the powdered chitosan membrane. The influence of the *DD* of the chitosan on inhibiting the growth of *S. aureus* was investigated by two methods: incubation using a mannitol salt agar medium and a conductimetric assay (**Figure 6**). In both methods, chitosan with a higher *DD* successfully inhibited growth of *S. aureus*. Our findings regarding the dominant role of the *DD* of chitosan will be useful for designing lasting, hygienic, membrane-based processes.

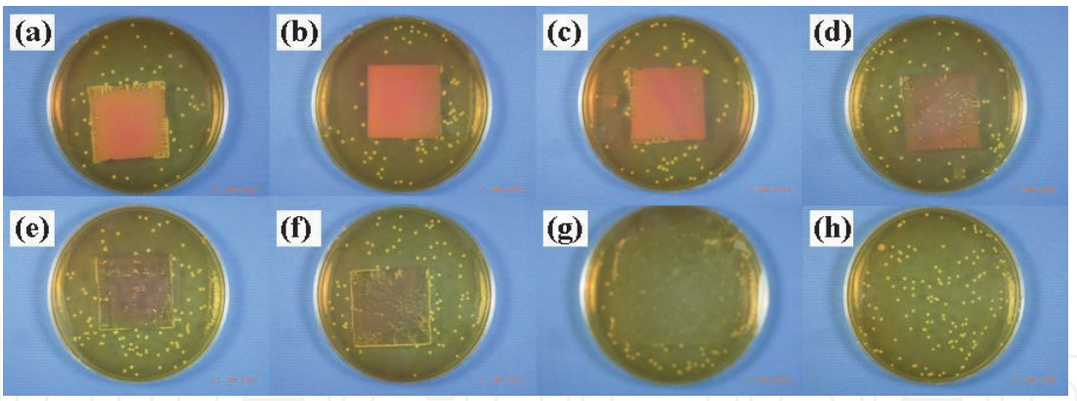


**Figure 6**
*Photographical evidence of antibacterial activity of chitosan membrane immersed in mannitol salt agar culture involved with degree of deacetylation: (a)* DD *92.2%; (b)* DD *90.1%; (c)* DD *88.0%; (d)* DD *83.9%; (e)* DD *79.7%; (f)* DD *75.5%; (g) PVC; (h) control [19] with permission from Elsevier.*

## 2.3 Molecular weight

The molecular weight of chitosan also plays a significant role in the properties of a prepared membrane. It was found that the tensile strength and elongation of membranes prepared from high molecular weight chitosan were higher than those prepared from low molecular weight chitosan; however, the permeability of membranes from high molecular weight chitosan is lower than those prepared from low molecular weight ones [38]. For convenient determination, the viscometric average molecular weight ($M$ [g mol$^{-1}$]) can be calculated from the intrinsic viscosity ($\eta$ [mL g$^{-1}$]) using the Mark-Houwink-Sakurada relationship as follows:

$$[\eta] = KM^{\alpha} \tag{4}$$

For a case, chitosan is dissolved in an acetate buffer composed of 0.2 M acetic acid +0.2 M sodium acetate at 25°C. The parameters in the above relationship were found as $K$ = 7.9 × 10$^{-2}$ [mL/g] and $\alpha$ = 0.796 [−] [27, 39, 40].

## 2.4 Cross-linker

Various types of cross-linkers are often employed for the fabrication of suitable chitosan membranes adapted to separation processes. Glutaraldehyde (GA) is usually used as cross-linker, because it is extremely reactive in cross-linking chitosan polymer chains *via* the Schiff reaction between aldehyde groups and amino groups to form covalent imine bonds [41–43]; however, GA is also toxic. Genipin, which is produced from the hydrolysis of geniposide extracted from the fruits of *Gardenia jasminoides Ellis*, is attracted as a biocompatible and low biohazardous cross-linker [44, 45]. Tetraethyl orthosilicate (TEOS) is also used as a covalent cross-linker, which serves to cross-link with chitosan polymers at their hydroxyl groups, to immobilize adsorbent particles, and to hydrophobize membrane surfaces [22, 46–48]. Jóźwiak and coworkers widely investigated the effect of the cross-linker type occurring covalent bond or ionic bond on the chitosan hydrogel prepared for ionic dye adsorption. In case of covalent agents, it is suggested that epoxide functional groups prefer to attack hydroxyl groups of chitosan during cross-linking, and the free amine groups formed are responsible for anionic dye adsorption [49].

## 3. Filtration processes with sieving to molecules

**Table 1** summarizes the recent studies of a filtration membrane consisting of chitosan or chitosan derivatives. In several membranes, the functional materials are additionally composed to improve the separation ability. The relevant studies are picked up and described below.

| Membrane body | Additive functionalizing material | Cross-linker | Target substances | Expected application | Ref. | Year |
|---|---|---|---|---|---|---|
| chitosan | phosphotungstic acid | *NA* | proton, methanol | DMFCs [a] | [60] | 2016 |
| chitosan/PSF[b] | MOFs[c] | *NA* | NaCl, $MgCl_2$, $CaCl_2$, $Na_2SO_4$ | nanofiltration | [50] | 2017 |
| chitosan | MWNT[d] | glycerin, PEGDE[e] | NaCl, $MgCl_2$ $MgSO_4$ | nanofiltration | [51] | |
| carboxymethyl chitosan/PVDF [f] | *NA* | *NA* | humic acid | nanofiltration | [52] | |
| chitosan/PTFE [g] | *NA* | TEOS [h] | methanol, toluene | pervaporation | [46] | 2018 |
| chitosan/PVA [i] | *NA* | adipic acid | NaCl aerosol | air filtration | [56] | |
| phosphorylated chitosan/PAN [j] | *NA* | GA [k] | $MgCl_2$ $Na_2SO_4$ MB [l] MO [m], AYR [n] | nanofiltration | [41] | |
| chitosan | polyester nonwoven fabric | sulfuric acid | $H_2O$ (vapor) | membrane drier | [61] | 2019 |
| chitosan/PVA [i] | motmorillonite | *NA* | Cr(VI) | nanofiltration | [53 | |
| chitosan | MWNT[d] | *NA* | NaCl, $MgSO_4$ | nanofiltration | [55] | 2020 |
| PVA [i] | microparticles of chitosan, phosphorylated chitosan, glycidol-modified chitosan, or sulphated chitosan | GA [k] | ethanol | pervaporation | [42] | |
| phosphorylated chitosan | graphene oxide | GA [k] | DB38 [o], PS [p], XO [q] | nanofiltration | [43] | |
| chitosan/PES [r] | MWNT[d] | *NA* | MG [s] | nanofiltration | [54] | |
| chitosan | *NA* | genipin | IPA [t] | pervaporation | [44] | |
| chitosan/PVA [i] | Cu-BTC [u] | *NA* | $CO_2$ (gas) | $CO_2$ separation | [57] | |
| carboxymethyl chitosan/ polyamidoamine | hydrotalcite | *NA* | $CO_2$ (gas) | $CO_2$ separation | [58] | |
| chitosan/ methoxy pectin | cutin | glycerol | $H_2O$ (vapor) | packaging film | [62] | |

| Membrane body | Additive functionalizing material | Cross-linker | Target substances | Expected application | Ref. | Year |
|---|---|---|---|---|---|---|
| **chitosan** | *NA* | **succinic acid** | **$H_2O$ (vapor)** | **packaging film** | **[63]** | **2021** |

[a]*DMFCs: direct methanol fuel cells*
[b]*PSF: polysulfone*
[c]*MOF: metal organic frameworks*
[d]*MWNT: multi-walled carbon nanotubes*
[e]*PEGDE: polyethyleneglycol diglycidyl ether*
[f]*PVDF: polyvinylidene fluoride*
[g]*PTFE: polytetrafluoroethylene*
[h]*TEOS: tetraethyl orthosilicate*
[i]*PVA: polyvinyl alcohol*
[j]*PAN: polyacrylonitrile*
[k]*GA: glutaraldehyde*
[l]*MB: Methylene Blue (dye)*
[m]*MO: Methyl Orange (dye)*
[n]*AYR: Alizarine Yellow (dye)*
[o]*DB38: Direct Black 38 (dye)*
[p]*PS: Ponceau S (dye)*
[q]*XO: Xylenol Orange (dye)*
[r]*PES: polyethersulfone*
[s]*MG: Malachite Green (dye)*
[t]*IPA: isopropanol*
[u]*Cu-BTC: copper-1,3,5-benzenetricarboxylic acid*

**Table 1.**
*Recent studies of a separation membrane consisting of chitosan.*

### 3.1 Nanofiltration

As a common procedure, many types of chitosan-based membranes are prepared using the casting method. Chitosan membranes prepared by the casting method usually has a highly compacted gel structure since hydrogen bonding derived from a lot of hydroxyl groups. The dense membranes are beneficial for the nanofiltration process separating small substances, for instance, salts, organic acids, or organic dyes [41, 43, 50–54]. For improving the separation ability, combination of organic–inorganic polymeric hybrid membrane is an innovative approach. Metal–organic frameworks (MOFs) were incorporated into the chitosan polymeric matrix to obtain a positively charged membrane surface for cation removal [50]. Montmorillonite clay, which is dispersed uniformly in a porous matrix, enhances chromium removal [53]. Also, carbon nanotubes are combined with the chitosan membrane for improving solution permeability and salt rejection [51, 55].

### 3.2 Gas separation

The chitosan membranes with dense polymeric structures can also be used for gas separation processes. It is reported that a chitosan/polyvinyl alcohol (PVA)-blended membrane exhibited high air filtration with antibacterial activity [56]. Nowadays, $CO_2$ separation technology has caught attention due to the increase in concerns related to climate change because of greenhouse gases. Chitosan-based membranes for $CO_2$ separation membranes are developed to immobilize active sites, such as copper-1,3,5-benzenetricarboxylic acid (Cu-BTC) [57] and hydrotalcite [58].

### 3.3 Hydrophilicity-based process

Due to a high hydrophilicity derived from abundant hydroxyl groups in glucosamine units, the polymeric membranes consisting of chitosan are suitable for

separation between water and organic solvents using pervaporation processes [42, 44, 46, 59]. From its hydrophilicity, the chitosan membrane has also been used as a separation membrane in direct methanol fuel cells (DMFCs) requiring blocking of methanol permeation as well as proton conductivity [60]. Regarding the hydrophilicity of chitosan, removal or blocking of water vapor was tested using a chitosan membrane for a part of a membrane drier apparatus [61] and packaging membrane [62, 63]. As a very recent issue, the interest in biodegradable films for packaging has recently been steadily increasing due to significant concerns on environmental pollution caused by nonbiodegradable packaging materials [64, 65].

## 4. Adsorption processes

Adsorption is frequently used in separation processes to recover worthy substances as well as water treatment due to its advantages, such as easy operation, high selectivity, and low operational costs [66, 67]. **Tables 2** and **3** summarize the recent studies of adsorption processes, which employed chitosan or chitosan derivatives as either main body of adsorbent or immobilizing media for other adsorbents (mainly fine particles).

As shown in **Figure 1**, chitosan has abundant functional groups. Thereby, chitosan and its derivatives also show adsorbent capabilities with various metal ions and organic substances depending on the pH and the concentration of ionic substances [13]. Hence, their adsorption properties are being widely researched [68]. A large portion of the study employed chitosan and its derivatives for adsorption processes devoted to remove heavy metal from polluted water [11, 13–16, 28, 69–71]. Considering organic compounds, chitosan can adsorb anionic dyes due to amine groups in a chitosan polymer chain [72–74]. It has been reported that nonionic compounds like naphthalene [22] are also adsorbed on chitosan. For improving the adsorption ability, fibrous membranes are fabricated to remove ionic substances or heavy metal ions [12–16, 71]. Fibrous membranes with the adsorption ability are beneficial due to not only large surface area but also high permeation flux. Chitosan gel have shown brilliant abilities as immobilizing media to various adsorbent substances not only to enhance absorption performance but also to enlarge coverage of adsorbates [20–21, 23, 25, 66, 73, 75–79].

Adsorption processes are liberally categorized into two types which are adsorption conducted in a batch process and adsorption in combination with filtration in a flow process.

### 4.1 Membrane-type absorbents adapting with filtration process

Chitosan have been focused as the media immobilizing various adsorbents since it is able to form stable gel structure through pH responsible [15, 20, 24, 71, 80]. From the advantages in both the adsorption process with selectivity and filtration with continuous operation, the membrane-type adsorbents have been recently developed to adopt the flow process. **Table 2** summarizes the studies of membrane-type adsorbents consisted of chitosan or chitosan derivatives.

#### *4.1.1 Characterization of chitosan membrane immobilizing adsorbent in batch process*

For a large portion of such studies, adsorption abilities are evaluated from isothermal adsorption in batch process to reveal adsorption mechanisms [24, 72]. As a typical case of an adsorption membrane immobilizing adsorbent particles, chitosan membrane incorporating Prussian Blue (PB) was developed for cesium

| Membrane body | Membrane type | Additional adsorbent | Cross-linker | Target substances | Ref. | Year |
|---|---|---|---|---|---|---|
| chitosan/PVA[a] | porous membrane | CNTs[b] | TEOS [c] | naphthalene | [22] | 2015 |
| chitosan/ polyester | fibrous membrane | *NA* | GA [d] | Cr(VI) | [14] | 2016 |
| chitosan/PET[e] | fibrous membrane | *NA* | GA [d] | Cu(II), Pb(II), Cd(II), Cr(VI) | [13] | 2017 |
| chitosan/cellulose | porous membrane | *NA* | GA [d] | Cu(II) | [69] | 2018 |
| chitosan | affinity membrane | multilayered molecularly imprinted | TEOS [c] | artemisinin | [47] | 2019 |
| chitosan/ polyacrylonitrile | fibrous membrane | zirconium MOF [f] | *NA* | Pb(II), Cd(II), Cr(VI) | [15] | |
| chitosan | dense membrane immobilizing adsorbents | PB [g] | *NA* | Cs | [20] | |
| chitosan | *NA* | *NA* | glyoxal | RO16[h], MO[i] | [72] | |
| oxidized chitosan/ PVA[a] | fibrous membrane | PHMG [j] | *NA* | Cu(II) | [16] | |
| chitosan/PVA[a] | dence membrane immobilizing adsorbents | ZIF-8 [k] | *NA* | MG [l] | [24] | 2020 |
| octyl-trimethoxysilane modified chitosan | dense membrane | *NA* | TEOS [c] | impurities from extracted artemisinin | [48] | |
| chitosan/PVA [a] | dence membrane immobilizing adsorbents | ZIF-8 [k] | *NA* | MG [l] | [80] | |
| chitosan/PVA[a] / PEI[m] | porous membrane | *NA* | *NA* | Cu(II) | [70] | |
| chitosan/PVDF[n] | fibrous membrane | ZIF-8 [k] | *NA* | BSA[o], Cr(VI) | [71] | |
| chitosan/ polyamide6 | fibrous membrane | *NA* | *NA* | Acid Fuchsin dye | [12] | |

*[a]PVA: poly(vinyl alcohol)*
*[b]CNTs: carbon nanotubes*
*[c]TEOS: tetraethyl orthosilicate*
*[d]GA: glutaraldehyde*
*[e]PET: polyethylene terephthalate*
*[f]MOF: metal–organic frameworks*
*[g]PB: Prussian Blue (dye used as adsorbent)*
*[h]RO16: Reactive Orange 16 (dye)*
*[i]MO: Methyl Orange (dye)*
*[j]PHMG: poly-hexamethylene guanidine*
*[k]ZIF-8: zeolitic imidazole framework-8*
*[l]MG: Malachite Green (dye)*
*[m]PEI: polyethyleneimine*
*[n]PVDF: polyvinylidene fluoride*
*[o]BSA: bovine serum albumin*

**Table 2.**
*Recent studies of adsorption membrane consisted of chitosan or its derivatives.*

removal from the aqueous phase [20]. The maximum adsorption capacity can be evaluated from the equilibrium adsorption amount of absorbate on absorbent ($q_e$ [mol $g^{-1}$]) and the equilibrium concentration of absorbate in aqueous phase

| Matrix body | Additional adsorbent | Cross-linker | Type of adsorbent media | Adsorption process | Target substances | Ref. | Year |
|---|---|---|---|---|---|---|---|
| chitosan | *NA* | GA [a], EGDE [b] | spherical beads | through packed bed | Cu(II), Pb, Zn(II), *E. coli, S. aureus* | [28] | 2016 |
| chitosan/PAM [c] | EDTA [d] | MBA [e] | cylindrical tablets | batch adsorption | Cu(II), Pb(II), Cd(II) | [75] | 2017 |
| Rayon fibers coated with chitosan | *NA* | PB[f] | rayon fibers | batch adsorption | Cs | [76] | |
| chitosan | *NA* | CIT [g], TPP [h], SSA [i], OA[j], ECH [k], GA[a], TTE [l], EGDE [b] | spherical beads | batch adsorption | RB5 [m] | [49] | |
| chitosan | graphene nanopalates | GA [a] | spherical beads | batch adsorption | MO[n], AR1 [o] | [66] | 2018 |
| chitosan | PDMAEMA [p] | *NA* | magnetic spherical beads | batch adsorption | AG25 [q], RB19[r] | [73] | |
| chitosan | CA-CD [s] | EDC [t], NHS[u] | particles | through packed column | RB49 [v] | [77] | |
| chitosan | CB[8] [w] | *NA* | powder | batch adsorption | Pb, RO5 [x], AB25 [y], RY145 [z] | [84] | 2019 |
| chitosan | bentnite, cobalt oxide | *NA* | powder | batch adsorption | CR [A], Cr(VI) | [21] | |
| *N*-allylthiourea chitosan | *NA* | *NA* | powder | batch adsorption | AS(III)[B] | [74] | |
| chitosan | $Fe_3O_4$ | *NA* | magnetic spherical beads | batch adsorption | AB [C] | [78] | |
| chitosan | CNTs[D] | *NA* | particles | batch adsorption | phenol | [23] | |
| chitosan | EDTA [d]-silane/mGO [E] | GA [a] | magnetic spherical beads | batch adsorption | Pb(II), Cd(II) | [25] | |
| oleoyl chitosan | *NA* | *NA* | nanoparticles | batch adsorption | Fe(II) | [11] | |

| Matrix body | Additional adsorbent | Cross-linker | Type of adsorbent media | Adsorption process | Target substances | Ref. | Year |
|---|---|---|---|---|---|---|---|
| chitosan | DES [F] (choline chloride + urea or chline chloride + glycerol) | *NA* | spherical beads | batch adsorption | MG [G] | [79] | 2020 |

[a]*GA: glutaraldehyde*
[b]*EGDE: ethylene glycol diglycidylether*
[c]*PAM: polyacrylamide*
[d]*EDTA: ethylenediaminetetra-acetic acid*
[e]*MBA: N,N-methylenebis(acrylamide)*
[f]*PB: Prussian Blue (dye used as adsorbent)*
[g]*CIT: trisodium citrate*
[h]*TPP: tripolyphosphate*
[i]*SSA: sulfosuccinic acid*
[j]*OA: oxalic acid*
[k]*ECH: epichlorohydrin*
[l]*TTE: trimethylpropane triglycidyl ether*
[m]*RB5: Reactive Black 5 (dye)*
[n]*MO: Methyl Orange (dye)*
[o]*AR1: Acid Red 1 (dye)*
[p]*PDMAEMA: poly(2-(dimethylamino)ethyl meth&$$$;*
[q]*AG25: Acid Green 25 (dye)*
[r]*RB19: Reactive Blue (dye)*
[s]*CA-CD: citric acid modified β-cyclodextrin*
[t]*EDC: 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide hydrochloride*
[u]*NHS: N-hydroxysuccinimide*
[v]*RB49: Reactive Blue (dye)*
[w]*CB[8]: cucurbit [8] uril*
[x]*RO5: Reactive Orange 5 (dye)*
[y]*AB25: Acidic Blue 25 (dye)*
[z]*RY145: Reactive Yellow 145 (dye)*
[A]*CR: Congo Red (dye)*
[B]*AS(III): Arsenazo III (dye)*
[C]*AB: Acid Blue (dye)*
[D]*CNTs: carbon nanotubes*
[E]*mGO: magnetic graphene oxide*
[F]*DES: deep eutectic solvents*
[G]*MG: Malachite Green (dye)*

**Table 3.**
*Recent studies of adsorption processes using chitosan or its derivatives (without membrane-type processes).*

($C_e$ [mol $g^{-1}$]) using the Langmuir adsorption isotherm (Eq. 5). The Langmuir isotherm explains both monolayer and homogeneous adsorption.

$$q_e = \frac{Q_{max}\, K\, C_e}{1 + K\, C_e} \tag{5}$$

Where, $Q_{max}$ and $K$ are the maximum adsorption capacity [mol $g^{-1}$] and the equilibrium adsorption constant [L $mol^{-1}$], respectively. **Figure 7a** displays the effect of the mass fraction of immobilized PB in chitosan membrane ($MF_{PB}$) on the maximum adsorption capacity for the membrane and for the PB immobilized in membranes. Immobilization in the chitosan membrane achieved to improve cesium adsorption without inhibition of the adsorption ability of PB.

The diffusivity of adsorbate molecules in adsorbent media strongly influences the adsorption rate [81]. The effective diffusion coefficient ($D_{eff}$ [$m^2$ $s^{-1}$]) of cesium ions in the chitosan membrane immobilizing PB was determined according to the mass transfer theory. **Figure 7b** depicts the effect of $MF_{PB}$ on the $D_{eff}$ of cesium ions in the initial period of isothermal adsorption. The obtained values of $D_{eff}$ were lower than that of the diffusion coefficient in the bulk aqueous phase, which was previously reported as $2.17 \times 10^{-9}$ $m^2$ $s^{-1}$ [82]. The structure of the membrane consisting of a chitosan polymer chain and PB were observed to suppress the diffusion of cesium ions into the membrane. The diffusion of cesium ions was inhibited considerably by the immobilized PB, which became dominant in comparison to the mass transfer resistance by the chitosan polymer chain [20].

### *4.1.2 Adsorbed separation in flow process*

The adsorbed separation in flowing process through the membrane immobilizing adsorbents is more efficiently than equilibrium adsorption in flask. Owing to the adsorption ability collaborated with molecular size screening ability, selective separation is exhibited higher than without adsorbent system. Moreover, adsorption capability of adsorbents is fully utilized in the adsorption in combination with filtration in a flow process whereas the batch adsorption is up to equilibrium by the decrease of solutes concentration in liquid phase [83].

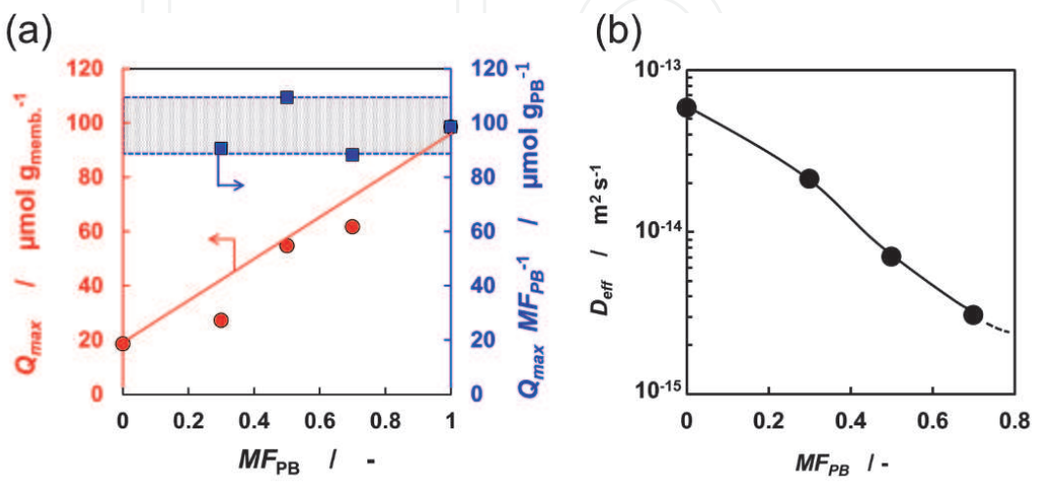


**Figure 7.**
*Effect of the mass fraction of PB on (a) the maximum adsorption capacity for the chitosan membrane (left axis) and for the PB immobilized in the membrane (right axis). (b) the effective diffusion coefficient of cesium ions in the initial period of isothermal adsorption (25°C). Reprinted from Fujisaki, Kashima, Hagiri, Imai [20] with permission from WILEY-VCH.*

Li and co-workers have prepared chitosan fibrous membrane and investigated the dynamic flow adsorption using filtration apparatus for removal of Cr (VI) from aqueous phase [14]. The study revealed the flow adsorption is advantageous than static batch adsorption for small concentration of feed Cr (VI). In addition, the flow rate of feed solution during the flow process directly influenced on the adsorption behavior. Lower flow rate increases the adsorbed amount of Cr (VI) nearly up to maximum adsorption capacity since the contact time between adsorbent and adsorbate increases.

Separation performance is strongly depended on the membrane structure as well as selection of absorbent. Khajavian and co-workers have demonstrated the removal of Malachite Green known as a cationic dye *via* flow process using chitosan/poly (vinyl alcohol) incorporating metal–organic frameworks (ZIF-8) [80]. The physical factors including membrane thickness and porous structure generated by pore generator (polyethylene glycol) inside membrane regulate water flux and dye rejection. The chitosan membrane adopting flow processes should be optimized for each system and target substances.

### 4.2 Other types of absorbent media composed of chitosan

**Table 3** shows the recent studies of adsorption processes using chitosan or its derivatives. Here, the adsorption studies in membrane-type process, which were already showed in **Table 2** are eliminated.

A spherical bead is the basis of the adsorbent media. Chitosan beads can be obtained easily *via* droplet method which a small portion of chitosan dissolved in acid solution is dropped to alkaline solution for neutralization. Bead-type absorbents can be applied to packed bed adsorption operated in a flow process [28].

Adsorbents composed of chitosan and its derivatives incorporated with inorganic substances have been developed to enhance adsorption performance [21, 75, 78, 84]. In addition, it is a notable trend that the studies about the removal of organic dye from aqueous are clearly increasing.

As a very recent approach, Sadiq and coworkers developed chitosan beads incorporating with deep eutectic solvents (DES) which are prepared from choline chloride with urea or glycerol for removing organic dye [79]. The DES is paid attention to possess biocompatibility and low toxicity regardless of similar characteristics with ionic liquids [79, 85, 86].

### 4.3 Highly porous aerogels

Highly porous media from chitosan gel which have large surface area contributing high adsorption ability have been developed recently [87–91]. Such highly porous media are frequently called aerogel. Chitosan aerogels can be prepared *via* freeze-drying of chitosan aqueous solution and stabilization using cross-linker, such as glutaraldehyde (GA). Yi and coworkers reported chitosan aerogel silylated with methyl trimethoxysilane which has spring-like structure leading to high oil absorption [89]. Yu and coworkers revealed preparation of chitosan aerogel incorporating graphene oxide and montmorillonite without cross-linker exhibited efficiently Cr (VI) adsorption [88].

Chitosan aerogel prepared with GA has highly porous structure showed by a field emission scanning electron microscopy (FE-SEM) (**Figure 8a**). The chitosan aerogel prepared with GA adsorbed anionic dye (Methyl Orange: 327 Da) rapidly although cationic dye of similar molecular weight (Methylene Blue: 320 Da) is not removed from their aqueous solutions (**Figure 8b**). Selective removal of anionic substrates in high adsorption rate can be achieved by static attraction derived

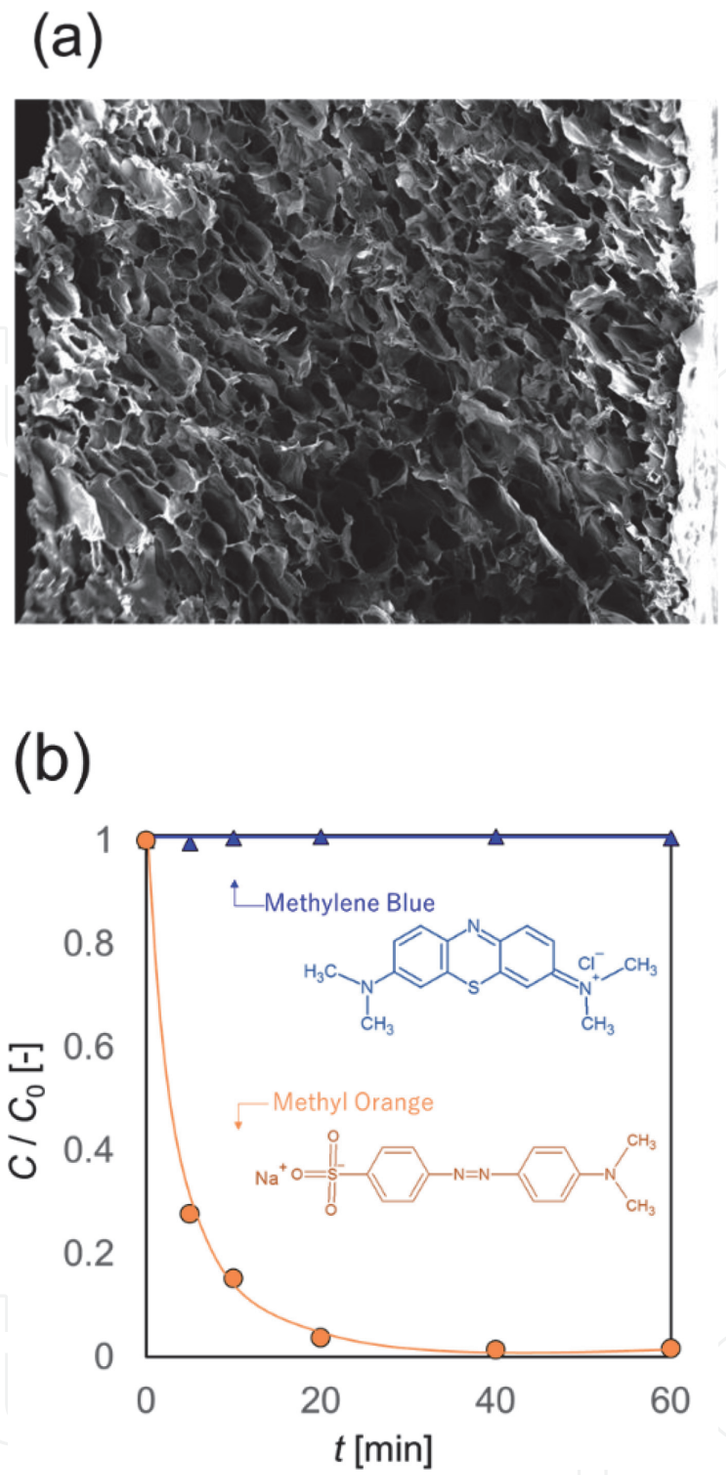


**Figure 8.**
*Chitosan aerogel prepared with glutaraldehyde as a cross-linker. (a) Morphology observed by FE-SEM. (b) Isothermal adsorption of anionic dye (methyl Orange) and cationic dye (methylene blue) onto chitosan aerogel.*

abundant amine and sufficient surface area. Moreover, chitosan aerogel can immobilize various functional particles hence it is expected to develop as new adsorbent media [88, 90].

## 5. Chitosan nanofiber

Recently, along with the expanding area of nanotechnologies, the area of nanofibers has been gaining interest [92]. Nanofibers with diameter in the range

from several micrometers down to tens of nanometers have useful properties such as high specific surface area, porosity, as well as biocompatibility [93, 94]. Nanofibers made from chitosan have not yet been fully established as compared with other nanofibers [95–97]. Chitosan nanofibers have diameter around 10 nm and amino groups on its surface with positive charge [98]. Accordingly, chitosan nanofibers possess unique characteristics and advantages that other nanofiber does not have, that has been expected to be used in various sorts of industrial fields, for instance, filtrations, recovery of metal ions, adsorption of proteins, drug release, enzyme carriers, wound healing, cosmetics, and biosensors have been developed [99–106].

In particular, chitosan nanofiber can be expected as an alternative to air filter media (**Figure 9**). The particle collection performance across chitosan nanofiber media decreased with increase in the amount of chitosan nanofibers on the polyethylene terephthalate (PET) non–woven, as shown in **Figure 10**. In addition, it was

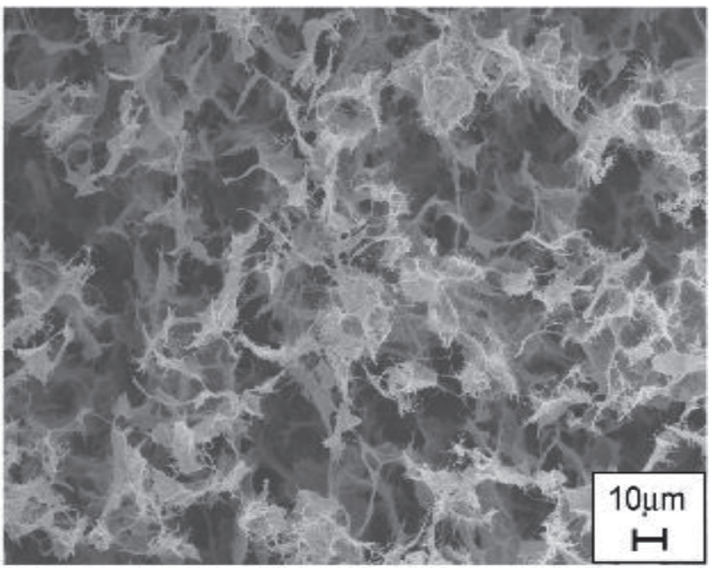


**Figure 9.**
*SEM photograph of chitosan nanofibers filter media.*

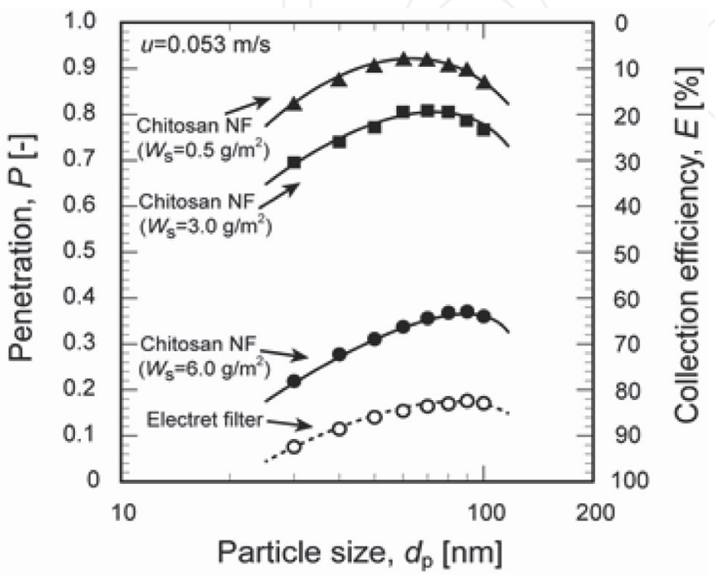


**Figure 10.**
*Comparison of the fractional penetrations of various sorts of test filter media.*

found that the particle collection performance of filter media with high weight of chitosan nanofibers (Weight of sheet ($W_s$) = 6.0 g/m$^2$) is compatible to commercial electret filter media.

## 6. Conclusion

This chapter described the promising development of utilizing chitosan and its derivatives with the focus on separation processes, and overviewed principal investigations. Reactive molecular chains of chitosan derived from hydroxyl and amino groups serve to form an attractive polymeric gel structure. In the filtration process, dense chitosan membranes separate small molecules of solvents, organic dyes, and toxic ions. The deacetylation degree is found to be the most notable factor for water permeation through membranes and its antibacterial ability. The complexity of chitosan-immobilizing systems with functional materials are found to be available in water purification processes. In recent years, adsorption combined with filtration is being developed for various types of water treatment. In this new type process, both the equilibrium adsorption capability and diffusivity in the absorbent matrix should be mentioned as describe in Section 4.1. Due to the easy operation, high selectivity, and low operational costs, adsorption process with chitosan gel is widely studied. As advanced separation media, highly porous chitosan aerogels and nanofibers, which possess large surface area that contribute to improved separation ability, have been developed recently.

Great demands of a novel biocompatible material and environmentally-friendly processes will continue to increase in future. Innovative separation technology utilizing chitosan obtained from bioresources is promising. The multifaceted development of chitosan-based system will lead to environmentally-friendly processes.

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## References

[1] Quirino JP, Alejandro FM, Bissember AC. Towards cleaner downstream processing of biomass waste chemical products by liquid chromatography: A review and recommendations, Journal of Cleaner Production. 2532020:119937. DOI: 10.1016/j.jclepro.2019.119937

[2] Cuellar MC, Straathof AJJ. Downstream of the bioreactor: advancements in recovering fuels and commodity chemicals, Current Opinion in Biotechnology. 62;2020:189-195. DOI: 10.1016/j.copbio.2019.11.012

[3] Moreira VR, Lebron YAR, Santos LVS, Coutinho de Paula E, Amaral MCS. Arsenic contamination, effects and remediation techniques: A special look onto membrane separation processes, Process Safety and Environmental Protection. 148;2021:604-623. DOI: 10.1016/j.psep.2020.11.033

[4] Johnson DJ, Hilal N. Can graphene and graphene oxide materials revolutionise desalination processes?, Desalination. DOI: 10.1016/j.desal.2020.114852

[5] Chiaramonte de Castro BJ, Sartim R, Guerra VG, Aguiar ML. Hybrid air filters: A review of the main equipment configurations and results. Process Safety and Environmental Protection. 144;2020:193-207. DOI: 10.1016/j.psep.2020.07.025

[6] İlk S, Ramanauskaitė A, Bilican BK, Mulerčikas P, Çam D, Onses MS, Torun I, Kazlauskaitė S, Baublys V, Aydın Ö, Zang L-S, Kaya M. Usage of natural chitosan membrane obtained from insect corneal lenses as a drug carrier and its potential for point of care tests, Materials Science and Engineering: C. 112;2020:110897. DOI: 10.1016/j.msec.2020.110897

[7] Menezes JESA, dos Santos HS, Ferreira MKA, Magalhães FEA, da Silva DS, Bandeira PN, Saraiva GD, Pessoa ODL, Ricardo NMPS, Cruz BG, Teixeira AMR. Preparation, structural and spectroscopic characterization of chitosan membranes containing allantoin, Journal of Molecular Structure. 1199;2020:126968. DOI: 10.1016/j.molstruc.2019.126968

[8] Kashima K, Nomoto R, Imai M. Novel application of oceanic biopolymers -strategic regulation of polymer characteristics for membrane technology in separation engineering. In: Perveen FK, editor. Recent Advances in Biopolymers. Croatia: InTech; 2016. p. 189–220. DOI: 10.5772/61998

[9] Nomoto R, Imai M. Dominant role of acid–base neutralization process in forming chitosan membranes for regulating mechanical strength and mass transfer characteristics, Journal of Chitin and Chitosan Science. 2;2014: 197–204. DOI: 10.1166/jcc.2014.1055

[10] Sun J, Yendluri R, Liu K, Guo Y, Lvov Y, Yan X. Enzyme-immobilized clay nanotube–chitosan membranes with sustainable biocatalytic activities, Physical Chemistry Chemical Physics. 19;2017:562-567. DOI: 10.1039/C6CP07450B

[11] Jaber N, Aiedeh K. Sorption behavior and release kinetics of iron (II) ions by oleoyl chitosan polymeric nanoparticles, Journal of Drug Delivery Science and Technology. 54;2019:101354. DOI: 10.1016/j.jddst.2019.101354

[12] Jalalian N, Nabavi SR. Electrosprayed chitosan nanoparticles decorated on polyamide6 electrospun nanofibers as membrane for Acid Fuchsin dye filtration from water, Surfaces and Interfaces. 21;2020:100779. DOI: 10.1016/j.surfin.2020.100779

[13] Li L, Zhang J, Li Y, Yang C. Removal of Cr (VI) with a spiral wound chitosan

nanofiber membrane module via dead-end filtration, Journal of Membrane Science. 544;2017:333-341. DOI: 10.1016/j.memsci.2017.09.045

[14] Li L, Li Y, Yang C. Chemical filtration of Cr (VI) with electrospun chitosan nanofiber membranes, Carbohydrate Polymers, 140;2016:299-307. DOI: 10.1016/j.carbpol.2015.12.067

[15] Jamshidifard S, Koushkbaghi S, Hosseini S, Rezaei S, Karamipour A, Jafari rad A, Irani M. Incorporation of UiO-66-$NH_2$ MOF into the PAN/chitosan nanofibers for adsorption and membrane filtration of Pb(II), Cd(II) and Cr(VI) ions from aqueous solutions, Journal of Hazardous Materials, 368; 2019:10-20. DOI: 10.1016/j.jhazmat.2019.01.024

[16] Chen S, Li C, Hou T, Cai Y, Liang L, Chen L, Li M. Polyhexamethylene guanidine functionalized chitosan nanofiber membrane with superior adsorption and antibacterial performances, Reactive and Functional Polymers. 145;2019:104379. DOI: 10.1016/j.reactfunctpolym.2019.104379

[17] Takahashi T, Imai M, Suzuki I. Water permeability of chitosan membrane involved in deacetylation degree control, Biochemical Engineering Journal, 36;2007:43-48. DOI: 10.1016/j.bej.2006.06.014

[18] Takahashi T, Imai M, Suzuki I. Cellular structure in an *N*-acetyl-chitosan membrane regulate water permeability, Biochemical Engineering Journal. 42;2008:20-27. DOI: 10.1016/j.bej.2008.05.013

[19] Takahashi T, Imai M, Suzuki I, Sawai J. Growth inhibitory effect on bacteria of chitosan membrane regulated with deacetylation degree, Biochemical Engineering Journal. 40; 2008:485-491. 10.1016/j.bej.2008.02.009

[20] Fujisaki T, Kashima K, Hagiri M, Imai M. Isothermal Adsorption Behavior of Cesium Ions in a Novel Chitosan-Prussian Blue-Based Membrane, Chemical Engineering & Technology. 42;2019:910-917. DOI: 10.1002/ceat.201800603

[21] Abukhadra MR, Adlii A, Bakry BM. Green fabrication of bentonite/chitosan@cobalt oxide composite (BE/CH@Co) of enhanced adsorption and advanced oxidation removal of Congo red dye and Cr (VI) from water, International Journal of Biological Macromolecules. 126;2019:402-413. DOI: 10.1016/j.ijbiomac.2018.12.225

[22] Bibi S, Yasin T, Hassan S, Riaz M, Nawaz M. Chitosan/CNTs green nanocomposite membrane: Synthesis, swelling and polyaromatic hydrocarbons removal, Materials Science and Engineering: C. 46;2015: 359-365. DOI: 10.1016/j.msec.2014.10.057

[23] Alves DCS, Gonçalves JO, Coseglio BB, Burgo TAL, Dotto GL, Pinto LAA, Cadaval Jr. TRS. Adsorption of phenol onto chitosan hydrogel scaffold modified with carbon nanotubes, Journal of Environmental Chemical Engineering. 7;2019:103460. DOI: 10.1016/j.jece.2019.103460

[24] Khajavian M, Salehi E, Vatanpour V. Chitosan/polyvinyl alcohol thin membrane adsorbents modified with zeolitic imidazolate framework (ZIF-8) nanostructures: Batch adsorption and optimization. Separation and Purification Technology, 241;2020: 116759. DOI: 10.1016/j.seppur.2020.116759

[25] Shahbazi A, Marnani NN, Salahshoor Z. Synergistic and antagonistic effects in simultaneous adsorption of Pb(II) and Cd(II) from aqueous solutions onto chitosan functionalized EDTA-silane/mGO, Biocatalysis and Agricultural

Biotechnology. 22;2019:101398. DOI: 10.1016/j.bcab.2019.101398

[26] Liu C, Wang G, Sui W, An L, Si C. Preparation and characterization of chitosan by a novel deacetylation approach using glycerol as green reaction solvent, ACS Sustainable Chemistry and Engineering. 5;2017: 4690-4698. DOI: 10.1021/acssuschemeng.7b00050

[27] Younes I, Rinaudo M. Chitin and chitosan preparation from marine sources. structure, properties and applications, Marine Drugs. 13;2015: 1133-1174. DOI: 10.3390/md13031133

[28] Tayel AA, El-Tras WF, Elguindy NM. The potentiality of cross-linked fungal chitosan to control water contamination through bioactive filtration, International Journal of Biological Macromolecules. 88;2016:59-65. DOI: 10.1016/j.ijbiomac.2016.03.018

[29] Hülsey MJ. Shell biorefinery: A comprehensive introduction, Green Energy & Environment. 3;2018:318-327. DOI: 10.1016/j.gee.2018.07.007

[30] Claesson PM, Ninham BW. pH-dependent interactions between adsorbed chitosan layers, Langmuir. 8; 1992:1406-1412. DOI: 10.1021/la00041a027

[31] Tsai C-C, Morrow BH, Chen W, Payne GF, Shen J. Toward understanding the environmental control of hydrogel film properties: How salt modulates the flexibility of chitosan chains, Macromolecules. 50;2017:5946–5952. DOI: 10.1021/acs.macromol.7b01116

[32] Kim MY, Kim J. Chitosan microgels embedded with catalase nanozyme-loaded mesocellular silica foam for glucose-responsive drug delivery, ACS Biomaterials Science and Engineering 3; 2017:572–578. DOI: 10.1021/acsbiomaterials.6b00716

[33] Aswathy NR, Palai AK, Mohanty S, Nayak SK. Freestanding electrically conducting flexible membranes based on novel chitosan/PANI/rGO nanocomposites, Materials Letters. 259; 2020:126777. DOI: 10.1016/j.matlet.2019.126777

[34] Kina K, Tamura K, Ishibashi N, Use of 8-anilino-1-naphthalene sulfonate as a fluorescent indicator in colloid titration, Japan Analist. 23;1974:1082-1083 (in Japanese). DOI: 10.2116/bunsekikagaku.23.1082

[35] Hattoti T, Colloidal Titration of Chitosan, Chitin and Chitosan Research. 5;1999:14-18 (in Japanese).

[36] Takahashi T, Imai M, Suzuki I, High-potential molecular properties of chitosan and reaction conditions for removal of *p*-quinone from the aqueous phase, Biochemical Engineering Journal. 25; 2005:7-13. DOI: 10.1016/j.bej.2005.02.017

[37] Takahashi T, Yamada A, Shono A, Otake K, Imai M. Gelation behavior with acetylation of chitosan for membrane preparation, Desalination and Water Treatment, 17;2010:150–154. DOI: 10.5004/dwt.2010.1711

[38] Chen RH, Hwa H-D. Effect of molecular weight of chitosan with the same degree of deacetylation on the thermal, mechanical, and permeability properties of the prepared membrane, Carbohydrate Polymers. 29;1996:353-358. DOI: 10.1016/S0144-8617(96)00007-0

[39] Rinaudo M. Chitin and chitosan: Properties and applications, Progress in Polymer Science. 31;2006:603-632. DOI: 10.1016/j.progpolymsci.2006.06.001

[40] Brugnerotto J, Desbrières J, Roberts G, Rinaudo M. Characterization of chitosan by steric exclusion chromatography, Polymer. 42;2001: 09921-09927. DOI: 10.1016/S0032-3861(01)00557-2

[41] Song Y, Hu Q, Li T, Sun Y, Chen X, Fan J. Fabrication and characterization of phosphorylated chitosan nanofiltration membranes with tunable surface charges and improved selectivities, Chemical Engineering Journal. 352;2018:163-172. DOI: 10.1016/j.cej.2018.07.010

[42] Dudek G, Turczyn R, Konieczny K. Robust poly(vinyl alcohol) membranes containing chitosan/chitosan derivatives microparticles for pervaporative dehydration of ethanol, Separation and Purification Technology. 234;2020: 116094. DOI: 10.1016/j.seppur.2019.116094

[43] Song Y, Sun Y, Chen M, Huang P, Li T, Zhang X, Jiang K. Efficient removal and fouling-resistant of anionic dyes by nanofiltration membrane with phosphorylated chitosan modified graphene oxide nanosheets incorporated selective layer, Journal of Water Process Engineering. 34;2020:101086. DOI: 10.1016/j.jwpe.2019.101086

[44] Du JR, Hsu LH, Xiao ES, Guo X, Zhang Y, Feng X. Using genipin as a "green" crosslinker to fabricate chitosan membranes for pervaporative dehydration of isopropanol, Separation and Purification Technology. 244; 2020: 116843. DOI: 10.1016/j.seppur.2020.116843

[45] Ma H-F, Meng G, Cui B-K, Si J, Dai Y-C. Chitosan crosslinked with genipin as supporting matrix for biodegradation of synthetic dyes: Laccase immobilization and characterization. Chemical Engineering Research and Design. 132;2018:664-676. DOI: 10.1016/j.cherd.2018.02.008

[46] Moulik S, Vani B, Chandrasekhar SS, Sridhar S. Chitosan-polytetrafluoroethylene composite membranes for separation of methanol and toluene by pervaporation, Carbohydrate Polymers. 193;2018:28-38. DOI: 10.1016/j.carbpol.2018.03.069

[47] Zhang Y, Tan X, Liu X, Li C, Zeng S, Wang H, Zhang S. Fabrication of Multilayered Molecularly Imprinted Membrane for Selective Recognition and Separation of Artemisinin, ACS Sustainable Chemistry and Engineering. 7;2019:3127–3137. DOI: 10.1021/acssuschemeng.8b04908

[48] Cao Y, Zhang Y, Zhang Y, Wang L, Lv L, Ma X, Zeng S, Wang H. Biodegradable functional chitosan membrane for enhancement of artemisinin purification, Carbohydrate Polymers. 246;2020:116590. DOI: 10.1016/j.carbpol.2020.116590

[49] Jóźwiak T, Filipkowska U, Szymczyk P, Rodziewicz J, Mielcarek A. Effect of ionic and covalent crosslinking agents on properties of chitosan beads and sorption effectiveness of Reactive Black 5 dye, Reactive and Functional Polymers. 114;2017:58-74. DOI: 10.1016/j.reactfunctpolym.2017.03.007

[50] Ma X-H, Yang Z, Yao Z-K, Xu Z-L, Tang CY. A facile preparation of novel positively charged MOF/chitosan nanofiltration membranes, Journal of Membrane Science. 525;2017:269-276. DOI: 10.1016/j.memsci.2016.11.015

[51] Alshahrani AA, Al-Zoubi H, Nghiem LD, M in het Panhuis. Synthesis and characterisation of MWNT/chitosan and MWNT/chitosan-crosslinked buckypaper membranes for desalination, Desalination. 418;2017:60-70. DOI: 10.1016/j.desal.2017.05.031

[52] Ekambaram K, Doraisamy M. Fouling resistant PVDF/Carboxymethyl chitosan composite nanofiltration membranes for humic acid removal, Carbohydrate Polymers. 173;2017:431-440. DOI: 10.1016/j.carbpol.2017.06.017

[53] Sangeetha K, Angelin Vinodhini P, Sudha PN, Alsharani FA, Sukumaran A. Novel chitosan based thin sheet nanofiltration membrane for rejection of heavy metal chromium, International

Journal of Biological Macromolecules. 132;2019:939-953. 10.1016/j.ijbiomac.2019.03.244

[54] Mousavi SR, Asghari M, Mahmoodi NM. Chitosan-wrapped multiwalled carbon nanotube as filler within PEBA thin film nanocomposite (TFN) membrane to improve dye removal, Carbohydrate Polymers. 237;2020: 116128. DOI: 10.1016/j.carbpol.2020.116128

[55] Alshahrani AA, Algamdi MS, Alsohaimi IH, Nghiem LD, Tu KL, Al-Rawajfehe AE, in het Panhuis M. The rejection of mono- and di-valent ions from aquatic environment by MWNT/chitosan buckypaper composite membranes: Influences of chitosan concentrations, Separation and Purification Technology. 234;2020: 116088. DOI: 10.1016/j.seppur.2019.116088

[56] Wang Z, Yan F, Pei H, Li J, Cui Z, He B. Antibacterial and environmentally friendly chitosan/polyvinyl alcohol blend membranes for air filtration, Carbohydrate Polymers. 198;2018:241-248. DOI: 10.1016/j.carbpol.2018.06.090

[57] Jiamjirangkul P, Inprasit T, Intasanta V, Pangon A. Metal organic framework-integrated chitosan/poly (vinyl alcohol) (PVA) nanofibrous membrane hybrids from green process for selective $CO_2$ capture and filtration, Chemical Engineering Science. 221; 2020:115650. DOI: 10.1016/j.ces.2020.115650

[58] Borgohain R, Mandal B. Thermally stable and moisture responsive carboxymethyl chitosan/dendrimer/hydrotalcite membrane for $CO_2$ separation, Journal of Membrane Science. 608;2020:118214. DOI: 10.1016/j.memsci.2020.118214

[59] Silvestre WP , Baldasso C, Tessaro IC. Potential of chitosan-based membranes for the separation of essential oil components by target-organophilic pervaporation, Carbohydrate Polymers, 247;2020: 116676. 10.1016/j.carbpol.2020.116676

[60] Wu Q, Wang H, Lu S, Xu X, Liang D, Xiang Y. Novel methanol-blocking proton exchange membrane achieved via self-anchoring phosphotungstic acid into chitosan membrane with submicro-pores, Journal of Membrane Science. 500;2016:203-210. DOI: 10.1016/j.memsci.2015.11.019

[61] Soontarapa K, Arnusan J. Dehydration of paddy rice in a chitosan membrane drier, Separation and Purification Technology. 209;2019: 401-408. DOI: 10.1016/j.seppur.2018.07.048

[62] Ye Y, Zeng F, Zhang M, Zheng S, Li J, Fei P. Hydrophobic edible composite packaging membrane based on low-methoxyl pectin/chitosan: Effects of lotus leaf cutin, Food Packaging and Shelf Life. 26:2020:100592. DOI: 10.1016/j.fpsl.2020.100592

[63] Gabriele F, Donnadio A, Casciola M, Germani R, Spreti N. Ionic and covalent crosslinking in chitosan-succinic acid membranes: Effect on physicochemical properties, Carbohydrate Polymers. 251; 2021:117106. DOI: 10.1016/j.carbpol.2020.117106

[64] Sun X, Wang Z, Kadouh H, Zhou K. The antimicrobial, mechanical, physical and structural properties of chitosan–gallic acid films, LWT - Food Science and Technology. 57;2014:83-89. DOI: 10.1016/j.lwt.2013.11.037

[65] Mujtaba M, Morsi RE, Kerch G, Elsabee MZ, Kaya M, Labidi J, Khawar KM. Current advancements in chitosan-based film production for food technology; A review, International Journal of Biological Macromolecules. 121;2019:889-904. DOI: 10.1016/j.ijbiomac.2018.10.109

[66] Zhang C, Chen Z, Guo W, Zhu C, Zou Y. Simple fabrication of Chitosan/ Graphene nanoplates composite spheres for efficient adsorption of acid dyes from aqueous solution, International Journal of Biological Macromolecules. 112;2018:1048-1054. DOI: 10.1016/j.ijbiomac.2018.02.074

[67] Bulut Y, Aydın H. A kinetics and thermodynamics study of methylene blue adsorption on wheat shells, Desalination. 194;2006:259–267. DOI: 10.1016/j.desal.2005.10.032

[68] Upadhyay U, Sreedhar I, Singh SA, Patel CM, Anitha KL. Recent advances in heavy metal removal by chitosan based adsorbents, Carbohydrate Polymers. 251;2021:117000. DOI: 10.1016/j.carbpol.2020.117000

[69] Urbina L, Guaresti O, Requies J, Gabilondo N, Eceiza A, Corcuera MA, Retegi A. Design of reusable novel membranes based on bacterial cellulose and chitosan for the filtration of copper in wastewaters, Carbohydrate Polymers. 193;2018:362-372. DOI: 10.1016/j.carbpol.2018.04.007

[70] Sahebjamee N, Soltanieh M, Mousavi SM, Heydarinasab A. Preparation and characterization of porous chitosan–based membrane with enhanced copper ion adsorption performance, Reactive and Functional Polymers. 154;2020:104681. DOI: 10.1016/j.reactfunctpolym.2020.104681

[71] Pishnamazi M, Koushkbaghi S, Hosseini SS, Darabi M, Yousefi A, Irani M. Metal organic framework nanoparticles loaded- PVDF/chitosan nanofibrous ultrafiltration membranes for the removal of BSA protein and Cr (VI) ions, Journal of Molecular Liquids. 317;2020:113934. DOI: 10.1016/j.molliq.2020.113934

[72] Jawad AH, Norrahma SSA, Hameed BH, Ismail K. Chitosan-glyoxal film as a superior adsorbent for two structurally different reactive and acid dyes: Adsorption and mechanism study, International Journal of Biological Macromolecules. 135;2019:569-581. DOI: 10.1016/j.ijbiomac.2019.05.127

[73] Xu B, Zheng H, Zhou H, Wang Y, Luo K, Zhao C, Peng Y, Zheng X. Adsorptive removal of anionic dyes by chitosan-based magnetic microspheres with pH-responsive properties, Journal of Molecular Liquids. 256;2018:424-432. DOI: 10.1016/j.molliq.2018.02.061

[74] Bondock S, El-Zahhar AA, Alghamdi MM, Keshk SMAS. Synthesis and evaluation of N-allylthiourea-modified chitosan for adsorptive removal of arsenazo III dye from aqueous solutions, International Journal of Biological Macromolecules. 137;2019:107-118. DOI: 10.1016/j.ijbiomac.2019.06.193

[75] Ma J, Zhou G, Chu L, Liu Y, Liu C, Luo S, Wei Y. Efficient removal of heavy metal ions with an EDTA functionalized chitosan/polyacrylamide double network hydrogel, ACS Sustainable Chemistry and Engineering. 5;2017:843–851. DOI: 10.1021/acssuschemeng.6b02181

[76] Dechojarassri D, Asaina S, Omote S, Nishida K, Furuike T, Tamura, H. Adsorption and desorption behaviors of cesium on rayon fibers coated with chitosan immobilized with Prussian blue, International Journal of Biological Macromolecules. 104;2017:1509-1516. DOI: 10.1016/j.ijbiomac.2017.03.056

[77] Zhao J, Zou Z, Ren R, Sui X, Mao Z, Xu H, Zhong Y, Zhang L, Wang B. Chitosan adsorbent reinforced with citric acid modified ⍺-cyclodextrin for highly efficient removal of dyes from reactive dyeing effluents, European Polymer Journal. 108;2018:212-218. DOI: 10.1016/j.eurpolymj.2018.08.044

[78] Xu P, Zheng M, Chen N, Wu Z, Xu N, Tang J, Teng Z. Uniform magnetic

chitosan microspheres with radially oriented channels by electrostatic droplets method for efficient removal of Acid Blue, Journal of the Taiwan Institute of Chemical Engineers. 104; 2019:210-218. DOI: 10.1016/j.jtice.2019.09.016

[79] Sadiq AC, Rahim NY, Suah FBM. Adsorption and desorption of malachite green by using chitosan-deep eutectic solvents beads, International Journal of Biological Macromolecules. 164;2020: 3965-3973. DOI: 10.1016/j.ijbiomac.2020.09.029

[80] Khajavian M, Salehi E, Vatanpour V. Nanofiltration of dye solution using chitosan/poly(vinyl alcohol)/ZIF-8 thin film composite adsorptive membranes with PVDF membrane beneath as support, Carbohydrate Polymers. 247; 2020:116693. DOI: 10.1016/j.carbpol.2020.116693

[81] Mihara Y, Sikder MT, Yamagishi H, Sasaki T, Kurasaki M, Itoh S, Tanaka S. Adsorption kinetic model of alginate gel beads synthesized micro particle-prussian blue to remove cesium ions from water, Journal of Water Process Engineering. 10;2016:9-19. DOI: 10.1016/j.jwpe.2016.01.001

[82] Sato H, Yui M, Yoshikawa H. Ionic diffusion coefficients of $Cs^{+}$, $Pb^{2+}$, $Sm^{3+}$, $Ni^{2+}$, $SeO^{2-}_{4}$ and $TcO^{-}_{4}$ in free water determined from conductivity measurements, Journal of Nuclear Science and Technology. 33;1996:950–955. DOI: 10.1080/18811248.1996.9732037

[83] Kashima K, Osawa K, Hagiri M, Imai M. High performance dye removal by embedded activated carbon in calcium alginate membrane combination with low-molecular-weight polyethylene glycol, In: Proceedings of The 5th Asian Conference on Innovative Energy and Environmental Chemical Engineering (ASCON-IEEChE 2016); 13-16 November 2016; Yokohama (Japan); 2016. p. 70-73.

[84] Li Z, Li L, Hu D, Gao C, Xiong J, Jiang H, Li W. Efficient removal of heavy metal ions and organic dyes with cucurbit [8] uril-functionalized chitosan, Journal of Colloid and Interface Science. 539;2019:400-413. DOI: 10.1016/j.jcis.2018.12.078

[85] Ozdemir N, Zengin H, Yavuz A. Electropolymerization of pyrrole from a deep eutectic solvent for supercapacitor applications, Materials Chemistry and Physics. 256;2020:123645. DOI: 10.1016/j.matchemphys.2020.123645

[86] Liu C, Mei G, Yu M, Cheng Q, Yang S. New applications of deep eutectic solvents for separation of quartz and magnetite, Chemical Physics Letters. 762;2021:138152. DOI: 10.1016/j.cplett.2020.138152

[87] Yang H, Sheikhi A, van de Ven TGM. Reusable green aerogels from cross-linked hairy nanocrystalline cellulose and modified chitosan for dye removal, Langmuir. 32;2016:11771–11779. DOI: 10.1021/acs.langmuir.6b03084

[88] Yu P, Wang H-Q, Bao R-Y, Liu Z, Yang W, Xie B-H, Yang M-B. Self-assembled sponge-like chitosan/reduced graphene oxide/montmorillonite composite hydrogels without cross-linking of chitosan for effective Cr(VI) sorption, ACS Sustainable Chemistry and Engineering. 5;2017:1557–1566. DOI: 10.1021/acssuschemeng.6b02254

[89] Yi L, Yang J, Fang X, Xia Y, Zhao L, Wu H, Guo S. Facile fabrication of wood-inspired aerogel from chitosan for efficient removal of oil from water, Journal of Hazardous Materials. 385; 2020:121507. DOI: 10.1016/j.jhazmat.2019.121507

[90] de Luna MS, Ascione C, Santillo C, Verdolotti L, Lavorgna M, Buonocore GG, Castaldo R, Filippone G, Xia H, Ambrosio L. Optimization of dye adsorption capacity and mechanical

strength of chitosan aerogels through crosslinking strategy and graphene oxide addition, Carbohydrate Polymers. 211;2019:195-203. DOI: 10.1016/j.carbpol.2019.02.002

[91] Keshipour S, Mirmasoudi SS. Cross-linked chitosan aerogel modified with Au: Synthesis, characterization and catalytic application, Carbohydrate Polymers. 196;2018:494-500. DOI: 10.1016/j.carbpol.2018.05.068

[92] Kalantari K, Afifi AM, Jahangirian H, Webster TJ. Biomedical application of chitosan electrospum nanofibers as a green polymer-review, Carbohydrate Polymers. 207 ;2019: 588-600. DOI: 10.1016/j.carbpol.2018.12.011

[93] Yoshimoto H, Shin YM, Terai H, Vacanti JP. A biodegradable nanofiber scaffold by electrospinning and its potential for bone tissue engineering, Biomaterials. 24;2003:2077-2082. DOI: 10.1016/S0142-9612(02)00635-X

[94] Min BM, Lee G, Kim SH, Nam YS, Lee TS, Park WH, Electrospinning of silk fibroin nanofibers and its effect on the adhesion and spreading of normal human keratinocytes and fibroblasts in vitro, Biomaterials. 25;2004:1289-1297. DOI: 10.1016/j.biomaterials.2003.08.045

[95] Sciffman JD, Schauer CL. Cross-linking chitosan nanofibers, Biomacromolecules. 8;2007:594-601. DOI: 10.1021/bm060804s

[96] Sciffman JD, Schauer CL. One-step electrospinning of cross-linked chitosan fibers, Biomacromelecules. 8;2007:2665-2667. DOI: 10.1021/bm7006983

[97] Shalumon KT, Binulal NS, Selvamurugan N, Nair SV, Menon D, Furuike T, Electrospinning of carboxymethyl chitin/poly (vinyl alcohol) nanofibrous scaffolds for tissue engineering applications, Carbohydrate Polymers. 77:2009:863-869. DOI: 10.1016/j.carbpol.2009.03.009

[98] Jayakumar R, Prabaharan M, Nair SV, Tamura H. Novel chitin and chitosan nanofibers in biomedical applications, Biotechnology Advances. 28;2010:142-150. DOI: 10.1016/j.biotechadv.2009.11.001

[99] Almasi H, Jafarzadeh P, Mehryar L. Fabrication of novel nanohybrids by impregnation of CuO nanoparticles into bacterial cellulose and chitosan nanofibers: Characterization, antimicrobial and release properties, Carbohydrate Polymers. 186;2018:273-281. DOI: 10.1016/j.carbpol.2018.01.067

[100] Zhang Y, Huang X, Duan B, Wu L, Li S. Yuan X. Preparation of electrospun chitosan/poly (vinyl alcohol) membranes, Colloid and Polymer Science. 285;2007:855-863. DOI: 10.1007/s00396-006-1630-4

[101] Amirabad LM, Jonoobi M, Mousavi NS, Oksman K, Kaboorani A, Yousefi H, Improved antifungal activity and stability of chitosan nanofibers using cellulose nanocrystal on banknote papers, Carbohydrate Polymers. 189; 2018:229-237. DOI: 10.1016/j.carbpol.2018.02.041

[102] Kawasaki T, Hirabayashi T, Masuyama K, Fujita S, Kitano H. Complex film of chitosan and carboxymethyl cellulose nanofibers, Colloids and Surfaces B: Biointerfaces. 139;2016:95-99. DOI: 10.1016/j.colsurfb.2015.11.056

[103] Aliabadi M, Irani M., Ismaeili J, Najafzadeh S. Design and evaluation of chitosan/hydroxyapatite composite nanofiber membrane for the removal of heavy metal ions from aqueous solution, Journal of the Taiwan Institute of Chemical Engineers. 45;2014:518-526. DOI: 10.1016/j.jtice.2013.04.016

[104] Tao F, Cheng Y, Shi X, Zheng H, Du Y, Xiang W, Deng H. Applications of chitin and chitosan nanofibers in bone regenerative engineering, Carbohydrate

Polymers. 230;2020:115658. DOI: 10.1016/j.carbpol.2019.115658

[105] Li Q-H, Dong M, Li R, Cui Y-Q, Xie G-X, Wang X-X, Long Y-Z. Enhancement of Cr(VI) removal efficiency via adsorption/photocatalysis synergy using electrospun chitosan/g-C3N4/TiO2 nanofibers, Carbohydrate Polymers. 253;2021:117200. DOI: 10.1016/j.carbpol.2020.117200

[106] Show PL, Ooi CW, Lee XJ, Yang C-L, Liu B-L, Chang Y-K. Batch and dynamic adsorption of lysozyme from chicken egg white on dye-affinity nanofiber membranes modified by ethylene diamine and chitosan, International Journal of Biological Macromolecules. 162;2020:1711-1724. DOI: 10.1016/j.ijbiomac.2020.08.065