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Review

A Review of Chitosan as a Coagulant of Health-Related Microorganisms in Water and Wastewater

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Abstract: The coagulation and flocculation properties of chitosan, an organic biopolymer derived from chitin, have been researched as an alternative to synthetic polymers and inorganic metal salt coagulants currently used in water and wastewater treatment. In an effort to encourage further research into the practical uses of chitosan as green chemistry in water and wastewater treatment and to promote the efficacious removal of microbial contaminants in drinking and wastewater, we have summarized the current state of research pertaining to the treatment of microorganisms in water and wastewater. A search of PubMed revealed 720 possible titles and abstracts, of which 44 full-text articles were identified as matching the eligibility criteria for inclusion in this systematic review. Results are presented based on the type of water matrix treated (i.e., drinking water, wastewater, and recreational waters) and a summary table providing details on the types and forms of chitosan utilized and the treatment mechanisms and processes described in the study. We find chitosan to be an effective coagulant, flocculant, and adsorbent for removing microbes from water and wastewater; some modified forms of chitosan can inactivate microbes and achieve disinfection, such as those containing metals like silver and antimicrobial chemicals like quaternary ammonium compounds or other strong oxidants, and use with filtration or electrochemical processes can achieve extensive reductions in microbes to meet performance targets of the World Health Organization.

Keywords: chitosan; water; microorganism; drinking water; wastewater; microbe; water treatment; waste treatment; coagulation; flocculation

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1. Introduction

Chitosan, a naturally occurring product derived from the chemical or enzymatic deacetylation of chitin, has been proposed as a low-cost, eco-friendly, and sustainable coagulant for use in water and wastewater treatment applications [1,2]. Chitosan has been proposed for a variety of practical uses due to its structural properties, including water solubility, molecular weight, degree of deacetylation, and various chemically created conformations [3,4]. Chitosan is a polysaccharide of repeating N-acetyl-D-glucosamine and D-glucosamine monomers that can be functionalized to increase its solubility in water and act as a cationic coagulant and adsorbent or, in some forms, a disinfectant. The protonation of amino groups of D-glucosamine forms cationic chitosan suspensions that are able to destabilize suspended colloids, induce floc formation, and adsorb negatively charged microorganisms, clay, and organic matter [5,6]. As an organic polyelectrolyte, chitosan is biodegradable, non-toxic, and widely available.

Despite these positive attributes, chitosan also has limitations related to its different chemical properties and poor solubility in neutral or alkaline aqueous solutions [7]. However, various physically and chemically modified forms of chitosan have been synthesized or modified and then evaluated for use in water and wastewater treatment.

In an effort to encourage more research and greater practical use, we compiled and examined the published scientific literature on chitosan in water and wastewater applications by conducting a systematic review of historical and current literature on the use of chitosan as a water and wastewater coagulant and adsorbent to capture and remove microbial contaminants. We examined the reported ability of different chitosans to remove microorganisms in water and wastewater directly or disinfect them, as well as their use in combination with other technologies such as sedimentation, filtration, and electrochemical processes. Historically, chitosans have been used in water and wastewater treatment for many decades, and a range of applications have been described. However, chitosans for water and wastewater treatment have not been widely adopted in practice or have become "mainstream" technologies. We consider chitosans for water and wastewater treatment to be a simple and effective but overlooked technology that deserves greater consideration for use in point-of-use technologies, as well as in small communities and larger municipal water and wastewater systems. In this brief review, we summarize the available literature on chitosan use in water and wastewater systems to control health-related microorganisms, with the goal of encouraging more widespread use in practice.

2. Materials and Methods

The objective of this review is to systematically gather and summarize the current research findings pertaining to chitosan applications for public-health-related microorganisms in water and wastewater. The scope of this review focuses solely on applications related to water or wastewater and microorganisms and does not cover chemical or other environmental remediation by chitosans, as these aspects have already been summarized in other reviews [8,9]. For this literature review, we searched the PubMed database using the following search terms: "chitosan AND (Water treatment [majr] OR Wastewater [majr]) (fft[Filter]))". PubMed was chosen as it is the largest database of biomedical and health science literature. Selection criteria were defined based on the scope of the review, and the inclusion and exclusion criteria are described in Table 1. Briefly, selected articles included original peer-reviewed papers in English reporting the use of chitosan as a coagulant in the removal and/or disinfection of at least one microorganism or the use of chitosan coagulation to reduce turbidity (as a surrogate for microorganisms). In this review, we have excluded studies that focused only on chemical removal as well as other review articles on other chitosan applications.

Table 1. Screening criteria for study inclusion and exclusion.

Inclusion and Exclusion Eligibility Criteria	
Include	**Exclude**
• Applications of chitosan to improve the quality of drinking water, surface water, stormwater, or wastewaters • Chitosan used for remediation of microorganisms in the marine environment • Research on chitosan or the properties of chitosan for reducing microorganisms in water • Research on chitosan for reducing chemical contaminants and microorganism in water • Research on chitosan or the properties of chitosan for coagulation/flocculation where turbidity is used as a proxy for or compared to microorganisms	• Chitosan remediation of contaminants not found in water • Chitosan for use in removal of non-microbial contaminants • Chitosan as a carrier for drug components or for medical applications • Chitosan reduces only chemical contaminants in water. • Chitosan for use in a clinical setting that is not related to treating water or waste • Chitosan used in food production that is not related to industrial waste

To conduct this review, we used Covidence, a web-based collaboration software platform that streamlines the production of systematic and other literature reviews (Covidence systematic review software, Veritas Health Innovation, Melbourne, Australia; available at www.covidence.org). Covidence software aided in managing the review process by organizing abstracts and paper reviews, logging reviewer responses, handling inclusion and exclusion criteria, and preventing repeats of effort. Each study title and abstract were screened by a minimum of two out of three reviewers based on the selection criteria (Table 1). In cases of disagreement for inclusion or exclusion, the third reviewer made a decision on the disposition of the study. Articles that did not meet the inclusion criteria were not considered for a full-text review. To determine the final articles for inclusion, papers that passed title–abstract screening were divided into topical sections and reviewed for inclusion by one author. Articles that did not meet inclusion criteria at this stage were reviewed again by another author for a final decision.

3. Results

3.1. Search Results

A total of 720 articles were identified by our search strategy, of which 672 were excluded during the title–abstract screening period. Four additional studies were excluded during the full-text review (Figure 1). A total of 44 articles were included in our full review (Table 2).

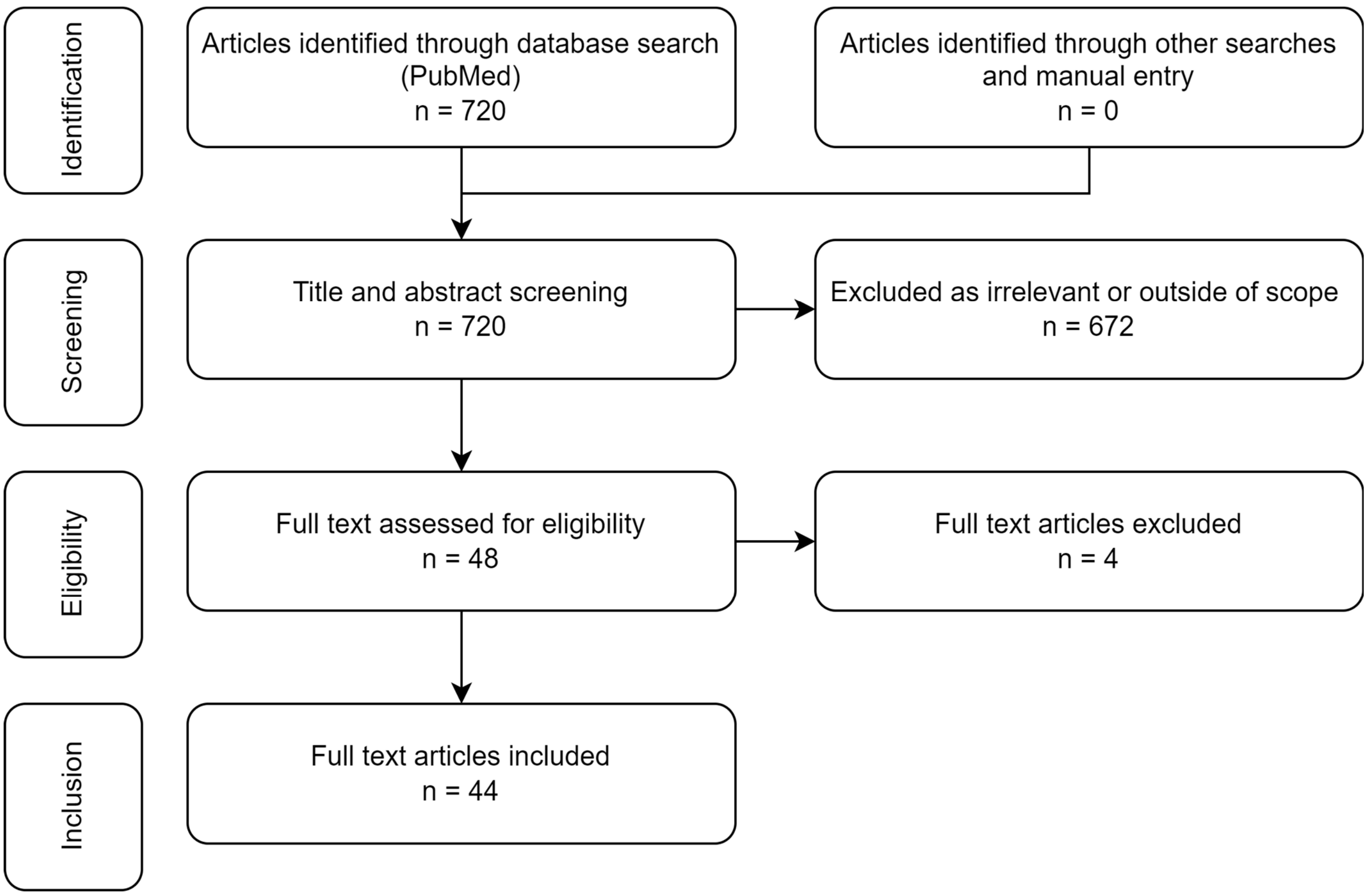

Figure 1. Flow diagram of the literature search process.

Table 2. Publications reporting microbial reductions using chitosan as a coagulant.

References	Treatment Topic			Treatment Mechanism		
	Water Type	**Microorganism**	**Other Contaminants**	**Type of Mechanism and Process**	**Type of Chitosan**	**Form of Chitosan**
Li et al., 2019 [10]	Drinking, Surface/ Recreational	*E. coli* and *S. aureus*	-	Direct use in solution and with sand filtration as composites; evidence of protein leakage from bacteria	Chitosan chloride-graphene oxide composites alone or combined with quartz sand	Used in water and also combined with modified quartz sand filter media to treat 2° wastewater effluent
Shukla et al., 2015 [11]	Reagent water	*Entamoeba invadens* cysts	-	absorption, adsorption and magnetic capture	Chitosan (>90% deacetylation degree) oligosaccharide-coated iron oxide nanoparticles	Chitosan-iron oxide nanoparticles
Soros et al., 2019 [12]	Drinking water with added total organic carbon and total dissolved solids	Clay turbidity as a surrogate for microorganisms	Kaolinite and bentonite clays	Coagulation, flocculation and sedimentation	Chitosans (11 total) with and without chemically different functional groups, deacetylation degrees and molecular weights	Chitosan powder dissolved in test waters; dosed at 1–30 mg/L concentrations in jar test flocculators
Cooper et al., 2013 [13]	Drinking	*S. aureus*	Polystyrene particles of 0.1, 0.3, and 1 micrometer	Membrane filter composites containing chitosan	Chitosan (85% deacetylation degree)-polycaprolactone nanofibrous membranes as mats for disinfection and filtration	Nanofibrous membrane mats to disinfect and remove particles
Morsi et al., 2017 [14]	Bacteria suspensions with added yeast extract; also raw and effluent sewage and sludges	*E. coli, S. aureus, A. flavus*	-	Chitosan composites of nanoparticles to disinfect bacteria and fungi suspensions with yeast extract and also raw and treated sewage and sludges	Chitosan composites of Ag or Cu nanoparticles or carbon nanotubes tested individually and when used as combinations	Composites tested individually or combined to disinfect microbe-yeast extract suspensions and wastewaters at contact times up to 30 min
Holmes et al., 2023 [15]	Surface (reservoir) water	*E. coli*, coliphage MS2	-	Coagulation, flocculation and sand filtration in columns	Chitosan acetate powder (85–95% deacetylation degree)	Dissolved in water at 2 g/L; dosed to test water at 3, 10 and 30 mg/L, then coagulated, flocculated, settled and sand filtered
Christensen and Myrmel., 2018 [16]	Untreated drinking water sources (surface water)	MS2 coliphage, Hepatitis A virus, Bovine norovirus, Bovine coronavirus	-	Coagulation, flocculation and filtration	KitoFlokk (commercial) (80% deacetylation degree); compared to polyaluminum chloride and zirconium oxychloride coagulants	Solution (2%) in 0.1 M HCl; dosed at 2–15 mg/L, coagulated, flocculated, centrifuged and filtered
Ferrero et al., 2014 [17]	Cultured bacteria diluted in water	*E. coli, S. aureus* and *Klebsiella pneumophila*; disinfection	-	Chitosan coated onto cotton gauze, UV irradiated and tested in suspensions and as filters to disinfect	Reagent chitosan (DD 75–85%) coated or cured onto cotton; tested as suspensions and by filtration	Chitosan coated or UV-cured onto cotton gauze fabric; used in suspension and as filters
Oza et al., 2022 [18]	Surface (reservoir) water only and with 10% pasteurized primary sewage	*E. coli*, MS2	Turbidity	Coagulation, flocculation and gravity filtration through 12 layers of cotton cloth	Chitosan acetate (93% deacetylation degree)	Chitosan powder dissolved in water, dosed at 10 mg/L to test water, flocculated, settled and then cloth filtered
Natarajan et al., 2016 [19]		Water bacteria (3 strains), individually and combined in minimal M9 culture medium	-	Contact disinfection of bacteria with chitosan/TiO_2 or Ag nanoparticles as thin film membranes	Chitosan (in 2% acetic acid) combined with TiO_2 or Ag nanoparticles creating thin film nanocomposites as contact disinfectants for water	Nanocomposites in contact with 3 strains of water bacteria separately or combined for 24 h at 37 °C
Brown and Emelko., 2009 [20]	Raw water (dechlorinated tap) with 2.5–5.0 NTU kaolinite turbidity, 300–330 mg/L $CaCO_3$ alkalinity, dissolved organic carbon (DOC)	*Cryptosporidium*	Microspheres	Chitosan compared to alum and ferric chloride for coagulation/ flocculation	Commercial chitosan polymer (Chitoclear®(Siglufjörður, Iceland) of unspecified chemical properties; powder dissolved in 0.1 M HCl and diluted in test water	Chitosan coagulant used alone and with granular media (sand and anthracite) filter columns, compared to alum and ferric chloride at 18–22 °C

Table 2. *Cont.*

References	Treatment Topic			Treatment Mechanism		
	Water Type	**Microorganism**	**Other Contaminants**	**Type of Mechanism and Process**	**Type of Chitosan**	**Form of Chitosan**
Fabris et al., 2010 [21]	Drinking water sources	Not specified (none reported)	Turbidity, dissolved organic carbon, color and UV absorbance	Coagulation, flocculation, sedimentation and then filtration	Chitosan powder (85% deacetylation degree) dissolved at 1% in 1% HCl and dosed at 4 and 12 mg/L; compared to polyaluminum chloride (PACl)	Chitosan alone compared to PACl; also used combined with ion exchange resin and activated carbon
Christensen et al., 2017 [22]	Raw drinking water source (river water)	*E. coli*, *Cryptosporidium*, MS2, and *S. typhimurium* 28B bacteriophage	Turbidity, TOC (total organic carbon) and color	Coagulation and sand filtration	KitoFlokk (Kløfta, Norway) (80% deacetylation degree)	Chitosan dose of 1 mg/L, diluted from 0.5% stock in 0.1M HCl
Correia et al., 2015 [23]	Drinking	*E. coli* and *S. aureus*	No other analytes	Absorption and adsorption for contact disinfection of diluted bacteria on scaffold surfaces	Chitosan (75–85% deacetylated, Mw = 190–310 kDa mol^{-1}) dissolved in 1% acetic acid (*v*/*v*) and then freeze-dried with other constituents to create 3D scaffold structures.	Oligo(2-methyl-2-oxazoline) quaternized with N,N-dimethyldodecylamine and grafted to a chitosan (CHT) scaffold (CHT-OMetOx-DDA)
Coleman et al., 2021 [24]	Raw surface water for drinking water, with or without added primary sewage at 1% volume	*E.coli*, *C. perfringens*, sewage derived *E. coli* and total coliforms, MS2, Qβ and ΦX174 coliphages, and sewage derived F+ and somatic coliphages	Turbidity	Filtration with ceramic filters preceded by coagulation and flocculation	Chitosan acetate (80–95% degree of deacetylation and molecular weight 30–200 kDa) or chitosan lactate (80–95% degree of deacetylation and molecular weight 30–500 kDa) in deionized water	Prepared from powder as 2% stocks and dosed to give 10 and 30 mg/L in test waters
Jin et al., 2017 [25]	Reservoir water, microfiltered	*Microcystis aeruginosa*	-	Coagulation and flocculation to capture and then storage to lyse cells	Chitosan quaternary ammonium salt, dissolved in distilled water to 1 mg/mL	Quaternary ammonium salt powder (20,000 g/mole molecular mass)
Cao et al., 2020 [26]	NaCl solution	*E. coli*	-	Electrochemical filtration and contact disinfection using composite membranes	3D cubic ordered mesoporous carbon with chitosan (85% deacetylation degree and 50 kDa molecular weight)	Used as activated carbon electrode in a recirculating system to disinfect saline water
Hu et al., 2019 [27]	Bacteria culture diluted in water	*E. coli*	-	Absorption, adsorption and disinfection	Chitosan-biochar-nanosilver composite made by high temperature carbonization	Synthesized chitosan composite dosed at 0.4–0.8 g/L into *E. coli* in water for up to 9 h contact times
Abebe et al., 2016 [28]	Phosphate buffered saline (PBS)	*E. coli*, MS2	Turbidity	Coagulation, flocculation, sedimentations and ceramic filtration	Chitosan acetate, HCl, and lactate (80–95% deacetylation degree) to treat water	Commercial chitosan sources, dissolved in PBS and tested for coagulation, flocculation and ceramic filtration
Wang et al., 2015 [29]	Reagent water with NaCl	*E. coli*, *Staphylococcus aureus* and *Candida albacans*	-	Absorption, adsorption and disinfection	Electrode of cationic nanohybrids of graphene oxide-graft-quaternized chitosan	Electrode in flowing water with NaCl to disinfect, followed by electrode regeneration
Strand et al., 2002 [30]	Reagent water with NaCl	*E. coli*, *Enterobacter cloacae*, *Serratia marcescens*, *Microccocus luteus*, *Bacillus megaterium*, *Pseudomonas putida*, and *Pseudomonas Rhodococcus* sp. 094	-	Coagulation, flocculation and sedimentation	Chitosans with 3 different degrees of deacetylation (high) moderate and low), as hydrochlorie salts	Chitosans in water containing NaCl and bacteria; stirred then sedimented
Mi et al., 2014 [31]		Porcine parvovirus and Sindbis virus	-	Adsorption and filtration from test water	Quaternized chitosan N-[(2-hydroxyl-3-trimethylammonium) propyl] chitosan (HTCC)	Chitosan (75–85% deacetylated and 190–310 kDa molecular weight) as quaternized nanofibers electrospun as a thin mat filter

Table 2. *Cont.*

References	Treatment Topic			Treatment Mechanism		
	Water Type	Microorganism	Other Contaminants	Type of Mechanism and Process	Type of Chitosan	Form of Chitosan
Lu et al., 2015 [32]	Swimming pool water	Cryptosporidium-sized polystyrene microspheres	-	Coagulation and filtration	Chitosan coagulation followed by sand filtration	SeaKlear (Syracuse, NY, USA) commercial chitosan product (properties unspedified and original company acquired or repeatedly resold)
Shao et al., 2012 [33]	Natural water plus *Microcystis aeruginosa* culture	*Microcystis aeruginosa* (a cause of harmful algal blooms)	Cell damage parameters indicative of disinfection	Coagulation, flocculation and sedimentation	Chitosan modified kaolinite added to *Microcystis aeruginosa* in culture medium plus natural water	Chitosan-kaolinite clay flocculant
Mandloi et al., 2004 [34]	Surface water	Total coliforms	Turbidity	Coagulation, flocculation and rapid sand filtration with chitosan, or *Moringa oleifera* or maize polymers	Chitosan (unspecified) dissolved in acetic acid, then diluted in water and dosed at 0.05 to 0.2 mg/L	Coagulation, flocculation with chitosan or other coagulant then rapid sand filtration in mini-columns
Habtemariam et al., 2021 [35]	Reservoir water	Cyanobacteria	Turbidity	Coagulation, flocculation and sedimentation	Commercial chitosan source dissolved in acidified water at 1 g/L	1–8 mg/L doses for coagulation–flocculation and sedimentation
Du et al., 2023 [36]	Chemically defined water representing surface water with algal blooms	Cultured cyanobacteria, green algae and diatoms	Extracellular organic material, turbidity and chlorophyl a	Coagulation, flocculation and dissolved air flotation of algae in bicarbonate buffered 1.8 mM NaCl	Nanochitosan grafted flocculant (PAD-g-MNC)	Branched chain chitin nanoparticles synthesized from chitin (deacetylation degree 80–95%) and high molecular weight
Chung et al., 2005 [37]	Eel aquaculture wastewater; biofiltered	HPC bacteria	Turbidity, suspended solids, BOD, COD, ammonia, phosphate	Coagulation, flocculation, sedimentation	Commercial chitosans (90% deacetylation degree) of high and low molecular weight (3.6×10^5, 4.7×10^4 & 6.2×10^3)	Chitosans dissolved in 0.2 M acetic acid
Chen et al., 2020 [38]	Surface/recreational	*Salmonella typhimurium*	Turbidity, as kaolinite clay in water plus *Salmonella* in boiled tap water	Coagulation, flocculation and disinfection	Nanochitosan-grafted flocculant	Chitosan (95% deacetylation degree) grafted to quaternary ammonium polyacrylamide, creating flocculant-disinfectant
Thongsamer et al., 2023 [39]	Surface water (canal water)	*E. coli*, HPC bacteria and ammonium oxidizing, nitrite oxidizing, denitrifying, polyphosphate accumulating and denitrifying phosphate-accumulating bacteria	Nutrient and bacteria removal	Absorption, adsorption, filtration	Coconut husk biochar pellets modified with commercial chitosan in 1% acetic acid; rinsed in water and dried.	Biochar-chitin columns as filters
Zhang et al., 2020 [40]	Wastewater	Bacteria including *Staphylococcus aureus* and *E. coli* O157:H7	Oily wastewater	Cellulose-chitin aerogel used to filter diluted bacteria cultures	Nanocrystalline cellulose and chitosan (50,000 MW and 90% deactylation degree)	Quaternized N-halamine siloxane polymer to form nanocrystalline cellulose and chitosan as aerogel
Parkpian et al., 2002 [41]	Wastewater	Fecal coliform bacteria	Heavy metals (Zn, Cu, Ni, Pb)	Absorption, adsorption, filtration and leaching	Chitosan and zeolite	Packed columns of sludge leached with tap water and sewage effluent
Sato et al., 2015 [42]	Primary effluent wastewater	Algae and *H. pluvialis*	Turbidity and suspended solids	Coagulation, Flocculation and sedimentation	Commercial chitosan, 1200 kDa molecular weight (Chitosan 500 from Wako Chemical (Richmond, VA, USA))	Chitosan powder in HCl dosed at 2 and 3 mg/L into primary effluent wastewater
Mohamed Hatta et al., 2023 [43]	Activated sludge	Not specified	Activated sludge dewatering	Coagulation, flocculation and sedimentation	Chitosan-like bioflocculant BF01314, chemically resembling chitosan; compared to commercial chitosan and a cationic polymer	N-acetylglucosamine and glucosamine- like polymer from *Citrobacter youngae* GTC 01314 and high molecular weight chitosan (319–375 kDa)

Table 2. *Cont.*

References	Treatment Topic			Treatment Mechanism		
	Water Type	**Microorganism**	**Other Contaminants**	**Type of Mechanism and Process**	**Type of Chitosan**	**Form of Chitosan**
Cainglet et al., 2020 [44]	Primary and secondary wastewater	Not specified	Turbidity, BOD, COD, suspended solids, nutrients	Coagulation, flocculation and sedimentation	Chitosan and 10 other coagulants	Low molecular weight chitosan in acetic acid and dosed at 7.5 mg/L
Guo et al., 2021 [45]	Anaerobically digested swine wastewater	Immobilized *B. subtilis* in chitosan-alginate composite as dried pellets	Ammonia removal from anaerobically digested swine wastewater	Biological treatment to reduce ammonia in batch reactors at 25 °C and optimum pH	Chitosan-sodium alginate composite with *Bacillus subtilis* as dried pellets to treat anaerobically digested swine wastewater	Immobilized bacteria pellets using chitosan-sodium alginate composites to decrease ammonia
de Godos et al., 2011 [46]	Algae and bacterial consortium from pig wastewater	Cultured green algae (3 species) and wastewater bacteria consortium	Removal efficiency of test algae and bacteria as percentages	Coagulation, flocculation and sedimentation	Commercial chitosan plus 6 other coagulants dosed at 5–250 mg/L in model algal-bacteria piggery wastewater	Commercial dry chitosan in 1% acetic acid to dose into model wastewater
Gani et al., 2017 [47]	Wastewater from treatment plant	Microalgae *Botryococcus* spp.	Algal biomass recovery	Coagulation, flocculation and sedimentation of test algae in wastewater	Chitosan source not specified	Chitosan (3% in acetic acid) dosed at 30–180 mg/L to determine optimum flocculation
Holder et al., 2017 [48]	Primary wastewater	Not specified because not studied	COD Removal	Filtration by composite membranes of chitosan-graphine oxide and phosphoric acid	Chitosan proton exchange membranes synthesized by crosslinking graphine oxide-phosphoric acid and chitosan (chitosan (200 kDa molecular weight and 67% deacetylation degree)	Commercial dry chitosan dissolved in 2% acetic acid and used to create composite membrane
Shitu et al., 2022 [49]	Synthetic aquaculture wastewater	Nitrogen metabolizing bacterial community of *Blastocatellia*, *Actinobacteria*, *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, and *Caldilineae*	Nitrogen metabolism for ammonium removal from organic wastewater by nitrification, denitrification and ammonium oxidation	Enhancement of bioreactor performance to degrade nitrogenous aquaculture wastewater	Chitosan-based natural sludge aggregates to enhance biofilm reactor performance in treating aquaculture effluent	Chitosan-based natural sludge aggregates of chitosan (95% deacetylation degree), acetic acid and activated sludge
Zhang et al., 2019 [50]	Stabilization pond wastewater sludge	Not specified	Moisture reduction, settlement ratio, % transmittance, sludge particle size and extracellular polysaccharides	Coagulation, flocculation and sedimentation	Chitosan (>90% deacetylation degree) 1% in acetic acid to dose sludge as grams/grams sludge total solids	Chitosan dosed to sludge in jar tests for coagulation, flocculation and sedimentation
Cainglet et al., 2023 [51]	Wastewater sludge	Not specified	Biogas production (methane and CO_2), nutrients (N and P) and inorganic metals removal	Anaerobic digestion and composting	Chitosan (low molecular weight), polyamine and polyaluminum chloride as coagulants/flocculants	Chitosan, polyamine and polyaluminum chloride dosed into sludge as treatments
Lin et al., 2023 [52]	Wastewater sludge (secondary)	Not specified	Sludge dewatering percentage, particle size, filtration resistance, compression, zeta potential and fractile dimension	Mechanical pressure filtration to dewater sludge	Chitosan (200,000 molec. wt.) and cationic polyacrylamide (10–12 million molecular wt.)	Mechanical pressure filtration of sludge pre-treated with chitosan or cationic polyacrylamide
Shafi et al., 2021 [53]	Model wastewater of inorganic salts and *E. coli*	*E. coli*	Inorganic salts	Nanofiltration by composite membrane	Nanofiltration membrane composed of chitosan (85% deacetylation degree and 120 Kda molec. wt.), acetic acid, piperazine, sodium polyphosphate, aqueous amine and trymesoyl	Chitosan-based thin film composite nanofiltration membrane

3.2. Chitosan in Drinking Water Treatment

Chitosan has been applied historically as well as more recently in drinking water and wastewater applications as a pre-treatment or partial treatment for the removal and/or disinfection of a variety of microbial contaminants, as well as turbidity as a microbial surrogate. The goal of these treatments is to increase the removal efficiency and/or disinfection of water and waste treatment technologies, particularly point-of-use (POU) and household water treatment technologies. For these types of technologies, the World Health Organization (WHO) has set performance targets that include "protective", and "highly protective" tiers for bacterial targets of two and four $\log_{10}$ reductions, respectively, and viral reductions of three and five $\log_{10}$, respectively [54]. In studies examining chitosan alone, such as coagulation, flocculation, and sedimentation, the "protective" level of protection was often but not always met for bacteria, viruses, and turbidity [12,16,21,28,30]. In studies examining other combination water treatment technologies, such as chitosan pre-treatment followed by sand filters or cloth filters, additional $\log_{10}$ reductions were possible under certain conditions, but only the protective performance category was met [15,18]. In contrast, when ceramic water filters were evaluated with chitosan pre-treatment, over a four $\log_{10}$ reduction in viruses and over a six $\log_{10}$ reduction in bacteria were possible, achieving the highly protective WHO performance level [24].

In studies examining other microorganisms or surrogates, such as protozoan parasites and surrogate microspheres, removals were based on the dose and loading of the chitosan itself. One study examined low-dose applications and determined that 3.0 mg/L resulted in extensive coagulation, whereas lower doses were not effective [20].

Advances in Chitosan Technology for Drinking Water

In addition to its use as a coagulant, chitosan has also been modified for advanced drinking water and wastewater treatment. One of these modifications includes the incorporation of metallic nanoparticles as a composite into the chitosan material. Several studies examined the use of silver, finding *E. coli* reductions of less than one $\log_{10}$ [19,27], and copper or carbon, which resulted in $\log_{10}$ reductions between one and two for *S. aureus*, *E. coli*, and *A. flavus* [14]. In an evaluation of chitosan-oligosaccharide-coated iron oxide nanoparticles, approximately 86% of entamoeba cysts were recovered from simulated water samples [11].

Another modification of chitosan included changing the structure of chitosan to form quaternized chitosan. Using this material, porcine parovirus and Sindbis virus were reduced by 3.5 and 4 $\log_{10}$, respectively [31]. Other studies evaluating quaternized chitosan found reductions of greater than five $\log_{10}$ for *E. coli* [29] and greater than four $\log_{10}$ for *S. aureus* [23].

Other modifications included (1) chitosan coated with activated carbon, which resulted in a four $\log_{10}$ reduction in *E. coli* [26] and (2) cotton gauze coated with chitosan, which was used to disinfect Gram-positive and -negative bacteria [17]. Microfiltration membranes, which include chitosan as a component of the membrane itself, were also effective [13,25]. Specifically, Cooper et al. (2013) reported a 50% *Staphylococccus aureus* reduction by contact disinfection with chitosan (85% deacetylation degree)-polycaprolactone nanofiber mats and also a 100% removal of polystyrene particles of 0.1-, 0.3-, and 1-micrometer diameter by mat filtration [13]. Also, Jin et al. (2017) reported *Microcystis aeruginosa* removal from drinking water by coagulation, flocculation, and sedimentation with chitosan (molecular mass of 20,000 g/mol; (deacetylation degree not reported), followed by a >95% disinfection in the floc stored for 6–8 days [25].

3.3. Surface and Recreational Water Treatment

Of the studies included in this review, nine focused on surface water treatment. Five studies investigated chitosan coagulation–flocculation alone [33,35–38], three studies utilized filtration after chitosan pre-treatment [22,32,34], and one study utilized a combination of adsorption and filtration [39]. Five [33,35–38] studies reported on chitosan coagulation–flocculation alone, of which three papers focused specifically on cyanobacteria or algae removal [33,35,36]. Shao et al. used 80 and 160 mg/L of chitosan-modified kaolinite loading to significantly reduce the levels of chlorophyll-a, carotenoids, phycocyanin, and allophycocyanin in water spiked with *Microcystis aeruginosa*, indicating that chitosan pre-treatment could lead to effective cell mortality [33]. Similarly, Habtemariam et al. (2021) optimized chitosan dosing at 4 mg/L to achieve a 59.9% reduction in chlorophyll-a and a 62.1% decrease in turbidity in turbid lake and reservoir waters [35]. Du et al. (2023) developed a nanochitosan-grafted flocculant (PAD-g-MNC), which achieved removal rates of 93.5%–95.4% for turbidity and 95.1–97.3% for chlorophyll-a at dosages of 4–5 mg/L of PAD-g-MNC [36].

Similar to Du et al., Chen et al. synthesized a dual-functioning nanochitosan-grafted flocculant (CPAM-g-NCS), which demonstrated significant flocculation and antibacterial performance, achieving a residual turbidity of 1.97 NTU in a low-turbidity *Salmonella* suspension [38]. Chung et al. utilized chitosan coagulation–flocculation to test the treatment of aquaculture wastewater [37]. The authors reported 99.998% removal of unspecified bacteria using 12 mg/L of high molecular weight chitosan (dissolved in 0.2 M acetic acid).

Four studies integrated coagulation–flocculation with subsequent filtration to enhance microbial removal [22,32,39]. Studies utilized a variety of filtration media, including sand [32,34], various other granular media [22], and one that was chitosan-modified [39].

Two studies that tested the use of chitosan pre-treatment followed by sand filtration resulted in a <1 $\log_{10}$ reduction [32,34]. Mandloi et al. (2004) found an optimum dose of 0.15 mg/L of unspecified chitosan flakes dissolved in a 1% acetic acid solution for the treatment of water with a turbidity of 15 NTU [34]. Pre-treatment was followed by sand filtration, which together resulted in total coliform reductions (MPN/100 mL) of 73.2% after 60 min and 87.5% after 120 min. Lu et al. (2016) studied the removal of cryptosporidium-sized microspheres from pool water by testing various doses of chitosan, ranging from 4.68 to 34.32 mg/L of SeaKlear commercial chitosan, followed by sand filtration. Pre-treatment followed by filtration could only remove <75% of the cryptosporidium-sized microspheres (compared to 20–63% with filtration alone) [32]. Christensen et al. (2017) reported pre-treatment using a 1 mg/l dose of KitoFlokk (diluted from a 0.5% stock solution in 0.1 M HCl) followed by dual-media contact filtration, which resulted in 2.5–3 $\log_{10}$ reductions in viruses and parasites (including cryptosporidium) and 4.5–5.0 $\log_{10}$ reductions in bacteria [22]. The reasons for microbial reduction performance differences among different studies are unclear, but they could be related to differences in chitosan properties and doses, the in-test water quality, and differences related to the specific test microbes.

Thongsamer et al. incorporated both filtration and adsorption mechanisms by using fixed-bed biofilters with chitosan-modified coconut husk biochar pellets, which achieved a 66–81% removal of *E. coli* from lake water; the effectiveness was not significantly different from other biofilter types [39].

3.4. Wastewater Treatment

Chitosan coagulants have shown significant promise as a green alternative to metallic and synthetic polymer coagulants for reducing inorganic and organic contaminants [10,40–50,52,53]. While much of the chitosan research focuses on the removal or reducing the bioavailability of heavy metals in wastewater treatment [51], several studies demonstrate its potential to reduce fecal pathogen concentrations in wastewater and sewage from wastewater treatment facilities [10,40,41,53]. Wastewater and sewage sludge contain high levels of fecal coliforms, with concentrations of 10^6 to 10^8 in raw sewage and even higher concentrations in various wastewater sludges [55–57].

The discharge, disposal, and possible beneficial use of treated wastewater effluents and sludges is based in part on achieving pathogen concentrations below the maximum allowable regulatory limits. The coagulation and flocculation of wastewaters and sludges with chitosan can aid in reducing fecal pathogen concentrations to meet pathogen reduction requirements based on fecal coliform (FC) or *E. coli* concentrations in the treated sludge (biosolids). The Environmental Protection Agency in the USA requires achieving Class A biosolids with <1000 for the most probable number (MPN) of FC/g of the total dry-weight solids and Class B to have no more than 2×10^6 FC MPN/G of the total dry-weight solids [41,58]. Globally, the World Health Organization specifies microbial quality guidelines for treated wastewater sludge as well as treated wastewater based on allowable levels of *E. coli* bacteria [59]. The *E. coli* limit for treated sludge is 1000 culturable organisms per gram of total solids. The *E. coli* limit for treated wastewater used for agriculture irrigation (food crop production) is 1000 organisms per liter for the unrestricted irrigation of crops eaten raw and a higher limit of 100,000 organisms per liter when other management methods are employed, such as a lengthy storage period before crop harvest to achieve further pathogen die-off.

In summary, this literature review revealed studies of treatments by chitosan and its various forms for a wide variety of microorganisms that were evaluated in labs and at wastewater treatment facilities. Chitosan has been shown to reduce microbial concentrations of culturable fecally derived bacteria [41], such as *E. coli* [10,40,53] and *S. aureus* [10,40], in wastewater and sludges. Cainglet et al. 2023 found that microbial community composition assessed through 16s rRNA-sequencing displayed composition changes between different stages of biological stabilization but did not display changes between the coagulant choice [44]. They also found a low overall abundance of genera of known pathogenic species and fecal indicators and found *Enterococcus* spp. in >1% of all coagulant-derived thickened raw sludge, while *Mycobacterium* had a relative abundance of >1% for chitosan and polyaluminum chloride sludge samples [51].

Research on chitosan has also investigated applications for the treatment of nutrient loads in wastewater using algae. For example, chitosan coagulants remove suspended solids and colloids to clarify the water and allow for the cultivation of microalgae such as *Botryococcus* spp. [47] and *Haematococcus pluvialis* [42] to aid in the removal of nitrogen and phosphorous, as well as to reduce unwanted algal biomass [46] when desired. Chitosans have found additional use as a constituent in forming membranes and in the development of biotechnologies to aid in the treatment of wastes. As an example, Guo et al., 2021 investigated ammonia removal by *Bacillus* spp. immobilized in chitosan beads and reported a 96.5% reduction [45].

4. Discussion

In this review, we found considerable evidence that chitosan enhances the reduction of microorganisms and related constituents in water, wastewater, and sludge in a variety of circumstances. In many cases, the combination of chitosan with point-of-use water treatment technologies allowed these treatment methods to reach the "protective" or "highly protective" performance levels specified by the WHO Household Water Treatment recommendations. In wastewater treatment, chitosan evaluated for the removal of both nutrient loads and bacteria was often extensive and better than alternative treatment alternatives.

This systematic review had a number of limitations. First, we limited the review to studies published online, so we may have missed early literature on chitosan coagulation or disinfection that was not available through online tools. Second, because our search strategy focused on studies that examined microbial removal using chitosan coagulation and flocculation, we may have missed important studies on chitosan performance that did not have documented microbial removal information but addressed the removal of other matrix contaminants or constituents. Our review suggests that chitosan and its various forms and derivatives are effective coagulants in surface water, wastewater, and sludges. However, there is a continued need to evaluate microbial removals or disinfection by the various chemical and physical forms and derivatives of chitosan to determine their most effective doses for coagulation and/or disinfection in each matrix type alone or in combination with other treatment processes, such as filtration.

A limitation of the available literature on chitosans for microbial reductions and disinfection is that all studies we found were lab-based or on a pilot scale. To our knowledge, there have not been large-scale field studies of chitosans for microbial reductions in full-scale municipal water or wastewater systems. This remains an unmet need that we recommend be filled by further studies.

Our findings demonstrate that chitosan is an effective resource in the coagulation and eventual removal and/or disinfection of microorganisms in water, wastewater, and sludge. Hence, we encourage chitosan to be considered an alternative coagulant and potential disinfectant in water, wastewater, and sludge treatment systems. As there is the potential for chitosan to be produced locally from the shells of shrimp and other crustaceans, as well as from certain insects, it presents a low-cost alternative to other inorganic and organic chemical coagulants currently used in water and waste treatment.

In recent work comparing the cost of traditional coagulants to chitosan, the cost of using alum for drinking water treatment ranged from 0.05 to 1.50 U.S. dollars (USD) [60,61] compared to 0.0025 for chitosan per meter cubed (m^3) [62]. Meanwhile, in wastewater, the cost for alum was USD 0.10 [62] compared to 0.015 for chitosan per m^3 [61]. Not all biocoagulants are less expensive than alum or traditional chemical coagulants, but chitin or chitosan does cost less, generally due to lower operational costs [62].

There are many opportunities to further improve and expand water and wastewater treatment systems using chitosan, especially in the developing world, where small businesses and entrepreneurs may be able to create and market such products and systems at affordable costs [24].

5. Conclusions

Based on our review of the currently available literature on chitosan coagulation and the disinfection of microorganisms in water, wastewater, and sludges, we have summarized compelling evidence that chitosan is an effective coagulant and potential disinfectant in these settings. From this examination, it is clear that there are opportunities to incorporate this neglected coagulant and potential disinfectant into water and wastewater treatment systems and to further explore them for removal and inactivation of microorganisms.

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References

1. Renault, F.; Sancey, B.; Badot, P.M.; Crini, G. Chitosan for coagulation/flocculation processes—An eco-friendly approach. *Eur. Polym. J.* **2009**, *45*, 1337–1348. [CrossRef]
2. Kumar, M.N.V.R.; Muzzarelli, R.A.A.; Muzzarelli, C.; Sashiwa, H.; Domb, A.J. Chitosan Chemistry and Pharmaceutical Perspectives. *Chem. Rev.* **2004**, *104*, 6017–6084. [CrossRef] [PubMed]
3. Strand, S.P.; Danielsen, S.; Christensen, B.E.; Vårum, K.M. Influence of chitosan structure on the formation and stability of DNA-chitosan polyelectrolyte complexes. *Biomacromolecules* **2005**, *6*, 3357–3366. [CrossRef] [PubMed]
4. Florea, B.I.; Thanou, M.; Junginger, H.E.; Borchard, G. Enhancement of bronchial octreotide absorption by chitosan and N-trimethyl chitosan shows linear in vitro/in vivo correlation. *J. Control. Release* **2006**, *110*, 353–361. [CrossRef]
5. Bielefeldt, A.R.; Kowalski, K.; Schilling, C.; Schreier, S.; Kohler, A.; Scott Summers, R. Removal of virus to protozoan sized particles in point-of-use ceramic water filters. *Water Res.* **2010**, *44*, 1482–1488. [CrossRef]
6. Strand, S.P.; Vårum, K.M.; Østgaard, K. Interactions between chitosans and bacterial suspensions: Adsorption and flocculation. *Colloids Surf. B Biointerfaces* **2003**, *27*, 71–81. [CrossRef]
7. Rinaudo, M. Chitin and chitosan: Properties and applications. *Prog. Polym. Sci.* **2006**, *31*, 603–632. [CrossRef]
8. Zhang, L.; Zeng, Y.; Cheng, Z. Removal of heavy metal ions using chitosan and modified chitosan: A review. *J. Mol. Liq.* **2016**, *214*, 175–191. [CrossRef]

9. Gerente, C.; Lee, V.K.C.; Cloirec, P.L.; McKay, G. Application of Chitosan for the Removal of Metals From Wastewaters by Adsorption—Mechanisms and Models Review. *Crit. Rev. Environ. Sci. Technol.* **2007**, *37*, 41–127. [CrossRef]
10. Li, X.; Sun, J.; Che, Y.; Lv, Y.; Liu, F. Antibacterial properties of chitosan chloride-graphene oxide composites modified quartz sand filter media in water treatment. *Int. J. Biol. Macromol.* **2019**, *121*, 760–773. [CrossRef]
11. Shukla, S.; Arora, V.; Jadaun, A.; Kumar, J.; Singh, N.; Jain, V.K. Magnetic removal of Entamoeba cysts from water using chitosan oligosaccharide-coated iron oxide nanoparticles. *Int. J. Nanomed.* **2015**, *10*, 4901–4917. [CrossRef] [PubMed]
12. Soros, A.; Amburgey, J.E.; Stauber, C.E.; Sobsey, M.D.; Casanova, L.M. Turbidity reduction in drinking water by coagulation-flocculation with chitosan polymers. *J. Water Health* **2019**, *17*, 204–218. [CrossRef] [PubMed]
13. Cooper, A.; Floreani, R.; Ma, H.; Bryers, J.D.; Zhang, M. Chitosan-based nanofibrous membranes for antibacterial filter applications. *Carbohydr. Polym.* **2013**, *92*, 254–259. [CrossRef] [PubMed]
14. Morsi, R.E.; Alsabagh, A.M.; Nasr, S.A.; Zaki, M.M. Multifunctional nanocomposites of chitosan, silver nanoparticles, copper nanoparticles and carbon nanotubes for water treatment: Antimicrobial characteristics. *Int. J. Biol. Macromol.* **2017**, *97*, 264–269. [CrossRef]
15. Holmes, E.B.; Oza, H.H.; Bailey, E.S.; Sobsey, M.D. Evaluation of Chitosans as Coagulants-Flocculants to Improve Sand Filtration for Drinking Water Treatment. *Int. J. Mol. Sci.* **2023**, *24*, 1295. [CrossRef]
16. Christensen, E.; Myrmel, M. Coagulant residues' influence on virus enumeration as shown in a study on virus removal using aluminium, zirconium and chitosan. *J. Water Health* **2018**, *16*, 600–613. [CrossRef]
17. Ferrero, F.; Periolatto, M.; Vineis, C.; Varesano, A. Chitosan coated cotton gauze for antibacterial water filtration. *Carbohydr. Polym.* **2014**, *103*, 207–212. [CrossRef]
18. Oza, H.H.; Holmes, E.B.; Bailey, E.S.; Coleman, C.K.; Sobsey, M.D. Microbial reductions and physical characterization of chitosan flocs when using chitosan acetate as a cloth filter aid in water treatment. *PLoS ONE* **2022**, *17*, e0262341. [CrossRef]
19. Natarajan, S.; Bhuvaneshwari, M.; Lakshmi, D.S.; Mrudula, P.; Chandrasekaran, N.; Mukherjee, A. Antibacterial and antifouling activities of chitosan/TiO2/Ag NPs nanocomposite films against packaged drinking water bacterial isolates. *Environ. Sci. Pollut. Res.* **2016**, *23*, 19529–19540. [CrossRef]
20. Brown, T.J.; Emelko, M.B. Chitosan and metal salt coagulant impacts on Cryptosporidium and microsphere removal by filtration. *Water Res.* **2009**, *43*, 331–338. [CrossRef]
21. Fabris, R.; Chow, C.W.; Drikas, M. Evaluation of chitosan as a natural coagulant for drinking water treatment. *Water Sci. Technol.* **2010**, *61*, 2119–2128. [CrossRef] [PubMed]
22. Christensen, E.; Nilsen, V.; Håkonsen, T.; Heistad, A.; Gantzer, C.; Robertson, L.J.; Myrmel, M. Removal of model viruses, E. coli and Cryptosporidium oocysts from surface water by zirconium and chitosan coagulants. *J. Water Health* **2017**, *15*, 695–705. [CrossRef] [PubMed]
23. Correia, V.G.; Ferraria, A.M.; Pinho, M.G.; Aguiar-Ricardo, A. Antimicrobial Contact-Active Oligo(2-oxazoline)s-Grafted Surfaces for Fast Water Disinfection at the Point-of-Use. *Biomacromolecules* **2015**, *16*, 3904–3915. [CrossRef] [PubMed]
24. Coleman, C.K.; Mai, E.; Miller, M.; Sharma, S.; Williamson, C.; Oza, H.; Holmes, E.; Lamer, M.; Ly, C.; Stewart, J.; et al. Chitosan Coagulation Pretreatment to Enhance Ceramic Water Filtration for Household Water Treatment. *Int. J. Mol. Sci.* **2021**, *22*, 9736. [CrossRef]
25. Jin, Y.; Pei, H.; Hu, W.; Zhu, Y.; Xu, H.; Ma, C.; Sun, J.; Li, H. A promising application of chitosan quaternary ammonium salt to removal of Microcystis aeruginosa cells from drinking water. *Sci. Total Environ.* **2017**, *583*, 496–504. [CrossRef]
26. Cao, C.; Wu, X.; Zheng, Y.; Chen, Y. Three-dimensional cubic ordered mesoporous carbon with chitosan for capacitive deionization disinfection of water. *Environ. Sci. Pollut. Res.* **2020**, *27*, 15001–15010. [CrossRef]
27. Hu, Z.; Zhang, L.; Zhong, L.; Zhou, Y.; Xue, J.; Li, Y. Preparation of an antibacterial chitosan-coated biochar-nanosilver composite for drinking water purification. *Carbohydr. Polym.* **2019**, *219*, 290–297. [CrossRef]
28. Abebe, L.S.; Chen, X.; Sobsey, M.D. Chitosan Coagulation to Improve Microbial and Turbidity Removal by Ceramic Water Filtration for Household Drinking Water Treatment. *Int. J. Environ. Res. Public Health* **2016**, *13*, 269. [CrossRef]
29. Wang, Y.; El-Deen, A.G.; Li, P.; Oh, B.H.L.; Guo, Z.; Khin, M.M.; Vikhe, Y.S.; Wang, J.; Hu, R.G.; Boom, R.M.; et al. High-Performance Capacitive Deionization Disinfection of Water with Graphene Oxide-graft-Quaternized Chitosan Nanohybrid Electrode Coating. *ACS Nano* **2015**, *9*, 10142–10157. [CrossRef]
30. Strand, S.P.; Nordengen, T.; Østgaard, K. Efficiency of chitosans applied for flocculation of different bacteria. *Water Res.* **2002**, *36*, 4745–4752. [CrossRef]
31. Mi, X.; Vijayaragavan, K.S.; Heldt, C.L. Virus adsorption of water-stable quaternized chitosan nanofibers. *Carbohydr. Res.* **2014**, *387*, 24–29. [CrossRef] [PubMed]
32. Lu, P.; Amburgey, J.E. A pilot-scale study of Cryptosporidium-sized microsphere removals from swimming pools via sand filtration. *J. Water Health* **2015**, *14*, 109–120. [CrossRef] [PubMed]
33. Shao, J.; Wang, Z.; Liu, Y.; Liu, H.; Peng, L.; Wei, X.; Lei, M.; Li, R. Physiological responses of Microcystisaeruginosa NIES-843 (cyanobacterium) under the stress of chitosan modified kaolinite (CMK) loading. *Ecotoxicology* **2012**, *21*, 698–704. [CrossRef] [PubMed]
34. Mandloi, M.; Chaudhari, S.; Folkard, G.K. Evaluation of Natural Coagulants for Direct Filtration. *Environ. Technol.* **2004**, *25*, 481–489. [CrossRef]

35. Habtemariam, H.; Kifle, D.; Leta, S.; Mucci, M.; Lürling, M. Removal of cyanobacteria from a water supply reservoir by sedimentation using flocculants and suspended solids as ballast: Case of Legedadi Reservoir (Ethiopia). *PLoS ONE* **2021**, *16*, e0249720. [CrossRef]
36. Du, B.; Tang, Q.; Chen, W.; Rong, X.; Zhang, K.; Ma, D.; Wei, Z.; Chen, W. Insight into the purification of algael water by a novel flocculant with enhanced branched nanochitosan structure. *J. Environ. Manag.* **2023**, *331*, 117283. [CrossRef]
37. Chung, Y.-C.; Li, Y.-H.; Chen, C.-C. Pollutant Removal From Aquaculture Wastewater Using the Biopolymer Chitosan at Different Molecular Weights. *J. Environ. Sci. Health Part A* **2005**, *40*, 1775–1790. [CrossRef]
38. Chen, W.; Rong, X.; Peng, J.; Tang, Q.; Luo, H.; Fan, L.; Feng, K.; Zheng, H. Assessment of a novel nanostructured flocculant with elevated flocculation and antimicrobial activity. *Chemosphere* **2020**, *239*, 124736. [CrossRef]
39. Thongsamer, T.; Vinitnantharat, S.; Pinisakul, A.; Werner, D. Fixed-bed biofilter for polluted surface water treatment using chitosan impregnated-coconut husk biochar. *Environ. Pollut.* **2023**, *334*, 122137. [CrossRef]
40. Zhang, Y.; Yin, M.; Li, L.; Fan, B.; Liu, Y.; Li, R.; Ren, X.; Huang, T.-S.; Kim, I.S. Construction of aerogels based on nanocrystalline cellulose and chitosan for high efficient oil/water separation and water disinfection. *Carbohydr. Polym.* **2020**, *243*, 116461. [CrossRef]
41. Parkpian, P.; Leong, S.T.; Laortanakul, P.; Poonpolwatanaporn, P. Environmental applicability of chitosan and zeolite for amending sewage sludge. *J. Environ. Sci. Health Part A* **2002**, *37*, 1855–1870. [CrossRef] [PubMed]
42. Sato, H.; Nagare, H.; Huynh, T.N.C.; Komatsu, H. Development of a new wastewater treatment process for resource recovery of carotenoids. *Water Sci. Technol.* **2015**, *72*, 1191–1197. [CrossRef] [PubMed]
43. Mohamed Hatta, N.S.; Lau, S.W.; Chua, H.B.; Takeo, M.; Sen, T.K.; Mubarak, N.M.; Khalid, M.; Zairin, D.A. Parametric and kinetic studies of activated sludge dewatering by cationic chitosan-like bioflocculant BF01314 produced from Citrobacter youngae. *Environ. Res.* **2023**, *224*, 115527. [CrossRef] [PubMed]
44. Cainglet, A.; Tesfamariam, A.; Heiderscheidt, E. Organic polyelectrolytes as the sole precipitation agent in municipal wastewater treatment. *J. Environ. Manag.* **2020**, *271*, 111002. [CrossRef]
45. Guo, J.; Chen, C.; Chen, W.; Jiang, J.; Chen, B.; Zheng, F. Effective immobilization of Bacillus subtilis in chitosan-sodium alginate composite carrier for ammonia removal from anaerobically digested swine wastewater. *Chemosphere* **2021**, *284*, 131266. [CrossRef]
46. de Godos, I.; Guzman, H.O.; Soto, R.; García-Encina, P.A.; Becares, E.; Muñoz, R.; Vargas, V.A. Coagulation/flocculation-based removal of algal–bacterial biomass from piggery wastewater treatment. *Bioresour. Technol.* **2011**, *102*, 923–927. [CrossRef]
47. Gani, P.; Mohamed Sunar, N.; Matias-Peralta, H.; Abdul Latiff, A.A.; Mohamad Fuzi, S.F.Z. Growth of microalgae Botryococcus sp. in domestic wastewater and application of statistical analysis for the optimization of flocculation using alum and chitosan. *Prep. Biochem. Biotechnol.* **2017**, *47*, 333–341. [CrossRef]
48. Holder, S.L.; Lee, C.-H.; Popuri, S.R. Simultaneous wastewater treatment and bioelectricity production in microbial fuel cells using cross-linked chitosan-graphene oxide mixed-matrix membranes. *Environ. Sci. Pollut. Res.* **2017**, *24*, 13782–13796. [CrossRef]
49. Shitu, A.; Zhang, Y.; Danhassan, U.A.; Li, H.; Tadda, M.A.; Ye, Z.; Zhu, S. Synergistic effect of chitosan-based sludge aggregates CS@NGS inoculum accelerated the start-up of biofilm reactor treating aquaculture effluent: Insights into performance, microbial characteristics, and functional genes. *Chemosphere* **2022**, *303*, 135097. [CrossRef]
50. Zhang, J.; Hu, Q.; Lu, J.; Lin, S. Study on the effect of chitosan conditioning on sludge dewatering. *Water Sci. Technol.* **2019**, *79*, 501–509. [CrossRef]
51. Cainglet, A.; Kujala, K.; Liimatainen, M.; Prokkola, H.; Piippo, S.; Postila, H.; Ronkanen, A.-K.; Heiderscheidt, E. The influence of coagulant type on the biological treatment of sewage sludge. *Sci. Total Environ.* **2023**, *869*, 161706. [CrossRef] [PubMed]
52. Lin, F.; Qin, W.; Wei, J.; Lv, J.; Yang, P. Effect of chemical regulation combined with mechanical filtration on deep dewatering and consolidation characteristics of sludge. *Environ. Sci. Pollut. Res.* **2023**, *30*, 89088–89100. [CrossRef] [PubMed]
53. Shafi, Q.I.; Ihsan, H.; Hao, Y.; Wu, X.; Ullah, N.; Younas, M.; He, B.; Rezakazemi, M. Multi-ionic electrolytes and *E. coli* removal from wastewater using chitosan-based in-situ mediated thin film composite nanofiltration membrane. *J. Environ. Manag.* **2021**, *294*, 112996. [CrossRef] [PubMed]
54. World Health Organization. *WHO International Scheme to Evaluate Household Water Treatment Technologies*; WHO: Geneva, Switzerland, 2014.
55. Rose, J.B.; Dickson, L.J.; Farrah, S.R.; Carnahan, R.P. Removal of pathogenic and indicator microorganisms by a full-scale water reclamation facility. *Water Res.* **1996**, *30*, 2785–2797. [CrossRef]
56. George, I.; Crop, P.; Servais, P. Fecal coliform removal in wastewater treatment plants studied by plate counts and enzymatic methods. *Water Res.* **2002**, *36*, 2607–2617. [CrossRef]
57. George, I.; Crop, P.; Servais, P. Use of beta-D-galactosidase and beta-D-glucuronidase activities for quantitative detection of total and fecal coliforms in wastewater. *Can. J. Microbiol.* **2001**, *47*, 670–675.
58. U.S. Environmental Protection Agency. NPDES Inspection Manual. In *CFR Part 503 Subpart D, EPA Method 1681*; U.S. Environmental Protection Agency: Washington, DC, USA, 2017.
59. World Health Organization. *Guidelines for the Safe Use of Wastewater, Excreta and Greywater—Volume 4: Excreta and Greywater Use in Agriculture*; World Health Organization, United Nations Environment Programme, Eds.; WHO: Geneva, Switzerland, 2013.
60. Keeley, J.; Jarvis, P.; Judd, S.J. An economic assessment of coagulant recovery from water treatment residuals. *Desalination* **2012**, *287*, 132–137. [CrossRef]

61. Hamawand, I.; Ghadouani, A.; Bundschuh, J.; Hamawand, S.; AAl Juboori, R.; Chakrabarty, S.; Yusaf, T. A Critical Review on Processes and Energy Profile of the Australian Meat Processing Industry. *Energies* **2017**, *10*, 731. [CrossRef]
62. Kangama, A.; Zeng, D.; Tian, X.; Fang, J. Application of Chitosan Composite Flocculant in Tap Water Treatment. *J. Chem.* **2018**, *2018*, 2768474. [CrossRef]