REVIEW

CHITOSAN-BIOSURFACTANT HYBRID SYSTEMS: FROM PHYSICOCHEMICAL INTERACTIONS TO FUNCTIONAL BIOFORMULATIONS

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Abstract

Chitosan-biosurfactant (CS-BS) systems represent an emerging class of hybrid bio-based materials with applications in environmental, biomedical, and agricultural fields. Despite extensive research on each component individually, their combined behaviour remains poorly understood, particularly regarding structure-function relationships. This review analyses the physicochemical interactions between chitosan and biosurfactants, focusing on electrostatic attraction, hydrophobic association, and interfacial activity. These interactions influence colloidal stability, delivery efficiency, and biological performance. Most CS-BS systems function as co-formulated hybrids rather than well-defined supramolecular complexes, although evidence of structured assemblies is emerging. The combination enhances antimicrobial activity, pollutant removal, and plant growth responses, with delivery-driven synergy identified as a key mechanism. However, limited structural characterisation and a lack of systematic parameter studies remain major challenges for rational design and scalable implementation.

Keywords: *chitosan, biosurfactants, polyelectrolyte-surfactant interactions, hybrid biomaterials, antimicrobial systems, plant biostimulants*

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1. Introduction

The increasing demand for biodegradable, sustainable, and multifunctional materials has intensified research focused on bio-based polymers and surface-active biomolecules. Among these, chitosan, a partially deacetylated derivative of chitin, has emerged as a versatile biopolymer due to its biodegradability, biocompatibility, and unique physicochemical properties, including cationic charge, film-forming ability, and metal-chelating capacity [1–3].

Biosurfactants are amphiphilic molecules produced by bacteria, yeasts and fungi that reduce surface and interfacial tension while exhibiting low toxicity, high biodegradability and excellent environmental compatibility. Depending on their chemical structure, biosurfactants are commonly classified into glycolipids (e.g., rhamnolipids and sophorolipids), lipopeptides (e.g., surfactin), phospholipids, polymeric biosurfactants, and particulate biosurfactants. Beyond their surface-active properties, biosurfactants participate in microbial motility, biofilm formation, hydrocarbon uptake, quorum sensing and plant-microbe interactions, making them attractive candidates for environmental, agricultural and biomedical applications [4, 5]. In recent years, biosurfactants have attracted increasing attention as sustainable alternatives to synthetic surfactants due to their low toxicity, biodegradability, and ability to perform under environmentally relevant conditions. Consequently, they have been investigated for applications ranging from environmental remediation and agriculture to drug delivery and biomedical formulations [6, 7].

Despite the extensive literature describing chitosan and biosurfactants individually, comparatively few studies have investigated their integration into multifunctional hybrid systems. This gap is particularly notable given the complementary and potentially synergistic nature of their functionalities. Chitosan provides structural integrity, bioadhesion, film-forming ability, and controlled release capacity [1, 8, 9], whereas biosurfactants contribute high interfacial activity, improved wettability, enhanced dispersion of hydrophobic compounds in aqueous and colloidal systems, thereby improving their bioavailability and delivery, as well as membrane-interacting properties [4, 10]. Although direct experimental evidence remains limited, the complementary physicochemical properties of chitosan and biosurfactants suggest that their combination may generate multifunctional systems. Based on current knowledge of polymer-surfactant interactions and the individual properties of both components, it may be hypothesised that their interaction is primarily governed by electrostatic attraction, hydrophobic association, and the formation of colloidal assemblies. However, these proposed mechanisms remain hypothetical and require direct experimental validation in integrated CS-BS systems [11].

Recent studies indicate that biosurfactants can act as functional modulators in polymeric systems, influencing particle size distribution, surface charge, ζ-potential, and interfacial activity [12–14]. Chitosan, in turn, provides structural integrity, controlled release capacity, and bioadhesive properties [15, 16]. For example, Bettencourt *et al.* [17] demonstrated that chitosan-based particles can serve as carriers for glycolipid biosurfactants, resulting in enhanced antimicrobial efficacy and stability of active compounds. More broadly, the integration of biosurfactants into colloidal and polymeric systems has been shown to improve dispersion, facilitate the encapsulation of hydrophobic agents, and enhance biological interactions, which are key factors in applications such as drug delivery, antimicrobial coatings, and environmental remediation [11, 18].

Importantly, the potential of chitosan-biosurfactant systems extends far beyond conventional applications such as plant growth stimulation [19]. Emerging evidence suggests that the synergistic combination of chitosan and biosurfactants may enable

advanced functionalities in areas such as bioremediation of contaminated environments [10], disruption of microbial biofilms [5, 17], development of responsive delivery systems [15, 17], and fabrication of functional biomaterials with tailored interfacial properties [20]. Nonetheless, the lack of understanding of the interaction mechanisms and the resulting structure-function relationships currently limits the design and wider implementation of such bioformulations.

In this context, the aim of this review is to analyse the physicochemical basis and application potential of chitosan-biosurfactant systems, with particular emphasis on the synergistic effects of combining these two components. The review focuses on the fundamental mechanisms of chitosan-biosurfactant interactions, formulation strategies, and emerging applications beyond plant growth promotion, including environmental, biomedical, and industrial applications. This review aims to provide a comprehensive perspective on this underdeveloped but highly promising field by synthesising the most relevant and representative literature available to date, including both seminal studies and recent advances, and by highlighting key directions for future research on CS-BS systems.

2. Structure-Function Relationships in CS-BS Systems

A fundamental understanding of CS-BS interactions is essential for the rational design of functional hybrid systems. Although direct studies of combined CS-BS biopreparations remain scarce, the underlying mechanisms can be proposed on the basis of principles of polyelectrolyte-surfactant interactions and biopolymer physics.

2.1. Insights from Model Polyelectrolyte-Surfactant Systems

Fundamental insights into the interaction between chitosan and amphiphilic molecules originate from studies of polyelectrolyte-surfactant systems. In these systems, chitosan behaves as a cationic polymer under acidic conditions due to protonation of its amino groups. The interaction capability of chitosan is predominantly determined by its molecular structure. The extent of deacetylation dictates the density of protonatable amino groups, thereby affecting the polymer's positive charge in acidic environments, while molecular weight impacts solution viscosity, chain conformation, and intermolecular interactions [2, 21]. Thus, these structural characteristics affect electrostatic interactions with oppositely charged surfactants and enhance the stability of the resultant complexes.

Many biosurfactants, particularly glycolipids such as rhamnolipids and sophorolipids, exhibit anionic or partially anionic character depending on pH and molecular structure [4]. This provides favourable conditions for the formation of polyelectrolyte-surfactant complexes (PESC). Experimental observations demonstrate that surfactant molecules can associate with the polymer chain, leading to the formation of polymer-surfactant complexes and significant changes in macromolecular conformation and solution properties. These studies further show that surfactant association may involve cooperative effects and aggregation phenomena along the polymer backbone [22].

It should be emphasised that these mechanisms have been directly demonstrated primarily in the case of synthetic, chemically homogeneous surfactants such as sodium dodecyl sulfate (SDS). Biosurfactants differ structurally from these model compounds in several important respects: they are typically produced as complex mixtures of congeners with varying chain lengths and degrees of glycosylation, their charge is strongly pH-dependent (e.g., the carboxylate group in rhamnolipids confers anionic character only

above pKa), and their amphiphilicity is more variable and context-dependent [23–25]. Similarly, sophorolipids exist in lactonic (non-ionic) and acidic (anionic) forms, whose relative proportions critically affect interfacial behaviour [23]. These structural complexities suggest that caution should be exercised when extrapolating interaction mechanisms established for synthetic polyelectrolyte-surfactant systems to biosurfactant-chitosan systems, owing to the greater structural heterogeneity and pH-dependent behaviour of microbial surfactants [26]. Their applicability to biosurfactant systems is therefore inferred on the basis of structural analogy, but requires independent experimental validation. Figure 1 illustrates potential interaction modes between chitosan and biosurfactants.

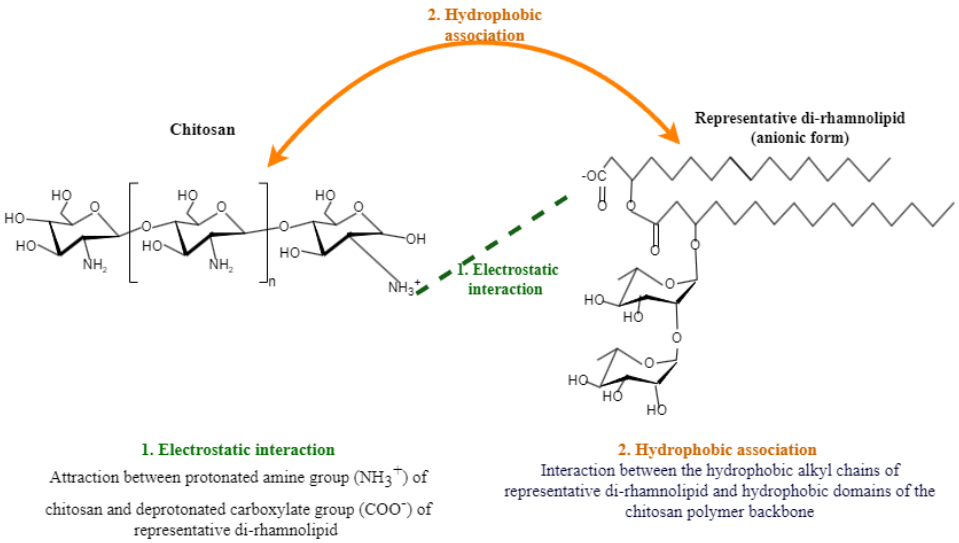

Figure 1. Schematic illustration of the proposed interactions between chitosan and biosurfactants (figure created based on [2, 20, 21, 27]). These interaction mechanisms remain hypothetical and have not yet been experimentally verified at the molecular level.

Figure 1 presents the currently proposed model for the interaction between chitosan and biosurfactants. The interaction is anticipated to consist of two complementary mechanisms. Under acidic conditions (typically below pH ~6.5), electrostatic attraction between the protonated amino groups of chitosan and the anionic headgroups of the biosurfactant is likely predominant, while at greater biosurfactant concentrations and during aggregate formation, hydrophobic interactions between the acetylated regions of the polymer and the hydrophobic chains of the biosurfactants gain significance. Hydrogen bonding can also stabilise these transient polymer-surfactant complexes. The resultant colloidal structures are estimated to be influenced by pH, ionic strength, the molecular attributes of chitosan (notably molecular weight and degree of deacetylation (DDA)), and composition of the biosurfactant mixture. At present, no experimental study has directly resolved these interaction pathways in CS-BS systems at the molecular level. The proposed model, therefore, represents a mechanistic hypothesis derived from current knowledge of biosurfactants, chitosan, and polyelectrolyte-surfactant systems.

2.2. Insights from Antimicrobial Systems

Although only a limited number of studies are currently available, experimental investigations of CS-BS combinations have generally demonstrated enhanced antimicrobial activity compared with the individual components [17, 28, 29]. Notably, Bettencourt *et al.* [17] demonstrated that rhamnolipid-chitosan nanoparticles (RLs-CS_p) achieved 99% inhibition of *Staphylococcus aureus* ATCC 25923 biofilms with a MIC of 130 μg/mL, outperforming the individual components, and confirmed cytocompatibility with human dermal fibroblasts.

Microscopic observations reported by Bettencourt *et al.* [17] suggest more extensive structural damage to microbial cells in the combined systems than with either component alone. A plausible working hypothesis is that biosurfactants may initially increase cell membrane permeability, facilitating subsequent interactions between chitosan and the cell envelope. Nevertheless, this proposed sequential mechanism remains hypothetical and should be regarded as a conceptual framework for future investigation rather than an established mechanism of action. Although rhamnolipid-induced membrane permeabilisation has been documented using flow cytometry and confocal laser scanning microscopy [30], while chitosan is known to depolarise bacterial membranes and induce potassium efflux [31], studies directly tracking the temporal sequence of these events in combined systems are currently lacking. This mechanistic gap is a priority for future research, as kinetic studies that examine the sequence and interdependence of these processes would substantially strengthen the proposed structure-function framework.

From a structural-functional viewpoint, the augmented antibacterial efficacy of chitosan-biosurfactant complexes is likely attributable to physicochemical alterations during complex formation, rather than the isolated effects of the two components. Electrostatic interactions between protonated chitosan and anionic biosurfactants can alter particle surface characteristics, whereas hydrophobic interactions may affect colloidal arrangement and interfacial dynamics [17, 29, 32, 33]. These modifications can enhance the distribution of formulations at biological interfaces, prolong residence time on microbial surfaces, and promote interactions between active ingredients and cell membranes [17, 32, 33]. While the specific contributions of each pathway are yet to be measured, existing evidence indicates that alterations in colloidal organisation and surface characteristics may significantly influence the apparent antibacterial activity [17, 32].

2.3. Insights from Nanoemulsion Systems

Nanoemulsion-based formulations may provide additional insight into chitosan interactions with biosurfactants. In such systems, biosurfactants primarily act as emulsifiers, creating stable oil-water systems, while chitosan contributes to surface charge and colloidal stability. For example, rhamnolipid-stabilised nanoemulsions have been successfully used to encapsulate hydrophobic bioactive compounds, improving colloidal stability and biological efficacy [13]. The subsequent deposition of a chitosan layer further increases the ζ-potential and storage stability through electrostatic stabilisation of the emulsion droplets [34]. In chitosan-based systems, the introduction of surfactants or emulsifiers often alters the ζ-potential, particle size distribution, and colloidal stability, which strongly influences the biological properties and release efficiency. Furthermore, electrostatic interactions between chitosan and amphiphilic molecules may enhance surfactant retention within polymer matrices, potentially reducing diffusion and prolonging residence time at target interfaces. This hypothesis is inferred

from studies of polymer–surfactant interactions and has not yet been directly demonstrated for CS-BS systems [26, 35].

Spectroscopic analysis by Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (FTIR-ATR) reveals changes in characteristic vibrational bands upon combining the components, suggesting the presence of interactions between chitosan and biosurfactants at the molecular level [17, 26]. However, FTIR data alone do not permit clear assignment of the type of interaction (e.g., electrostatic or hydrophobic), because band shifts may reflect changes in hydrogen bonding, ionic association, or conformational rearrangement. Resolving the nature of these interactions would require complementary techniques with higher specificity and resolution. Isothermal titration calorimetry (ITC) could quantify binding enthalpies and stoichiometry, and has been successfully applied to chitosan-surfactant systems containing SDS [36]. Solution-state Nuclear Magnetic Resonance (NMR, e.g., NOESY - Nuclear Overhauser Effect Spectroscopy, or diffusion-ordered spectroscopy) could provide evidence for close spatial contacts between specific molecular groups [37]. These methods have not been applied to CS-BS systems to date, representing an important methodological gap. The existence of interactions at the molecular level is thus confirmed, but their precise nature and thermodynamic driving forces remain unresolved.

In the currently available chitosan–biosurfactant nanoemulsion systems, enhanced antimicrobial and antibiofilm activity has been associated with alterations in microbial cell surface properties and disruption of the extracellular polymeric substance (EPS) matrix [38]. These observations suggest that the colloidal organisation of the formulation contributes significantly to its biological performance. In particular, smaller droplets provide a larger contact area with microbial cells, while chitosan-rich positively charged interfaces may enhance adhesion to negatively charged cell surfaces, thereby increasing the effectiveness of the formulation. Similar relationships between colloidal organisation and functional performance have also been demonstrated in CS-BS coacervate systems. A particularly relevant example of direct structural characterisation of biosurfactant-polyelectrolyte complex formation comes from work on sophorolipid-chitosan systems: complex coacervation of bolaform sophorolipid micelles with chitosan oligosaccharide lactate was studied by a combination of dynamic light scattering (DLS), cryo-transmission electron microscopy (cryo-TEM), and small-angle X-ray scattering (SAXS), demonstrating pH-dependent formation of structured colloidal assemblies and confirming that sophorolipid micelles maintain their structural integrity after binding to the cationic polyelectrolyte [20]. This study, although performed on a specific sophorolipid form, provides direct structural evidence of colloidal complex formation between a biosurfactant and chitosan, thereby establishing a methodological benchmark for future work on rhamnolipid- and surfactin-based biopreparations.

2.4. Linking Physicochemical Interactions to Functional Performance

In summary, the available evidence indicates that CS-BS systems operate through a combination of interfacial, colloidal, and biological effects that are strongly influenced by their physicochemical properties. Biosurfactants primarily modulate interfacial behaviour, including membrane permeability and dispersion, whereas chitosan contributes to adsorption, charge neutralisation, and structural destabilisation of biological systems [39].

As discussed in the preceding sections, the performance of chitosan-biosurfactant systems is governed by several interconnected physicochemical variables, including the DDA and molecular weight of chitosan, pH-dependent protonation, and the molecular

structure and aggregation state of biosurfactants [40]. Together, these parameters are expected to influence colloidal organisation, interfacial behaviour, and ultimately biological performance. Polymer-surfactant theory further suggests that electrostatic and hydrophobic interactions may drive the formation of transient or semi-structured assemblies, thereby influencing system stability, transport, and biological activity [41]. However, direct experimental evidence for such assemblies in CS-BS systems remains scarce.

Current findings suggest the observed synergy may be more plausibly interpreted not as the consequence of a single, well-defined molecular mechanism, but as an emergent property resulting from the coupling of interfacial activity, biopolymer-mediated interactions, and multiscale system organisation. These considerations highlight the need for systematic studies combining advanced physicochemical characterisation techniques, including DLS, ζ-potential analysis, and calorimetric or scattering methods, with functional assays to establish quantitative structure-function relationships in CS-BS systems. Such an integrated approach has been proposed as essential for understanding complex polymer-based colloidal systems, but remains largely unexplored for chitosan-biosurfactant formulations [42]. In particular, SAXS and small-angle neutron scattering (SANS) would allow characterisation of the mesoscale organisation of CS-BS complexes in solution - including aggregate geometry, interparticle spacing, and the preservation of biosurfactant micellar structure upon complexation - parameters that cannot be fully resolved using light scattering or FTIR alone [43]. Cryo-TEM would complement these data by providing direct nanoscale imaging of colloidal structures in their native hydrated state, avoiding fixation and drying artefacts inherent to conventional TEM [43]. ITC could provide thermodynamic parameters, including binding enthalpy, stoichiometry, and association constants, enabling discrimination between predominantly electrostatic and entropically driven association mechanisms [44]. Integrating these techniques with established functional assays: antimicrobial, antibiofilm, and encapsulation efficiency, would enable the first systematic mapping of structure-activity relationships in CS-BS systems, and would transform the current descriptive framework into a predictive, design-oriented one.

3. Formulation Strategies and Material Architectures

The practical efficacy of CS-BS combinations is fundamentally dependent on the method by which they are integrated into a functional material form. The selection of formulation approach dictates the physicochemical characteristics of the final biopreparation, including particle size, surface charge, colloidal stability, and encapsulation efficiency, which in turn affects biological performance, delivery kinetics, and application range. Three primary material architectures have been identified in the literature. The first comprises nanoparticulate systems, in which biosurfactants are integrated into or co-assembled with chitosan matrices during particle synthesis. The second includes nanoemulsion systems, in which biosurfactants serve as the primary emulsifiers, while chitosan modulates interfacial characteristics and colloidal stability. The third consists of complex coacervate and Pickering emulsion systems formed through electrostatic interactions between anionic biosurfactant micelles and cationic chitosan chains [45]. Each architecture exhibits distinct physicochemical and functional characteristics.

3.1. Nanoparticulate Systems

Ionotropic gelation, based on the spontaneous cross-linking of protonated chitosan chains by multivalent anions such as sodium tripolyphosphate (TPP), is the most widely used technique for preparing chitosan nanoparticles [40, 46] and has been successfully applied to the preparation of CS-BS nanoparticulate systems [17, 29]. The process is carried out under mild, aqueous, and organic solvent-free conditions, which is particularly advantageous when incorporating biosurfactants that may be sensitive to heat or organic solvents [47]. In chitosan-biosurfactant formulations, ionotropic gelation typically yields nanoparticles ranging from approximately 150 to 400 nm with a positive ζ-potential, although these properties strongly depend on formulation parameters such as chitosan molecular weight, chitosan/TPP ratio, pH, biosurfactant type, and preparation conditions [17, 29, 48].

Marangon *et al.* [29] showed that incorporation of rhamnolipid during ionic gelation reduces particle size and polydispersity while maintaining positive surface charge, indicating surface exposure of chitosan amino groups. The resulting nanoparticles exhibited enhanced antimicrobial activity and complete biofilm eradication at low concentrations [29].

Bettencourt *et al.* [17] extended this framework by separately preparing sophorolipid-chitosan nanoparticles (SLs-CS_p) and rhamnolipid-chitosan nanoparticles (RLs-CS_p) via the same ionic gelation approach, and comparing their physicochemical and biological properties. Encapsulation efficiency differed markedly between the two biosurfactants: RLs-CS_p achieved 74.2 ± 1.3% encapsulation efficiency with a mean diameter of 329.6 ± 8.0 nm, whereas SLs-CS_p showed only 41.1 ± 8.8% encapsulation efficiency and a smaller diameter of 210.0 ± 15.7 nm [17]. Notably, a synergistic antimicrobial effect was confirmed only for RLs-CS_p (99% biofilm inhibition against *S. aureus*), and HPLC-MS/MS confirmed sustained release of intact rhamnolipid from the nanoparticle matrix [17].

A source of chitosan that has received relatively little attention in formulation research is fungal biomass, which represents a promising alternative to conventional crustacean-derived chitosan. The physicochemical properties of fungal chitosan, including molecular weight and degree of deacetylation (DDA), depend strongly on the fungal species and extraction method. Nevertheless, several studies have reported fungal chitosan preparations with relatively low molecular weight and high DDA compared with many commercially available crustacean-derived chitosans [49–51].

Karamchandani *et al.* [28] extracted high-purity chitosan from *Cunninghamella echinulata* NCIM 691 and used it to prepare fungal chitosan nanoparticles (FCHNPs) by ionic gelation, which were subsequently combined with commercial rhamnolipid in synergy studies against phytopathogens. Nanoparticle Tracking Analysis (NTA) confirmed a mean particle size of 144.33 ± 10.20 nm - notably smaller than analogous nanoparticles prepared from commercial crustacean chitosan under the same conditions (256.33 ± 18.80 nm). The authors attributed these differences to the lower molecular weight and more uniform DDA of the fungal polymer. Combined application of rhamnolipid and FCHNPs reduced the minimum inhibitory concentration against *Xanthomonas campestris* NCIM 5028 from 256 μg/mL (rhamnolipid alone) and > 1024 μg/mL (FCHNPs alone) to 128/4 μg/mL, respectively - a reduction consistent with a synergistic, rather than merely additive, interaction as evaluated by the fractional inhibitory concentration index. Moreover, rhamnolipid combined with FCHNPs inhibited spore germination of *Fusarium* spp. by 61–90% in a dose-dependent manner, with activity at concentrations as low as 0.015–0.020% (w/v) [28].

Core-shell chitosan-coated rhamnolipid nanoparticles exhibited high encapsulation efficiency (~90%) while preserving the polymer structure. These observations suggest physical stabilisation by the chitosan coating rather than extensive molecular co-assembly, although the molecular organisation of the interface remains to be elucidated [52].

Collectively, these nanoparticulate studies reveal several formulation-relevant principles. First, the physicochemical outcome of ionic gelation is sensitive to the identity and form of the incorporated biosurfactant. For example, rhamnolipids generally exhibit higher encapsulation efficiency than sophorolipids under comparable preparation conditions, whereas differences in molecular geometry and charge influence particle size, surface properties, and release behaviour [17]. Second, the molecular weight and DDA of chitosan substantially influence nanoparticle formation. Existing evidence indicates that lower-molecular-weight chitosan may favour the formation of smaller nanoparticles because shorter polymer chains can form more compact cross-linked networks during ionotropic gelation. Conversely, higher-molecular-weight chitosan has frequently been associated with larger particle sizes, likely owing to increased solution viscosity and reduced chain mobility. However, this relationship is not universal and depends strongly on formulation parameters, including polymer concentration, chitosan/TPP ratio, pH, and preparation conditions [29, 47, 48]. Third, the architecture of the nanoparticle (co-assembled matrix vs core-shell coating) may determine release kinetics and the relative contributions of chitosan and the biosurfactant to the observed biological activity. These variables require systematic optimisation and should be explicitly reported in future formulation studies.

3.2. Nanoemulsion Systems

In nanoemulsion-based formulations, biosurfactants fulfil their classical role as surface-active agents by stabilising oil-in-water emulsions through reduction of interfacial tension, whereas chitosan acts as a secondary stabiliser, contributing a positive surface charge and modulating droplet-droplet interactions [53, 54]. Taken together, the available evidence suggests that this functional division is likely to generate colloidal systems with physicochemical and biological properties that differ from those of formulations containing either component alone. Yet the contributions of each component and the molecular mechanisms underlying their interactions remain poorly understood.

Rhamnolipid-stabilised nanoemulsions have been shown to exhibit enhanced wettability, improved biological uptake, and induced plant defence responses compared with rhamnolipid suspensions of equivalent concentration, effects that are thought to result from more efficient delivery of biosurfactants to biological interfaces rather than from changes in their chemical composition [55]. When chitosan is incorporated as a secondary stabiliser, electrostatic interactions between the positively charged polymer and the biosurfactant-coated droplet interface can modify the physicochemical characteristics of the nanoemulsion. These interactions may increase ζ-potential, may improve colloidal stability by reducing droplet aggregation, and enhance adhesion to negatively charged biological surfaces [52, 53]. However, the relative contribution of each mechanism has not yet been experimentally resolved, and this interpretation should be regarded as a working hypothesis.

It is worth noting that there is currently little direct experimental evidence in the scientific literature that clearly contrasts the efficacy of biosurfactant nanoemulsions with and without chitosan against biological targets. The enhanced activity may result from either chitosan-mediated adhesion or changes in droplet size, surface tension,

or biosurfactant release. This requires independent validation using suitable adhesion and desorption assays.

3.3. Complex Coacervate and Pickering Emulsion Systems

A more recently explored architecture exploits the electrostatic interaction between anionic (or pH-dependent) biosurfactant micelles and cationic chitosan chains to form PESC coacervates. Ben Messaoud *et al.* [20] demonstrated that bolaform acidic sophorolipids - whose micellar charge is negative above their apparent pKa of approximately 5.5 - combine with chitosan oligosaccharide, lactate and other polycations to form colloidal complex coacervates, the structure of which depends on pH and the biopolymer-to-biosurfactant ratio [20]. At acidic pH, electrostatic attraction can promote the formation of compact coacervate particles, while an increase in pH may cause structural rearrangement and ultimately disintegration of the complexes. Similarly, changes in the biopolymer-to-biosurfactant ratio may affect particle morphology and colloidal stability by changing the degree of charge neutralisation and intermolecular association [20]. Combined DLS, cryo-TEM, and SAXS characterisation revealed that sophorolipid micelles maintain their internal structural integrity after binding to the polyelectrolyte, transitioning between spherical coacervate particles and aggregated structures as a function of the coacervation stage. This is a qualitatively different behaviour from that observed in conventional polymer-surfactant systems with synthetic surfactants such as SDS, where micellar structure is typically disrupted upon strong polyelectrolyte binding [20].

In a subsequent development, the same group demonstrated that these PESC coacervates could function as Pickering emulsion stabilisers, producing pH-responsive oil-in-water emulsions in which the stability of the emulsion can be reversibly altered by pH adjustment. This property has potential applications in triggered release, stimuli-responsive formulations and the production of soft materials [45]. The formation of multilamellar wall vesicles as a result of pH-stimulated phase transitions in chitosan-glycolipid biosurfactant systems further illustrates the diversity of structured colloidal phases available through this approach [43].

From a formulation perspective, coacervate architectures may offer several potential advantages over ionotropically cross-linked nanoparticles. First, the process does not require additional cross-linking agents such as TPP, thereby simplifying the formulation. Second, the pH-dependent association between chitosan and biosurfactants may enable reversible assembly and stimuli-responsive behaviour. These features are inferred from current knowledge of biosurfactant self-assembly and polymer-surfactant complexation and remain to be systematically evaluated for CS-BS delivery systems [20, 23, 41]. The main current limitation is that most structural characterisation has been performed with sophorolipid in specific molecular forms (predominantly bolaform acidic sophorolipid from *Starmerella bombicola*) [20]. Whether comparable coacervate architectures form with rhamnolipids, surfactin, or other biosurfactants of different structural classes has not yet been systematically established.

3.4. Critical Formulation Parameters and Their Mutual Dependencies

Across all three architectural approaches, a set of formulation parameters recurs as critical determinants of the physicochemical and functional outcomes of CS-BS systems.

The molecular weight and DDA of chitosan determine chain flexibility, solution viscosity, and the density of protonated amino groups available for electrostatic association with anionic biosurfactants, thereby influencing particle formation, colloidal

stability, and the efficiency of interaction with biosurfactant molecules. The source of chitosan (crustacean vs. fungal) is an underexplored variable: chitosan derived from *Cunninghamella* sp. produces nanoparticles that are approximately 40–45% smaller than those from crustacean sources under the same preparation conditions [28], a difference that directly affects cellular uptake, biological activity and colloidal stability.

The pH of the formulation medium regulates the protonation state of chitosan (pKa 6.2–6.8) [2] and the charge of biosurfactants with ionizable carboxylate groups (pKa 4.5–5.5 for rhamnolipids [56]; pH-dependent for sophorolipids [57]). Above the pKa of chitosan, the progressive deprotonation of amino groups causes a decrease in the positive charge density of the polymer, thus weakening the electrostatic interactions involved in the formation of complexes. For formulations intended for physiological applications (pH ~7.4), this pH-dependent behaviour should be carefully considered, as chitosan may become partially or extensively deprotonated depending on its DDA and molecular weight [3]. The biosurfactant concentration relative to its critical micelle concentration (CMC) is another critical variable [20, 23]. Below the CMC, interactions are expected to occur predominantly between individual biosurfactant molecules and chitosan chains, whereas above the CMC, self-assembled micelles may associate with the polymer, giving rise to more complex colloidal architectures and potentially different physicochemical properties [20, 23]. The relative concentration of chitosan and biosurfactant, usually expressed as a charge or mass ratio, determines the degree of charge neutralisation and, consequently, whether soluble complexes, stable colloids or precipitates will form, as well as the total surface charge of the resulting particles. Despite the recognised importance of these variables, systematic studies mapping the phase behaviour of CS-BS systems as a function of pH, composition, and biosurfactant concentration remain lacking for most biosurfactant classes.

Table 1 summarises the principal formulation architectures reported for CS-BS preparations, together with the preparation methods, key physicochemical parameters, and functional outcomes documented in the primary literature.

3.5. Scalability, Stability, and Translational Constraints

Considering the biological potential of CS-BS nanoformulations evidenced in laboratory conditions, many translational limitations must be acknowledged. Ionic gelation generates nanoparticles with inherent pH sensitivity; as pH approaches the chitosan pKa, particle enlargement and aggregation may occur, reducing colloidal stability under physiological conditions [48]. Lyophilisation using a variety of cryoprotectants (e.g., trehalose, mannitol) is a widely used technique to enhance the long-term storage stability of chitosan nanoparticles; nevertheless, its impact on the integrity and preservation of biosurfactants within the matrix has not been systematically assessed for CS-BS systems [58, 59].

Converting ionotropic gelation from batch to continuous production presents challenges related to mixing homogeneity and the reproducibility of droplet generation, both of which influence particle size distribution and polydispersity [60]. Although these challenges have been described for chitosan nanoparticle production, their impact on CS-BS formulations has not yet been systematically evaluated.

Microfluidic techniques provide enhanced regulation of these parameters and have been effectively utilised in the synthesis of chitosan nanoparticles [61]. However, their adaptation to biosurfactant-containing formulations remains largely unexplored. The regulatory status of biosurfactants in pharmaceutical and food applications varies according to both the producing microorganism and the regulatory jurisdiction.

Table 1. Summary of formulation architectures, preparation methods, and key outcomes reported for CS-BS systems.

Source	BS	Formulation	Preparation	Physicochemical parameters	Functional outcome	[Ref]
squid pens chitosan (*Doryteuthis* spp.)	RL	CS/RL-NPs	ionotropic gelation (tpp)	reduced particle size compared to CS-NPs; increased ζ-potential; increased surface exposure of $–NH_2$ groups	MIC (*S. aureus*) 14/19 µg/mL; complete biofilm eradication at 58/75 µg/mL; low cytotoxicity	[29]
commercial chitosan	RL	RLs-CSp	ionotropic gelation	size 329.6 ± 8.0 nm; EE 74.2 ± 1.3%	RLs-CSp: MIC 130 µg/mL vs *S aureus*; 99% biofilm inhibition; cytocompatible with human dermal fibroblasts	[17]
commercial chitosan	SL	SLs-CSp Nps	ionotropic gelation	size 210.0 ± 15.7 nm; EE 41.1 ± 8.8%	cytocompatible	[17]
fungal chitosan (*Cunninghamella echinulata*)	RL	FCHNPs	ionic gelation	size 144.33 ± 10.20 nm; spherical morphology	RH-BS + FCHNPs synergistic: MIC for *X. campestris* reduced from 128 to 4 µg/mL; spore germination inhibition 61–90% (*Fusarium* spp.)	[28]
commercial chitosan	RL	core-shell NPs	solvent emulsification	size 150–300 nm; EE 90.2%; LC 42-50%; biopolymer molecular organisation preserved	enhanced antioxidant and antimicrobial activity; high biocompatibility; stable *in vitro*	[52]
chitosan oligosaccharide lactate	BASL	complex coacervates / pickering emulsions	pH-induced coacervation	pH-dependent phase boundary (coacervation pH 5-9); sophorolipid micelle structure preserved in complex	stable Pickering o/w emulsions; pH-switchable colloidal disassembly; biosurfactant micellar integrity maintained	[11, 20, 45]

Note. BS, biosurfactant; RL, rhamnolipid; di-RL, di-rhamnolipid; SL, sophorolipid; BASL, Bolaform acidic sophorolipid; NPs, nanoparticles; FCHNPs, fungal chitosan nanoparticles; EE, encapsulation efficiency; LC, loading capacity.

For example, rhamnolipids produced by *Pseudomonas aeruginosa* raise biosafety concerns because of the pathogenic nature of the producer strain, whereas sophorolipids from *Starmerella bombicola* and surfactin from *Bacillus subtilis* are generally considered more suitable candidates for food-related and pharmaceutical applications. Nevertheless, regulatory acceptance remains product- and jurisdiction-specific [62].

The relatively high production cost of biosurfactants compared with synthetic surfactants remains a major limitation for their large-scale application. However, advances in fermentation optimisation and the utilisation of agro-industrial waste streams as renewable carbon substrates are expected to improve the economic feasibility of biosurfactant production [14, 24]. Formulations that integrate biosurfactants with chitosan, primarily derived from low-cost crustacean processing waste, are expected to maintain a reasonable material cost for high-value applications such as biomedical coatings, precision agriculture, and functional food packaging, despite not being immediately competitive in commodity markets [14].

4. Emerging Applications of CS-BS Systems

The synergistic integration of chitosan (CS) with biosurfactants (BS) is a scarcely documented but versatile platform technology across several sectors [14]. The molecular complementarity between a cationic, film-forming polysaccharide and an amphiphilic, self-assembling biosurfactant results in nanostructured complexes with functional properties, such as surface charge modulation, antimicrobial and anti-adhesive activity, and improved interfacial behaviour, that may exceed those of each individual component [20]. In recent years, a few strategic applications have garnered substantial scientific and industrial focus, namely: the purification of emerging micropollutants in aquatic ecosystems, advanced biopesticide and biocontrol formulations [28], active coatings for post-harvest preservation [63], and ecological restoration of degraded soils [38]. Taken together, each domain exploits specific physicochemical synergies of CS-BS systems, forming a coherent research landscape that underpins this review [17, 20].

4.1. Bioremediation and Environmental Detoxification

CS-BS biopreparations offer a promising approach for the remediation of environments contaminated with heavy metals and hydrophobic organic pollutants. Based on the complementary properties of the individual components, chitosan may contribute through adsorption and chelation of metal ions [3], whereas biosurfactants may enhance the solubilization and mobilisation of hydrophobic contaminants [14]. This complementary functionality may enable the simultaneous mobilisation and capture of pollutants. Based on the complementary properties of both components, such systems are expected to improve remediation efficiency, although direct experimental evidence remains limited.

A key demonstration of this synergy comes from the development of rhamnolipid-functionalized chitosan magnetic nanoparticles (Rh-cMNPs). The resulting sorbent achieved 96.7% removal of acetaminophen with an adsorption capacity of 96.3 mg/g within 60 min at pH 5.0, outperforming analogous unfunctionalized sorbents [64]. More broadly, studies of biosurfactant-assisted bioremediation have demonstrated that biosurfactant production can be maintained under moderate salinity, thereby enhancing hydrocarbon bioavailability for microbial degradation [65]. Although these findings do not directly concern CS-BS systems, they illustrate the potential environmental robustness of biosurfactant-based remediation strategies. Based on the complementary functions of

chitosan and biosurfactants, incorporating biosurfactants into a chitosan matrix may be a promising strategy for sustained delivery of biosurfactants to contaminated sites. Nevertheless, this concept remains hypothetical and has not yet been experimentally validated in integrated CS-BS systems. Furthermore, studies conducted in real wastewater matrices containing complex pollutant mixtures, together with investigations of biosurfactant retention and desorption during sorbent regeneration, remain scarce and represent important directions for future research.

4.2. Biopesticide and Biocontrol Formulations

The available literature suggests that CS-BS formulations may act through three complementary mechanisms: direct antimicrobial activity, induction of systemic plant resistance, and improved biological stability through encapsulation. Based on studies of the individual components and the currently available CS-BS formulations, biosurfactants primarily disrupt microbial membranes, whereas chitosan interacts electrostatically with negatively charged cell envelope components [14, 17]. Biosurfactants present at the nanoparticle interface may also improve wetting of biological surfaces by reducing the contact angle, thereby facilitating particle-pathogen interactions and increasing the local concentration of active components [66]. Nonetheless, the relative contribution of these mechanisms has not yet been experimentally dissected in integrated CS-BS systems. The proposed mode of action is therefore inferred from studies on the individual components and related polymer-surfactant systems.

Karamchandani *et al.* [28] provide a representative example of the enhanced antimicrobial efficacy that can be achieved through CS-BS formulations, demonstrating synergistic activity against bacterial phytopathogens together with effective inhibition of fungal spore germination [28]. Four structurally distinct biosurfactants - surfactin, rhamnolipid, sophorolipid, and spiculisporic acid - were shown to reduce the severity of grape downy mildew and strawberry anthracnose while upregulating defence-related genes (beta-1,3-glucanase, class IV chitinase), with each activating a distinct resistance signalling pathway [67]. Incorporation of these biosurfactants into chitosan-based delivery systems could potentially prolong their residence time on plant surfaces and thereby sustain their biological activity; yet, this hypothesis remains to be experimentally verified.

Encapsulation of biological control agents (BCAs) within chitosan matrices represents a promising extension of chitosan-based delivery systems. For example, *Bacillus subtilis* encapsulated in chitosan gel beads retained viability for up to 180 days while enabling controlled release in the rhizosphere [68]. Because *B. subtilis* can produce biosurfactants under appropriate environmental conditions, encapsulated strains could combine controlled delivery with in situ biosurfactant production upon release. Functionally, the encapsulated *B. subtilis* system exhibited pronounced antifungal activity against phytopathogens, including *Fusarium avenaceum* and *Rhizoctonia solani*. Under the experimental conditions investigated, no clear dependence of antifungal efficacy on chitosan molecular weight was observed [68]. These findings demonstrate that chitosan can serve as an effective carrier for biological control agents by maintaining bacterial viability and enabling controlled release. Importantly, field-scale ecological risk assessment of CS-BS systems, particularly their impact on non-target soil organisms, remains largely unexplored and represents an important regulatory challenge [69].

4.3. Active Coatings for Postharvest Preservation

Postharvest losses of fruits and vegetables reach 20–50% of harvested volume globally, with pathogenic decay and uncontrolled transpiration as primary drivers [70, 71]. Chitosan's triple mode of action in postharvest protection, namely, eliciting host defence

responses, direct antimicrobial activity, and semi-permeable film formation, has been reported to be confirmed, but its efficacy may be limited by poor adhesion to hydrophobic epicuticular wax surfaces and inhomogeneous film distribution during spray application [72]. The introduction of biosurfactants has the potential to address both of these limitations by improving surface wettability and enabling more uniform coating distribution, although this effect has not been systematically evaluated in different post-harvest systems and still remains unexplored.

At sub-CMC concentrations, rhamnolipids and sophorolipids have been shown to reduce the contact angle of chitosan coating solutions, thereby improving wetting and promoting more uniform spreading on hydrophobic produce surfaces [73]. More uniform film formation is expected to improve coating performance, although this relationship depends on formulation composition and processing conditions [63]. The incorporation of biosurfactants into chitosan matrices has also been reported to improve film flexibility and reduce brittleness. These effects are thought to arise from partial disruption of intermolecular interactions between adjacent chitosan chains, although the molecular basis of this behaviour remains incompletely understood [74, 75]. Because chitosan and sophorolipids have been reported to activate distinct plant defence pathways, their combination may provide complementary biological effects. However, this possibility has not yet been explored in integrated CS-BS formulations [7, 67]. The emulsifying properties of biosurfactants may facilitate the incorporation of hydrophobic essential oils into chitosan-based formulations and contribute to improved formulation stability through enhanced interfacial stabilisation [53].

Nevertheless, the safety of biosurfactant residues on coated products following chronic oral exposure has not yet been comprehensively assessed, representing an important challenge for regulatory approval and wider implementation of this approach.

4.4. Plant Growth Promotion

CS-BS biopreparations have been reported to promote plant growth, resulting in improved shoot elongation, root biomass, chlorophyll content, and nutrient use efficiency [76]. Nonetheless, the mechanisms responsible for these effects have not yet been elucidated, and any contribution of enhanced interfacial interactions or improved distribution of chitosan across plant tissues remains speculative [19].

These findings are further supported by recent studies on endophyte-derived biosurfactant-chitosan biopreparations, where optimised formulations (e.g., 0.2%) significantly enhanced root and shoot growth across multiple crop species, with increases reaching up to 316% relative to controls in selected experimental variants [19]. Such effects may be attributed to the complementary biological activities of both components and may involve improved nutrient availability, root development, and stress resilience. In line with previous reports, biosurfactants can promote germination and nutrient mobilisation, while chitosan and its oligomers activate plant defence and growth pathways.

In the available studies, the combined CS-BS formulation generally outperformed the individual components [27], indicating a delivery-driven synergy: biosurfactants improve spray atomization and surface coverage, potentially increasing the probability of chitosan-receptor interactions. This mechanism may operate at the formulation level and complements the direct biological effects of each component. Collectively, these findings suggest that CS-BS systems represent promising multifunctional biostimulants [77]; however, validation under field conditions and across diverse soil-microbiome contexts remains essential for practical implementation.

5. Conclusions

CS-BS systems represent a promising but still insufficiently understood class of hybrid bio-based materials. Current evidence indicates that their performance arises from the coupling of interfacial activity, electrostatic interactions, and colloidal organisation rather than from a single dominant mechanism. While significant functional enhancements have been demonstrated across antimicrobial, environmental, and agricultural applications, the lack of systematic structure-function studies limits predictive formulation design. Future progress in this field will depend on integrating advanced physicochemical characterisation with application-oriented studies to enable rational and scalable development of these systems.

6. Future Perspectives

Future research on CS-BS systems should address several interconnected priorities. A systematic exploration of the parameter space governing CS-BS interactions: pH, ionic strength, DDA, and biosurfactant molecular structure, is needed to establish quantitative structure-function relationships that would enable rational rather than empirical formulation design. This effort should be supported by advanced characterisation techniques, particularly ITC, SAXS/SANS, and cryo-TEM, which together can elucidate interaction mechanisms across multiple length scales. Beyond structural understanding, the development of stimuli-responsive CS-BS matrices incorporating pH-, temperature-, or enzyme-triggered release behaviour represents a promising path toward smart delivery systems and adaptive antimicrobial materials. At the translational level, scalability, reducing production costs through the use of agro-industrial substrates, and regulatory compliance remain key challenges that need to be considered alongside laboratory-scale innovation. Finally, integrating computational modelling and machine learning approaches can significantly accelerate formulation optimisation by predicting composition-performance relationships, thereby reducing experimental burden and shortening the scaling-up process. In summary, the combination of biopolymer science and biosurfactant technology offers a promising opportunity to develop next-generation sustainable materials with broad societal impact.

7. References

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