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Specific Features of Chitosan Depolymerization by Chitinases, Chitosanases, and Nonspecific Enzymes in the Production of Bioactive Chitooligosaccharides (Review)

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Abstract—The data on features of the hydrolytic cleavage of chitosan by different groups of specific and nonspecific enzymes are summarized. Alternative approaches to the production of chitooligomers and their derivatives are also briefly considered.

Keywords: chitosanase, chitinase, chitosan, chitooligosaccharides, enzymatic depolymerization, chitin deacetylase, transglycosylation

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INTRODUCTION

Chitosan is a linear aminopolysaccharide that mainly consists of D-glucosamine residues linked by β-1,4-glycosidic bonds and N-acetyl-D-glucosamine units, the content of which varies from 0 to 50% [1]. The term "chitosan" usually refers to soluble polymers that differ in their deacetylation degree (**DD**), polymerization degree (**n**), molecular weight (**MW**), polydispersity index (**Ip**), and the distribution pattern of N-acetyl-D-glucosamine residues in the molecule [2]. The unique physicochemical properties and multiple biological activities of chitosan, its biocompatibility, and biodegradability lead to the extensive use of this polysaccharide in agriculture, medicine, cosmetics, wastewater treatment, etc. [3–10]. The great interest in the study of chitosan and its derivatives is reflected in the literature; more than 20000 publications appeared by 2011 [10]. The annual growth rate of the number of articles devoted to the study of this subject that are indexed in Scopus has steadily increased from 500 articles in 2000 to about 7500 in 2014 [5]. In addition to chitosan, water-soluble products of its depolymerization, which have a wide spectrum of biological activity, are also of great practical and theoretical interest [2]. The biological activity and areas of potential applications of low molecular weight derivatives of chitosan and chitooligosaccharides (**COS**s) have been considered in detail in reviews [2, 4, 5, 11–14]. However, the action mechanism of chitosan and COSs remains unknown, although, in some cases, their activities have been experimentally confirmed on the cellular and molecular level [2, 15]. The biologically active components of chitooligomer mixtures are often unidentified [2]. Moreover, the results of the evaluation of a particular type of activity in COSs are unreproducible.

It is known that commercial chitosan that is produced by alkaline deacetylation of chitin consists of polymer chains with a considerable MW (300–3000 kDa) and forms highly viscous solutions in dilute acids. Partial depolymerization of chitosan solves the problem of its high viscosity and low solubility in water and makes it possible to produce chitooligomers with improved functional characteristics. Functionally significant products of chitosan hydrolysis can be loosely grouped by their MW or polymerization degree. The groups typically comprise a so-called "low-molecular" chitosan with the MW of ~16–50 kDa; oligochitosan, which is represented by oligomers with a polymerization degree n = 13–100 (MW ~ 2–16 kDa); and chitooligomers (MW ≤ 2 kDa and n = 2–12) [14–18]. The products of deep depolymerization of chitosan can also include its monomer, D-glucosamine, which is widely used in medicine and the pharmaceutical industry [19, 20]. Depolymerization of chitosan is carried out by physical, chemical, and enzymatic methods [2]. There are variants of a combined application of physical and enzymatic or chemical-enzymatic methods of chitosan hydrolysis [21]. For example, one study [22] demonstrates the effectiveness of the action of linearly polarized light on the rate of the enzymatic hydrolysis of chitin and chitosan. This method can be used for the production of oligochitosans with a MW of 10–100 kDa; however, an effective conversion of

the initial polymer to lower COSs ($n = 2–12$) can only be performed by enzymatic or chemical methods [2]. The advantage of enzymatic methods is that they provide the ability to control the degradation process at relatively low temperatures and near-neutral pH values. The enzymatic degradation yields no by-products, which are formed in acid or oxidative depolymerization of chitosan as the result of an uncontrolled modification of its functional groups. When endochitosanases are used, a small amount of monomer is formed as a by-product. With the development of enzymatic methods and substrate selection, it becomes possible to control the process of chitosan depolymerization and to perform directed synthesis of biologically active chitooligomers that differ in their MW, deacetylation degree, and positions of D-glucosamine and N-acetyl-D-glucosamine residues. Another advantage of the enzymatic destruction of chitosan is the environmental safety of the process, which is due to the limited use of aggressive agents and media and to the absence of toxic waste.

The results of numerous studies carried out over the past 10–15 years show that both specific [2, 11, 23, 24] and nonspecific enzymes [2, 25–28] can be successfully used to produce functionally active chitooligomers, the products of controlled hydrolysis of chitosan. Specific enzymes include endochitosanases (EC 3.2.1.132), which—according to the classification based on amino acid sequence similarities—belong to GH46, GH75 and GH80 families and bifunctional chitosanases belonging to glycosyl hydrolases family GH8 [29–31]. Chitinases of the families GH18 and GH19 can be effectively used in the hydrolysis of partially N-acetylated chitosan with DD ≤ 40–50% [2]. Depolymerization of chitosan is often performed with lysozyme, which is related to chitosanases [32, 33]. Among the other hydrolases, many cellulases, hemicellulases, pectinases, lipases, and proteases (papain) exhibit nonspecific chitosan-degrading activity [25–28, 34, 35].

This review considers currently available data on the action of different groups of specific and nonspecific enzymes towards chitosan in the production of chitooligosaccharides. Moreover, the review briefly describes alternative approaches to the enzymatic preparation of COSs: these alternatives are based on the use of chitindeacetylases, chitin synthases, and certain groups of chitinolytic enzymes that can catalyze reverse transglycosylation. However, the production of chitin oligomers by chitin depolymerization will not be discussed here, because this process has its own specifics and should be considered separately.

Aspects of chitosan depolymerization by specific enzymes. There are indications that chitosan-degrading enzymes differ not only in their substrate and reaction specificity but also in their action mode. The action of many of these enzymes has the following feature: they specifically bind with substrate regions that differ in their sequences and distribution patterns (alternating, random, or block distribution) of acetylated residues in the molecules. This determines the multiphase kinetics of chitosan degradation and significant differences in the composition of the hydrolysis products obtained by the end of each phase [2]. Thus, both the choice of the initial chitosan and the choice of the enzyme have a significant effect on the spectrum of resulting depolymerization products, which makes it possible to effectively control this process.

The most extensively studied is the depolymerization of chitosan under the effect of well-characterized chitinases ChiA, ChiB, and ChiC from the gram-negative bacterium *Serratia marcescens*, which, in turn, is considered as model object for the study of the process of microbial chitin degradation [36]. The aforementioned chitinases belong to the GH18 family of glycosyl hydrolases and have similar specificity to the binding subsites of the substrate (Table 1). This family is the most numerous and widespread chitinase group (Table 2). Based on phylogenetic analysis of their primary structure, which was found from data on the genomic sequencing of different organisms [37], the family was divided into three main clusters: A, B, and C [37]. The bacterial chitinases ChiA and ChiB, which were previously distinguished by the amino acid sequence similarity, belong to cluster A, whereas the ChiC chitinase is in cluster B [29].

The action of the chitinases ChiA and ChiB on chitin follows the exomechanism, whereas the action of the ChiC chitinase follows the endomechanism. The main difference between the chitinases ChiA and ChiB lies in their orientation relative to the polymer molecule of the substrate; moreover, the enzymes move in mutually opposite directions during chitin degradation [38]. It was noted that the chitinases ChiA and ChiB predominantly act on chitosan as endoprocessive enzymes. For instance, HPLC profiles of the products that were formed at the early stages of substrate degradation are characterized by the dominance of oligomers with an even number of units [38]. The products of chitin degradation are mostly dimers, whereas chitosan degradation can yield longer oligomers ($GlcN_2$–$GlcN_{12}$) comprised of an even number of residues. As the reaction continues, "even" oligomers become less predominant, because long molecules of the substrate can no longer be found and the enzyme is mostly involved in rebinding and subsequent degradation of the oligomers formed at the previous stage [2]. The hydrolysis of chitosan under the action of the chitinase ChiC is of a different nature, and the composition of the resulting products turns out to be typical of nonprocessive enzymes with the endomechanism of action. The ChiC chitinase degrades chitosan

Table 1. General characteristics of the most studied groups of chitinases and chitosanases and specific features of their action on chitosan [2]

Enzyme (family of glycosyl hydrolases)	Chitinase subgroup/chitosanase cluster*	Mechanism of action on chitin/chitosan**	Processivity and its degree***	Presence of carbohydrate-binding modules	Specificity of subsites in the active site in the binding of sugar residues		
					–2	–1	+1
Chitinase ChiA *Serratia marcescens (GH18)*	A	Exo/endo-	+ (N = 9.1)	Fn_3 (fibronectin3-like) module	GlcNAc/GlcN	GlcNAc	GlcNAc/GlcN
Chitinase ChiB *S. marcescens* (GH18)	A	Exo/endo-	+ (N = 3.4)	Chitin-binding module CMB5	GlcNAc/GlcN	GlcNAc	GlcNAc/GlcN
Chitinase ChiC *S. marcescens* (GH18)	B	Endo/endo-	—	—****	GlcNAc/GlcN	GlcNAc	GlcNAc/GlcN
Human chitotriosidase (GH18)	A	Exo/endo-	+ (N = 2.5)	Chitin-binding module CMB14	GlcNAc/GlcN	GlcNAc	GlcNAc/GlcN
Chitinase ChiG *Streptomyces coelicolor* A3(2) (GH19)	—	Endo/endo-	—	—	GlcNAc	GlcNAc/GlcN	GlcNAc
Chitosanase ScCsn *S. coelicolor* A3(2) (GH46)	A	Endo-	—	—	GlcN/ GlcNAc	GlcN/ GlcNAc	GlcN/ GlcNAc
Chitosanase SaCsn *Streptomyces avermitilis* (GH75)	—	Endo-	—	—	GlcN/ GlcNAc	GlcNAc/GlcN	GlcN

* For chitinases of the GH18 family, subgroups are given based on the amino acid sequence similarities of their catalytic domains [23]. In the case of chitosanases, the subdivision into clusters according to their primary structure is known only for the GH46 family [30].

** The mechanism of action on two substrates is provided only for chitinases.

*** N is the average number of cleaved bonds per one binding.

**** Upon its synthesis, the ChiC chitinase contains a Fn3-module and a chitin-binding module of the CMB12 family. In the mature protein, these modules are cleaved by proteolytic enzymes.

Table 2. Structural and functional features of the main groups of specific chitin- and chitosan-degrading enzymes and their occurrence

Groups of chitinolytic enzymes, EC number*	Families of glycosyl hydrolases**	Mechanism of action on chitosan; hydrolyzable substrates; the presence of transglycosidase activity (+/–)***	Catalytic mechanism	Anomeric configuration of the products of chitin/chitosan hydrolysis	Spatial structure of the catalytic domain	Occurrence
Chitinases (EC 3.2.1.14)	**GH18, GH19** (only chitinases)	Endoprocessive and nonprocessive. Chitin and chitosan (DD 30–70%) ±	Substrate (GH18) and acid-base (GH19)	β- (retention) for GH18 and α- (inversion) or GH19	$(\beta/\alpha)_8$-barrel (GH18) and α+β-lysozyme (GH19)	GH18—in all organisms (from viruses and archaea to vertebrates); GH19—only in plants and actinobacteria
β-N-Acetyl-hexosaminidases (EC 3.2.1.52)/ β-N-Acetylglucosaminidase	**GH20**	Inactive against chitosan. N-acetyl-glucosides/galactosides, chitin dimers, and chitin (exo) +	Substrate	β- (retention)	$(\beta/\alpha)_8$-barrel	Archaea, bacteria, fungi, invertebrates
Chitosanases (EC 3.2.1.132)	**GH3, GH5, GH7, GH8—**include various groups of enzymes: mainly endo- and exogluconases, xylanases, glucosidases, mannanases, and galactanases, cellulases, and others	Endomechanism Characterized by the presence of bifunctional enzymes that act on various forms of chitosan, insoluble cellulose, Na-CMC, lichenan, branched β-glucans, etc. ±	Acid-base— for all the enzymes	β- (retention) for GH3, GH5, and GH7 α- (inversion) for GH8	Variable for GH3, $(\beta/\alpha)_8$-barrel for GH5, β-sandwich (β-Swiss roll) for GH7, $(\alpha/\alpha)_6$-barrel for GH8	GH3—cyanobacteria GH5—bacteria; GH7—mainly, fungi; GH8—mainly, bacteria.
	GH46, GH75, GH80 (include only chitosanases)	Highly specific chitosanases; hydrolyze chitosan (DD 65—100%) by endo-mechanism —	Acid-base mechanism for GH46 and GH75; unknown mechanism for GH80	α-(inversion) for all enzymes	α+β-lysozyme for GH46 and GH80; unknown for GH75	GH46—bacteria****, archaea, viruses GH75—mainly, fungi *****; GH80—Gram-negative bacteria
Exo-1,4-β-D-glucosaminidase (EC 3.2.1.165)	**GH2** (includes various groups of β-galactosidases, β-mannosidase, β-glucuronidase, etc.)	Exomechanism (monomer is cleaved from the nonreducing end). Chitosan (DD—50–100%) and oligomers from $GlcN_2$ and higher, with a maximum for $GlcN_5$–$GlcN_6$ +	Acid-base	β- (retention)	$(\beta/\alpha)_8$-barrel	Fungi, bacteria, archaea

* According to the IUBMB biochemical nomenclature.
** According to the classification given in [31].
*** The presence of transglycosidase activity (+), its absence (–), and its presence in individual representatives (±).
**** Gram-positive *Bacillus* bacteria and actinobacteria.
***** The family also includes a few representatives of actinobacteria.

to a continuous series of oligomers of different sizes, and the main substrate disappears at the early stages of the depolymerization reaction [2]. At the start of the reaction, the action of the ChiC chitinase does not lead to the formation of dimers or other oligomers with an even number of units. This indicates that the enzyme attacks its substrate in a random manner and not by a stepwise cleavage of similar fragments.

The products of chitosan hydrolysis by chitinases differ significantly in the arrangement of acetylated residues [2]. It should be noted that the effect of the aforementioned chitinases was studied on chitosan with a high acetylation degree (65%). Since chitinases are specific to the GlcNAc residue, it can be assumed that an increase in the acetylation degree of the initial substrate will be accompanied by an increase in the relative content of longer oligomers [39]. However, it was noted that such chitinases as ChiB can efficiently hydrolyze chitosan with DD ~ 90% [2]. A two-dimensional model of the hydrolysis of different chitosans by the ChiB chitinase was developed, making it possible to accurately predict the distribution of oligomeric products at different DDs of the initial substrate [40]. Based on this model, the optimal parameters can be determined for substrate hydrolysis and the efficient production of COSs of the desired size.

The action of human chitotriosidase (HCT) of the GH18 family on chitosan is described in detail. This enzyme was originally found in the blood plasma and the macrophages of a patient with Gaucher's disease and used as a biochemical marker [41, 42]. The physiological function of HCT is in the innate immunity against chitin-containing pathogens [43]. The molecule of this enzyme has a two-domain structure [44] and consists of a catalytic domain and a C-terminal chitin-binding domain (Table 1). However, there is also an active HCT isoform with no chitin-binding domain [45]. Study of the HCT action on chitosan is important for an understanding of the processes of chitosan degradation in the human body and for the development of specific inhibitors of its chitinases [42]. Although specific features of the catalytic core of HCT define it as a processive enzyme with an exomechanism of action, the depolymerization of highly acetylated chitosan (DD ~ 38%) under its action follows an endomechanism. Moreover, this reaction is accompanied by a rapid decrease in the viscosity of the substrate solution, which is also observed under the action of *Serratia marcescens* chitinases ChiA and ChiB [46]. This is due to the fact that, in such crystalline polymers as chitin, terminal and amorphous regions of the substrate molecule are more accessible for the enzyme action. Unlike chitin, the molecule of soluble chitosan has internal regions that are available for binding with the enzyme. The number of such internal regions is much higher than the number of available terminal units in the polymer chain. Consequently, enzymes that commonly act according to the exomechanism interact with chitosan as endoenzymes: at the initial stages of depolymerization, the reaction yields chitooligosaccharides that consist of an even number of residues [42]. When acting on chitosan, HCT has a moderate processivity: on average, it performs 2.5 cleavages per enzyme–substrate complex (Table 1). The depolymerization efficiency decreases with an increase in the DD of the substrate, because the enzyme preferably binds to N-acetyl-D-glucosamine residues at subsites –2 and –1. HCT is similar to ChiB chitinase in its ability to hydrolyze chitosans of different DD and in the composition of the resulting chitooligosaccharides [42].

In the study of the properties of the bacterial chitinase ChiG from *Streptomyces coelicolor* A3 (2) (an enzyme of the GH19 family), different results of chitosan depolymerization were obtained [47]. Chitinases of this family of glycosyl hydrolases were initially found in plants and only later discovered in streptomycetes [48]. It turned out that the ChiG chitinase hydrolyzes chitosans of high DD less efficiently than the ChiB chitinase of the GH18 family. It is proposed that several aromatic amino acid residues that are present in GH18 chitinases in the region of the substrate-binding cleft can come into contact with the hydrophobic surfaces of sugars according to the principle of stacking interactions [49]. As was shown for the GH19 chitinases, such interactions do not depend on the presence of N-acetyl groups. Thus, in contrast with the GH18 chitinases, the chitinase ChiG can degrade chitosan to oligomers with a more diverse arrangement of acetylated residues [2]. A change in the DD value of the initial substrate has a key effect on the molecular weight distribution of the reaction products and on the degree chitosan degradation. As noted above, the GH19 chitinases are more sensitive than the GH18 chitinases to chitosan deacetylation [2]. These features were confirmed for other chitinases of the GH19 family, including the *Streptomyces griseus* HUT6037 chitinase and rice chitinase (*Oryza sativa* L.) [50, 51].

Unlike chitinases, chitosanases specifically hydrolyze chitosan. However, in contrast with the chitinases ChiA, ChiB, and ChiC, the effect of chitosanases has not been studied in detail. Specific features of chitosan depolymerization by chitosanases of the families GH46 and GH75 have been studied in [52, 53]. According to [10], the known chitosanases belong to seven different families of glycosyl hydrolases but the greatest attention is paid to the families GH8 and GH46 (Table 2). Most chitosanases of these families are produced by gram-positive bacteria from two different evolutionary branches: bacilli (e.g., *Bacillus* and *Paenibacillus*) and actinobacteria (*Streptomyces* or

Kitasatospora) [30]. Similar to the GH18 chitinases, chitosanases of the GH46 family are the most numerous and extensively studied. Based on the phylogenetic analysis of the primary structures of 58 known chitosanases, the members of the GH46 family are distributed into five clusters: A, B, C, D, and E [30]. Just like GH46, the GH75 and GH80 families contain only chitosanases of a narrow specificity, whereas the families GH3, 5, 7, and 8 contain many enzymes with other activities [54]. The chitosanases of these groups are typically bifunctional and often have broad specificity (Table 2). A feature of the GH46 chitosanases is the presence of a strongly negatively charged substrate-binding cleft: this is due to the relatively high content of glutamic and aspartic acid residues in this part of the molecule. This may be the reason behind the high specificity of the GH46 chitosanases and their weak affinity for highly acetylated chitin substrates [24, 55, 56]. In bifunctional chitinases of the GH8 family, several aromatic amino acid residues participate in substrate recognition via stacking interactions with the pyranose rings of chitosan. This enables them to exhibit not only the chitosanase activity but also the hydrolytic activity against β-1,4-glucans [57]. Some GH46 enzymes contain a single or double discoidin-like domain, which is a carbohydrate-binding module of the CMB32 family [30, 58]. Such modules are characterized by a 50–55% similarity to the modules that are present in the GH8 chitosanases and that enable specific binding with chitosan [30, 56, 57]. A number of chitosanases of clusters C and E of the GH46 family also contain putative peptidoglycan-binding modules and N-terminal domains of unknown function [30].

Chitosanases are divided into three subclasses by their reaction specificity mechanism, i.e., the ability to hydrolyze mixed types of bonds in chitosan [59]. There are chitosanases that hydrolyze all types of mixed bonds in chitosan [60]. There are data on the deep hydrolysis of chitosan with DD = 65–75% by the enzyme of the *Streptomyces* sp. N174 strain [61]. In addition to fully deacetylated GlcN, $(GlcN)_2$, and $(GlcN)_3$, various hetero-oligomers with $n = 2–5$ were identified among the products of the hydrolysis. However, the kinetics of the action of this chitosanase, the effect of the DD value of chitosan on its activity, and the composition of the reaction products have not been studied. The study of other chitosanases of the GH46 family (namely, the enzyme of the *Streptomyces coelicolor* A3(2) strain, Table 1) has shown that these enzymes efficiently depolymerize chitosans of a wide range of DDs yielding oligomers that contain both N-acetylated and deacetylated residues on the reduced ends of the molecules [52]. It was established by ^{1}H NMR spectroscopy that the ScCsn46A *S. coelicolor* A3(2) chitosanase cleaves three types of bonds and represents the fourth class of specificity (see above, [60]). This makes it possible to reach a significant depth of hydrolysis(α) of chitosans with a wide range of DD. For instance, chitosan with DD = 99.2% is hydrolyzed by 59%, whereas chitosan with DD = 68% is hydrolyzed by 44%. A study on the kinetics of hydrolysis with the ScCsn46A chitosanase showed that it hydrolyzes the substrate via a nonprocessive endomechanism. This leads to the formation of a continuous set of oligomers that contain even and odd numbers of residues. The dominant products of the deep degradation of the substrate (DD = 68%) were GlcN–GlcN and GlcN–GlcNAc dimers. A similar mechanism was observed in the study of the action of the same chitosanase on completely deacetylated chitosan; deep hydrolysis of the chitosan resulted in a good yield of completely deacetylated oligomers. Thus, for the ScCsn46A chitosanase, it was shown that the yield of a specific oligomer with the desired chain length can be optimized by controlling the degree of chitosan degradation [52].

The SaCsn75A *Streptomyces avermitilis* chitosanase of the GH75 family has a narrower specificity (Table 1). As compared with the ScCsn46A chitosanase, this enzyme inefficiency degraded chitosan with DD of 69% [53]. The molecular weight distribution of the depolymerization products showed that, like the chitinases ChiC and ChiG, the chitosanases SaCsn75A and ScCsn46A follow the nonprocessive endomechanism. At a low degree of chitosan degradation (DD = 69%), there was a decrease in the accumulation of dimers and trimers. This pattern differed significantly from an increase in the content of the same oligomers that were formed under the action of the *S. avermitilis* chitosanase on the same substrate. The molecular weight distribution of the products at the maximum degradation of the chitosan indicated the predominance of dimers, trimers, and tetramers, although oligomers with $n > 10$ were also present. The maximal degree of chitosan hydrolysis by this enzyme decreased with increasing DD from $\alpha = 0.42$ for chitosan with DD = 99.2% to $\alpha = 0.11$ for chitosan with DD = 37% [53]. The molecular weight distribution of the degradation products of the latter chitosan demonstrated a shift towards the formation of longer oligomers, the majority of which were oligomers with $n > 50$. As the DD of chitosan increased to 48%, the formation of large oligomers ($n > 40$) almost stopped but the yield of COSs with $n < 10$ was relatively high. The composition of these COSs was characterized by a high content of internal acetylated (including double-acylated) residues. This pattern reflected that uncleaved bonds between repeating GlcNAc residues accumulated when the *S. avermitilis* chitosanase acted on highly acetylated chitosan.

Both chitinases and chitosanases can be effectively used for the directed production of biologically active COSs. The main difference between the action of chitinases and chitosanases is their specificity to the N-acetyl-D-glucosamine and D-glucosamine residues located near the cleavable glycosidic bond. Both groups of enzymes are characterized by overlapping substrate specificities; therefore, like chitosanases, chitinases can effectively hydrolyze chitosans with a moderate degree of acetylation [2]. To date, a lot of experimental data have been accumulated on the use of different chitosanases (particularly those from the *Bacillus* bacteria) for the production of COSs and oligomeric chitosan (Table 3) [11]. The hydrolysis of chitosan by many chitosanases is characterized by the formation of a mixture of low molecular weight COSs, the polymerization degree of which is shifted in one direction or another ($2–5 \le n \le 4–7$ or higher). However, the depolymerization conditions, characteristics of the initial polymer, and the depth of its hydrolysis have a significant effect on the final composition of the COSs. Therefore, depending on the goal, the key factors in the production of the desired product can be the DD of chitosan, the depth of its hydrolysis, and the choice of a suitable enzyme. This fact is illustrated by the scheme in Fig. 1.

Depolymerization of chitosan by nonspecific enzymes. Such nonspecific enzymes as papain and cellulases are widely used for the depolymerization of chitosan [11, 25, 28, 34, 35]. As far back as the 1990s, hydrolytic activity to chitosan was demonstrated in a wide range of enzymes (including 10 types of glycanases, 21 types of proteases, 5 types of lipases, and tannase) from various bacterial, fungal, plant, and animal sources [34]. It was later found that cellulases, papain, and pectinases possess chitosan-degrading activity [25, 62]. Since crude enzyme preparations of fungal and vegetable origin are commonly used for these purposes, there is a certain doubt as to which enzymes are actually responsible for the chitosan-degrading activity. Among lipases, the activity was found in the lipase from wheat germ [63, 64], the recombinant lipase B from *Candida antarctica* [65], the *C. cylindracea* lipase [66], and the porcine pancreatic lipase [34, 64]. The nonspecific activity of lipases is considered, on the one hand, as a consequence of their contamination by chitosanase and, on the other hand, as the result of the similarity between the active sites of these enzymes [67]. For instance, proteins with endochitosanase and lipase activities were found in the *Mucor circinelloides* fungi: these proteins are of a very similar MW (43 and 42 kDa) and are poorly separable [68]. However, the presence of chitosanase as an impurity is completely ruled out in the case of the recombinant *C. antarctica* lipase [65]. A new enzyme with exochitinase (chitobiosidase) and exochitosanase activity was found in the preparation of the commercial lipase from *Aspergillus oryzae*. The enzyme was identified as a member of the GH18 family. It has been established that the bifunctionality of this enzyme is due to the presence of two separate catalytic domains with partially overlapping active sites [35].

It is suggested that almost all microbial cellulases are capable of degrading chitosan to chitooligomers. One of the reasons for the high activity of cellulases towards chitosan is the wide prevalence of bifunctional enzymes with cellulase and chitosanase activities [25]. These enzymes can have either one catalytic domain that participates in depolymerization of two substrates or two domains that specifically interact with chitosan and cellulose [69, 70]. Bifunctional cellulases–chitosanases mostly belong to the GH8 family, although a few of them belong to the families GH5 and GH7 (Table 2). Cellulases from various sources can significantly reduce the viscosity of chitosan solutions at an early stage of the interaction. This feature points at a possible endomechanism of their chitosanase activity. The mechanism is supported by the predominant formation of a diverse mixture of COSs with $n = 2–24$. Commercial cellulases from *Trichoderma viride* and *T. reesei* have the highest chitosanase activity: they decrease chitosan viscosity by 99% [25]. Interestingly, endochitosanase activity in the cellulolytic complex of some *T. reesei* representatives is exhibited not by endoglucanase but by exocellulase (particularly by cellobiohydrolase I, which belongs to the same family, GH7) [71]. In recombinant expression in *Aspergillus oryzae*, the activity of this enzyme towards chitosan was ten times higher than its activity towards CMC. Chitosanase activity was found in the cellobiohydrolases of other micromycetes, particularly *Aspergillus aculeatus* [71]. Some cellulolytic *T. reesei* strains that are overproducers of cellulases can synthesize exochytosanases in the absence of induction [72]. Among domestic commercial cellulases, the action of Celloviridin G20X on chitosan is the most extensively studied; it has been shown that Celloviridin G20X is effective in the production of chitosan oligomers [73]. Bifunctional cellulases of different origin differ in their substrate specificity and the reaction specificity of their action on chitosan: they can form a wide range of hydrolysis products [25], which, like in the case of chitinolytic enzymes, is determined by the MW and DD of the initial substrate. Cellulases of some *T. viride* strains are mostly active against chitosan with DD = 80–90%; a decrease of its DD below 80% is accompanied by a significant decrease (~40%) in the rate of its hydrolysis [74]. However, it has been shown that the acetylation degree of the substrate has almost no effect on the efficiency of enzymatic hydrolysis by Celloviridine G20X [75]. A bifunctional enzyme, which was isolated from the preparation of the *T. viride* commer-

Table 3. Catalytic characteristic of different groups of chitosanases that are used in the production of chitooligosaccharides

Source	Type of the enzyme, molecular weight, GH family	Optimal conditions for chitosan hydrolysis (°C, pH), enzyme–substrate ratio (when available)	Kinetic properties of the enzyme (K_M, V_{max})	Additional hydrolytic activity, % of chitosanase activity	Main products of chitosan degradation (DD)	Reference
Bacillus sp. MET 1299	Constitutive chitosanase 52 kDa GH8	60°C, pH 5.5 (Colloidal chitosan, DD 75–90%)	ND	Hydrolysis of β-glucan (24%)	$GlcN_{5-6}$ at the initial stages; 1 h into the reaction, also $GlcN_{2-4}$	[76]
Bacillus circulans WL-12	Bifunctional chitosanase/β-1,3-1,4- glucanase 40 kDa (GH8)	37°C, pH 5.0, 0.04 U/mg, 24 h (soluble chitosan, DD 73%)	ND	Hydrolysis of lichenane (141%), CMC (12%), glycol-chitin (7%), and colloidal chitin (1.4%)	COSs with $n = 2–4$	[77]
Matsuebacter chitosanotabidus 3001	Chitosanase 34 kDa (GH80)	30–40°C, pH 4.0 colloidal chitosan with DD 90%	ND	Hydrolysis of glycol-chitosan (113%) and CM-chitosan (6%)	COSs with $n = 2–6$	[94]
Bacillus cereus S-1	Chitosanase 45 kDa GH(?)	60°C, pH 6.0 (soluble chitosan, DD = 75–100%)	ND	Hydrolysis of colloidal chitosan (32%), colloidal chitin (7%), CMC (32%), and crystalline cellulose (1%)	Dimer, trimer and tetramer after 24-h hydrolysis of soluble chitosan with DD = 100%	[95]
Bacillus subtilis IMR-NK1	Chitosanase 41 kDa GH(?)	45°C, pH 4.0 1 : 3	ND	Hydrolysis of glycol-chitosan (11%), colloidal chitin (26%), and CM-chitin (8%)	COSs with = 2–7 (CTS 60–94%)	[96]
Bacillus sp. KCTC 0377BP	Bifunctional chitosanase- glucanase 45 kDa GH8	60°C, pH 4.0–6.0 (soluble chitosan, DD 39–94%)	K_M = 1.10 mg/mL (soluble chitosan, DD 94%)	Hydrolysis colloidal chitin (2,8%) and CMC (2,5%)	$GlcN_{2-9}$—at 0.2 U/mg chitosan; oligochitosans (MW ~1.5–21.5 kDa)—at 0.002 U/mg chitosan	[97]
Aspergillus fumigatus S-26	Exo-β-D-glucosaminidase 104 kDa	50–60°C, pH 3.0–6.0	K_M = 1 mg/mL V_{max} = 7.8×10^{-2} μM/s × mg chitosan (DD ~99.5%, MW ~200 kDa)	Hydrolysis of colloidal chitin (~3%), powder chitin (~1%), glycol-chitin (~6.5%), and CMC (3.5%)	COSs with $n = 2–7$, GlcN	[98]
Bacillus cereus D-11	Bifunctional antifungal chitosanase 41 kDa GH8	60°C, pH 6.0	K_M = 7.5 mg/mL $V_{max} = 2{,}15 \times 10^{-7}$ M/mg/s	CMCase—76%	$(GlcN)_{2-4}$ in the hydrolysis of chitosan with DD 86%	[99]

Table 3. (Contd.)

Source	Type of the enzyme, molecular weight, GH family	Optimal conditions for chitosan hydrolysis (°C, pH), enzyme–substrate ratio (when available)	Kinetic properties of the enzyme (K_M, V_{max})	Additional hydrolytic activity, % of chitosanase activity	Main products of chitosan degradation (DD)	Reference
Gongronella sp. JG	Chitosanase 28 kDa GH75	55–60°C, pH 5.6 0.1 mg enzyme/mg chitosan	K_M = 8.86 mg/mL (colloidal chitosan, DD 85%)	Hydrolysis of soluble chitosan with DD of 85% (14%) and glycol-chitosan (4%)	COSs with n = 2–4, where $GlcN_3$ is the main product of hydrolysis of colloidal chitosan	[100]
Shoot sheath of the *Bambusa oldhami* bamboo	Thermostable chitosanases A (24,5 kDa) and B (16,4 kDa)	70°C (A) and 60°C (B), pH 3.0–4.0, soluble chitosan, DD ~60%, MW 1100 (A) and 750 kDa (B)	K_M = 0.539 μg/mL (A) V_{max} = 0.262 μM/min/mg (A) K_M = 0.183 μg/mL (B) V_{max} = 0.092 μM/min/mg (B) chitosan, DD = 92%	Hydrolysis of glycol-chitin (30% for A, 14% for B), CM-chitin (42% for A, 17% for B), and insoluble chitin (8% for A, 11% for B)	Oligochitosans (MW ~12–29 kDa) for isoforms A and B	[101]
Anabaena fertilissima	Antifungal endo-chitosanase (GH3?)	27°C, pH 7.5	K_M = 0.89 mg/mL (glycol-chitosan)	Hydrolysis of CM-chitosan (30%) and colloidal chitin (5%)	COSs with n > 2	[102]
Bacillus subtilis 168	Recombinant chitosanase 30 kDa GH46	40–50°C, pH 5.0–6.0 0.5 μg enzyme/mg chitosan	K_M = 1.57 mg/mL V_{max} = 530 μM/s mg (low molecular weight CTS, DD 75-85%)	—	COSs with $n \geq 2$–6	[103]
Aspergillus sp. W-2	Chitosanase 28 kDa GH75	55°C, pH 6.0	K_M = 7.10 mg/mL V_{max} = 1.58 mM/L/min (CTS 92% DD)	Hydrolysis of chitosan with DD of 54% is 1.5% of the hydrolysis degree of chitosan with DD of 90%	COSs with n = 2–6 (for CTS 92%)	[104]
Milky sap of dwarf ficus (*Ficus pumila* var. awkeotsang)	Chitosanase 20.5 kDa	50°C, pH 4.5 0.014 U/mg chitosan derivatives	K_M = 0.089 mg/mL V_{max} = 0.69 μM/min/mg	Hydrolysis of glycol-chitin (96%) and colloidal chitin (23%)	Oligomers (MW 8–11 kDa) in the hydrolysis of EG*, CM**, AE*** -substituted derivatives	[105]
Bacillus mycoides TKU038	Chitosanase 48 kDa	50°C, pH 6.0, 10.0	K_M = 0.098 mg/mL V_{max} = 1.336 U/min, chitosan with DD ~60%	ND	COSs with n = 3–9 at 2 U/mg chitosan	[106]

* EG is glycol-chitosan.
** CM is carboxymethyl-chitosan.
*** AE is aminoethyl-chitosan.

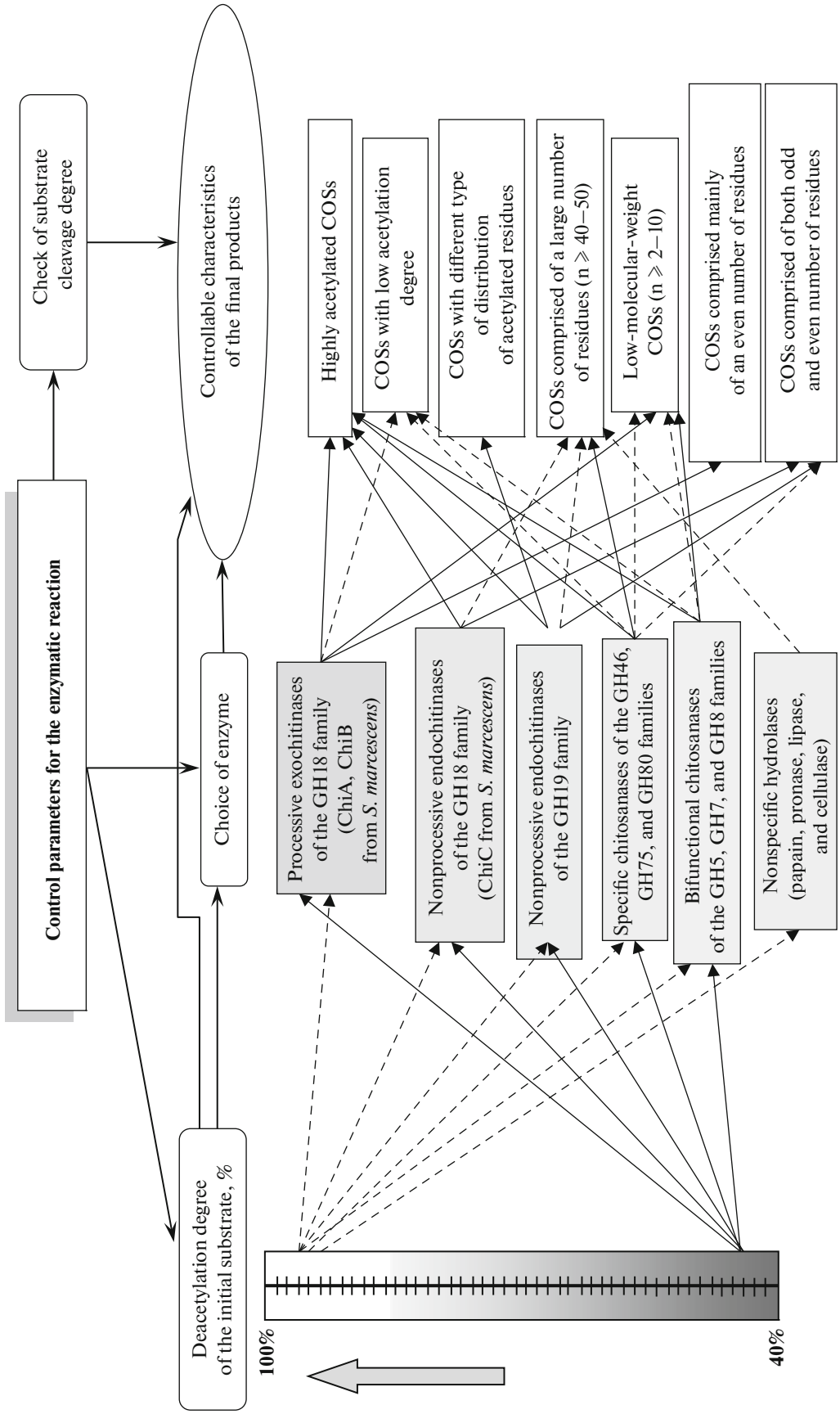

Fig. 1. Possibilities for the directed synthesis of the desired COS with predetermined characteristics via control of the key parameters of enzymatic depolymerization of chitosan and the selection of different enzymes. At the start of the process (except for the cases of specific enzymes), high (85–95%) and low (40–50%) deacetylation degrees of the initial chitosan are shown by dotted and solid arrows, respectively. At the end of the process, we show the products that are formed during the hydrolysis of highly and low-acetylated substrate.

cial cellulase and purified, exhibited cellulase and chitosanase activity and degraded chitosans with a wide DD range [70]. The analysis of the depolymerization products demonstrated the ability of this cellulase to hydrolyze all three types of bonds in chitosan. Bifunctional cellulases–chitosanases of bacterial origin are mostly described in representatives of the genus *Bacillus*. The hydrolytic activity of these enzymes increases with an increase in the chitosan DD and peaks at DD ~ 90% [74]. To express these enzymes, certain strains of bacilli require the medium to contain substrates with ≥3 D-glucosamine or N-acetylglucosamine residues. It has been established that N-acetylglucosamine residues play an important role in the recognition of the substrate by these enzymes, which implies certain differences in the nature of their interaction with chitosan and cellulose [76].

Some bifunctional enzymes that were originally described as chitosanases have higher activity towards chitosan. These enzymes are characterized by the following features: (1) the enzymes are secreted in the presence of β-glucan or CMC; only a few of the enzymes are secreted in the presence of chitosan; (2) the enzymes are bifunctional; they have cellulase and chitosanase activity; (3) the primary structure of these enzymes is highly homologous to that of glucanases of the GH8 family, which mainly contains cellulases, xylanases, lichenases, etc., instead of strictly specific chitosanases of the GH46 family. Two chitosanases (with an MW of 33 and 40 kDa) of the *Bacillus circulans* WL-12 strain were assigned to this family. These chitosanases had been initially described as enzymes with β-1,3–1,4-glucanase activity [77]. The properties of this group of enzymes turned out to be similar to those of bifunctional chitosanases of the well-known strains *Streptomyces griseus* HUT6037, *B. cereus* S1, and *Myxobacter* sp. AL-1. The latter enzymes belong to the GH5 family of glycosyl hydrolases, and their chitosanase activity tends to significantly exceed their cellulase activity (by a factor of 3–5) [25]. The presence of chitosanase activity in bifunctional cellulases was also confirmed for purified enzymes; the determination of the mechanism of their action is traditionally based on the data of HPLC, TLC, kinetic analysis, NMR, and mass spectrometry [25]. In comparison with chitosanases, commercial preparations of fungal cellulases are considered to be more cost-efficient, which makes it possible to use them widely as an alternative to chitosanases for the obtainment of bioactive COSs (n = 2–12) and oligomeric chitosan (MW ~10–20 kDa) under production conditions [25].

Nonspecific chitosanase activity was found in the homogeneous isoform of *A. niger* pectinase, which cleaves the bonds between GlcN-GlcN and GlcNAc-GlcN residues [26]. The efficiency of this enzyme was demonstrated in the production of chitosan oligomers with an MW of ~6–20 kDa at a pH of 3.0 and 47°C. Unlike cellulases, however, pectinases have been studied in less detail, and the mechanism of their nonspecific activity remains unclear.

Hydrolytic activity towards chitosan was also found in many commercial preparations of proteolytic enzymes [27, 28, 34, 78]. Highly purified preparations of papain and pronase from *S. griseus* were used to produce oligochitosan comprised of a mixture of COSs (n = 2–6); the yield of COSs was 14–19% [28]. A rather stark difference was observed in the pH profiles of pronase (20 kDa) when the catalysis employed its proteolytic (pH-optimum ~ 7.0–7.5) and chitosanolic (pH-optimum ~ 3.5) activity [27]. This fact and the partial inhibition (30%) of the chitosanase activity by a specific protease inhibitor suggest that a serine residue in the active site of the pronase participates in the nonspecific degradation of chitosan [27]. Nevertheless, the question of the presence of a second active site or a second catalytic domain, as is common for other bifunctional enzymes, in proteases remains open. The nature of the solvent, as well as the MW and DD of the initial substrate, are of great importance for the manifestation of proteases activity towards chitosan [27]. The action of pronase follows the endomechanism; the reaction is accompanied by a rapid decrease in the viscosity of the substrate solution, and the enzyme has higher specific activity towards high molecular weight chitosan (71–600 kDa) as its acetylation degree increases from 15 to 26% [27]. A comparative assessment of the efficiency of chitosan depolymerization by different commercial protease preparations showed that pepsin had the highest relative activity, which consistently decreased in papain, pronase, and the protease from *A. niger* [27]. It was shown in [79] that pepsin was the most efficient of the other nonspecific enzymes in the production of monomer-free oligomers with a relatively high DD (~93%) by chitosan hydrolysis. Although proteases (in particular, pronase) have a much lower specific activity than *S. griseus* chitosanase (a 43-fold decrease for pronase), it is considered that proteases are easier to use in the production of oligochitosans that do not contain significant amounts of lower oligomers and monomers as byproducts [27]. Another advantage of commercial protease preparations is their low cost, which is more than two orders of magnitude (per unit of activity) lower than the cost of chitosanase [27]. Table 4 demonstrates the applications of certain nonspecific enzymes in the depolymerization of chitosan.

Alternative methods of enzymatic production of COSs. The use of chitin deacetylase (**CDA**, EC 3.5.1.41) can be a promising but still problematic method for the directed production of COSs with a controlled number of acetylated residues. This enzyme hydrolyses the

Table 4. Catalytic activity of certain groups of nonspecific enzymes (including commercial preparations) that were used in chitosan depolymerization and the resulting reaction products

Source	Type of enzyme, molecular weight	Optimal conditions for chitosan hydrolysis (°C, pH), enzyme–substrate ratio	Kinetic properties of the enzyme (K_M, V_{max})	Additional hydrolytic activity, % of the chitosanase activity	Main products of chitosan degradation (DD)	Reference
Aspergillus niger	Pectinase (commercial)	47°C, pH 3.0	K_M = 3.12 mg/mL V_{max} = 0.154 μM/mg/ min	Hydrolysis of CM-chitin (<50%)	Oligochitosans with MW 6–20 kDa GlcN, GlcNAc (CTS 86%, 100kDa)	[26]
Streptomyces griseus	Pronase 20 kDa (commercial)	37°C, pH 3.5, 100 : 1	K_M = 5.21 mg/mL V_{max} = 0.139 μM/mg/min	—	Oligochitosans with MW 9–9.5 kDa, COSs with n = 2–6, GlcN (soluble chitosan 75%, 71 kDa)	[27]
Trichoderma viride	Bifunctional enzyme from commercial cellulase preparation, 66 kDa	60°C, pH 5.2	K_M = 10 mg/mL V_{max} = 0.164 μM/min/mL (chitosan, DD 90%)	Hydrolysis of CMC (800%)	COSs with $n \geq 2$–4	[70]
Trichoderma reesei	Bifunctional cellobiohydrolase CBH1 (GH7)	50°C, pH 5.0 0.1–0.4 mg enzyme/mg chitosan	ND	Glycosidase (cellobiase) activity ~8%; Hydrolysis of CMC (10%), avicel (10%), swollen cellulose (73%), and glycol-chitosan (<1%)	COSs with n = 2–5 (after 4 h), where $GlcN_2$ is the main product of the hydrolysis of CTS with DD 98%	[71]
Laticifer of papaya (*Carica papaya*)	Papain 22 kDa	37°C, pH 3.5 (soluble chitosan, DD = 84–85% and MW ~ 71 kDa) 100 μg enzyme/mg chitosan	K_M = 7.14 mg/mL V_{max} = 0.289 μM/min/mg (soluble chitosan, DD 84%)	Protease activity (hydrolysis of hemoglobin ~560%)	Oligochitosans with MW ~4.1–5.6 kDa (75-82%) and COSs with n = 2–5 (8–10%) in 1–5 h	[78]
Porcine gastric mucosa	Pepsin (commercial)	40°C, pH 4.5 1 : 100	ND	—	Oligochitosans with MW > 5kDa and COSs with n ~16–17 (CTS 93%)	[79]
Aspergillus niger	Lipase A (commercial)	40°C, pH 3.0 1 : 100	ND	—	COSs with n_{av} ~12 (CTS 93%)	[79]
Trichoderma vitride	Celloviridine G20x (commercial cellulase)	55°C, pH 5.5 1 : 400	ND	—	Oligochitosans with MW 2–8 kDa	[107]
Lysobacter sp. IB-9374	Bifunctional cellulase Cel8A 41 kDa (GH8)	60°C, pH 5.0, pH 7.0 (colloidal chitosan, DD 85%)	ND	Hydrolysis of CMC (~255%), glycolic chitosan (DD ~40%)—38%, colloidal chitin—5%	COSs with n = 2–4	[108]

bond between the acetyl and amino groups of the N-acetyl-D-glucosamine residues in the chitin molecule. As intracellular and extracellular enzymes, chitin deacetylases are found mostly in chitosan-containing fungi, marine bacteria, and certain insects [80, 81]. Intracellular chitin deacetylases are involved in the formation processes of the cell wall: they deacetylate de novo synthesized chitin chains [80, 81]. It should be noted that these enzymes are almost inactive towards insoluble forms of chitin. They can, however, deacetylate its water-soluble derivatives, including glycol-chitin, chitosan (with DD ≤ 88%), and chitooligomers with $n = 2–7$. The rate of the enzymatic deacetylation of small oligomers ($n \leq 10$) is determined by their sizes and increases with an increase in the polymerization degree [11]. According to [11], when CDA acts on the dimer of N,N'-diacetyl-β-D-chitobiose, it deacetylates only one residue at the nonreducing end of the dimer molecule, whereas trimers and tetramers are converted into completely deacetylated products. The combined action of CDA and endochitinase from *A. nidulans* makes it possible to obtain chitosan oligomers with $n = 2–6$ from chitin [82]. The *Vibrio* sp. marine bacteria degrade chitin using a similar mechanism [80]. There are currently many methodological obstacles in the implementation of the technology for the directed enzymatic deacetylation of chitin, its derivatives, and COSs, which limits the use of CDA to the laboratory studies [11].

The use of transglycosylation activity, which is exhibited by some of the chitinolytic enzymes, can be one approach to the directed synthesis of COSs with a predetermined size and of new stereospecific oligomers [11, 32, 83–85]. This approach is used for the synthesis of both chitin and chitosan oligomers. Oligomers with a lower polymerization degree ($n = 2–3$) are usually used as the starting substrate for such reactions [86]. With transglycosylation reactions catalyzed by lysozyme, it was possible to synthesize under aqueous conditions oligomers with the polymerization degree ranging from 6 to 15 from the $(GlcNAc)_3$ trimer [87]. Artificial derivatives of COSs were also successfully synthesized with the use of these enzymes. For instance, with the use of purified N-acetylglucosaminidase from *Penicillium funiculosum* KY616, n-butyl-β-D-glucosaminide was synthesized as a transglycosylation product from a mixture of chitosan oligomers and n-butanol; the yield of the product significantly increased as the polymerization degree of the initial oligomers increased from $n = 2$ to 4 [88]. An unusual N-acetyl-D-glucosamine dimer linked by a β-1,6 bond was synthesized from a mixture of COSs ($n = 2–6$) by the chitinase of the *Alteromonas* sp. OK2607 marine bacterium [89]. The use of transglycosylation reactions is promising for the small-scale production of glycoside derivatives of COSs with a high added value for medicine and biotechnology. A biosynthetic method for the production of chitin oligomers with $n = 4–6$ using the transglycosylation reaction has been previously studied [90]. Risobial N-acetylglucosaminyltransferase (EC 2.4.1.16), which participates in the synthesis of chitooligosaccharide molecules of Nod-factors, was used as an enzyme. Its genes were cloned from the *Rhizobium sp.* GRH2 and *Mesorhizobium loti* bacteria to into *E. coli*. COSs were synthesized in vivo by cultivation the bacteria in medium containing 0.01% N-acetyl-D-glucosamine. For gene constructs from the *Rhizobium sp.* GRH2 and *M. loti* strains, the yield of $(GlcNAc)_5$ and $(GlcNAc)_6$ ranged from 20–50 to 100–200 mg per 1 L medium, respectively [90].

Transglycosylation was used to produce and modify COSs by a method of combined chemical and enzymatic synthesis such as chemical-enzymatic glycosylation [11, 91, 92]. Chitin oligomers are the main acceptor substrates for these reactions, whereas chitinases of *Bacillus* origin are most commonly used as an enzyme that catalyzes these reactions. It should be noted that, as of now, these approaches are not applied, because chemical glycosylation of chitosan oligomers remains a very effective and widely used method [93].

The accumulated data on the structural and functional diversity of specific and nonspecific enzymes that can hydrolyze chitosan and the current state of knowledge on the specific features of their hydrolytic action significantly increases the possibilities of controlling the chitosan depolymerization process in the directed production of functional chitooligosaccharides and make it possible to control the characteristics of the target products (Fig. 1). Endochitinases GH46, GH75, and GH80, which have a very narrow specificity of action, perform a rather deep destruction of chitosan to lower COSs with a polymerization degree of 2–10, although, even in this case, the degradation degree of the substrate can be controlled by the duration of the process. When acetylated forms of chitosan with acetylation degrees (ADs) ranging from 30 to 50% are used as the substrate for these enzymes, the reaction yields longer oligomers and their AD will also be increased. Chitinase, bifunctional chitosanases, and nonspecific hydrolases, including cellulases and proteases, can be effectively used in the limited hydrolysis of chitosan with high DD to produce COSs with a higher polymerization degrees. The advantage of commercial preparations of nonspecific enzymes over chitinases and chitosanases is their low cost. However, these preparations are significantly inferior to chitosanases in their specific activity and kinetic characteristics. As a result, increased amounts of an enzyme preparation must be used, and this may affect the

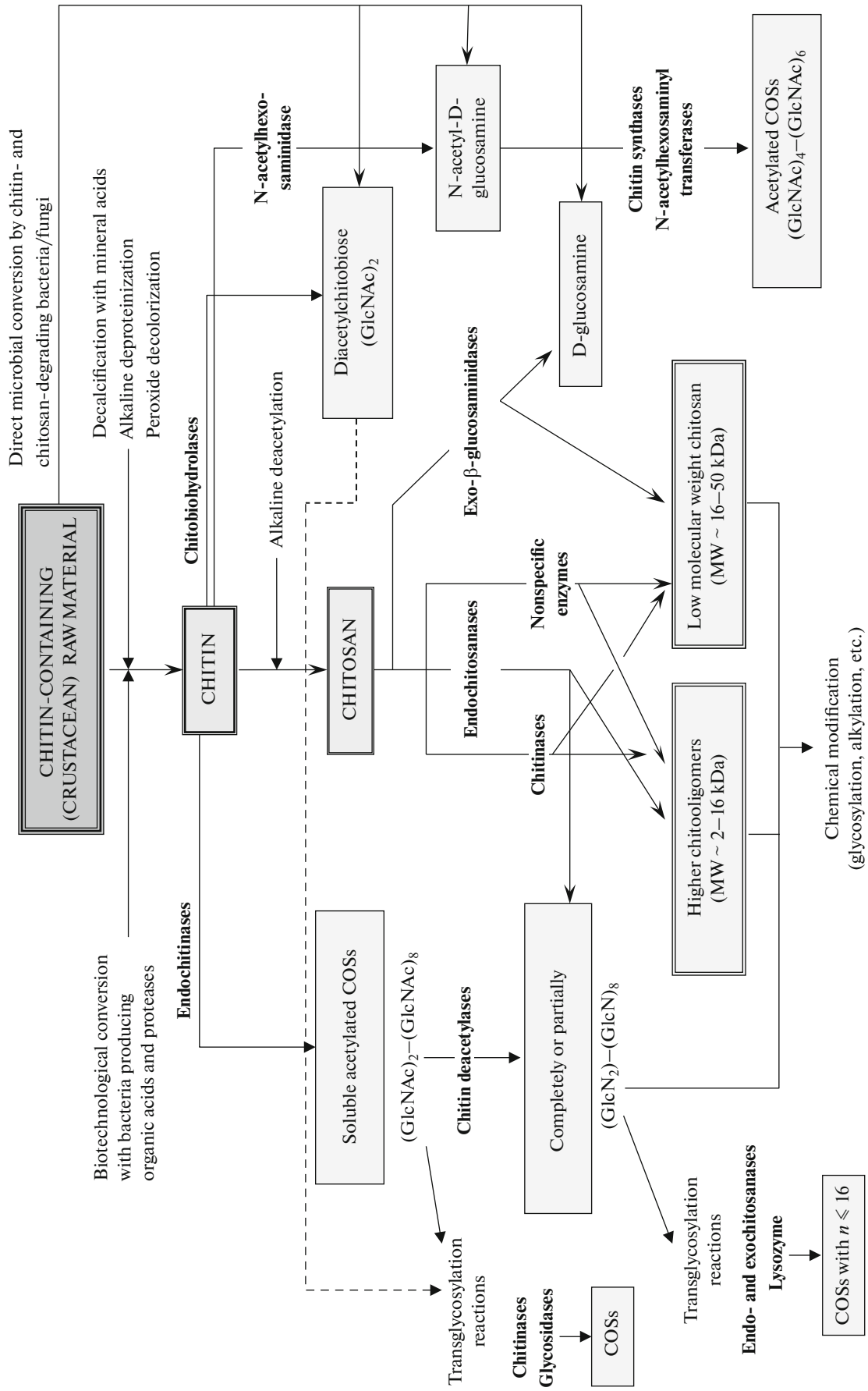

Fig. 2. Methods of enzymatic production of biologically active COSs and oligochitosans from chitin-containing raw materials.

quality of the final product, which is important for medicine and pharmacology.

In this regard, the strategy of using certain enzymes and enzyme preparations will be determined by the field of application of the desired COSs. Chitinases of the GH18 and GH19 families can be effectively used in the depolymerization of N-acetylated chitosan to produce partially acetylated COSs. Products of the enzymatic degradation of chitosan are potential substrates for further modifications performed by enzymatic, chemical–enzymatic, and chemical methods. A variety of enzymatic methods for the preparation of chitooligosaccharides is shown in Fig. 2 in the form of a general scheme of the processes of chitin and chitosan conversion. It should be noted that the approaches to the enzymatic preparation of various forms of oligomeric chitosan are mostly of a theoretical nature. Therefore, further research should be focused on the development of specific methods of directed enzymatic chitosan degradation to oligochitosans with predetermined MW values with the use of different groups of specific and nonspecific enzymes.

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