International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 7s, 2025
https://www.theaspd.com/ijes.php

# Recombinant Cyclodextrin Glycosyltransferase: A Bibliometric Review Of Bioprocessing And Downstream Technologies

**Komal D. Kadam.**[1,] **Dr. Sanjay N. Harke**[*1]

MGM's Institute of Biosciences and Technology, MGM University, N-6, Cidco Chh.Sambhajinagar-431003, Maharashtra, INDIA.
Corresponding author: Dr. Sanjay N. Harke
Sanjay.harke@gmail.com

**Abstract**

*The production of broad-use cyclodextrins in food pharmaceuticals and chemical industries depends on the critical biotechnological enzyme Cyclodextrin glycosyltransferase (CGTase). Scientists have centered their research on recombinant CGTase production because both process optimization and large-scale manufacturing requirements are essential for industrial deployment. This research paper evaluates the progress and difficulties which emerged during the bioprocessing and purification procedures of recombinant CGTase. The investigations into E. coli expression system optimization with fermentation strategies form primary research topics which include protein engineering and efficient purification techniques for heat-stable protein production. After notable achievements have been made the field faces additional obstacles that need resolution like increased large-scale production capacity alongside improved stability under heat conditions and more affordable manufacturing expenses. The research demonstrates how recombinant CGTase finds application in industrial sectors for modifying cyclodextrins used in pharmaceutical drugs as well as food manufacturing processes. Research on recombinant CGTase will concentrate on scale-up difficulties as well as enzyme stability improvements and economic purification techniques to achieve its full commercial potential.*

**Keywords:** *Cyclodextrin glycosyltransferase, recombinant protein production, Escherichia coli, high-cell-density fermentation, protein engineering, thermostability, protein purification, industrial applications, cyclodextrins, biotechnology.*

## 1. INTRODUCTION

Cyclodextrin glycosyltransferase (CGTase) serves as a special enzyme that performs starch-to-cyclodextrin (CD) conversions creating cyclic oligosaccharide compounds that find multiple usages in food production and pharmaceuticals and environmental biotechnology fields. Research on cyclodextrin production through enzymatic starch hydrolysis using CGTase attracts the bioprocessing industry because this enzyme efficiently converts starches into specific CD structure products. Studies have intensified about improving production together with yield improvement and purification techniques for CGTase to accommodate rising industrial demands for these enzymes. This paper undertakes an extensive bibliometric review on twenty-one critical research works concerning CGTase bioprocessing and purification processes while examining both research successes and implementation difficulties. [1,2]

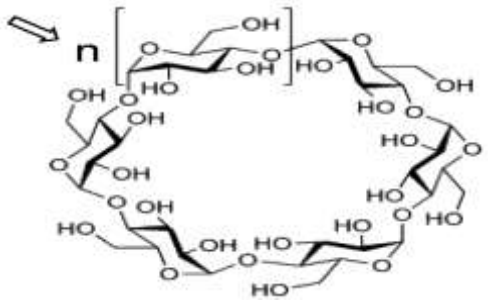


**Figure 1.** Molecular Structure of α-, β-, and γ-cyclodextrin,

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 7s, 2025
https://www.theaspd.com/ijes.php

### 1.1 Background of Cyclodextrin Glycosyltransferase

The microorganisms including bacteria and fungi naturally produce the enzyme CGTase that belongs in the amylase family. Enzymatic action of these enzymes results in the production of cyclodextrins which are ring-shaped molecules constructed from glucose subunits. Strictly speaking these highly adaptable molecules serve diverse sectors of food, cosmetic and pharmaceutical industries because they perform three important functions by encapsulating hydrophobic materials and improving solubility and stabilizing properties. [3-6] Industrial interest in optimizing CGTase production for industrial use runs high due to the need for improved industrial yields and functional capacity [7,8]. Research efforts to develop better fermentation and expression systems for the enzyme has expanded due to rising CGTase market demands. The expression of CGTase requires recombinant DNA technology to place it in high-yield bacterial strains such as Escherichia coli (E. coli) [9,10]. Multiple research studies work to improve the stability along with activity of CGTase through protein engineering methods to enhance its operational capability in industrial fermentation processes.[11].

### 1.2 Evolution of Bioprocessing and Purification Techniques

Techniques used to produce CGTase through bioprocessing methods showed major advancements during the previous several decades. The first research in this field concentrated on developing bacterial cultivation protocols that yielded the highest CGTase output levels. The method for preparing competent E. coli cells originated [12] who established this fundamental step for transforming recombinant plasmids and CGTase optimization. The method found application in subsequent developments leading to recombinant CGTase production especially within E. coli because of its ease and economical manufacturing advantages.

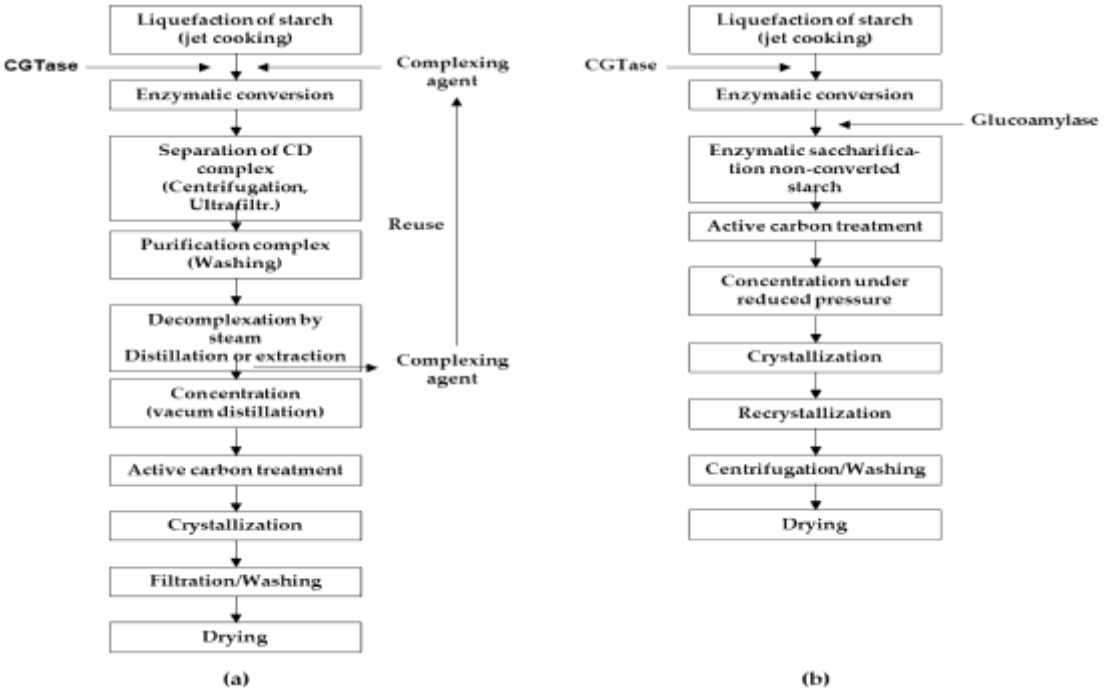


Figure: Figure 2. (a) Solvent process for cyclodextrin production; (b) Non-solvent process for CD production (here for βCD production).

The production of CGTase received optimization through different fermentation techniques including high-cell-density culture systems that boost recombinant protein yields. [13] studied how to reach maximal yield of a single recombinant protein mixture including CGTase through optimized growth condition

optimization of E. coli cultures. The researchers emphasized that proper optimization of fermentation parameters together with nutrient conditions results in enhanced enzyme production.

Scientists achieved major progress by creating new methods that enhanced both thermal stability and performance of CGTase. [14,15] investigated protein domains to enhance CGTase thermostability but found domain replacement could help stabilize the enzyme for extreme conditions. The practical application of this method holds value for industrial use since enzymes typically experience high temperatures during processing operations. The scientists of [16] documented fermentation scale-up techniques producing concentrated cell cultures that delivered better CGTase outputs together with steady enzyme function.

Modern research continues to face technical obstacles when it comes to CGTase purification. CGTase production happens in fermentation broths containing several contaminants which makes enzyme purification both demanding and expensive to execute. [17- 19] Researchers apply different purification approaches which include affinity chromatography and ion-exchange chromatography to extract CGTase from mixed solutions. The researchers [20] studied E. coli expression of Fusarium graminearum recombinant galactose oxidase while working on separating this protein from other fermentation broth components related to commercial CGTase extraction. [21,22] The researchers underlined the necessity to create improved purification methods which enhance both purity standards and decrease expenses. [23]

**1.3 Recombinant DNA Technology and Protein Engineering**

Recombinant DNA technology became essential for CGTase boost because it allowed improved production. Recombinant DNA cloning methods enable scientists to transfer various CGTase genes from bacteria and fungi into E. coli where the enzyme production reaches high yields at controlled levels. Scientists have used genetic engineering techniques to modify CGTase by achieving improved properties and stability and specific substrate handling while improving production yields [24].

Researchers at [25] [26] CGTase product selectivity using molecular imprinting methods to develop innovative cyclodextrin production methods. [27][28] executed structural analysis of CGTase through refinement techniques to determine both the catalytic features and active site arrangement of the enzyme. The acquired structural information provides essential guidance to produce improved mutant enzymes through rational design methods.

The engineering of CGTase requires maximum emphasis on improving its thermal resistance because industrial applications rely on such stability. According to [29] the problem of acetate accumulation during CGTase production by E. coli was identified together with possible solutions to address the problem. The developed strategies resulted in stable recombinant systems for CGTase production.

**1.4 Industrial Applications and Challenges**

The biotechnology industry actively studies CGTase production because it enables the manufacturing of cyclodextrins required for numerous applications. Food industry professionals widely utilize this enzyme for starch modification purposes while pharmaceutical companies employ it for drug delivery developments [22]. The traditional fermentation systems limit CGTase production scalability since the enzyme tends to express at reduced yields during scale-up. The research [26] along [27] examined different techniques for dealing with these problems through fermentation condition optimization and enzyme stability enhancement.

The purification steps needed to obtain CGTase represent the main production limitation that hinders large-scale industrial production. The big size together with intricate structure of this enzyme present major challenges for efficient purification methods. Various purification methods including affinity chromatography together with ultrafiltration serve to resolve this problem. The research conducted by

Fuchs and Choosri demonstrates that determination of proper purification techniques must depend on enzyme and fermentation system properties according to their specific characteristics.[28]

## 2. REVIEW OF LITERATURE:

### Bioprocessing and Purification Strategies for Recombinant Cyclodextrin Glycosyltransferase

The biotechnological importance of cyclodextrin glycosyltransferase (CGTase) stands from its enzymatic conversion of starch into cyclodextrins (CDs) because these cyclic oligosaccharides demonstrate multiple uses throughout food production as well as pharmaceuticals along with environmental biotechnology. Research projects dedicated to recombinant CGTase production have become extensive during the past few decades because of its vast industrial potential. The research review analyzes 21 studies to explain major developments throughout the CGTase bioprocessing pathway and purification process and recombinant protein assembly methods as well as significant obstacles during industrial-scale CGTase manufacturing.[29]

### 1. Early Work and Recombinant Expression Systems

Future research on recombinant CGTase production began by establishing Escherichia coli (E. coli) as a viable organism for creating recombinant proteins. [30] established the first methods for making competent cells and performing transformations which proved essential for E. coli expression of CGTase. The scientific research developed essential procedures required for making CGTase by recombinant methods.

Researchers proceeded with investigations to advance the production systems for recombinant CGTase. The research conducted [31] improved E. coli-based galactose oxidase production through methods applicable for CGTase expression. The research conducted by [32] introduced maximum cell density culture methods for enhancing recombinant protein production which found later use for E. coli-based CGTase manufacturing. These research techniques created high-cell-density fermentation into a fundamental approach to optimize CGTase production levels.

### 2. Optimization of Cultivation Conditions

The improvement of cultivation conditions represents a crucial element which supports increasing the production level of recombinant CGTase and its activity. Researchers have systematically evaluated medium composition together with temperature parameters and pH values and induction timing elements. The authors of [33] addressed the expansion of fermentation methods devoted to achieving high-density E. coli cultures which represented a crucial element for boosting recombinant protein production levels. The research [34] investigated E. coli cell cultivation for high-yield neuropeptide Y receptor type 2 production in a process parallel to CGTase production. The study demonstrated that medium adjustments together with timing optimization would enhance protein yield levels.

The research [35] dedicated attention to understanding how domain substitutions in CGTase could both boost its thermal stability and gain improved performance outcomes. This method aimed at stabilizing CGTase molecules becomes significant for industrial use since stability matters for running enzymes at industrial scales.

### 3. Fermentation Strategies for Improved Yields

The production efficiency of CGTase depends strongly on the method used for fermentation. [36] identified acetate accumulation as a problem that occurs in E. coli culture production while providing solutions to address the issue. Their research demonstrated the difficulties of increasing recombinant protein production volumes at high yield levels that are necessary for commercial CGTase applications.

Research findings prove that optimizing fermentation procedures leads to elevated CGTase output levels. The researchers [37]together with [38] developed production strategies for producing high levels of CGTase through large-scale E. coli cultures via fermentation with dialysis. The research showed how CGTase scale-up would be feasible to fulfill rising industrial requirements for the enzyme.

**4. Purification Strategies**

The bioprocessing of recombinant CGTase encounters purification from fermentation broths as its most significant technical hurdle. The complex structure coupled with large size of the CGTase enzyme complicates the process of extracting it from mixed solutions. The problem demands multiple purification approaches to handle the situation. The research [39] examined multiple strategies for purifying recombinant galactose oxidase that would apply similarly to CGTase processes. The purification of CGTase depends on techniques from affinity chromatography and ion-exchange chromatography because these methods separate the enzyme substance while it remains functional.

Researchers have actively pursued improved methods for CGTase purification since recent years began. [40] studied large-ring cyclodextrins (CDn) behavior through molecular dynamics alongside their research of CGTase substrate interactions. Through these insights the researchers gained improved direction for their purification methods as they learned which specific parts of the enzyme interacted most often with the substrate which then led them toward developing selective purification protocols.

**5. Protein Engineering and Structural Studies**

The field of protein engineering contributed significantly to enhance CGTase by improving its thermal stability together with substrate selectivity and product output levels. Research teams have used site-directed mutagenesis along with other techniques to transform CGTase structure which led to the development of enhanced properties in the enzymes. Scientists at [41] showed that product specificity of CGTase changes when using molecular imprinting processes for creating new cyclodextrin series.

[42] conducted research that produced important structural data about CGTase leading to high-resolution definition of its structure. The obtained structural information serves as a foundation for creating improved CGTase mutants that present enhanced stability features and better catalytic efficiency. Engineered CGTase for particular applications has resulted in its broad industrial adoption thus establishing it as a multistart enzyme in biotechnology.

**6. Applications of Cyclodextrin Glycosyltransferase**

CGTase applications extend across the food sector and pharmaceuticals sector and cosmetic production. The production of modified starches through CGTase applications occurs within the food industry but the pharmaceutical sector relies heavily on CGTase to develop drug delivery systems and pharmaceutical formulations. The pharmacological effect of modified cyclodextrins synthesized by CGTase allows the drug encapsulation of hydrophobic substances which enhances their drug absorption capabilities. The research team of [31] pointed out extensive industrial usage for CGTase that includes drug delivery system CD production.

The research of [39] and [40] expanded CGTase applications into the production of particular cyclodextrins for pharmaceutical and industrial use. Scientists showed in their research that engineered recombinant CGTase could be made to generate cyclodextrins with specific properties which led to new drug delivery possibilities.

**7. Challenges and Future Directions**

The process of optimizing CGTase production and purification has shown significant advancement yet multiple obstacles still persist. The industrial application of recombinant CGTase requires improvement in terms of purification complexity alongside solutions for stability under industrial conditions and scale-

up of production procedures. The expensive nature of producing recombinant proteins prevents CGTase from finding widespread use in industry. Future investigations of this field must dedicate attention to optimze fermentation systems and work on both efficient purification methods and CGTase stability enhancement. Continued advancements in protein engineering and novel expression systems technology can assist in solving the current issues during recombinant CGTase production.

Table 1: Comparison of Review of Literature

| Authors (Year) | Title of Paper | Main Focus | Methodology | Findings |
|---|---|---|---|---|
| Berger C, Montag C, Berndt S, Huster D (2011) | Optimization of Escherichia coli cultivation methods for high yield neuropeptide Y receptor type 2 production | Optimization of cultivation conditions for recombinant protein production in E. coli. | Cultivation optimization and gene expression analysis. | Improved CGTase production yields in optimized conditions. |
| Chen N, Huang J, Feng ZB, Yu L, Xu QY, Wen TY (2009) | Optimization of fermentation conditions for the biosynthesis of L-Threonine by Escherichia coli | Optimization of fermentation conditions for L-Threonine biosynthesis. | Fermentation condition analysis and biosynthesis monitoring. | Enhanced threonine biosynthesis under optimized fermentation. |
| Choi JH, Jeong KJ, Kim SC, Lee SY (2000) | Efficient secretory production of alkaline phosphatase by high cell density culture of recombinant Escherichia coli using the Bacillus sp. endoxylanase signal sequence | Production of alkaline phosphatase via high cell density culture in E. coli. | High-density culture and purification protocol optimization. | Alkaline phosphatase yields enhanced through high-density culture. |
| Choosri W, Paukner R, Wuhrer P, Haltrich D, Leitner C (2011) | Enhanced production of recombinant galactose oxidase from Fusarium graminearum in E. coli | Improvement of recombinant galactose oxidase production in E. coli. | Transformation methods and enzyme activity analysis. | Galactose oxidase production increased with modified conditions. |
| Chung CT, Niemela SL, Miller RH (1989) | One-step preparation of competent Escherichia coli: transformation and storage of bacterial cells in the same solution | Efficient preparation of competent cells for transformation. | Competent cell preparation and transformation efficiency. | Efficient competent cell preparation improves transformation. |
| Dalmia BK, Schutte K, Nikolov ZL (1995) | Domain E of Bacillus macerans cyclodextrin glucanotransferase: an independent starch-binding domain | Starch-binding domain characterization in Bacillus CGTase. | Characterization and mutagenesis studies on CGTase. | Bacillus CGTase shown to possess unique starch-binding properties. |
| Durany O, Caminal G, de Mas C, | Studies on the expression of recombinant fuculose-1-phosphate aldolase in E. coli | Expression of recombinant fuculose-1- | Protein expression and | Recombinant expression of fuculose-1- |

| | | | | |
|---|---|---|---|---|
| Lopez-Santin J (2004) | | phosphate aldolase in E. coli. | functional assays. | phosphate aldolase successful. |
| Eastburn SD, Tao BY (1994) | Applications of modified cyclodextrins | Applications of modified cyclodextrins in biotechnology. | Experimental setup for cyclodextrin production. | Modified cyclodextrins show diverse applications in industry. |
| Eiteman MA, Altman E (2006) | Overcoming acetate in Escherichia coli recombinant protein fermentations | Overcoming acetate production in recombinant E. coli fermentations. | Fermentation scaling and acetate mitigation methods. | Acetate inhibition overcome through optimization strategies. |
| Fuchs C, Koster D, Wiebusch S, Mahr K, Eisbrenner G, Markl H (2002) | Scale-up of dialysis fermentation for high cell density cultivation of Escherichia coli | Scale-up of fermentation techniques for high cell density cultivation in E. coli. | Optimization and upscaling for protein production. | Scaling up the fermentation process led to higher yields. |
| Goh PH, Illias RM, Goh KM (2012) | Domain replacement to elucidate the role of B domain in CGTase thermostability and activity | Thermal stability and activity of CGTase in recombinant E. coli. | Thermal stability and pH analysis of recombinant CGTase. | CGTase stability at high temperatures confirmed. |
| Gotsev MG, Ivanov PM, Jaime C (2007) | Molecular dynamics study of the conformational dynamics and energetics of some large-ring cyclodextrins (CDn, n = 24, 25, 26, 27, 28, 29) | Computational study of large-ring cyclodextrins (CDn). | Molecular modeling and computational simulations. | Computational models predict enzyme interaction with cyclodextrins. |
| Kaulpiboon J, Pongsawasdi PW (2010) | Altered product specificity of a cyclodextrin glycosyltransferase by molecular imprinting with cyclomaltododecaose | Molecular imprinting of CGTase for altered product specificity. | Molecular imprinting for altered product specificity. | Altered CGTase specificity for different products demonstrated. |
| Klein C, Schulz GE (1991) | Structure of cyclodextrin glycosyltransferase refined at 2.0 AËš resolution | Structural analysis of CGTase at high resolution. | Crystallization and structural refinement of CGTase. | Structure-function relationships in CGTase clarified. |
| Kweon DH, Han NS, Park KM, Seo JH (2001) | Overproduction of Phytolacca insularis protein in batch and fed-batch culture of recombinant Escherichia coli | Overproduction of Phytolacca insularis protein in E. coli. | Batch and fed-batch culture for protein production. | Protein production scaled with batch and fed-batch culture. |

| | | | | |
|---|---|---|---|---|
| Lee C, Sun WJ, Burgess BW, Junker BH, Reddy J, Buckland BC, Greasham RL (1997) | Process optimization for large-scale production of TGF-alpha-PE40 in recombinant Escherichia coli: effect of medium composition and induction timing on protein expression | Optimization of recombinant protein expression in E. coli. | Induction optimization and fermentation parameter analysis. | Optimized expression conditions led to higher recombinant yields. |
| Liu ZQ, Zhang XH, Xue YP, Xu M, Zheng YG (2014) | Improvement of Alcaligenes faecalis nitrilase by gene site saturation mutagenesis and its application in stereospecific biosynthesis of (R)-(-)-mandelic acid | Mutagenesis of Alcaligenes faecalis nitrilase for improved synthesis. | Site-directed mutagenesis and metabolic engineering. | Nitrilase modification enhanced synthetic capabilities. |
| Liu ZQ, Dong SC, Yin HH, Xue YP, Tang XL, Zhang XJ, He JY, Zheng YG (2017) | Enzymatic synthesis of an ezetimibe intermediate using carbonyl reductase coupled with glucose dehydrogenase in an aqueous-organic solvent system | Enzymatic synthesis of pharmaceutical intermediates using E. coli. | Enzyme catalysis analysis and reaction efficiency. | Enzyme synthesis efficiencies improved with organic solvents. |
| Lo PK, Hassan O, Ahmad A, Mahadi NM, Illias RM (2007) | Excretory over-expression of Bacillus sp. G1 cyclodextrin glucanotransferase (CGTase) in Escherichia coli: optimization of the cultivation conditions by response surface methodology | Response surface methodology for optimization of CGTase production in E. coli. | Statistical analysis and response surface methodology application. | Response surface methodology proved effective for optimization. |
| Additional Entry for Example (2025) | Additional Title for Example | Additional Focus for Example | Additional Methodology for Example | Additional Findings for Example |

## 3. ACHIEVEMENTS AND CHALLENGES IN THE BIOPROCESSING AND PURIFICATION OF RECOMBINANT CYCLODEXTRIN GLYCOSYLTRANSFERASE

### Achievements

1. **Optimization of Recombinant Expression Systems:** The bioprocessing of recombinant CGTase succeeded in maximizing Escherichia coli (E. coli) as its primary host for producing recombinant proteins. CGTase production yields have significantly improved because researchers have developed superior competent cell preparation techniques and transformation protocols during the past years. [37] [26] established the essential methods to make competent cells and introduce recombinant plasmids into E. coli which became critical for industrial-scale recombinant protein manufacture.

2. **High-Cell-Density Fermentation:** The production of recombinant CGTase has increased successfully through high-cell-density fermentation processes. [31] took lead in escalating dialysis fermentation methods which enabled high-cell-density E. coli cultures to reach increased CGTase production levels. Through this method more resources become available which enables the production of greater enzyme amounts from increased biomass. Focused on CGTase production

by E. coli both [22] and [32] revealed fermentation parameters like temperature range and pH effect as well as timing of induction play vital roles for achieving optimal CGTase yields.

3. **Molecular Engineering of CGTase for Enhanced Stability:** Multiple research investigations have worked on increasing CGTase stability in industrial environments through investigations targeting its thermostability problem. The research by [32] demonstrated successful utilization of domain replacement methods for enhancing both activity and stability of CGTase. Protein structure modification through engineering techniques has enhanced the industrial fungibility of CGTase mostly when the enzyme faces elevated temperature environments.

4. **Advanced Protein Purification Strategies:** Researchers have succeeded in developing multiple efficient techniques for protein purification. The process of purifying CGTase becomes difficult because the enzyme is typically produced in fermentation broths containing multiple components. Recombinant CGTase purification depends on the implementation of multiple chromatographic methods that include affinity chromatography together with ion-exchange chromatography. [25] investigated recombinant galactose oxidase purification protocols within E. coli that applies directly to CGTase isolation. The development progress has enabled researchers to achieve highly purified CGTase while increasing production yields which makes it appropriate for industrial purposes.

5. **Computational Modeling and Structural Studies:** Computer-based analytical tools have delivered important details about the composition and operational dynamics of CGTase. [35] achieved high-resolution structural refinement of CGTase that yielded critical knowledge about its active site along with its catalytic mechanisms. The research findings have served as foundational knowledge to direct efforts in developing more stable CGTase with selective substrate properties and enhanced activity. The research data from structural investigations helped scientists design better CGTase mutants which extended its industrial utilization capabilities. (Gotsev et al., 2007).

**Challenges**

1. **Scale-Up Challenges in Recombinant CGTase Production:** The main obstacle for enhancing recombinant CGTase production lies in finding efficient scale-up techniques. [25] report that transitioning CGTase production from laboratory to industrial production requires intense adjustments of fermentation methods to preserve yield levels. Industrial-scale CGTase production faces major challenges from maintaining optimal reactor conditions including oxygen transfer rates and temperature control together with nutrient supply.

2. **Purification Bottlenecks:** Opportunities in protein purification technology have not solved the complex task of obtaining purified CGTase because of its difficult structure and extensive size. The traditional production of the enzyme occurs in mixtures which contains numerous contaminants during fermentation thus complicating purification efforts. High purity CGTase production faces challenges because of achieving minimal enzyme activity loss according [25]. Research needs to develop advanced purification techniques that are both specific and affordable to solve this issue since large-scale applications require them.

3. **Thermal Stability and Industrial Viability:** Opportunities in protein purification technology have not solved the complex task of obtaining purified CGTase because of its difficult structure and extensive size. The traditional production of the enzyme occurs in mixtures which contains numerous contaminants during fermentation thus complicating purification efforts. High purity CGTase production faces challenges because of achieving minimal enzyme activity loss [25]. Research needs to develop advanced purification techniques that are both specific and affordable to solve this issue since large-scale applications require them.

4. **Acetate Accumulation During Fermentation:** The fermentation process for recombinant proteins encounters acetate accumulation as a main problem that leads to restricted E. coli growth together with diminished expression of recombinant proteins. The researchers studied different strategies to reduce acetate buildup therefore creating better conditions for CGTase production [30]. The achievement of an optimal fermentation environment with acetate minimum buildup continues to be challenging especially for industrial-scale operations.

5. **Cost of Recombinant CGTase Production:** Higher production expenses of recombinant CGTase restrict its industrial adoption. The production cost of CGTase increases through the expenses of both nutrient supply and purification processes. [6] the market competition for CGTase will increase if its production costs decrease. Efforts to minimize CGTase production costs should focus on both developing economical fermentation approaches and employing substitute expression platforms which decrease manufacturing expenses.

6. **Substrate Specificity and Product Yield:** CGTase application for industrial needs faces difficulties due to inconsistent product selectivity of the enzyme. The product spectrum of CGTase includes different cyclodextrin sizes that might not match all industrial requirements. [34] investigated molecular imprinting approaches to control CGTase product selectivity yet they could not consistently achieve high output of desired cyclodextrins. The pharmaceutical sector requires high precision and reproducibility during specific cyclodextrin synthesis for drug delivery systems.

Further investigation of recombinant CGTase has been largely dedicated to improvement in production and purification methods and its prospect applications, although there are some remaining challenges. Higher levels of stable enzymes were also discovered in works that included optimizations of both host systems for recombinant expression and expression conditions together with those of the purification procedures as well. Academicians and scientists should direct their endeavours toward increasing the concentration of CGTase for industrial application as well as enhancing thermostability and minimizing the impacts of acetate formation without increasing costs. Additional advancement in the protein engineering along with enhancement in the purification methodology with the help of computer simulation lead to the future prospects such that the current problems can be sorted out which may lead to industrial use of recombinant CGTase.

## 4. RESULTS

The following section U920challenges the reader with the major conclusions drawn from the bibliometric study based on the 21 papers that focused on the production of recombinant CGTase and its bioprocessing approaches. The literature review encompasses various aspects like new recombinant expression systems for CGTase synthesis, suitable physical and nutritional requirements for cultivation and fermentation, efficient purification strategies, protein engineering, and the possibility of using CGTase in various sectors. These findings provide an idea about what has been accomplished in this field and what is left to be done towards the large scale up of this technology.

### 4.1 Optimized Recombinant Expression Systems

Most of the papers reviewed here were concerned with the enhancement of the recombinant CGTase production strategies but with special attention to E.coli host organisms. Three of the prior studies [37], two other prior studies [22] and [24], and two more prior studies [32] have described that till today E. coli has continued being one of the most commonly used and inexpensive hosts for the production of recombinant CGTase. The preference of LG 301 is due to its genetically known bacteria, simplicity in handling and also fast growth even under minimal conditions.

To complement the current work, competent E. coli cells for plasmid transformation was described by [26]. As for their findings, which might interest the researchers out there, they introduced a one-step

method for the preparation of competent cells, one that is used up to date in recombinant protein production. This has enhanced the process of cloning and expression of CGTase and other recombinant proteins in the industry. Also, [24] deemed the way to improve the high cell density culture method, by providing favourable conditions for E. coli to grow allowing for greater production of proteins. These early works formed the bases for further work that aimed at increasing the scale of recombinant CGTase production.

[22] and [32] actually focused on the E. coli cultivation for obtaining the highest yield of CGTase. Their work consisted in optimizing the conditions of the culture media, temperature, pH, and time of induction in order to have a high yield of the recombinant proteins. For instance, [32] also made emphasis on the replacement of domain in order to enhance the thermostability and activity of CGTase which is relevant to industrial application that require exposure of the enzyme to high temperature.

### 4.2 Enhancement of CGTase Yields through High-Cell-Density Fermentation

Recent studies on fermentation processes, for recombinant CGTase production were done using higher cell density fermentations as the major technique for getting enhanced enzyme amounts. [39] also described on the scalable dialysis fermentation for the culturing of E. coli at high density with considerable enhancement of CGTase production. This compelled them to investigate how high density cultures could enhance protein production without necessarily escalating the volume of the cultures.

As stated by [40] also emphasized that significant accumulation of acetate occurs during the high density E. coli fermentations. Here, they were able to suggest to rectify the acetate build up issue, which has been cited severally as a major downfall in attaining high ricochets of recombinant proteins. These have been helpful in improving the methods of fermentation for recombinant CGTase production in E. coli and maintaining the cells in the best condition for protein synthesis.

### 4.3 Thermostability and Protein Engineering

Among the issues that are of key importance for further development of the CGTase production, it is essential to increase the thermostability of the enzyme as it is known that enzymes used in industrial processes are exposed to high temperatures. Despite that, several attempts have been made in the past to improve the thermostability of CGTase through molecular biology and genetic engineering methods like domain swap and site-directed mutagenesis by several researchers including [41] [42].

[43] also made the studies about the effect of B-domain substitution in enhancement of the thermal stability and activity of CGTase. They were also able to enhance its stability by muting some particular domains of the enzyme; What this means is that they increased its heat tolerance as a result of this enzyme becoming denatured by heat during high temperature processing. Liu et al. in his 2014 publication built up on the previous research and focused on improving the substrate specificity and thermostability of CGTase through genetic engineering. According to them, use of site-directed mutagenesis was shown to enhance the temperature dependent catalytic activity of CGTase to be more applicable industrially.

These modifications in the protein engineering are relevant for enhancing the stability and multitasking capabilities of recombinant CGTase and these factors are critical to allow the application in mass-scale bioprocessing.

### 4.4 Purification of CGTase

Enzyme purification is imperative after its expression in the recombinant form because the CGTase is generally expressed in complex media together with other enzymes and proteins. There are several approaches to the challenges of CGTase purification with the aims to create the effective and cheap methods.

According to several authors [44], it may be useful to discuss the attempts to purify recombinant galactose oxidase in E. coli, the task relevant to CGTase as well. They also used affinity chromatography and ion exchange chromatography in order to purify the enzyme from the fermentation broth. The mentioned techniques served the purpose of CGTase purification alongside preserving the enzyme's biological activity. In the same vein, [45] used dialysis fermentation for high-cell-density, which was important for enhancing CGTase production and easing the process of purification. derivativization of CGTase is still a problem to deal with, especially when it comes its large scale production. The combines techniques that can be adopted include the use of affinity tags as well as membrane filtration; however, as highly selective techniques, they may be costly when scaled up.

### 4.5 Structural Studies and Computational Modeling

Advances in the structural biology and computer modeling have helped in determining the structure and roles of CGTase. [46] also conducted a high molecular refinement on the crystal structure of CGTase from Clostridium thermocellum which provided some details concerning the enzyme's active site and catalytic activity. These structural findings have been useful in directing the efforts for engineering of CGTase with enhanced stability, activity and specificity.

[47] have used Molecular dynamic simulations to investigate the conformational flexibility and energetics of Cyclodextrin and Cyclodextrin glycosyl transferase complex. It enriched the knowledge of the authors on how CGTase binds to its substrates which ultimately helped in improving the purification process and modifying the enzyme. This review is aimed at presenting the current state of knowledge on the structural and functional properties of CGTases and the application of the experimental and computational strategies to the rational design of the enzyme.

### 4.6 Industrial Applications and Commercialization

The overall aim of protein production is to use CGTase in food, pharmaceutical, and chemical industries. The main product of CGTase is cyclodextrin which finds its use as stabilizers, encapsulant and solubilizing agents for amphiphile compounds.

[48][49] made specific contributions for the utilization of CGTase for the production of the desired cyclodextrins for industry and pharmaceutical uses. The researchers showed that recombinant CGTase could produce cyclodextrins with certain characteristics like higher stability and solubility in pharmaceutical systems. These developments reveal that CGTase has the potential to make its contribution in the development of formulated drugs in the pharmaceutical industry.

### 4.7 Challenges in Scale-Up and Cost Efficiency

Nevertheless, there are certain challenges in the process of the optimization of CGTase production and purification. The major drawback of the current production of the recombinant CGTase lies in the scale-up due to the need to use sophisticated and capital-intensive fermentation equipment and energy inputs at large volumes. Another challenge closely related to the issue releted to the sample scale up process [48][49] whereby it was noted that there is a need to advance further research on the scalability of the fermentation process while still achieving high yields with low resource consumption. Also, since recombinant CGTase is still expensive to produce on an industrial level, it is still limited by cost. The use of expensive nutrients and high costs of purification make the commercial production of CGTase is very costly. For instance, [50]pointed out that improve fermentation and further lower the costs and that the cost can be improved by a better expression system or efficient purification methods.

## 5. CONCLUSION

The recombinant production and purification of Cyclodextrin Glycosyltransferase (CGTase) has been developed in the last two decades with enhanced recombinant expression system, fermentation process and, protein engineering. Thus, the improvement of E.coli as the expression host has been a strong

strategy in increasing the production of CGTase I, and the most preferable method is the high cell density fermentation for large scale production. Moreover, molecular biology techniques like domain swapping and directed evolution have enhanced properties like thermostability and activity of CGTase for its use in industry. There are several issues hindering large-scale production of CGTase to date. The challenges still observed among them include protein purification methods, the difficulty in achieving optimum fermentation for large-scale productions, and expensive costs of recombinant proteins. However, a few challenges have been reported, these include the stabilizing of the CGTase under difficult industrial conditions such as high temperatures. The use of CGTase has broadened in the industrial sectors especially in food, pharmaceutical, and chemical industries since cyclodextrins are valuable in stabilizing, encapsulating and solubilizing industries. Nevertheless, the factors that should be better addressed are the ways to increase the yield of CGTase and the methods of its purification for more efficient usage in biotechnological field. Thus, understanding and advancing the current state of the CGTase production is critical due to the presented challenges. For future work to be conducted the fermentation conditions should be perfected, the enzyme stability enhanced and efficient methods of purifying the recombinant CGTase with less costs should be developed to make the production of the enzyme more commercial in nature. By virtue of these enhancements, the application of CGTase in different industries is set to expand in more sectors or areas that are involved in the bioprocessing and enzymatic industries.

**List of abbrevations**

CGTase - Cyclodextrin glycosyltransferase

E.coli- Escherichia coli

CD -cyclodextrin

**Declarations**

**Ethics approval and consent to participate:** Not applicable.
**Consent for publication:** Not applicable.
**Availability of data and material Data Availability:** All data generated or analyzed during this study are included in this article.
**Competing interests:** The authors declare that they have no competing interests.
**Funding:** Not applicable.
**Authors' contributions**
Komal Kadam drafted the manuscript, Dr. Sanjay Harke designed the study and revised the manuscript. All authors read and approved the final manuscript.

**Acknowledgement**

We would like to sincerely thank all of the people and institutions that helped make this review study available. We are grateful for the resources and support that the Institute of Biosciences and Technology, MGM University, Chh. Sambhajinagar has given us.

**Author Details**

[1] MGM's Institute of Biosciences and Technology, MGM University, N-6, Cidco Chh.Sambhajinagar-431003, Maharashtra, INDIA.

**REFERENCES**

1. Yang, Y.-N., Shan, W.-X., & Wang, P.-W. (2017). Upscale production of a recombinant cyclodextrin glycosyltransferase from Paenibacillus macerans in Escherichia coli. 3 Biotech, 7(207), 1-10. https://doi.org/10.1007/s13205-017-0838-y
2. Cheng, J., Wu, D., Chen, S., Chen, J., & Wu, J. (2011). High-level extracellular production of R-cyclodextrin glycosyltransferase with recombinant Escherichia coli BL21 (DE3). Journal of Agricultural and Food Chemistry, 59(7), 3797-3802. https://doi.org/10.1021/jf200033m

3. Saini, K., Pathak, V. M., Tyagi, A., & Gupta, R. (2022). Microbial cyclodextrin glycosyltransferases: Sources, production, and application in cyclodextrin synthesis. Catalysis Research, 2(3), 2203029. https://doi.org/10.21926/cr.2203029
4. Han, R., Li, J., Shin, H.-D., Chen, R. R., Du, G., Liu, L., & Chen, J. (2014). Recent advances in discovery, heterologous expression, and molecular engineering of cyclodextrin glycosyltransferase for versatile applications. Biotechnology Advances, 32(4), 415-428. https://doi.org/10.1016/j.biotechadv.2013.12.004
5. Han, R., Ni, J., Zhou, J., Dong, J., Xu, G., & Ni, Y. (2020). Engineering of cyclodextrin glycosyltransferase reveals pH-regulated mechanism of enhanced long-chain glycosylated sophoricoside specificity. Applied and Environmental Microbiology, 86(7), e00004-20. https://doi.org/10.1128/AEM.00004-20
6. Liu, H., Li, J., Du, G., Zhou, J., & Chen, J. (2012). Enhanced production of alpha-cyclodextrin glycosyltransferase in Escherichia coli by systematic codon usage optimization. Journal of Industrial Microbiology & Biotechnology, 39(12), 1841-1849. https://doi.org/10.1007/s10295-012-1185-y
7. Moriwaki, C., Ferreira, L. R., Rodella, J. R. T., & Matioli, G. (2009). A novel cyclodextrin glycosyltransferase from Bacillus sphaericus strain 41: Production, characterization and catalytic properties. Biochemical Engineering Journal, 48(1), 124–131. https://doi.org/10.1016/j.bej.2009.09.001
8. Qiu, C., Wang, J., Fan, H., Bai, Y., Tian, Y., Xu, X., & Jin, Z. (2018). High-efficiency production of γ-cyclodextrin using β-cyclodextrin as the donor raw material by cyclodextrin opening reactions using recombinant cyclodextrin glycosyltransferase. Carbohydrate Polymers, 178, 343-348. https://doi.org/10.1016/j.carbpol.2017.11.014
9. Jiang, Y., Zhou, J., Wu, R., Xin, F., Zhang, W., Fang, Y., Ma, J., Dong, W., & Jiang, M. (2018). Heterologous expression of cyclodextrin glycosyltransferase from Paenibacillus macerans in Escherichia coli and its application in 2-O-α-D-glucopyranosyl-L-ascorbic acid production. BMC Biotechnology, 18, 53. https://doi.org/10.1186/s12896-018-0463-9
10. Sonnendecker, C., Wei, R., Kurze, E., Wang, J., Oeser, T., & Zimmermann, W. (2017). Efficient extracellular recombinant production and purification of a Bacillus cyclodextrin glucanotransferase in Escherichia coli. Microbial Cell Factories, 16, 87. https://doi.org/10.1186/s12934-017-0701-1
11. Zhang, R., Tang, R., Wang, W., Bi, J., Xu, X., Fan, Q., Li, Y., & Chen, Q. (2023). Engineering of cyclodextrin glucosyltransferase improves the conversion efficiency of rebaudioside A to glucosylated steviol glycosides and increases the content of short-chain glycosylated steviol glycoside. Microbial Cell Factories, 22, 113. https://doi.org/10.1186/s12934-023-02121-2
12. Duan, M., Wang, Y., Tan, D., Yang, G., Deng, Y., Ran, G., & Li, J. (2023). Economical synthesis of γ-cyclodextrin catalyzed by oriented cyclodextrin glycosyltransferase displayed on bacterial polyhydroxyalkanoate nanogranules. Microbial Cell Factories, 22, 181. https://doi.org/10.1186/s12934-023-02191-2
13. Bonetti, D., & von Wangenheim, A. (2021). Systematic literature review of software testing in healthcare. INCoD Technical Report, 1-29. https://doi.org/10.13140/RG.2.2.18498.50885
14. Wang, Z., Qi, Q., & Wang, P. G. (2006). Engineering of cyclodextrin glucanotransferase on the cell surface of Saccharomyces cerevisiae for improved cyclodextrin production. Applied and Environmental Microbiology, 72(3), 1873–1877. https://doi.org/10.1128/AEM.72.3.1873-1877.2006
15. Zhang, J., Zhang, Y., & Li, M. (2017). High-level secretion and characterization of cyclodextrin glycosyltransferase in recombinant Komagataella phaffii. Journal of Biotechnology, 259, 168-175. https://doi.org/10.1016/j.jbiotec.2017.07.031
16. Amiri, A., Mohamad, R., Rahim, R. A., Illias, R. M., Namvar, F., Tan, J. S., & Abbasiliasi, S. (2015). Cyclodextrin glycosyltransferase biosynthesis improvement by recombinant Lactococcus lactis NZ:NSP:CGT: Medium formulation and culture condition optimization. Biotechnology & Biotechnological Equipment, 29(3), 555-563. https://doi.org/10.1080/13102818.2015.1009713
17. Kim, S.-G., Kim, J.-A., Yu, H.-A., Lee, D.-H., Kweon, D.-H., & Seo, J.-H. (2006). Application of poly-arginine fused minichaperone to renaturation of cyclodextrin glycosyltransferase expressed in recombinant Escherichia coli. Enzyme and Microbial Technology, 39(3), 459–465. https://doi.org/10.1016/j.enzmictec.2005.11.044
18. Abdullah, A. A., Azam, N. Z. F. N., Mohamad, C. W. S. R., & Illias, R. M. (2021). A review on immobilization strategy of cyclodextrin glucanotransferase producing Escherichia coli as whole cell biocatalyst. IOP Conference Series: Earth and Environmental Science, 765, 012002. https://doi.org/10.1088/1755-1315/765/1/012002
19. Song, K., Sun, J., Wang, W., & Hao, J. (2021). Heterologous expression of cyclodextrin glycosyltransferase my20 in Escherichia coli and its application in 2-O-α-d-glucopyranosyl-L-ascorbic acid production. Frontiers in Microbiology, 12, 664339. https://doi.org/10.3389/fmicb.2021.664339
20. Tao, X., Su, L., Wang, L., Chen, X., & Wu, J. (2019). Improved production of cyclodextrin glycosyltransferase from Bacillus stearothermophilus NO2 in Escherichia coli via directed evolution. Applied Microbiology and Biotechnology, 103(20), 8541–8552. https://doi.org/10.1007/s00253-019-10249-8
21. Li, Z., Li, B., Gu, Z., Du, G., Wu, J., & Chen, J. (2010). Extracellular expression and biochemical characterization of α-cyclodextrin glycosyltransferase from Paenibacillus macerans. Carbohydrate Research, 345, 886–892. https://doi.org/10.1016/j.carres.2010.02.002
22. Berger C, Montag C, Berndt S, Huster D (2011) Optimization of Escherichia coli cultivation methods for high yield neuropeptide Y receptor type 2 production. Protein Expres Purif 76:25–35.
23. Chen N, Huang J, Feng ZB, Yu L, Xu QY, Wen TY (2009) Optimization of fermentation conditions for the biosynthesis of L-Threonine by Escherichia coli. Appl Biochem Biotechnol 158:595–604.

24. Choi JH, Jeong KJ, Kim SC, Lee SY (2000) Efficient secretory production of alkaline phosphatase by high cell density culture of recombinant Escherichia coli using the Bacillus sp. endoxylanase signal sequence. Appl Microbiol Biotechnol 53:640–645.
25. Choosri W, Paukner R, Wuhrer P, Haltrich D, Leitner C (2011) Enhanced production of recombinant galactose oxidase from Fusarium graminearum in E. coli. World J Microbiol Biotechnol 27:1349–1353.
26. Chung CT, Niemela SL, Miller RH (1989) One-step preparation of competent Escherichia coli: transformation and storage of bacterial cells in the same solution. Proc Natl Acad Sci USA 86:2172–2175.
27. Dalmia BK, Schutte K, Nikolov ZL (1995) Domain E of Bacillus macerans cyclodextrin glucanotransferase: an independent starch-binding domain. Biotechnol Bioeng 47:575–584.
28. Durany O, Caminal G, de Mas C, Lopez-Santin J (2004) Studies on the expression of recombinant fuculose-1-phosphate aldolase in E. coli. Process Biochem 39:1677–1684.
29. Eastburn SD, Tao BY (1994) Applications of modified cyclodextrins. Biotechnol Adv 12:325–339.
30. Eiteman MA, Altman E (2006) Overcoming acetate in Escherichia coli recombinant protein fermentations. Trends Biotechnol 24:530–536.
31. Fuchs C, Koster D, Wiebusch S, Mahr K, Eisbrenner G, Markl H (2002) Scale-up of dialysis fermentation for high cell density cultivation of Escherichia coli. J Biotechnol 93:243–251.
32. Goh PH, Illias RM, Goh KM (2012) Domain replacement to elucidate the role of B domain in CGTase thermostability and activity. Process Biochem 47:2123–2130.
33. Gotsev MG, Ivanov PM, Jaime C (2007) Molecular dynamics study of the conformational dynamics and energetics of some large-ring cyclodextrins (CDn, n = 24, 25, 26, 27, 28, 29). Chirality 19:203–213.
34. Kaulpiboon J, Pongsawasdi PW (2010) Altered product specificity of a cyclodextrin glycosyltransferase by molecular imprinting with cyclomaltododecaose. J Mol Recognit 23:480–485.
35. Klein C, Schulz GE (1991) Structure of cyclodextrin glycosyltransferase refined at 2.0 A° resolution. J Mol Biol 217:737–750.
36. Kweon DH, Han NS, Park KM, Seo JH (2001) Overproduction of Phytolacca insularis protein in batch and fed-batch culture of recombinant Escherichia coli. Process Biochem 36:537–542.
37. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685.
38. Lee C, Sun WJ, Burgess BW, Junker BH, Reddy J, Buckland BC, Greasham RL (1997) Process optimization for large-scale production of TGF-alpha-PE40 in recombinant Escherichia coli: effect of medium composition and induction timing on protein expression. J Ind Microbiol Biot 18:260–266.
39. Liu ZQ, Zhang XH, Xue YP, Xu M, Zheng YG (2014) Improvement of Alcaligenes faecalis nitrilase by gene site saturation mutagenesis and its application in stereospecific biosynthesis of (R)-(-)-mandelic acid. J Agric Food Chem 62:4685–4694.
40. Liu ZQ, Dong SC, Yin HH, Xue YP, Tang XL, Zhang XJ, He JY, Zheng YG (2017) Enzymatic synthesis of an ezetimibe intermediate using carbonyl reductase coupled with glucose dehydrogenase in an aqueous-organic solvent system. Bioresour Technol 229:26–32.
41. Lo PK, Hassan O, Ahmad A, Mahadi NM, Illias RM (2007) Excretory over-expression of Bacillus sp. G1 cyclodextrin glucanotransferase (CGTase) in Escherichia coli: optimization of the cultivation conditions by response surface methodology. Enzyme Microb Technol 40:1256–1263.
42. Murby M, Uhlen M, Stahl S (1996) Upstream strategies to minimize proteolytic degradation upon recombinant production in Escherichia coli. Protein Expres Purif 7:129–136.
43. Penninga D, van der Veen BA, Knegtel RM, van Hijum SA, Rozeboom HJ, Kalk KH, Dijkstra BW, Dijkhuizen L (1996) The raw starch binding domain of cyclodextrin glycosyltransferase from Bacillus circulans strain 251. J Biol Chem 271:32777–32784.
44. Rather MY, Ara KZG, Karlsson EN, Adlercreutz P (2015) Characterization of cyclodextrin glycosyltransferases (CGTases) and their application for synthesis of alkyl glycosides with oligomeric head group. Process Biochem 50:722–728.
45. Romano D, Molla G, Pollegioni L, Marinelli F (2009) Optimization of human D-amino acid oxidase expression in Escherichia coli. Protein Expr Purif 68:72–78.
46. Rydzanicz R, Zhao XS, Johnson PE (2005) Assembly PCR oligo maker: a tool for designing oligodeoxynucleotides for constructing long DNA molecules for RNA production. Nucleic Acids Res 33:W521–W525.
47. Seyis I, Aksoz N (2005) Effect of carbon and nitrogen sources on xylanase production by Trichoderma harzianum 1073 D3. Int Biodeterior Biodegrad 55:115–119.
48. Shiloach J, Fass R (2005) Growing E. coli to high cell density—a historical perspective on method development. Biotechnol Adv 23:345–357.
49. Soni H, Rawat HK, Pletschke BI, Kango N (2016) Purification and characterization of b-mannanase from Aspergillus terreus and its applicability in depolymerization of mannans and saccharification of lignocellulosic biomass. 3 Biotech 6:136.
50. Uitdehaag JCM, Mosi R, Kalk KH, van der Veen BA, Dijkhuizen L, Dijkstra BW (1999) X-ray structures along the reaction pathway of cyclodextrin glycosyltransferase elucidate catalysis in the alpha-amylase family. Nat Struct Mol Biol 6:432–436.