Vol.64: e21180747, 2021
https://doi.org/10.1590/1678-4324-2021180747
ISSN 1678-4324 Online Edition

Review - Agriculture, Agribusiness and Biotechnology

Overviewing the Application of β-Galactosidase "Immobilized on Nanoparticles" in Dairy Industries

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Editor-in-Chief: Paulo Vitor Farago
Associate Editor: Paulo Vitor Farago

Received: 2019.01.21; Accepted: 2019.07.13.

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HIGHLIGHTS

- Nanoparticles (NPs) exhibited greater performance of immobilized enzyme.
- They influence the sensitivity, selectivity, loading, mobility and stability of enzymes.
- β-galactosidase immobilized on NPs retained greater activity against various physical and chemical denaturants.

Abstract: Owing to the excellent catalytic potential, β-galactosidase (EC: 3.2.1.23) has been exploited as an important industrial enzyme for obtaining galactooligosaccharides (GOS) and lactose-free products in dairy industries. Moreover, novel technologies have been implemented in the recent past for preparing and modifying nanoparticles (NPs) for immobilizing therapeutically and industrially important enzymes. Nanoparticles based enzyme immobilization (NBEI) offered more stability and robustness to the enzymes due to their fixed conformation and hence extend their applications in broader areas. A quick overview of the results exhibited greater activity for the enzymes immobilized on NPs as compared to enzyme immobilized on 2-D matrices. Based on these findings, this review was aimed to emphasize the recent development achieved for immobilizing β-galactosidase on NPs with their specific utilization in obtaining dairy products. These studies includes β-galactosidases from various sources that were immobilized on various NPs for hydrolyzing lactose in batch and continuous reactors, and for the production of GOS in biotechnology industries. NBEI of β-galactosidase offered profound stability for transporting substrate and product for enzymatic reactions, apart from cost effective advantage due to reusable nature of immobilized enzyme.

Keywords: β-galactosidase; immobilized nanobiocatalyst; dairy industries; nanoparticles; surface modification.

INTRODUCTION

β-galactosidase

Milk based regular dairy products such as cheese, yogurt and whey are extensively used by human beings due to their considerable nutritional value. However, significant proportion of human population is not able to consume these health-promoting dairy products because of their inability to metabolize lactose present in these food products [1,2]. This condition commonly known as 'lactose intolerance' results from the lower availability of an enzyme called *β*-galactosidase (lactase) in the gastrointestinal tract. β-galactosidase (EC 3.2.1.23) is one of the most frequently used enzymes in the food industry because it hydrolyses lactose to release glucose and galactose. The reduction in lactose content has nutritional and technological advantages, and it increases the sweetness of dairy products. It also catalyzes transgalactosylation reactions to produce galactosides and oligosaccharides. *β-galactosidase* is responsible for breaking down lactose (disaccharide) into galactose and glucose (monosaccharides) by hydrolyzing glycosidic linkage. Additionally, in lactose-intolerant people, the presence of excessive amounts of lactose in large intestine adversely affects digestive processes including water and calcium absorption thereby leading to tissue dehydration, calcium deficiency as well as bloating, flatulence, blanching, cramps and watery diarrhea [3,4]. Owing to its hygroscopic nature, lactose showed greater tendency to absorb flavors and odors. Hence, it is responsible for causing several defects in refrigerated foods like development of sandy/gritty texture and crystallization in dairy products. Another major application of this enzyme involves the transglycosylation of lactose for producing galacto-oligosaccharides (GOS). They represents the class of oligosaccharides which are non-digestible and possesses low- sweetness, caloric values and cariogenicity. They undergo selective fermentation by beneficial intestinal bacteria as they pass onto the colon [5,6]. Traceable amount of GOS is available in cow milk and honey, and various fruits and vegetables. Due to their health benefits, whey lactose can be used to manufacture GOS by enzymatic transgalactosylation of *β*-galactosidase [7,8]. The brief utility of β-galactosidases in various fields have been discussed below.

Health

The consumption of dairy products is limited to 75% of world's adult population which is affected by lactose intolerance. This problem is overcomed by the hydrolysis of lactose into galactose and glucose. This reaction also favored the formation of GOS which acts as indigestible and dietary fibres. They support intestinal bifid bacterial growth which are pre-requisite for efficient functioning of liver and intestine [9,10].

Food technology

Frozen milk products like whey spreads, condensed milk and ice-cream contains greater amount of lactose. It leads to the formation of gritty and sandy texture of products as a result of excessive lactose crystallization. Hence, β-galactosidase is used to decrease the lactose concentrations of these products to the desired values. This technology leads to the improvement of these dairy items by increasing their creaminess, digestibility and softness [11]. The temperature control is the main method to avoid crystallization for producing "dulce de leche" in biotechnology industries. Hence, feasibility of controlling lactose crystallization in "dulce de leche" can be improved by β-galactosidase immobilized on (surface modified) nanoparticles. Such methods can improve the stability of the enzyme against higher temperature ranges [12].

Environment

Since majority of the whey produced globally every year is disposed of as waste, there are several environmental and economic problems that have been found to be associated with the production of lactose as waste in cheese industry. This is due to the fact that uncertain solubility of lactose leads to its association with high COD and BOD. Hence, whey lactolysis by β-galactosidase leads to the production of sweet syrup that can be used for commercial purposes like baking, confectionery, soft drinks and dairy industries [13].

Industrial applications

The batch and continuous reactors of various configurations have been operated to obtain enzymatic hydrolysis of lactose from whey and milk. However, due to the high cost of β-galactosidase, only continuous reactors have been mainly utilized as the enzyme can be reused again [14]. Moreover, small dairy plants witnessed the utilization of inexpensive batch reactors which uses cheap and crude form of β-galactosidase for preparing lactose free dairy products [15]. Hence, immobilization of β-galactosidase had been suggested which aimed to offer numerous advantages over free form in the choice of batch and continuous reactors. It also favors the formation of product in a controlled manner, ease of enzyme removal from the reaction mixture, adaptability to various engineering designs and rapid termination of reactions [16].

Development of biosensors

β-galactosidase has been used for the construction of reliable and robust biosensors that exhibited high response rate, increased detection limit and enriched useful lifetime. Several alternative strategies to immobilize β-galactosidase for their utilization in biosensors have been developed in recent years in the quest of maximum utility by controlling the defects seen in the previous biosensors. Hence, there is an urgent need for developing a direct and low-cost method to determine the concentration of substrate with possible impact in various types of industry [17].

Application of nanoparticles for enzyme immobilization

NPs are particles between 1 to 100 nm with a surrounding interfacial layer. These characteristics impart them distinctive chemical and physical properties as a result of greater surface area to volume ratio gained by their nanoscale magnitude. The size of NPs reflects their optical properties which is responsible for imparting different colors as a result of absorption in the visible region. Moreover, their toughness and reactivity also depends on their structure, shape and unique size which is required for distinctive mechanical, optical and magnetic properties as compared to their bulk counterparts [18,19]. Hence, they are extensively used for waste water treatment, environmental applications, imaging and enzyme catalysis [20,21].

Immobilization of β-galactosidase on nanoparticles

Nanobiocatalyst has emerged as an innovative field that combines biotechnology and nanotechnology with an aim of improving enzyme stability in biotechnological applications. They have also been obtained in the form of nanosheets, nanofiber scaffolds, nanotubes and nanocomposites for acquiring nanomatrices with large surface area. Additionally, their surface can be easily regulated for ligand introduction in order to assemble β-galactosidase [22,23]. In this regard, effectiveness of ZnO-NPs as nanobiocatalyst was observed for *Lactobacillus plantarum* HF571129 β-galactosidase by adsorption and crosslinking technology. The temperature and pH-optima was broadened from 50-60 °C and pH 5-7.5, respectively, for the enzyme

retained on ZnO-NPs. Moreover, the immobilized enzyme exhibited an increase in K_m and V_{max} value from 6.64 to 10.22 mM and 147.5 to 192.4 µmol/min/mg, respectively, as compared to the free enzyme. It may be attributed to diffusion of high molecular weight substrate in a limited manner. Additionally, immobilized β-galactosidase (IβG) preserved 90% enzyme activity even after 1 month of its storage under refrigerated conditions as compared to 74% activity by the native enzyme under identical storage conditions. Moreover, IβG showed 88% activity even after its third repeated use. The half-life and activation energy of the prepared biocatalyst was reported as 130 min at 35 °C and 24.33 kcal/mol, respectively. The batch reactor and packed bed reactor showed the rate of lactose hydrolysis at 0.023 and 0.04 per min for the prepared immobilized nanobiocatalyst [24]. Table I depicts the utility of various NPs that were involved for β-galactosidase immobilization. Another study witnessed the synthesis and modification of nanofibers prepared by polystyrene and silica NPs via chemical oxidation and fabrication by one-pot sol-gel technology. β-galactosidase was immobilized on these nanocarriers for bioconversion of lactose to GOS to obtain excellent biocatalytic activity, stability and functionality. β-galactosidase immobilized on these nanobiocatysts exhibited excellent stability, functionality and biocatalyst activity. The results suggested that polystyrene nanofibers were able to adsorb β-galactosidase 8-fold higher than on aminated silica NPs, and showed exceptional catalytic ability by favoring transgalactosylation over hydrolysis. Hence, the formation of GOS was significantly improved from 19% to 28% apart from substantial reduction of undesirable products during lactose hydrolysis. β-galactosidase retained on the functionalized silica NPs were reported to yield 25% GOS under similar operating conditions [25]. The utility of ZnO-NPs in preparing nanobiocatalyst was extended for immobilizing β-galactosidase from *Aspergillus oryzae*. 85% immobilization yield was obtained on ZnO-NPs as compared to 60% on bulk ZnO. The pH-optima was same (i.e. pH 4.5) for both free and immobilized enzyme. However, the immobilized nanobiocatalyst exhibited 73% activity even at pH 7.0 against 32% and 47% activity by the free enzyme and the enzyme adsorbed on bulk ZnO at same operating conditions.

Temperature-optima was broadened from 50°C to 60°C for the immobilized nanobiocatalyst. The prepared nanobiocatalyst also showed improved activity against galactose mediated product inhibition. The commercial aspect of the nanobiocatalyst was analyzed by observing its stability for subsequent reuse. It revealed 75% activity even after its 7^{th} repeated use. The advantages associated with the immobilized biocatalyst was observed and compared with free enzyme and bulk ZnO in terms of producing lactose-free dairy products. Soluble, bulk ZnO and ZnO-NPs, β-galactosidase- favored 54%, 63% and 71% lactose hydrolysis respectively from milk in laboratory scale batch reactors after 9 h. This conversion was 61%, 68% and 81% for hydrolyzing lactose under similar operation conditions, respectively [26].

Table 1. Immobilization of β-galactosidase on different types of nanoparticles

Source of β-galactosidase	Nanoparticles used for immobilization	Efficiency	Biotechnological applications	Reference
Lactobacillus plantarum HF571129	ZnO	The rate of lactose hydrolysis was 0.023 and 0.04 per min. $t_{1/2}$ was 30.13 and 17.325 min for batch and packed bed, respectively.	Lactose hydrolysis	24
Aspergillus oryzae	Fe_3O_4-chitosan nanoparticles	Bioconversion determined by these nanocarriers showed 59 % yield.	GOS production	25
Aspergillus oryzae	ZnO	Maximum lactose hydrolysis obtained by ZnO-NPs-β galactosidase was 71% in 8 h.	Lactose hydrolysis from whey and milk	26
Aspergillus oryzae	Graphene-iron oxide nanocomposites	Genotoxicity assay revealed that this system exhibited negligible toxicity to pBR322 DNA plasmid and human lymphocytes.	Construction of biosensors, production of lactose-free dairy products and large scale enzyme catalyzed applications	27
Aspergillus oryzae	Chitosan	Hydrolysis was over 80% even after 50 cycles of reuse.	Lactose hydrolysis	28
Agaricus bisporus	Polyaniline nanofiber, DEAE cellulose fiber, CM cellulose fiber and polystyrene nanofiber	PANI-, PAMP-, and DEAE-lactase exhibited greater percentage of lactose conversion (100%, 47% and 12%, respectively).	Lactose hydrolysis	29
Aspergillus oryzae	Core-shell silica nanoparticles	After nine cycles of hydrolytic reaction, the encapsulated β-galactosidase retained 94% of its initial activity.	Lactose hydrolysis	30

Another study witnessed 94% immobilization efficiency for *Aspergillus oryzae* β-galactosidase immobilized on graphene-iron oxide nanocomposites. Although the pH-optima and temperature-optima did not change for the developed nanobiocatalyst, but it exhibited significant enhancement in its pH stability at both acidic and basic pH, and showed improved tolerance against higher temperature ranges. Reusability experiments confirmed 83% activity of the immobilized enzyme even after its eighth reuse. Moreover, the assessment of immobilized nanobiocatalyst by genotoxic measure demonstrates its negligible toxicity for human lymphocytes and pBR322 DNA plasmid. The developed nanobiocatalyst can be extensively used to construct biosensors and produce lactose-free products for lactose intolerant patients due to its non-toxicity, excellent reusability and stability, and easy production [27]. Additionally, Klein and co-workers developed chitosan based nanobiocatalyst for immobilizing β-galactosidase to improve the enzyme stability and load. It was observed that chitosan NPs

retained 204.2 mg protein/g of the developed nanobiocatalyst and was able to hydrolyze 76% lactose even after 50 runs of reuse at 37 °C [28].

Nanofibers of different polymeric composition have been tested by several researchers for immobilizing β-galactosidase for improving its reusability and stability. PANI nanofiber and magnetic- carboxymethyl cellulose fiber (CM), DEAE cellulose fiber and polyaniline nanofiber (PAMP), and polystyrene nanofiber (PSNF) were exploited to immobilize lactase. K_{cat} of free enzyme was observed as 4.0 while for magnetic DEAE-cellulose, PAMP and PANI were analyzed as 0.042, 0.59 and 2.05 mM/min/mg protein, respectively. The reusability was excellent for the polyaniline based biocatalyst. It exhibited minor decline in enzyme activity even after long storage at room temperature. The efficiency of lactose conversion was 100% by the enzyme immobilized on PANI while for DEAE and PAMP- bound lactase, it exhibited 12% and 47%, respectively of lactose conversion after 1 hour. The residual activities after the 10^{th} run for the prepared nanobiocatalyst was 96%, 97% and 98% for PAMP-, DEAE- and PANI-lactase, respectively [29]. PAMP- and PANI-lactase were quite stable with over 90% activity even after 3 months of rigorous shaking conditions. PANI-, PAMP- and DEAE-lactase showed greater lactose conversion (100%, 47% and 12%) after 1 h. Immobilized lactases were easily recovered and recycled after the reaction. Their residual activities after recycling 10 times were 98%, 96% and 97%, respectively. Hence, such immobilized systems could be used in lactose analysis and biosensors to detect the lactose concentration. Moreover, Wu et al. developed novel method for immobilizing *Aspergillus oryzae* β-galactosidase by encapsulation in core-shell silica NPs. The developed nanobiocatalyst exhibited excellent stability against higher pH and temperature ranges, and significant stability upon storage for longer periods. The reusability study showed 94% activity even after 9 runs of hydrolysis, thereby indicating its excellent reusability after encapsulation [30].

Immobilization of β-galactosidase on surface modified nanoparticles

The advancement in technology extends the utilization of NPs by functionalization them with several functional groups like −COOH, $-NH_2$, −CHO, −OH, etc. This process brings significant improvement in the performance of enzyme catalysis in terms of specificity and sensitivity by promoting favorable accessibility, orientation and bioactivity of enzymes on the functionalized NPs [31-34]. Moreover, surface modification of NPs for the immobilization of β-galactosidase improves their monodispersity, stability and biocompatibility [Fig 1]. This section summarizes the surface modification strategies that have been developed till date for the immobilization of β-galactosidases on several NPs for improving their performance in various biotechnological applications [Table 2].

In this regard, a plant based lectin, concanavalin A (Con A) was earlier used to modify the surface of Al_2O_3-NPs and ZnO-NPs by Ansari and co-workers for immobilizing lactase. The method developed herein was cheap and simple, and exhibited high enzyme yield. In case of enzyme immobilized on Con A modified Al_2O_3-NPs, 6% loss in weight was observed by TGA at 600 ^oC while the thermal decomposition was observed at 350 ^oC by DTA. AFM monitored the greater surface area of the prepared nanobiocatalyst while the lactase binding on the matrix was observed by FTIR spectroscopy at 3220.61 cm^{-1} and 3447.27 cm^{-1}. Stability studies suggested that pH and temperature tolerance was improved significantly for the obtained nanobiocatalyst. The competitive inhibition offered by galactose was less for immobilized nanobiocatalyst and retained 85% enzyme activity after the 6^{th} run [35]. However, in case of enzyme immobilized on Con A modified ZnO-NPs, TGA exhibited loss in weight as 4% at 600 ^oC and thermal decomposition at 530 ^oC, and immobilization yield of 89%. The prepared nanobiocatalyst showed insignificant change in tail length of comet

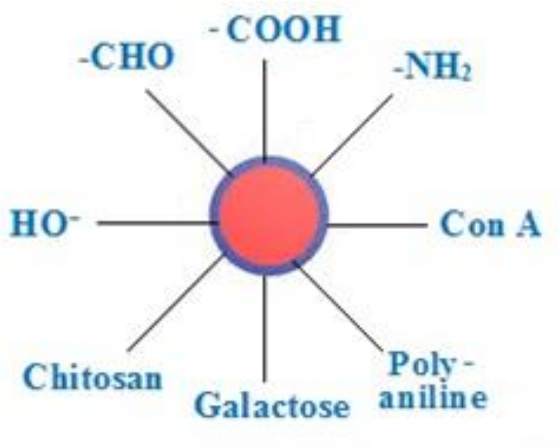

Figure 1. Ligands used to modify the surface of NPs for the immobilization of β-galactosidase for dairy industry application

When treated with lymphocytes, and negligible change in pUC19 plasmid band intensity. Km was increased 2.5 fold while Vmax decreased slightly for the immobilized enzyme. Reusability experiments confirmed 86% activity of the immobilized biocatalyst after the 6th run and showed pronounced stability against denaturants of physical and chemical nature [36].

Table 2. Immobilization of β-galactosidase on the ligand modified nanoparticles

Source of β-galactosidase	NPs	Ligand	Efficiency	Application	Reference
Kluyveromyces lactis	Al_2O_3	Concan avalin A	Comet assay exhibited negligible change in tail length of comet after treating the lymphocytes with nanosupport.	Biomedical/ biotechnological industries	35
Aspergillus oryzae	ZnO	Concan avalin A	No significant change was observed in the band intensity of pUC19 plasmid upon nanosupport treatment.	Biomedical/biotechnological/biosensors	36
Aspergillus oryzae	Magnetic nanoparticles	Amine	Ability to hydrolyze substrates during multiple cycles of use.	Continuous production of lactose hydrolyzed products	37
Kluyveromyces lactis	Multi-walled carbon nanotubes	Glutaral dehyde	Functionalized MWCNTs retained greater biocatalytic activity at higher concentration of galactose.	Lactose biosensors	39
Aspergillus oryzae	Nanodiamonds	Glutaral dehyde	Modified NDs retained 7420 U/gm enzyme and exhibited improved lactolysis in batch processes even after 10 h.	Hydrolysis of lactose from solution in batch processes	40

Aspergillus oryzae	AgNPs	Nitric acid/sulfuric acid	Immobilized enzyme yielded greater amount of galacto-oligosaccharides at higher temperatures from 0.1 mol/L lactose solution at pH 4.5	GOS production	41
Aspergillus oryzae	AgNPs	Glutaraldehyde	The remarkable bioconversion rates of lactose promoting its use in producing lactose-free dairy products.	Production of lactose free dairy products in batch reactors	42
Aspergillus oryzae	Graphene	Ethanol	Maximum GOS content registered an increase in lactose conversion.	GOS production	43
Aspergillus oryzae	Agarose nanoparticles	Galactose	The developed nanosystem hydrolyzed greater amount of lactose at higher temperatures, thereby suggesting its application in large scale dairy industries.	Production of lactose-free dairy products at large scale	44
Kluyveromyces lactis	Silicon dioxide	Glutaraldehyde	Maximum lactose hydrolysis by immobilized enzyme was achieved after 8 h.	Bioremediation for cleaning and converting whey into value added products	45
Aspergillus oryzae	Silica nanoparticles	Glutaraldehyde	The rate of lactose and whey hydrolysis by immobilized enzyme was 1.5 and 2.5 times higher as compared to the free enzyme, respectively	Lactose and whey hydrolysis	46
Aspergillus oryzae	Fe_3O_4-NPs	Chitosan	Maximum GOS yield was observed as 50.5% from 36% w/v lactose on a dry weight basis.	GOS production	47
Aspergillus oryzae	Cobalt/ Multi-walled carbon nanotubes	Polyaniline	The bound enzyme retained 92% activity after its 10^{th} repeated, and negligible change in the band intensity of pBR322 plasmid.	Biosensors	48

Additionally, magnetic NPs were modified by amino group for lactase immobilization to achieve higher immobilization efficiency of 58 µg/mg on the modified nanomatrix. Immobilized β-galactosidase exhibited excellent reusability after several repeated uses, and facilitated the continuous conversion of lactose in dairy industries in much more cheaper and convenient way [37]. Owing to the excellent properties of glutaraldehyde that were exploited for stabilizing immobilized enzyme systems [38], a highly effective *Kluyveromyces lactis*

β-galactosidase nanobiocatalyst was designed from multi-walled carbon nanotubes (MWCNTs) pretreated by glutaraldehyde. XRD and UV-vis spectroscopy reveled the dispersive nature of MWCNTs in aqueous solution. The stability of immobilized enzyme was increased against various pH and temperature ranges. Nevertheless, it exhibited greater biocatalytic activity against the competitive inhibition mediated by galactose. It offered excellent reusability for lactose conversion in obtaining dairy products at commercial level [39]. The utility of glutaraldehyde in functionalizing the nanomatrices and imparting profound stability to β-galactosidase from *Aspergillus oryzae* was extended for diamond NPs. Enzyme activity of this immobilized biocatalyst was 7420 U/gm. The temperature and pH-optima was similar for soluble and immobilized enzyme (50 °C and pH 4.5), but significant enhancement was noticed for the immobilized enzyme at the lower and higher ranges under the effect of these chemical denaturants. The obtained nanobiocatalyst was more stable even after two months and at greater galactose concentration. Reusability studies revealed its greater potential as observed by its repeated uses in order to exploit for hydrolyzing lactose batch processes for 10 h [40].

Another simple and inexpensive method was achieved for modifying AgNPs via carboxylation for strong binding of *Aspergillus oryzae* β-galactosidase. The stability of the immobilized nanobiocatalyst was significantly improved in buffers of various pH values. Temperature-optima exhibited broadening in peaks from 50 ºC to 60 ºC for IβG. Moreover, kinetic parameters suggested slight decrease in Vmax and 2.5 fold increase in Km value for immobilized enzyme. At 4% galactose concentration, immobilized enzyme showed 70% activity and promoted greater amount of GOS production at higher temperatures from 100 mM lactose solution [41]. This study was extended for obtaining high yield enzyme immobilization by modifying the surface of silver NPs with glutaraldehyde. Lactase bound to glutaraldehyde functionalized AgNPs showed profound stability against alkaline and acidic pH, and improved tolerance against temperature variations. A 3-fold increase in K_m was observed for the nanobiocatalyst while V_{max} value was decreased to 0.495 mM/min. The prepared nanomatrix imparts greater resistance to the enzyme in terms of competitive inhibition offered by the generated products especially galactose. The biocatalytic activity of the immobilized enzyme was retained even after several repeated rums. The incredible conversion of milk lactose from the immobilized biocatalyst in batch reactors further disclose its excellent catalytic efficiency in producing lactose-free dairy products on large commercial scale [42]. Ethanol was later on used by researchers for modifying the surface of graphene to immobilize β-galactosidase. This biocatalyst was investigated for producing GOS in higher yields. The chemical interactions involved in binding lactase to the designed nanobiocatalyst was confirmed by FTIR and bioinformatics analysis. The stability of immobilized enzyme was considerably improved against mild denaturing agents. A noticeable change was observed for K_m value of immobilized enzyme while insignificant change in V_{max} value was obtained for the enzyme upon immobilization. GOS yield was obtained maximally for the immobilized biocatalyst at a specified time and temperature [43]. Galactose was used to impart stability to β-galactosidase by modifying the surface of agarose NPs. The researchers obtained 91% activity for the enzyme upon immobilization and greater stability of the immobilized nanobiocatalyst against various pH and temperature ranges, and at higher galactose concentration. Moreover, 81% activity was obtained after its 7^{th} reuse for the nanobiocatalyst. Significant amount of lactose was converted by the immobilized nanobiocatalyst at higher temperatures which favored it use in large scale production of lactose-free products in dairy industries [44].

Verma and co-workers have developed nanobiocatalyst for lactase from *Kluyveromyces lactis* by modifying the surface of silicon dioxide NPs via glutaraldehyde and the interaction involved in enzyme binding was observed by FTIR and SEM. Immobilized biocatalyst showed excellent activity at both higher and lower

pH ranges. The broadening in peak for temperature-optima was observed from 35-40 °C as a result of multipoint covalent attachment of the enzyme to the nanomatrix. Kinetic constants were changed significantly for the immobilized nanobiocatalyst. It exhibited improved performance under high thermal denaturation conditions and retained 50% enzyme activity even after eleventh cycle reuse [45]. The utility of this nanobiocatalyst was investigated for immobilizing β-galactosidase from other source to convert lactose from whey and milk [46]. The developed nanomatrix showed 94% immobilization efficiency. The immobilized nanobiocatalyst exhibited pronounced activity against different pH buffers, greater temperature ranges and at higher concentration of galactose. An increased catalytic efficiency and a lower value of K_m specified higher affinity for affinity and reactivity as a result of attachment of enzyme on the developed nanobiocatalyst. Moreover, it showed greater activity even after its 14^{th} reuse and was able to promote 50% lactose bioconversion form whey after 6 h. Lactose hydrolysis achieved was 1.5 and 2.5 fold for soluble and immobilized nanobiocatalyst, respectively. Hence, such type of immobilized β-galactosidase system may explored commercially for effective bioremediation of dairy waste and constructing biosensors for environmental technology, conversion of whey into value-added products and analytical tools for food. Several workers have exploited chitosan to coat Fe_3O_4-NPs for immobilizing β-galactosidase to produce GOS from lactose. The developed nanobiocatalyst had shown the improvement of enzyme at broader pH and temperature ranges, apart from exhibiting greater thermal stability. On the basis of dry weight, 50% GOS was obtained from 36% w/v lactose [47]. Polyaniline was used by researchers to modify cobalt-multiwalled carbon nanotubes for immobilizing *Aspergillus oryzae* β-galactosidase by glutaraldehyde pretreatment. The functional groups involved in binding of enzyme on the prepared nanobiocatalyst was analyzed by SEM and FTIR techniques. Immobilized nanobiocatalyst showed remarkable stability against higher and lower, pH and temperature ranges. K_i was increased ten times for glutaraldehyde modified immobilized nanobiocatalyst which showed its improved resistance against the competitive inhibition mediated by galactose. Moreover, the immobilized nanobiocatalyst preserved 92% activity even after its 10^{th} successive run. The researchers have demonstrated the utility of various other nanobiocatalysts for constructing cheap and convenient biosensor for detecting lactose concentration and for producing GOS in large scale [48-50].

The different immobilized systems have their own pros and cons as can be seen by their yield, recyclability, productivity, enzyme stability against various physical and chemical denaturants, and adsorption capability. These immobilized β-galactosidase systems promised greater potential for obtaining the desired products in highly significant yields and offered sustainable green waste management, but with their own limitations. Hence, the translation of these bench-scale technologies can be achieved in major commercial practices due to their bioengineering performance and potential for scaling-up and reutilization.

Future prospects

β-galactosidase immobilized on novel surface modified NPs can be employed for obtaining lactose-free dairy products and GOS without microbial contamination hazard which may crept as a result of prolonged operation times at ambient temperatures. Such nanoparticle based immobilized β-galactosidase systems when operated at high temperatures can prevent clogging of ultrafiltration membranes from milk proteins by using whey permeate as deprotonated substrates. In view of the novel technology implemented for synthesizing and functionalizing NPs, other industrially important enzymes can be stabilized against chemical and physical denaturants as a result of immobilization, which may then be exploited for the construction of enzyme-based analytical devices in various biomedical applications.

CONCLUSION

Selection of NPs to immobilize enzyme for preparing highly effective nanobiocatalyst is influenced by its recyclability, productivity, enzyme stability and adsorption capability. Since the high density of NPs can separate them easily by centrifugation/sedimentation, their biocatalyst recovery can be obtained easily in the downstream process. Hence, the translation of these bench-scale technologies can be achieved due to their bioengineering performance and potential for scaling-up and reutilization in major commercial practices. These immobilized β-galactosidase systems promised greater potential for obtaining the desired products in highly significant yields and offered sustainable green waste management. The studies on NBEI of β-galactosidase displayed excellent results and reaction profiles thereby supporting their utility in transgalactosylation and lactose hydrolysis, thereby suggesting their utility in bioprocessing applications.

Conflicts of Interest: The authors declare that they have no conflict of interest.

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