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# Beta-galactosidase Structure, Sources, Function and Application in Food Industry: Review

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**Abstract**

This review mainly focuses on the function, characteristics, and industrial use of β - galactosidase in lactose intolerance. Galactosidase is a food-based enzyme that hydrolyzes lactose into glucose and galactose. It is most frequently utilized in the dairy manufacturing business. The primary goal of this catalyst is to enhance non-fermented dairy products' technological, textural, and scientific qualities while also creating new and innovative products with hydrolyzed lactose that can be suitable for people who cannot tolerate lactose. Galactosidase, a converting enzyme belonging to the hydrolase family and generated from the group of saccharides They are widely dispersed throughout the various organic life systems. Due to lactose's restricted solubility range, enzymatic hydrolysis of lactose is also recommended in food-based technologies. In fermented dairy products including ice cream, butter, cheese curd, and yogurt, the amount of lactose was discovered to be particularly high. This can cause severe lactose crystallization, which results in the passage of the products through a rough, abrasive surface. Dairy products' enhanced flexibility and overall richness are also benefits of lactose hydrolysis. These goods are particularly palatable. Galactosidase enzyme use prior to the condensing operation can also achieve this goal by lowering the lactose concentration to a level where lactose is no longer an issue. Utilization of β-galactosidase in industry. Due to the beneficial and beneficial impact on the intestinal bacterial microbiota, the galactosidase enzyme has a variety of industrial applications.

**Keyword**: β-galactosidases, Function, Pharmaceutical Industry, Purification

# إنزيم البيتا كلاكتوسايديز ،التركيب، الوظيفة والتطبيق في الصناعات الغذائية

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**الخلاصة**

تركز هذه المراجعة بشكل أساسي على التركيب والوظيفة والتطبيق في الصناعات الغذائية لإنزيم -β galactosidaseفي ظاهرةعدم تحمل سكراللاكتوز. إنزيم البيتاكلاكتوسايديز و هو إنزيم غذائي يلعب دورا حيويا في عملية الهضم البايلوجية حيث يقوم بتحليل السكر الثنائي اللاكتوز الى جزيئات أبسط هما سكر الكلكوز والكلاكتوز. يتم استخدامه بشكل شائع في تصنيع الألبان. الهدف الأساسي لاستخدام هذا الانزيم هو تعزيز الصفات التكنولوجية والتركيبية لمنتجات الألبان غير المخمرة مع إنتاج منتجات جديدة ومبتكرة تحتوي على اللاكتوز المتحلل والتي يمكن أن تكون مناسبة للأشخاص الذين لا يستطيعون تحمل سكر اللاكتوز. كلاكتوسايديز ، هو من إنزيمات التحلل المائي والذي ينتج من قبل العديد من الكائنات الحية بما في ذلك البكتريا وهو منتشر على نطاق واسع في جميع أنحاء أنظمة الحياة العضوية المختلفة. نظرًا لمحدودية ذوبان اللاكتوز يوصى أيضًا بالتحلل المائي الأنزيمي للاكتوز في التقنيات الغذائية، وفي منتجات الألبان المخمرة بما في ذلك الآيس كريم والزبدة والجبن والزبادي، تم اكتشاف أن كمية اللاكتوز المرتفعة يمكن أن تسبب تبلور اللاكتوز ، مما يؤدي إلى ظهور المنتجات بشكل محبب. يعد الاتساق في النسجة والقوام لمنتجات الألبان من فوائد تحلل اللاكتوز المائي مما يجعل هذه المنتجات مستساغة بشكل خاص. يمكن أيضًا أن يحقق استخدام إنزيم الكالاكتوسايديز قبل عملية التكثيف هذا الهدف عن طريق خفض تركيز اللاكتوز الذي يمكن ان يسبب طعما غير مرغوب فيه. لذلك فإن إنزيم البيتا كلاكتوسايديز يلعب دورا مهما في تحسين نوعية وكفاءة المنتجات الغذائية مما يجعله أحدالادوات الحيوية في الصناعات الغذائية الصناعية.

**الكلمات المفتاحية:** β-galactosidase ، الوظيفة ،الاسخلاص والتنقية ، الصناعات الصيدلانية والدوائية

## 1.Introduction

Enzymes are biological catalysts that accelerate chemical reactions in living organisms, as they are extracted from cells and used to catalyze a wide range of processes of commercial interest [1]. β-galactosidases are involved in the synthesis and breakdown of carbohydrates [2] Because of their crucial function whenever glycosides are hydrolyzed, the formation of oligosaccharides, polysaccharides, and glycoconjugates, as well as other advantages like simple, inexpensive substrates in one-step reactions, glycosidases (EC 3.2.1) have received greater attention in recent years[3]. Galactosidase (EC 3.2.1.23), often known as lactase, is one of the most frequent water-soluble enzymes found higher echelons of plants, animals, and microorganisms. Today, β-galactosidases from a number of organisms, including bacteria, Plants, animals, yeast, fungi, are purified and identified[4].

Active β-galactosidases throughout a wide pH range, according on extraction sources. For instance, fungal enzymes are active in the pH range of 2.5 to 5.4, whereas In the pH range, yeast and bacterial enzymes are active range of 6.0 to 7.0[5]. Lactose intolerance is the term used to describe the lack of or insufficient production of lactase in humans[6]. Lactose-intolerant people can eat fermented items made from dairy that have minimal to no lactose [7] β-Galactosidases can be used to break down lactose to milk alleviate the issues likewise discomforts brought on thanks to lactose intolerance. These Enzymes are a isolated from several sources, such as bacteria and fungus [8] . The yearly β-galactosidase production is expected to be 5,750,000 tons. The transgalactosylation activity of β-galactosidase has received a lot of interest lately. In addition to enhancing the impact Impact human health of intestinal flora, GOS that are formed by β-galactosidase's transglycosylation activity, also stimulate the proliferation of beneficial intestinal bacteria [9]. Probiotics improve human health by reducing the amount of harmful bacteria, strengthening the function of the immune system, and preventing cancer [10] .

The reason β-galactosidases are used so often in the processing It is essential that β-galactosidases be able to produce various glycosides or oligosaccharides besides galactooligosaccharides (GOS)As a result, their substrate specificity is wide ranging.
[11] .β-Galactosidases play an important role in the production of ABO blood group determinants and cancer related carbohydrate antigens[12]. Additionally, β -Galactosidases are thought to be a useful tool for glycosylating antibiotics and natural compounds [12]. A biosensor is created using certain -galactosidases to precisely measure the lactose in dairy products[13]. The β -galactosidase is a fascinating reporter for identifying a recombinant protein due to its capacity to release chromogenic or fluorogenic groups [12].

## 2-β -GALACTOSIDASE SOURCES

Although β- Galactosidase, an enzyme, can be extracted from a variety of range of organisms, including animals, plants, fungus, yeast, plants, and organism, each organism has its own specific properties [14]. The use is not of β-galactosidases limited to the creation of products devoid of lactose. They could also utilized in the synthesis of prebiotics and whey [4]. owing to their great effectiveness and resilience to temperature and pH, Lactase derived from yeast and fungal sources are more frequently utilized in industry.

**Table 1-** Properties of yeast and bacterial β –galactosidases

| **Microoganisms** | **Location of enzyme** | **Temperature (°C)** | **pH** | **References** |
|---|---|---|---|---|
| *Lactobacillus fermentum* | Intracellular | (35°C) | (7.0) | Mahadevaiah *eta l*( 2020) |
| *Lactiplantibacillus plantarum* GV54 | Intracellular | (37°C) | (7.0) | Vasudha, M., & Gayathri, D. (2023) |
| *Bacillus aryabhattai* GEL-09 | Intracellular | (45 °C) | (6.0) | Luan, S., & Duan, X. (2022). |
| *Aspergillus oryzae* | extracellular | (40°C) | (7.5) | Sass, A. C., & Jördening, H. J. (2020) |
| Saccharomyces fragilis IZ 275 | Intracellular | (37°C) | (6.0) | Setti *etal*.,(2022) |
| Kluyveromyces marxianus SLDY – 005 | Intracellular | (40°C) | (6.0) | Afolabi, F. T., Adewale, O. P., & Adeyemo, S. M. (2022) |

*2-1 extracted β-galactosidases from bacterial sources*

β-galactosidase is an enzyme commonly found in bacterial sources. It plays a crucial role in breaking down lactose, a sugar found in milk and other dairy products, into glucose and galactose. This enzyme is widely used in various biotechnological applications, including the dairy industry, food processing, and molecular biology research[21].The process of extracting β-galactosidases from bacterial sources typically involves the followi steps:

**Bacterial Culture**: Bacterial sources that naturally produce β-galactosidase, such as Escherichia coli (E. coli) or Lactobacillus spp., are selected. These bacteria are then cultured in a suitable growth medium under controlled conditions to allow the production of the enzyme.

**Harvesting:** Once the bacterial culture reaches a specific growth phase, it is harvested. The cells are separated from the growth medium, usually through centrifugation, filtration, or other cell separation techniques.

**Cell Disruption:** To release the β-galactosidase enzyme from the bacterial cells, a cell disruption step is employed. This process involves breaking open the cell walls and membranes to release the intracellular enzymes. Various methods like mechanical disruption, sonication, or enzymatic lysis can be used.

**Enzyme Extraction:** The cell lysate, containing the β-galactosidase, is collected. The enzyme is then extracted from the cell debris and other cellular components using separation techniques such as centrifugation, filtration, or chromatography.

**Purification:** After extraction, the β-galactosidase is further purified to obtain a highly concentrated and pure enzyme. Purification methods may include techniques like chromatography, electrophoresis, or salting-out.

**Enzyme Characterization:** The purified enzyme is characterized to determine its activity, stability, substrate specificity, and other relevant properties. This step ensures that the extracted enzyme meets the desired quality standards for its intended application.

Once the β-galactosidase is successfully extracted and purified, it can be used in various applications. For example, in the dairy industry, it is used to convert lactose in milk into its constituent sugars, making it suitable for lactose-intolerant individuals and to produce lactose-free dairy products. In molecular biology research, β-galactosidase is widely used as a reporter enzyme to assess gene expression in various genetic studies.

### 2-2 β-Galactosidases from fungal and yeast sources

β-Galactosidases can also be found in fungal and yeast sources, and the process of extracting these enzymes from these organisms is similar to that of bacterial sources[22]. Here's a general outline of how β-galactosidases are extracted from fungal and yeast sources:

**Fungal or Yeast Culture:** Fungal strains (e.g., *Aspergillus*, *Penicillium*) or yeast strains (e.g., *Saccharomyces cerevisiae*) that produce β-galactosidase are selected and grown in a suitable culture medium[23]. The growth conditions are optimized to encourage enzyme production .

**Harvesting:** When the fungal or yeast culture reaches the appropriate growth phase, the cells are harvested. Similar to bacterial sources, this can be achieved through centrifugation, filtration, or other cell separation methods.

**Cell Disruption:** To release the β-galactosidase from the fungal or yeast cells, cell disruption methods are used. These methods break open the cell walls and membranes, allowing the enzyme to be extracted. Common techniques include mechanical disruption, sonication, or enzymatic lysis.

**Enzyme Extraction:** The cell lysate containing the β-galactosidase is collected after cell disruption. The enzyme is then extracted from the mixture using separation techniques like centrifugation, filtration, or chromatography.

**Purification:** Similar to the extraction process from bacterial sources, the extracted enzyme from fungal or yeast sources undergoes further purification to obtain a highly concentrated and pure form of β-galactosidase. Purification methods may include chromatography, electrophoresis, or salting-out.

**Enzyme Characterization:** Once purified, the β-galactosidase is characterized to determine its activity, stability, substrate specificity, and other relevant properties. This step ensures that the enzyme meets the desired quality standards for its intended applications.

**Application:** The purified β-galactosidase from fungal or yeast sources can be used in various industrial processes, such as in the dairy industry for lactose hydrolysis to produce lactose-free products, or in food processing to improve the digestibility and nutritional value of certain foods.The choice of fungal or yeast source may depend on factors such as enzyme yield, production cost, and the specific application of the β-galactosidase. Different strains and species may have varying enzyme properties, which can be advantageous for specific uses. As with any biotechnological process, optimizing the extraction and purification steps is essential to obtain high yields of active and pure β-galactosidase [23].

## 3- Structure of Bacterial B-Galactosidases

Bacterial β-galactosidases are enzymes that play a crucial role in breaking down lactose into glucose and galactose. They are encoded by the lacZ gene in bacteria and are commonly found in species such as Escherichia coli [24] The structure of bacterial β-galactosidases is well-studied and has been determined through X-ray crystallography and other structural biology techniques. Here's an overview of the general structure of bacterial β-galactosidases,Quaternary Structure: Bacterial β-galactosidases typically exist as homo-tetramers, meaning they consist of four identical subunits that come together to form a functional enzyme. Each subunit is composed of a specific arrangement of amino acids.Catalytic Domain: The β-galactosidase enzyme has a catalytic domain that is responsible for the enzymatic activity[25] . This domain contains the active site where lactose and other substrates bind and undergo the enzymatic reaction.Substrate Binding Site: The active site in the catalytic domain contains specific amino acid residues that interact with the lactose substrate. These residues facilitate the hydrolysis of lactose into glucose and galactose.Lactose-Binding Site: Apart from the catalytic site, β-galactosidase may also have a lactose-binding site, which helps in the proper positioning of the lactose substrate for catalysis.N-terminal Domain: The enzyme often possesses an N-terminal domain that is involved in the tetramerization of the subunits, forming the functional homo-tetramer.

C-terminal Domain: The C-terminal domain may have regulatory functions and is involved in the overall stability of the enzyme.Metal Ion Binding Site: Some bacterial β-galactosidases require metal ions, such as zinc ormagnesium, for proper enzymatic activity. These metal ions may be coordinated by specific amino acid residues within the enzyme's structureThe overall 3D structure of bacterial β-galactosidases is quite complex, and the enzyme's active site has a pocket-like structure where the catalysis takes place. The quaternary structure of the tetramer provides stability to the enzyme and contributes to its functionality.It's important to note that the structure of β-galactosidases can vary slightly among different bacterial species, but the general features and functions are conserved across most bacterial sources. The knowledge of the enzyme's structure is critical for understanding its catalytic mechanism and for engineering modifications to enhance its industrial applications, such as in lactose hydrolysis for lactose-free dairy products. inducer of the lac operon [26].

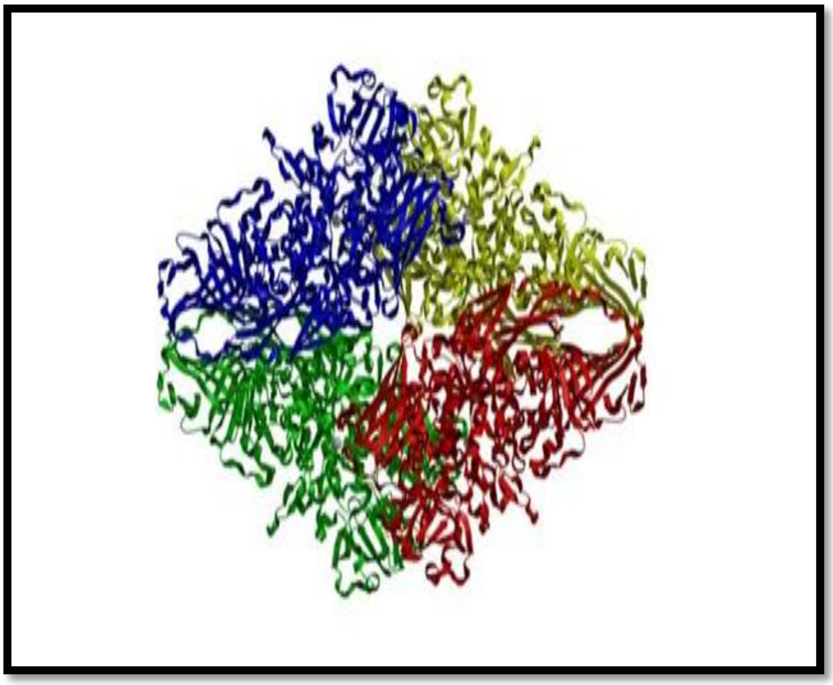

**Figure -1** depicts the general structure of the β–galactosidase [21]

## 4- Reaction Mechanism of β-galactosidase

The reaction mechanism of β-galactosidase involves the hydrolysis of the disaccharide lactose into its constituent monosaccharides, glucose, and galactose [27]. This reaction takes place in the active site of the enzyme and involves several steps. Here's a simplified overview of the reaction mechanism:Substrate Binding: The lactose substrate binds to the active site of the β-galactosidase enzyme. The enzyme has specific amino acid residues that interact with the substrate, positioning it in the correct orientation for the reaction to occur.Nucleophilic Attack: One water molecule acts as a nucleophile and attacks the glycosidic bond between glucose and galactose in the lactose molecule. This nucleophilic attack results in the formation of a covalent intermediate between the enzyme and the glucose moiety of lactose.Formation of a Glycosyl-Enzyme Intermediate: The glycosidic bond is temporarily broken, and the glucose moiety becomes covalently attached to the enzyme, forming a glycosyl-enzyme intermediate.Galactosyl Transfer: The galactose moiety is released from the enzyme's active site as galactose.Hydrolysis of the Intermediate: Another water molecule enters the active site and hydrolyzes the glycosyl-enzyme intermediate, breaking the covalent bond between the glucose and the enzyme. This hydrolysis releases the glucose as a free monosaccharide.Regeneration of the Enzyme: After the reaction, the enzyme is regenerated and is ready to bind to another lactose molecule and repeat the process.Overall, the reaction mechanism involves two hydrolysis steps: one between the enzyme and glucose, forming a glycosyl-enzyme intermediate, and the other between the enzyme and the glycosyl-enzyme intermediate, releasing glucose as a product.It's important to note that β-galactosidases are glycoside hydrolases, and their mechanism of action is common among other enzymes involved in breaking glycosidic bonds in various carbohydrates. The ability of β-galactosidase to break down lactose dairy industry to produce lactose-free milk and other dairy products makes it a critical enzyme in lactose metabolism and is widely used in the dairy industry to produce Lactose-free milk and other dairy products [17].

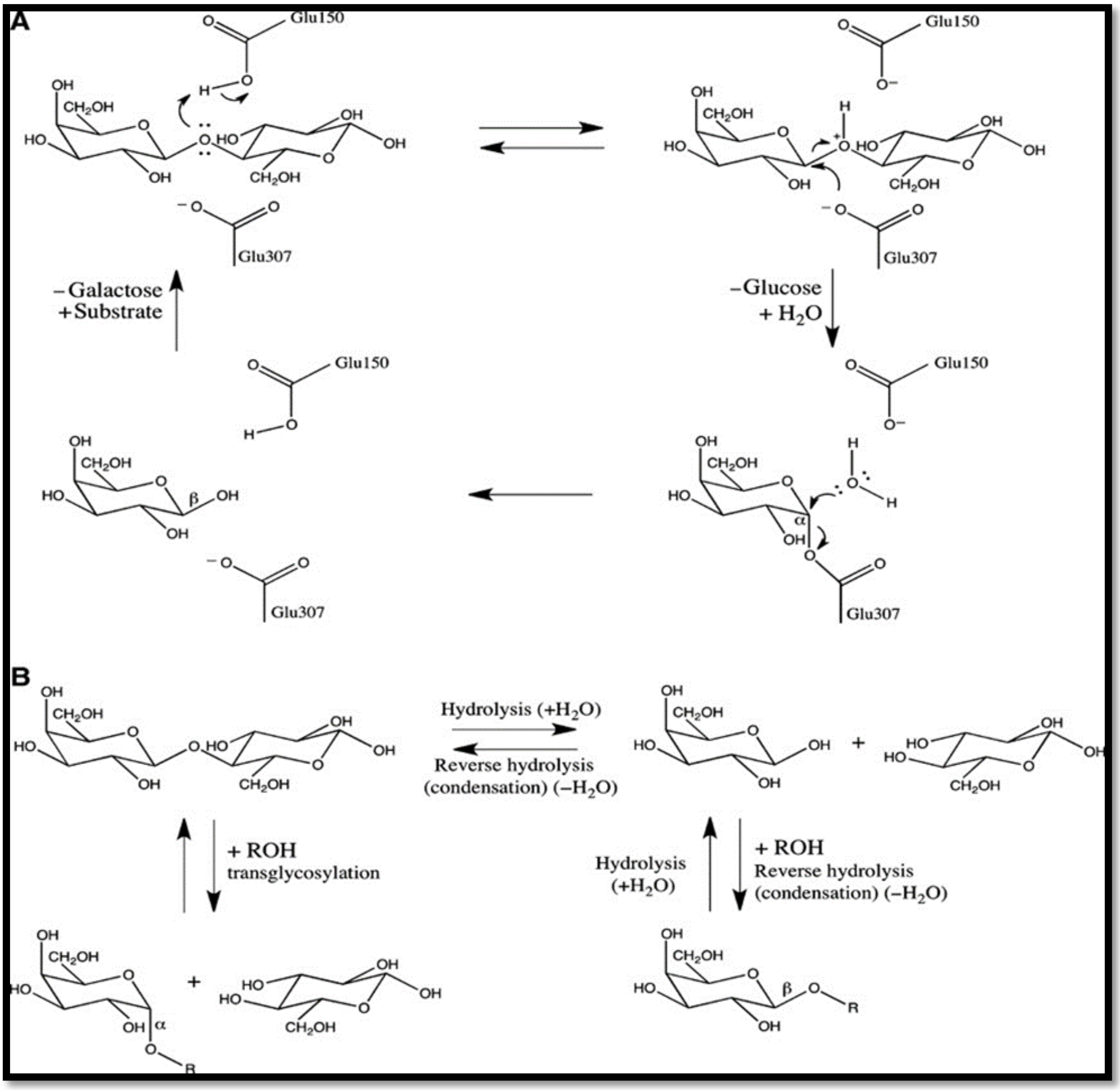


**Figure -2** Reaction Mechanism of β- galactosidase [28]

## 5- β-galactosidase Extraction by Different Methods

Extracting β -galactosidase can be achieved using different methods, depending on the source of the enzyme and its intended use. Here are some common methods for β-galactosidase extraction such us Cell Lysis and Homogenization, Permeabilization, Protein Precipitation, Affinity Chromatography, Ion Exchange Chromatography, Gel Filtration Chromatography (Size Exclusion Chromatography), Ultrafiltration.The choice of extraction method depends on factors such as the source of the enzyme, the scale of extraction, purity requirements, and intended applications. Additionally, different organisms and cell types may require specific adaptations of these general methods to optimize β-galactosidase extraction [4].

## 6- Applications of β-Galactosidase

*6-1 Dairy Industry*

One of the primary applications of β-galactosidase is in the dairy industry. Lactase is used to break down lactose in milk and dairy products into glucose and galactose. This process is particularly important for lactose-intolerant individuals who lack sufficient lactase in their digestive systems. Lactose-free dairy products are produced using β-galactosidase to enhance their digestibility[17].

*6-2 Food Industry*

β-Galactosidase is used in food processing to reduce lactose content in various products, such as baked goods, beverages, and infant formula. It helps improve the quality of these products by preventing lactose crystallization and extending their shelf life [4] .

*6-3 Pharmaceutical Industry*

In pharmaceuticals, β-galactosidase can be utilized to modify certain drugs or drug delivery systems, allowing for more effective absorption or targeted drug release [29] .

*6-4 Genetic Research*

β-Galactosidase is used as a reporter gene in genetic research and molecular biology. It is incorporated into DNA constructs or plasmids to indicate the presence of the gene or specific promoter activation. When a target gene is expressed, β-galactosidase converts a colorless substrate (X-gal) into a blue product, indicating successful gene expression[30] .

*6-5 Diagnostic Assays*

Enzyme-based assays employing β-galactosidase are used in diagnostic testing to detect specific substances or analytes. These assays are essential in medical diagnostics, research, and biotechnology [31].

*6-6 Environmental Monitoring*

β-Galactosidase activity can be used as an indicator of fecal contamination in water and environmental samples. This is particularly relevant in assessing water quality and potential health risks [32] .

## 7. Conclusion

In this review, focused on the In conclusion, β-Galactosidase is a vital enzyme with various applications, particularly in the food industry. It is responsible for breaking down lactose into glucose and galactose, making it beneficial for individuals who are lactose intolerant. This enzyme is widely used in the production of lactose-free and low-lactose dairy products, as well as in the brewing industry to create lactose-free beer. Its function extends beyond the food industry, as it is also employed in pharmaceuticals, genetic research, diagnostic assays, and environmental monitoring. Furthermore, the enzyme finds applications in other food products, like baked goods, infant formula, and beverages, to reduce lactose content, enhance flavor, and increase product stability.

Overall, β-galactosidase's role in the food industry is essential for ensuring that lactose-intolerant individuals can enjoy a variety of dairy and non-dairy products without adverse effects. Additionally, its versatility in other industries makes it a valuable tool in biotechnology, genetics, and environmental monitoring.

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