Review on Biowaste as a Sustainable Source for Inulinase Production Prospects and Industrial Integration

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Abstract

Inulinase enzymes, key in hydrolyzing inulin into fructose and fructooligosaccharides (FOS), hold significant industrial potential due to their applications in food, pharmaceuticals, bioethanol, and cosmetics. This review explores the diverse types and mechanisms of inulinase, focusing on microbial sources, including fungi, bacteria, and yeasts, and their ability to produce these enzymes efficiently. The study emphasizes the use of agro-waste substrates, such as banana peels, garlic peels, and corn cobs, for cost-effective and eco-friendly inulinase production through solid-state and submerged fermentation. Optimization strategies for enhanced yield, including bioreactor modeling and thermotolerant enzyme development, are discussed. Applications span from prebiotic production to biofuel synthesis, highlighting the enzyme's role in sustainable and circular industrial practices. Despite challenges like low yields and limited microbial diversity, advancements in microbial engineering and fermentation technologies promise a bright future for inulinase's industrial applications.

Keywords

Inulinase, biowaste recycling, Microbial enzyme production, Cost-effective substrates, Biofuel production

1.1. Overview of inulinase: Types and Mechanisms

Inulinases are crucial hydrolyzing enzymes that specifically act on the β-2,1 linkages of inulin, leading to the production of fructose or fructooligosaccharides.[1] Inulinase is found in a variety of sources. It can be secreted by plants, as well as various microorganisms that are present in soil, water, and the digestive tracts of animals. There are many types of inulinase obtained from microorganisms, and these enzymes exhibit good thermal stability, making them suitable for fermentation production. Inulin is effectively decomposed by two distinct types of inulinases: exoinulinase (β-D-fructohydrolase) and endoinulinase (2,1-β-D-fructan fructanohydrolase) [2].

Types and Mechanisms of Inulinase Enzymes

1.1.1 Endo-Inulinase

Endo-inulinase cleaves the internal β-2,1 linkages of inulin in a random manner, resulting in the formation of inulooligosaccharides [3].

- **Substrate Binding**: Endo-inulinase enzymes possess an active site that accommodates longer inulin chains. The configuration of the enzyme's binding pocket is designed to identify and attach to the internal linkages present in the inulin molecule. This specificity allows the enzyme to skip cleaving the ends of the chain, which are designated for exo-inulinase.

- **Catalytic Cleavage**: After binding, endo-inulinase facilitates the hydrolysis of β-2,1-glycosidic bonds within the polymer, effectively "snipping" the chain at random points along the inulin backbone. This action generates shorter fructooligosaccharides (FOS), which are small fructose chains that offer beneficial prebiotic effects in the human digestive system.
- **Resulting Products**: The cleavage results in a blend of oligosaccharides instead of individual fructose units, as the enzyme's internal cleavage produces fragments of the polymer rather than releasing single sugar molecules.
- Endo-inulinase is typically sourced from various microbial organisms, including fungi and bacteria, and plays a crucial role in the production of fructooligosaccharides (FOS) for prebiotic purposes.

1.1.2 Exo-Inulinase

Exo -inulinase efficiently releases fructose molecules sequentially from the non-reducing ends of inulin, showcasing its potent ability to transform inulin into valuable sugars [4].

- **Substrate Recognition**: Exo-inulinase has a high affinity for the terminal (non-reducing) ends of the inulin polymer, which is where it initiates its action. The enzyme's active site is specifically shaped to interact with these terminal fructose residues, allowing it to repeatedly bind to the end of the molecule as it cleaves each fructose unit.
- **Catalytic Hydrolysis**: The enzyme's catalytic domain then performs hydrolysis, breaking the β-2,1-glycosidic bond connecting the terminal fructose unit to the rest of the chain. This process is **sequential**, meaning that after each bond is cleaved, the enzyme binds to the next available terminal fructose and repeats the process.
- **Product Formation**: Exo-inulinase continues hydrolyzing the inulin chain until all that remains are individual fructose molecules. Exo-inulinase is especially useful in applications where pure fructose is desired, such as in high-fructose syrup production.

1.2 Importance of inulinase in industrial applications

Inulinase enzymes play a crucial role in various industries as they can hydrolyze inulin into valuable products like fructose and fructooligosaccharides (FOS). These products are used in the food, pharmaceutical, and bioethanol manufacturing.

Meeting the increasing demand for d-fructose and fructooligosaccharides (FOS) as sweeteners and prebiotics is essential. The enzyme plays a crucial role in the production of ultra-high fructose syrup, biofuels, lactic acid, citric acid, and single-cell oil [1]. One significant application of inulinase is in the production of high-fructose syrup, which is extensively used in the food industry [5] [6].In addition, Inulinase has shown potential for developing a consolidated bioprocessing (CBP) strategy for biofuel production from Jerusalem artichoke tubers [7]. Its ability to efficiently hydrolyze inulin makes the enzyme valuable for various industrial applications. Inulinase extracted from Nocardiopsis species has demonstrated antibacterial properties against various pathogenic bacteria, highlighting its potential applications in the pharmaceutical industry [8]. Furthermore, the creation of magnetically insolubilized inulinase has demonstrated effectiveness in producing a sturdy, stable, and recyclable biocatalyst suitable for industrial applications [9]. Recent advancements in inulinase production and modification techniques have greatly enhanced its industrial applicability, making it a versatile and valuable enzyme for various biotechnological processes.

2 Bio-waste as a Source for Inulinase Production

- **Banana peels are** Banana peels are highly rich in sugars and are particularly advantageous for microbial fermentation. This study clearly demonstrates that banana peels are a cost-effective feedstock for inulinase production, with submerged fermentation yielding significantly higher results than solid-state fermentation [10].
- **Garlic peels and corn cobs** are rich in inulin and carbohydrates. the production of inulinase by Bacillus species using agro-waste substrates such as banana peels, garlic, and corn cobs. It highlights the recycling potential of these waste materials for enzyme production, emphasizing an eco-friendly approach to generate valuable bioproducts[11].

- **Onion peel** – contain inulin and other fructans
- onion peels as a valuable and cost-effective substrate for producing microbial inulinase through solid-state fermentation (SSF). This research emphasizes not only the efficiency of using onion peels but also the promotion of sustainable practices in enzyme production.[12]
- **Chicory roots** – A major source of inulin..
- **Jerusalem artichoke residues** – Rich in inulin..
- **Agave waste** – Contains fructans, which help promote the production of enzymes..
- **Leek and asparagus residues** – Byproducts that are rich in inulin..

3.0 Microbial Strain for Inulinase Production

Microbial strains used for inulinase production include various fungi, bacteria, and yeasts, which are recognized for their ability to produce extracellular inulinases. These microorganisms efficiently hydrolyze inulin from plant-based substrates.

3.1 Fungal strains (e.g., *Aspergillus*, *Penicillium*)

- Aspergillus strains are widely used in the production of inulinase, an enzyme with significant industrial applications. Various species of Aspergillus, including *A. niger*, *A. terreus*, *A. niveus*, and *A. japonicus*, have been studied for their ability to produce inulinase [5] [13] [14] [15]. Interestingly, the production of inulinase by Aspergillus strains can be optimized through various methods. For instance, solid-state fermentation using substrates like wheat bran, palm, and cassava peel has shown promising results [5] [14]. In conclusion, Aspergillus strains are valuable sources of inulinase, with potential applications in the food and pharmaceutical industries.
  Penicillium strains, specifically *Penicillium oxalicum* and *Penicillium subrubescens*, are effectively utilized in the production of inulinase [6] [16].

3.2 Bacterial strains (e.g., *Bacillus*, *Pseudomonas*)

Bacterial strains are essential for inulinase production using biowaste as a substrate. Numerous studies have identified and characterized various bacterial species that can efficiently produce inulinase.

- Bacillus species are prominent inulinase producers, with *B. safensis, B. licheniformis, and B. velezensis* showing significant potential [17] [18]. *B. safensis* demonstrated maximum inulinase activity of 12.56 U/mL after 20 hours of incubation at 37°C [17]. *B. licheniformis* and *B. velezensis* exhibited even higher inulinase activities of 401.18 EU/mL and 344.61 EU/mL, respectively, under optimized conditions [18]. Other Bacillus species, such as B. subtilis and *B. tequilensis*, have also been identified as thermophilic inulinase producers [19].
- *Pseudomonas species*, particularly *P. putida* and *P. aeruginosa*, have been studied for their ability to produce various compounds and degrade different substrates. For instance, these strains can produce polyhydroxyalkanoates (PHAs) using plant oils and glycerol as carbon sources [20] [21]. P. putida strains are being developed as microbial production hosts for a range of amphiphilic and hydrophobic biochemicals [22].

3.3 Yeast strains (e.g., *Kluyveromyces*, *Candida*)

Kluyveromyces marxianus strains have been extensively studied for inulinase production. Four *K. marxianus* strains (CBS 6397, DSM 70792, ATCC 36907, and IZ 619) were selected based on their enzyme activity and growth capacity at low pH and high temperature, with *K. marxianus* ATCC 36907 ultimately chosen for inulinase production [23].

Candida species, C. kutaonensis sp. nov. (Strain KRF1T), was found to produce an extracellular exoinulinase with strong acid resistance, notable thermostability, and high affinity for inulin [24]. This enzyme showed unique characteristics compared to previously reported inulinases, making it a potential candidate for industrial applications. Another marine-derived yeast, *Candida membranifaciens subsp. flavinogenie* W14-3, was also found to produce inulinase, and its gene was successfully cloned and expressed in *Saccharomyces sp*. W0 [25].

4.0 Production Methods of Inulinase

Using natural inulin sources like Jerusalem artichoke tubers, garlic bulbs, dahlia tubers, and chicory roots, yeast isolates such as *Candida catenulata, Sarocladium kiliense, Galactomyces candidum,* and *Scopulariopsis brevicaulis* can produce inulinase. After 48 hours of incubation on particular media, the maximum amount of enzymes was produced [26]. When cultivated in Luria Bertani medium containing 1% inulin, bacterial strains such as *Bacillus aquimaris*, which was isolated from Jerusalem artichokes, can also produce inulinase [27].

4.1 Solid-state fermentation (SSF)

A biotechnological process known as "solid-state fermentation" (SSF) involves growing microorganisms on a solid substrate without or almost without free water. The substrate, which serves as a source of carbon and energy, can be synthetic materials, agro-industrial byproducts, or agricultural residues. This method is particularly suited for fungi, yeast, and some bacteria that thrive in low-moisture conditions. SSF is widely used to produce enzymes, bioactive compounds, organic acids, and fermented foods due to its cost-effectiveness, high product yields, and minimal water requirements. Even though a lot of microbes have been investigated for their ability to produce inulinase through Solid-State Fermentation (SSF), finding new inulinase-producing microbes is still crucial. SSF is a sustainable and cost-effective process because it frequently uses cheap agro-industrial residues as substrates. Optimizing SSF processes, investigating underutilized resources, and developing microbial inulinase production including its purification, characterization, and industrial applications are the main goals of current research.[1]

4.2 Submerged fermentation (SmF)

Submerged Fermentation (SmF**)** is a biotechnological process where microorganisms grow and produce desired products in a liquid nutrient medium. This medium contains dissolved nutrients, and the microorganisms are either suspended in the liquid or attached to solid supports that are submerged in the liquid. The inulinases were produced using a variety of substrates. In submerged fermentation, inulin and inulin-rich raw materials are primarily used as a carbon source. Since pure inulin is costly, alternative, less expensive raw substrates that contain inulin are used to produce microbial inulinase. Reports indicate inulinase production using various low-cost substrates such as agave, wheat bran, rice bran, banana peel, and orange peel [2].

5.0 Optimization of Inulinase Production

It involves fine-tuning various factors to maximize enzyme yield efficiently and cost-effectively. This process is crucial for enhancing productivity in industrial applications.

The isolated enzyme demonstrates optimal performance at a pH of 4.0 and a temperature of 60 degrees Celsius, making these conditions ideal for its effectiveness. Furthermore, the isolated enzyme was highly stable at 60 °C. In conclusion, the current work provides a cost-effective method for producing inulinase from *Rhizopus oryzae.* [18]. An isolated from a rotten garlic sample, a fungal culture producing inulinase demonstrated strong potential for industrial biotechnology. The enzyme production was high under optimal conditions, and the crude enzyme exhibited excellent thermal tolerance, with peak activity at 45°C. This reduces the need for strict temperature control, enhances substrate solubility, and helps minimize contamination. [28].

6.0 Applications of Inulinase

Inulinase production has several key applications across industries, primarily in food, pharmaceuticals, and biotechnology.

i. **Food industry**: Fructose syrup production, prebiotics, low-calorie sweeteners
   In the food industry, inulinases are used to create fructooligosaccharides (FOS) through transfructosylation of sucrose, which can extend the shelf life and flavor of products while offering beneficial characteristics. [29]. FOS and inulin also serve as prebiotics, selectively fermented by beneficial gut bacteria to impart health benefits [30].
ii. **Bioethanol production**: The conversion of inulin into fermentable sugars is a process that transforms inulin into sugars suitable for fermentation, which can then be used to produce bioethanol. In the bioethanol sector, inulinases play a crucial role in developing consolidated bioprocessing strategies for biofuel production from inulin-rich substrates, such as Jerusalem artichoke tubers [7]. Additionally, inulinases support efforts for the circular economy and waste valorization. For example, in an

integrated strategy, pineapple waste can be utilized to produce both bioethanol and proteolytic enzymes [31].

iii. **Pharmaceutical industry**: Synthesis of functional oligosaccharides
Inulinases are used in the production of high-fructose syrups, an essential sweetener. This offers beneficial effects for individuals with diabetes [2].

iv. **Cosmetic industry**
Microbial pigments, which can be derived from inulin-based fermentation, have applications in cosmetics due to their photoprotection, antioxidant, and anti-aging properties [32].

v. **Other applications**: Bioremediation, biosensors, and sustainable industrial processes
Additionally, inulin shows promise in biorefinery applications, where inulin-containing waste can be converted into biofuels and electricity [33].

7 Challenges and Future Prospects

The production of inulinase through microbial processes faces several notable challenges, but it also has exciting future growth opportunities. One hurdle is enhancing and refining the production methodology to achieve greater enzyme yields. Additionally, ensuring the economic feasibility of this production remains an important issue. As researchers explore the microbial mechanisms at play, they discover inventive approaches that may boost efficiency and lower costs, opening broader applications for inulinase in various sectors. Solid-state fermentation (SSF) offers benefits such as reduced water consumption, lower wastewater management needs, and increased yield. However, there is still a pressing need for extensive investigation into untapped sources and the isolation of new microbes for inulinase production.

Recent studies suggest that bacteria can be important contributors to inulinase production. For instance, newly identified strains of *Bacillus licheniformis* and *Bacillus velezensis* have demonstrated remarkable inulinase activities of 401.18 EU/mL and 344.61 EU/mL, respectively [18].

The outlook for microbial inulinase production is promising. Higher yields are expected as bioreactor modeling progresses and as physical and chemical parameters in SSF are carefully monitored [1]. It has been shown that the utilization of low-cost substrates, such as paneer whey, can enhance inulinase production in stirred tank reactors [34]. Furthermore, the development of thermo-tolerant and metal-resistant microorganisms capable of producing inulinase could broaden the enzyme's application across various biotechnological processes [28].

As research continues to evolve in the discovery of new microbial sources and the improvement of various production conditions, we anticipate a significant rise in the use of inulinase across multiple industries. This enzyme, essential for converting inulin into fructose, shows great potential for applications in high-fructose syrup production, biofuel development, and the food sector. The increasing demand for sustainable and efficient practices in these fields will likely propel the broader adoption of inulinase, fostering greater productivity and innovation.

Conclusion

Inulinase production has become a crucial biotechnological process, utilizing agro-waste recycling to produce valuable enzymes for various industries. The implementation of advanced fermentation methods and diverse microbial sources highlights the enzyme's flexibility in generating high-value products like fructose syrups, prebiotics, and biofuels. Although challenges persist in optimizing production effectiveness and identifying new microbial strains, ongoing advancements in microbial engineering, substrate use, and process optimization promise solutions. The growing significance of inulinase in industry, in alignment with sustainability objectives, establishes it as a key factor in promoting eco-friendly and cost-efficient bioprocesses for the future.

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