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REVIEW ARTICLE

Review of inulinase production using solid-state fermentation

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Abstract

The purpose of the present study is to critically analyze the recent literature covering the production of inulinase enzyme from various sources by solid-state fermentation and discuss various approaches to increase its production in solid-state fermentation, purification, and its properties. The review deals with the solid-state fermentative production of inulinase production. Inulinases have many applications in industries, such as for the production of ultra-high fructose syrup, biofuels, lactic acid, citric acid, and single-cell oil. Solid-state fermentation (SSF) is more economic, requires smaller vessels, lowers water intake, reduces waste-water treatments, higher product yield, lesser chance of bacterial contamination, and lowers energy consumption. Furthermore, the crude products obtained from SSF can be directly used as the source of enzyme for biotransformation. Although many reports are available on a wide range of microbes which produces inulinases by SSF, it is important to isolate novel microbes for its production. Also, extensive research is going on to exploit unexplored sources for SSF. Higher yield of inulinases can be achieved by bioreactor modeling and proper monitoring of physical and chemical parameters in SSF.

Keywords Inulin · Inulinase · Optimization · Substrate · Solid state fermentation

Introduction

Inulin

Inulin is a fructan polymer which mainly comprises of linear β-2, 1-D-fructofuranose linked with a glucose unit at the terminal end (Pandey et al. 1999). It ranks second in being the most abundant storage carbohydrate after starch. It is a part of dietary fiber and does not get digested. It is classified as a unique oligosaccharide or polysaccharide depending on the length of its chain. It is made up of D-fructosyl subgroups linked together by β(2 → 1) glycosidic bonds which generally ends with a (1 ↔ 2)-bonded α-D-glucosyl group as shown in Fig. 1. The length of these chains of fructose ranges and varies from 2 to 60 monomers. Inulin containing a maximum of 10 fructose molecules is referred as oligofructose. Inulin generally contains 2 to 140 fructose units and is heterodisperse. There is no sugar ring in its backbone (Zhu et al. 2016).

Large quantities of inulin is found in plants like Jerusalem artichoke, chicory root, garlic, asparagus root, burdock root, coneflower, yacon root, camas root, jicama, salisfy, and dandelion root. Inulin is also present in common vegetables and fruits like garlic, banana, onion, leek, wheat, rye, and barley (Mensink et al. 2015).

Inulinases

Inulinases are the class of enzymes which hydrolyze β-2,1 glycosidic linkage to produce fructose, inulo-oligosaccharides, and glucose. Inulinases are produced by fungi, bacteria, yeast, actinomycetes, and molds (Neagu and Bahrim 2011). Inulinases are of two types based on their action pattern namely exoinulinase and endoinulinase. Exoinulinase removes the terminal fructose units from inulin and produces fructose as the main product. Endoinulinase hydrolyzes the internal linkages of inulin into inulo-oligosaccharides (Singh and Singh 2010).

Inulinases are widely used for the production of ultra-high fructose syrup (Lima et al. 2011), fructose (Ricca et al. 2009), ethanol (Ge and Zhang 2005), lactic acid (Petrova et al. 2015),

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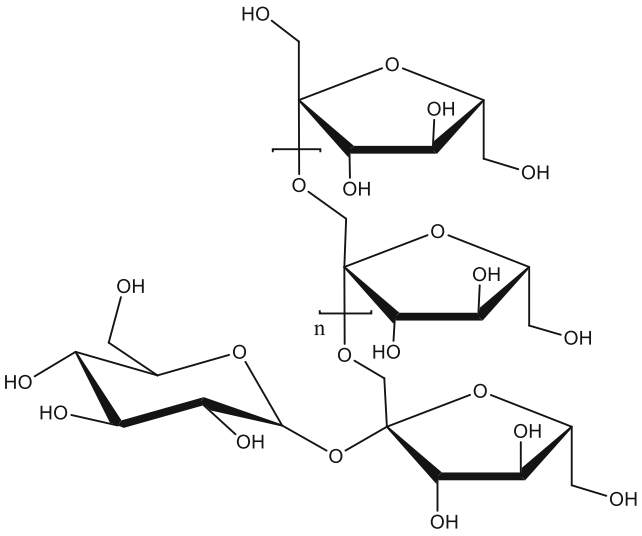

Fig. 1 Structure of inulin

citric acid (Yan Liu et al. 2010), and single-cell oil (Zhao et al. 2010).

Fructose is produced by a traditional method in which amylolysis of starch takes place with α-amylase and amyloglucosidase followed by glucose isomerase which converts glucose into fructose. However, only about 45% of fructose is produced by this method (Singh and Gill 2006). The enzymatic reaction using inulinase is a single-stage method for higher yield of fructose production which leads to the production of pure fructose (95%) after enzymatic hydrolysis of inulin (Danial et al. 2015).

Inulinase versus Invertase activity

The quantity of enzyme that releases 1 μmol of fructose per minute from inulin is defined as one unit of inulinase activity whereas the amount of enzyme that hydrolyzes 1 μmol of sucrose per minute is one unit of invertase activity (Sharma et al. 2006). Exoinulinases shows invertase activity coupled with inulin hydrolytic activity whereas endoinulinases lacks invertase activity. Generally, one can distinguish between inulinase and invertase from the I/S ratio (relative activities with inulin and sucrose). When the I/S ratio is greater than 10^{-2}. it indicates high production of inulinase in culture, whereas when it is less than 10^{-4}, it indicates higher invertase production (Pessoni et al. 2007).

Solid-state fermentation

Solid-state fermentation (SSF) is a process in which a solid substrate such as wheat bran or sugarcane bagasse is deposited on flatbeds. It is then cultivated with microbes and incubated in a temperature-controlled room for some days (Singhania et al. 2009).

SSF has gained its popularity over a decade because of its high productivity. It has many industrial applications and utilizes abundantly available agro-industrial residues (Bhargav et al. 2008). SSF is important for the manufacture of biomolecules used in pharmaceutical, food, cosmetic, fuel, and textile industry. These biomolecules are generally metabolites produced by microorganisms in the absence or near absence of free-flowing water on a solid support (Al-Dabbagh and Mahmood 2015). The important factors involved in SSF are the substrate, size of substrate particle, water activity, temperature, moisture, pH, agitation, and aeration. Among these, particle size affects the total surface area of the substrate to the substrate volume ratio, and thus helps in the characterization of the substrate (Chen 2013). As the particle size of substrate decreases, the ratio of surface area to volume increases. Besides, the void space is also determined by the substrate particle size which further affects the rate of oxygen transfer. When the particle size is small, the microbes get a larger surface area for its growth. Very small particles lead to the reduction in growth and clumping of substrates in comparison to particles larger in size which provides a better exchange of oxygen and heat. Hence, a suitable size of the substrate particle should be maintained to enhance better microbial growth and mass transfer (Singh et al. 2017). Enzyme activity in SSF is represented in the units of activity/g of dry substrate (U/gds). One unit of enzyme activity (U) is defined as the amount of enzyme, which forms 1 μmol of the product per minute per gram of dry substrate (Dilipkumar et al. 2014).

Inulinase-producing microorganisms in SSF

Microorganisms are used as potential sources for inulinase production because they can be easily cultured and result in high enzyme yields. Microbial inulinases are stable at high temperatures, prevent microbial contamination, and have a high solubility for the substrates. The major class of microbes engaged in inulinase production using SSF are fungi and yeast. Fungi can grow on particle surfaces by penetrating their hyphae into the spaces between the particles and thereby inhabiting solid substrates, thus they are considered to be the most suitable organisms for SSF. The most preferred and commonly used strains for inulinase production by SSF are the fungal strains belonging to *Kluyveromyces* (Mazutti et al. 2006, 2007) and *Aspergillus* (Romero-Gómez et al. 2000; Al-Dabbagh and Mahmood 2015) genus. The first report on successfully employing SSF for bacterial inulinase production was from *Staphylococcus* sp. (Selvakumar and Pandey 1999). The first report on inulinase production using garlic and onion peels by this method was from *Xanthomonas campestris*, a bacterial species (Ayyachamy et al. 2007). *Streptomyces* species is an actinomycete also produced inulinase by SSF using copra waste (Dilipkumar et al. 2013b), pressmud (Dilipkumar

et al. 2011a), and garlic bulb powder (Dilipkumar et al. 2011b) as shown in Tables 1 and 2.

Substrates used for the production of inulinase enzyme in SSF

In SSF, the various factors involved in substrate selection for enzyme production are mainly related to availability and cost of the substrate. Thus, numerous agro-industrial residues can be screened. In this process, the supply of essential nutrients by the solid substrate assists in the growth of microbes and also provides support for the cells (Kapilan 2015). SSF differs from submerged fermentation (SmF) culturing since the product is formed by the microbes at the surface of the solid substrate particle having a low moisture content or near to it. Besides, water plays an important role in the physical and chemical properties of the solids which affect the complete process productivity (Chen 2013).

Inulinase enzyme has been produced from a large number of agro-industrial residues by cultivating numerous microbes. The most commonly used substrates which are cheap and non-soluble for microbial inulinase production are wheat bran, sugarcane bagasse, pressmud, rice bran, garlic, onion peels, etc. (Singh et al. 2017). Non-soluble substrates have a low thermal conductivity which results in accumulation of heat thereby further influencing the formation of final product (Singh and Chauhan 2016). Copra waste using a bacteria, *Streptomyces* sp., also proved to give a high inulinase activity of 131 U/gds (Dilipkumar et al. 2013b) whereas it gave an activity of 239 U/gds using a fungus, *Penicillum rugulosum* (MTCC-3487) species (Dilipkumar et al. 2014). Marine yeast *Cryptococcus aureus* G7a gave an activity of 420.9 U/gds using a mixture of wheat bran and rice husk (Sheng et al. 2009). *Aspergillus niger* gave an activity of 200 U/gds using banana peel as substrate (Narayanan et al. 2013). *Kluyveromyces marxianus* NRRL Y-7571 gave 586 U/gds of inulinase activity using bagasse and soybean meal in a fed-batch reactor using saturated air that led to decrease in temperature of solid media by evaporative cooling, thereby highlighting the importance of moisture in SSF (Astolfi et al. 2011). Another suitable substrate for inulinase

Table 1 Fungi and yeast employed for inulinase production by SSF

Microorganisms	Substrate	Maximal activities	References
Aspergillus niger	Jerusalem artichoke and bean	11.13 U/gds	Al-Dabbagh and Mahmood (2015)
Aspergillus niger	Banana peel Rice bran	200 U/gds 137.2 U/gds	Narayanan et al. (2013))
Aspergillus niger AUMC 9375	Sunflower tubers and lettuce roots Lettuce roots	0.232 U/gds 0.0879 U/gds	Housseiny (2014))
Aspergillus parasiticus	Sugarcane bagasse Artichoke leaves	1.773 ± 0.627 U/gds 0.177 ± 0.125 U/gds	Abd El Aty et al. (2014))
Aspergillus terreus	Garlic wastes Artichoke leaves	0.022 ± 0.031 U/gds 4.433 ± 0.121 U/gds	Abd El Aty et al. (2014))
Aspergillus versicolor	Chicory roots Orange rinds	0.177 ± 0.125 U/gds 1.917 ± 0.016 U/gds	Abd El Aty et al. (2014))
Cryptococcus aureus G7a	Wheat bran and rice husk	420.9 U/gds	Sheng et al. (2009))
Geotrichum candidum	Leek powder	412.1 U/gds	Canli and Kurbanoglu (2012))
Kluyveromyces S120	Wheat bran	409.8 U/gds	Xiong et al. (2007))
Kluyveromyces marxianus ATCC-52466	Wheat bran (coarse) Corn flour	106.72 U/gds 21.23 U/gds	Selvakumar and Pandey. (1999)
Kluyveromyces marxianus NRRL Y-7571	Sugarcane bagasse + cane molasses + soybean bran	463 U/gds	Mazutti et al. (2010a)
Kluyveromyces marxianus NRRL Y-7571	Sugarcane bagasse	390 U/gds	Mazutti et al. (2006))
Kluyveromyces marxianus NRRL Y-7571	Soybean bran and sugarcane bagasse	436.70 U/gds	Mazutti et al. (2010b)
Kluyveromyces marxianus NRRL Y-7571	Soybean bran and sugarcane bagasse	250 U/gds	Mazutti et al. (2007))
Kluyveromyces marxianus NRRL Y-7571	Sugarcane bagasse and soybean meal	586 U/gds	(Astolfi et al. (2011))
Penicillium brevicompactum	Artichoke leaves Garlic wastes	1.241 ± 0.877 U/gds 0.665 ± 0.156 U/gds	Abd El Aty et al. (2014))
Penicillum rugulosum (MTCC-3487)	Copra waste	239 U/gds	Dilipkumar et al. (2014))
Pichia guilliermondii	Wheat bran and Rice husk	291.0 U/gds	Guo et al. (2009))
Saccharomyces sp.	Wheat bran Orange peel	78.29 ± 0.13 U/gds 22.47 ± 0.01 U/gds	Onilude et al. (2012))

Table 2 Bacteria and actinomycetes employed for inulinase production by SSF

Microorganisms	Substrate	Maximal activities	References
Staphylococcus sp.	Wheat bran (fine) Corn flour	96.77 U/gds 16.11 U/gds	Selvakumar and Pandey (1999))
Streptomyces sp.	Copra waste	131 U/gds	Dilipkumar et al. (2013b)
Streptomyces sp. MTCC-3119	Garlic bulb powder	76 U/gds	Dilipkumar et al. (2011b)
	Pressmud	89 U/gds	Dilipkumar et al. (2011a)
Xanthomonas campestris pv *phaseoli*	Garlic peel Onion peel	117 IU/gds 101 IU/gds	Ayyachamy et al. (2007))

production from *Penicillium oxalicum* BGPUP-4 by SSF was carrot pomace which gave an inulinase activity of 322.10 IU/gds after optimization (Singh et al. 2018b).

Approaches to increase microbial Inulinase production in SSF

In latest studies, extensive research has been applied for developing the production of inulinases. Numerous strategies, such as improvement of strain, metabolic engineering, heterologous or recombinant inulinase expression, types of a bioreactor, and also modeling of SSF are gaining prominence for the microbial inulinase production in SSF.

Strain improvements by metabolic engineering

To expand the production of inulinase, optimization of culture conditions along with appropriate strain improvement should be carried out. Mutagenic agents can be a good source to bring an improvement in strain. Canli and Kurbanoglu (2012) applied 7mT magnetic field on *G. candidum* OC-7, using leek as the substrate which gave an inulinase activity of 535.2 U/gds. Guo et al. (2009) isolated overproducers of inulinase by mutating cells of *Pichia guilliermondii*, a marine yeast strain 1by using UV light and $LiCl_2$. They obtained one mutant (M-30) with enhanced inulinase production. The medium compositions and cultivation conditions were optimized. The mutant strain M-30 produced maximum inulinase at the following conditions: inoculum (2.5%), initial moisture (60.5%), ratio of wheat bran to rice bran (0.42), pH (6.50), and temperature (30 °C). After optimizing the conditions, the mutant strain gave 455.9 U/gds of inulinase activity whereas its parent strain produced only 291.0 U/gds under the same conditions.

Screening and optimization of inulinase production by statistical methods

In order to obtain the enhanced yield in an SSF process, the media components and the operating conditions should be optimized. One of the most relevant methods of optimization is a statistical method which includes full factorial and fractional factorial designs (FFD), Plackett-Burman designs (PBD), and response surface methodology (RSM) (Behera and Ray 2016).

Full factorial and fractional factorial designs

The initial step in the optimization of a fermentation process is the screening of significant factors which can be performed by factorial design. The interaction between the factors can be identified by factorial design experiments which are not possible in conventional experimental methodologies .

Normally, the factorial design experiments are performed by assigning a low level and a high level to all the factors (k) taken for the study and thus named as 2^k designs. In full factorial designs, all possible factor level combinations are considered and the total number of experiments will be 2^k. However, as the number of factors increases, the total number of experiments will also increase proportionally which eventually demand more resources and time. Therefore, fractional factorial design experiments are suggested to decrease the number of experiments which consist of fractions of corner experiments. The fractional factorial design follows 2^{k-1}, 2^{k-2}, and 2^{k-4} number of experiments which correspond to ½, ¼, and 1/8 fraction of full factorial design.

Plackett-Burman design

Since many nutrient factors and trace elements affect the inulinase enzyme production, the number of experiments based on FFD will be very high and will not be economical. In such cases, screening of the significant factors can be done by using the Plackett-Burman design. It is very economical with the total number of experiments with a multiple of four. Dilipkumar et al. (2011b) screened 18 nutrient components by using this design for inulinase production using garlic. Among the 18 media components, only four nutrient constituents namely NH_4NO_3, $MnSO_4 \cdot 7H_2O$, soya bean cake, and K_2HPO_4 influenced the production of inulinase to a greater extent which was then optimized using central composite design (CCD) and achieved an inulinase activity of 76 U/gds.

Similarly, the 18 medium components for inulinase production using copra waste was screened using PBD by the same research group. Only three factors ($MgSO_4.7H_2O$, soya bean cake and $(NH_4)_2SO_4$) significantly affected the inulinase activity. These three variables were finally optimized using CCD and an activity of 131 U/gds was achieved (Dilipkumar et al. 2013b). On the other hand, 11 media components were screened using PBD for inulinase production from *Aspergillus terreus* using artichoke leaves as the solid substrate, and from this design, only four components (pH, temperature, KH_2PO_4 and Ca^{2+}) were found to influence the inulinase activity (Abd El Aty et al. 2014).

Response surface methodology

Response surface methodology comprises a set of empirical methods which reveals the relationship between the input and output variables (Sivapathasekaran and Sen 2017). The analysis of RSM is based on the multiple regression analysis and analysis of variance (ANOVA). There are two types of response surface methodologies namely, central composite design (CCD), and Box-Behnken design (BBD). The following methods help in determining the optimum process conditions in SSF (Dinarvand et al. 2013).

The design matrix of CCD was used to optimize the production of inulinase by employing bagasse as a solid substrate from *Kluyveromyces marxianus* NRRLY-7571. Under the optimum conditions of 36 °C and 20% (wt) of CSL, a maximum inulinase activity of 391.9 U/g was obtained (Mazutti et al. 2006).

Sheng et al. (2009) achieved a high inulinase production from *Cryptococcus aureus* G7a, a marine yeast by varying the process parameters for optimization using CCD. He found that the size of inoculum, moisture, temperature, the ratio of amount of wheat bran to rice husk, and pH had great influence on inulinase production. Of inulinase activity, 420.9 U/gds was achieved under the optimized conditions.

Dilipkumar et al. (2014) optimized the production of inulinase using copra waste as a substrate. They first screened the nutrients using PBD and then optimized the selected nutrients using CCD to enhance the production of inulinase. A packed-bed bioreactor was used to carry out the experiments to optimize the process variables like packing density, rate of air flow, moisture content, and the particle size which showed an 18% increase in inulinase production. In this study, a maximum productivity of 239 U/gds was obtained. Trivedi et al. (2012) produced inulinase using wheat bran as substrate and corn steep liquor as the moistening agent. BBD was used for optimizing the process parameters viz. size, fermentation time period, percentage moisture content, inoculum, and pH of the medium, and a yield of 257 ± 11.4 U/g was achieved. A summary of various screening and optimization methods employed for inulinase production by SSF is shown in Table 3.

Fermenter operation for Inulinase production by SSF

Past many years, inulinase production in SSF systems has been carried out by different types of bioreactors. Laboratory-based experiments are normally performed in beakers, Roux bottles, jars, Erlenmeyer flasks, and glass tubes. Drum, deep tank, or tray fermenters have been used for carrying out for large-scale fermentations (Mitchell et al. 2006). Both batch and fed-batch mode of operations can be carried out for inulinase production by SSF processes, although the better yield is given by fed-batch operation. The most commonly used bioreactor used to evaluate inulinase production by SSF of agro-residual wastes with different microorganisms is Packed Bed Bioreactor (PBB). Mazutti et al. (2010a) studied the production of inulinase and cell growth by *Kluyveromyces marxianus* NRRL Y-7571 in a packed-bed bioreactor. They verified that the rate of volumetric air flow rate and the temperature of the inlet air influenced the process dynamics. The best condition to carry out the inulinase production was volumetric flow rate ($3m^3/h$) and inlet air temperature (30 °C) achieving an activity of about 463 U/gds. In another study, the production of inulinase by *Kluyveromyces marxianus* NRRL Y-7571 in a packed-bed bioreactor by SSF was examined. Three kilograms (dry basis) of sugarcane bagasse, soybean bran, and previously treated cane molasses were used as substrates (Mazutti et al. 2010b). RSM was used for optimizing the temperature, initial cell mass, and flow rate of air. The following parameters viz. temperature of inlet air (30 °C), cells for fermentation (22 g), and air flow rate ($2.2m^3/h$) gave an optimum activity of inulinase (436.7 ± 36.3 U/gds) and a high productivity of 18.2 U/gds, thus proving that inulinase production by SSF in a PBB is technically feasible. Astolfi et al. (2011) used both batch and fed-batch mode in a fixed-bed reactor to study the production of inulinase by SSF. The reactor had a working capacity of two kilograms of dry substrate. Generally, metabolic heat is generated during the microbial growth in SSF. They studied different approaches to perform evaporative cooling by feeding inlet air in the bioreactor as a substitute to remove the generated heat. They achieved a maximum enzyme activity (586 ± 63 U/gds) after 24 h of fermentation in the fed-batch mode using saturated air.

Purification methods and properties of microbial inulinases in SSF

Purification and characterization of an enzyme are necessary to analyze its nature, determine its physicochemical characteristics, and obtain a good biocatalyst. The important factors

Table 3 Different screening and optimization methods employed for inulinase production by SSF

Statistical method	Variables optimized	Activity	References
PBD, CCD	$MnSO_4{\cdot}7H_2O$, NH_4NO_3, soya bean cake, and K_2HPO_4	76 U/gds	Dilipkumar et al. (2011b)
PBD, CCD	Soya bean cake, $MgSO_4{\cdot}7H_2O$ and $(NH_4)_2SO_4$	131 U/gds	Dilipkumar et al. (2013b)
BBD	Flow rate of air, packing density, and size of particle	300.5 U/gds	Dilipkumar et al. (2013a)
CCD	Temperature and corn steep liquor	391.9 U/g	Mazutti et al. (2006))
PBD, BBD	Inulin, corn steep liquor and $(NH_4)_2SO_4$	409.8 U/g	Xiong et al. (2007))
PBD, CCD	Yeast extract, $FeSO_4.7H_2O$, and NH_4NO_3	89 U/gds	Dilipkumar et al. (2011a)
CCD	Temperature, initial mass of cells and air flow rate	436.7 ± 36.3 U/gds	Mazutti et al. (2010b)
PBD, CCD, and BBD	K_2HPO_4, $ZnSO_4{\cdot}7H_2O$ and soya bean cake and packing density, air flow rate, moisture content, and particle size	239 U/gds	Dilipkumar et al. (2014))
CCD	Initial moisture, inoculum, wheat bran/rice bran, temperature, pH	455.9 U/g	Guo et al. (2009))
CCD	Initial moisture, inoculum, wheat bran/rice bran, temperature, pH	420.9 U/g	Sheng et al. (2009))
BBD	Moisture content, inoculum size and pH	257.2 ± 11.4 U/g	Trivedi et al. (2012))
CCRD	Moisture, incubation time, pH	77.95 IU/gds	Singh et al. (2018a)

required for carrying out successful purification are the source of the enzyme, enzyme complexity, distribution of charge, and physicochemical properties. Purification leads to the separation of different types and isoforms of inulinases. Different techniques of purification have been considered for the purification of inulinases based on polarity, size, ligand interactions, solubility, etc. (Fernandes and Jiang 2013). Different techniques of purification like salt precipitation, solvent precipitation, ion exchange, and gel filtration chromatography have been used individually or in combination for purification of fungal inulinases by SSF.

Based on the source and growth conditions, inulinases can be different in their structure and mode of action. Ettalibi and Baratti (1987) purified two endoinulinases (Endo-I and II) and five exoinulinases (Exo-I, II, III, IV, and V) from *Aspergillus ficuum* using ammonium sulfate precipitation, ion exchange, and fast protein liquid chromatography (FPLC). Golunski et al. (2017) used a simple and cheap purification method of changing the ionic strength of the medium by addition of NaCl and $CaCl_2$, followed by alcohol precipitation. A 4.8-fold purification was achieved by this process resulting in a specific inulinase activity of 516.6 U/mg. Housseiny (2014) purified an endoinulinase from *Aspergillus niger* AUMC 9375 using solvent precipitation, ion exchange chromatography (IEC), and gel filtration chromatography (GFC). He achieved a 51.11-fold purification with a specific inulinase activity of 11,883.33 U/mg.

Till date, there are no reports on purification of bacterial inulinases produced by SSF.

The two important properties of microbial inulinases considering the industrial requirements are pH and thermal stability. A higher temperature increases the solubility of the substrate and also prevents any cross-contamination. pH stability is essential to maintain interactions between the linkages of protein and restore their structural conformity (Singh and Chauhan 2016). Fungal strains have an optimum temperature in the range of 45–55 °C and pH of 4.5–7, respectively (Kango and Jain 2011). However, Chen et al. (2009) reported two forms of endoinulinases (Endo-I and II) and three forms of exoinulinases (Exo-I, II, and III) from *Aspergillus ficuum* having a pH 4–8 and stability below 50 °C. This shows the occurrence of different inulinases, their synergistic effect, and a wide range of stability. Bacterial strains have a temperature stability in the range of (10–80 °C) and optimum pH (3.5–9.0). An important physical factor of an enzyme is its molecular weight. It helps to understand the heteromeric structure of an enzyme and any variations in its conformation. Generally, the molecular weight of fungal inulinases ranges between 30 and 175 kDa and that of bacterial inulinases between 45 and 600 kDa (Cho and Yun 2002). Metal ions such as Fe^{3+}, Cu^{2+}, Mn^{2+}, Co^{2+}, Mg^{2+}, Ag^{+}, and Na^{+} may be present as a part of the catalytic site of the enzyme. These may act as coenzymes or may affect the activity of the enzyme by various means. Most of the metal ions participate with the enzyme to increase the reaction rate, either by acting as cofactors or prosthetic groups (Sarup et al. 2007).

Challenges in solid-state fermentative production of inulinases

Although SSF has several benefits in industrial applications, scale-up of the process is limited due to monitoring problems and regulating the different process parameters. But the problems can be overcome by controlling the physical and chemical factors in the process of SSF. The effects of heat and mass transfer, temperature, oxygen transfer, and humidity can be controlled on-line in the system by measuring the temperature

build-up, oxygen, and carbon dioxide (Manan and Webb 2017). Steady aeration through the substrate can be controlled by forced aeration. Substrates with a suitable particle size should be used for proper enzymatic action. For a successful SSF process, the substrate should maintain a suitable water activity for the growth of microbes, 0.60–0.70 for fungus and yeast while 0.90–0.99 for bacteria (Chen 2013). Heat removal in SSF is generally achieved by evaporative cooling (Krishna 2005).

Currently, the literature available on the current status of industrial production of inulinases by SSF is based on the reduction in fermentation time from 96 to 24 h, thus improving the process productivity. Currently, there is no data available on the annual production and cost of inulinase production via SSF. When the fermentation time reduces, chances of contamination is reduced and the scale-up of SSF is aided since the mass gradients and the temperature reduces. However, all the SSF studies on inulinase published so far were performed on a small scale (Leelaram et al. 2016). The reports on inulinase production by SSF were carried out using very less quantity of substrate in tray bioreactors. Since the capacity of tray bioreactors is limited with poor aeration control, other bioreactors like PBB and rotating drum bioreactor have been used as discussed earlier in section 5.3. Packed-bed reactors with feedback control schemes and proper modeling are effective for inulinase production as they improve productivity and saves sheer sensitive microbes from damage.

On an industrial scale, the production capacity of SSF is several times greater as compared to SmF. SSF also has a positive impact on the environment as the wastes produced can be used in other operations in future, leading to an increase in its value at the industrial level. By monitoring all the important physical and chemical parameters in SSF process and by proper modeling of bioreactors, inulinases can be produced at a higher level.

Conclusion

Inulin and its various sources represent an inexhaustible, inexpensive, and abundantly available raw material for bioprocess industries. The products obtained after hydrolyzing inulin by exo and endoinulinases are fructose and inulo-oligosaccharides. These acts as a raw material for a wide range of applications in food industry, production of biofuel, obtaining single cell oil and protein, citric acid, and other production of chemicals. Although a wide range of microbes has been reported to produce inulinases by SSF, it is important to isolate novel microbes which produce inulinases. Numerous agro-industrial residues are employed for inulinases production by SSF which are cost-effective and abundantly available. Research is going on to exploit unexplored sources for SSF. This literature portrays a critical study of the overview of SSF process, recent advances in microbial inulinase production by SSF, various optimization techniques, purification, and properties of inulinases and their applications.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Research involving human participants and/or animals There was no involvement of human and animal participation in the research.

Informed consent All the authors have gone through the manuscript and well informed about the research.

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