

Agro-Industrial Residues as Solid Substrate for α -Amylase Production Using Solid State Fermentation by Filamentous Fungi: A Review

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ABSTRACT

Now a days, many industries like textile, food and pharmaceuticals use solid dry wastes as substrate for fermentation. In the solid-state fermentation (SSF), solid surface matrix in the absence or nearly in the absence of available free water used to carry out fermentation process. In this, microorganisms are grown in the absence of free flowing aqueous phase. A traditional way for the production of α -amylase in the past was the use of submerged fermentation (SmF) which is now replaced with SSF. The alternative of SmF is SSF in a variety of industrial production like enzymes, biofuels, single cell proteins, organic acids, antibiotics, aroma, biopesticides etc. Earlier, SSF was the choice of fermentation process only where the choice of microorganisms is fungi, but now variety of bacterial strains are also being used in such processes. SSF facilitates the natural habitat of microorganisms and has a better choice due to its simplicity, relatively low capital investment and operating cost and less water output, etc. SSF with an agro-industrial residues have also replaced the high cost media used in submerged fermentation for α -amylase production. Agro-industrial residue utilization by filamentous fungi provides an alternative source for utilization of these substrates as a solid substrate for production of amylase and other useful industrial products. In this review we tried to study various solid substrates used for fungal amylase production, their potential, and optimization strategies

KEY WORDS: SOLID SUBSTRATE, SSF, BIO-PROCESSING, AGRICULTURAL WASTE, α -AMYLASES.

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INTRODUCTION

Among many industrially important enzymes, one of the most commonly used enzymes is α -amylases, which is required for the production of many fermented foods. Apart from food and starch industries α -amylases are also having a wide application in other industries such as paper pulp and textile, etc. (Gupta et al., 2003). Amylase enzymes having a wide variety of industrial applications ranging from the conversion of starch to sugar, corn and maltose syrup for food and productions of cyclodextrins. (Kunamneni et al., 2005). Demand of amylases is also increasing in paper and pulp industry, textile industry. These enzymes account for about 30% of the world's total enzyme productions. Due to increasing demand in various industries, there is enormous interest in developing amylase with better properties, thermal stability and suitability for industrial application by using cost effective production techniques (Pandey et al., 2000). α -amylase are produced by microorganisms plants and animals, where they play a key role in carbohydrate metabolism. From centuries amylases from plant and microbial origin containly being used as food additives. Amylases isolated from barley have been used in brewery industries and fungal amylases have been widely used in the preparation and processing of oriental foods (Reddy et al., 2003).

Barley amylases have been used in brewery industries and fungal amylases have been widely used in the preparation and processing of oriental foods (Reddy et al., 2003). Amylases from microbial sources are used for the industrial production of various useful products due to the cost effectiveness, consistency, thermal stability and ease of process modifications and optimization. Amylases play key role in the carbohydrate metabolism and are produced by microbe, plants as well as animals also. From centuries amylases from plant and fungal origin are being constantly used as food additives. Bacteria and filamentous fungi such as *Aspergillus* are used for the production of α -amylase. (Ajayi et al., 2003). However the amylase of fungal origin has been found to be most stable than the bacterial enzyme. Filamentous fungi are attractive host organism for the production of commercially useful enzymes. Coproduction of two industrially important enzyme amylase and protease through SSF by *Rhizopus oryzae* using bread waste as a solid substrate have been studies and effect of various physical and chemical parameters were studied (Benabda et al., 2019).

Amylase production using *Bacillus subtilis* as a bacterial strain using solid residues such as wheat bran, rice bran, banana peel and other fruit peel for optimize the production of enzyme with minimal amount of supplementary carbon and nitrogen source (Almanaa et al., 2020). Combined effect of solid state fermentation to submerged fermentation was studied using the edible fungus *Neurospora intermedia* to biotransformation of ethanol and this study may be useful for the production of other industrially important products (Gmoser et al, 2019).

All these types of fermentation processes agro-industrial solid wastes can be efficiently used in the form of solid substrates for SSF. The purpose of this review article is to summarize the information related to SSF for α -amylase production, which is distributed across various literatures to help scientific community. Various different types of solid substrates are used in this purpose and each substrate used to have their own merits and demerits. Hence this article will felicitate the researchers with the information regarding various solid substrates used for fungal amylase production, their potential, and optimization strategies.

2. Solid State Fermentation strategies for amylase production:

In solid state fermentation solid matrix is used in presence of minimal water content and sometimes in absence of water to support growth and metabolic activity of microorganism. Solid matrix could be either used as support material or simply a source of nutrient content allows the growth and metabolism of microorganism. SSF provides natural habitats to the microorganisms therefore considered as a preferred choice over submerged fermentation. SmF is considered as a violation to natural living of microorganism, especially unfavorable for the growth of fungi. Scientific data reveals that more than 98% of microbial isolates from marine habitat have been obtained from the underwater surfaces of solid substrates (Kelecom et al., 2002, Holker et al., 2004).

This indicates that SSF is well adapted suited for the growth and metabolism of fungi. At laboratory level studies many research article have been published for observing various physical and chemical factors that affects on fungal metabolism. SSF provides better economic feasibility for production of amylase and other high end products by using low cost agricultural residues and resulting in solving the problem of waste disposal. Solid substrate not only supply nutrient for fungal growth it also play a key role as a support material which helps in effective growth of fungus due to its porosity, moisture holding capacity etc. These agricultural byproducts are rich in starch and lignocellulosic compound based on their origin in agricultural practices or agro-industrial sources (Pandey et al., 1992).

In present time enzyme production strategies are centered in the selection and screening of suitable solid substrate for maximum enzyme production and optimization of various physical and chemical parameters process parameters that affects the growth of fungus and enzyme production. Industrial production of amylase using SSF having advantages for growth of fungus which have capability to penetrate these solid substrate effectively. (Ramachandran et al., 2004). In addition to the utilization of these agro-industrial wastes as solid substrate in amylase production as solid substrate as well as in solving the waste disposal problem. With the improved production of lignocellulose degrading enzyme such as xylanase and cellulase SSF becoming most favorable process for cost effective production

of industrially important enzymes and biofuels etc (Taherzadeh et al., 2019).

With the increasing harmful effect of traditional chemical pesticides now researchers are working on the production of biological control agents through SSF by using agro-industrial waste as substrates. Production of entomopathogens of fungal origin is being produced by SSF for adopting sustainable production method and for effective biological control method to reduce the pest eradication in the agricultural practices (Sala et al., 2019). Now novel approaches are developing for agricultural waste treatment and further being used for up scaling of SSF processes using bacterial culture for the production of poly γ glutamic acid in continuously stirred solid state bioreactors (Fang et al., 2020)

3. Role of filamentous fungi for amylase production:

However many fungal strains are commercially used for production of amylase but researchers are constantly trying to isolate novel fungal strains for amylase production. A team of researchers of Brazil isolated new fungus from the Atlantis forest region having ability to produce higher glucoamylase using agro-industrial waste as solid substrate under standardized conditions. The isolated fungi *A. carbonarius* showed maximum production of amylase at pH 6.0, temperature 30°C and incubation time 96 hours when supplemented with 1% starch in the media (Pasin et al., 2020).

Since fungi have very efficient enzymatic system therefore fungi having ability to degrade lignocellulosic waste resulting in the formation of many valuable compounds that may be useful for production vast variety of useful products. Two types of extracellular enzymatic system are recognizable in fungi i.e. hydrolytic and ligninolytic system. Lignocellulosic residues from agricultural and municipal solid wastes are particularly abundant in nature and have a potential for bioconversion (Abdel-Azeem et al., 2020).

Filamentous fungi have unique and excellent property to degrade cellulosic waste and resulting in the production of α -amylases and other industrially important enzymes, amino acids, antibiotics, organic acids etc. using SSF for past several decades. As these moulds are known for the production of extracellular products such as enzymes. Therefore filamentous fungi are widely exploited for various SSF processes for the production of a variety of industrial products including α -amylase. Production of enzymes as well as other industrially important products by solid-state fermentation (SSF) using agricultural wastes through moulds turned to be a cost-effective production technique and surprisingly most of these processes are optimized. Detailed literature is available on various fungal sources for the productions of amylases are given in the table.

Table 1. Different types of agro-substrate used for α -amylase production using SSF by Filamentous fungus

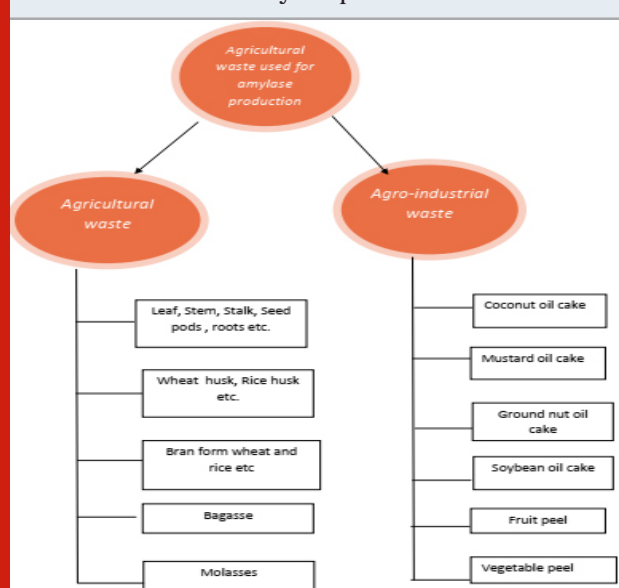
S. No	Organism	Substrate	Activity(U/g)	References
1	<i>Aspergillus oryzae</i>	Spent brewing grain	6583	Francis et al., (2003)
2	<i>Aspergillus oryzae</i>	Coconut oil cake	3388	Vishwanathan et al., (2001)
3	<i>Aspergillus flavus</i>	Amaranthus grains	1920	Ramachandran et al., (2004)
4	<i>Thermomyces lanuginosus</i>	Wheat Bran	534	Kunamneni et al., (2005)
6	<i>Aspergillus oryzae</i>	Ground nut oil cake	9196	Ramachandran et al., (2004)
7	<i>Aspergillus oryzae</i>	Wheat Bran	15095	Gangadharan et al., (2007)
8	<i>Aspergillus awamori</i>	Corn streep liquor	738	Prakasham et al., (2006)
9	<i>Penicillium fellutanum</i>	Soluble starch with sea water	157	Kathiresan & Manivannan 2006
10	<i>Penicillium expansum</i>	Loquat kernel flour	1012	Erdal & Taskin (2010)
11	<i>Aspergillus sp.</i>	wheat bran	164	Chimata et al., (2010)
12	<i>Penicillium brevicompactum</i>	Wheat bran	666.6	Balkan & Ertan (2010)
13	<i>Trichothecium roseum</i>	Wheat bran	1048	Balkan et al., (2011)
	<i>Aspergillus niger</i>	Ipomoea batatas	450	Sunder et al., (2012)
15	<i>Aspergillus niger</i>	Rice Bran	334.51	Rajasekar et al., (2013)
16	<i>Aspergillus fumigatus</i>	Pomegranate peel	341.7	Singh et al., (2014)
	<i>Aspergillus awamori</i>	Casava Peel	31.64	Kalaiaarasi & Pavatham (2015)
17	<i>Aspergillus oryzae</i>	Agro-waste	750	Naili et al., (2016)
18	<i>Aspergillus terreus</i>	pearl millet	19.19	Sethi et al., (2016)
19	<i>Pleurotus ostreatus</i>	Potato peel waste	2503.6	Ergun et al., (2017)
20	<i>Rhizopus deleamar</i>	Apple pomace	21.03	Pathania et al., (2018)
21	<i>Rhizopus oryzae</i>	Bread waste	100	O Benabda et al., (2019)
22	<i>Aspergillus carbonarius</i>	Wheat and brewing residues	404	Pasin et al.,(2019)
23	<i>Aspergillus oryzae</i>	soybean husk and flour mill	47000	Melnichuk et al.,(2020)

4. Agricultural by products in amylase production:

Microbial enzymes are versatile in nature and relatively more stable compared to plant and animal enzymes and having wide range of industrial applications. Since these agricultural residues are rich in cellulosic content along with other biologically active compounds. Therefore these agricultural wastes can be used as alternative solid substrate in SSF for the production of industrially important compounds like biofuel production, animal feed, mushroom cultivation, as well as for the production of industrial products such as enzymes, organic acids, amino acids etc.

These agro industrial wastes can be used in industrial processes for the production of high value industrial products to reduce the production cost which also reduces the pollution load from the environment these agro industrial residues consist of molasses, rice husks, rice bran and wheat bran, bagasse, leaves, straw, stalk, shell, pulp, peel, roots, etc. These substrate traditionally used for animal feed, soil quality improvement, biofertilizers etc. from ancient times, But due to scientific innovations and technology development in agricultural practices huge amount of field residues are generated. Disposal of underutilized agricultural waste is problematic and non eco friendly practice. In oil exploration industries, huge amount of processed residues in the form of oil cake are produced. These residues contain high concentration of fat, carbohydrate suspended solids and as well as dissolved solids are also present. Oil cake is of different types like canola oil cake, sunflower oil cake, coconut oil cake, sesame oil cake, mustard oil cake, soy bean cake, groundnut oil cake etc. (Ramachandran et al., 2007). Different types of agricultural by products which have been used as solid substrate for production of various industrial products including enzymes in fermentation process are summarized in Fig-1

Figure 1: Different types of agricultural waste used in SSF as solid substrate for amylase production



These agricultural wastes are generated throughout the year through various agricultural practices and having various limiting nutrients for the growth of microorganism. Therefore, these substrates having an unlimited prospective to be used as alternative solid substrates in SSF. Researchers are constantly using these agro industrial residues for α -amylase production by using various microbial strains to optimize fermentation process parameters for enhanced production. A similar type of study was carried out by using wheat bran as solid substrate for single cell protein and amylase production by using *A. Niger* as microbial strain. Maximum biomass was produced when media was supplemented with NH_4Cl , NaNO_3 , KNO_3 and $(\text{NH}_4)_2\text{SO}_4$ as additional nitrogen source for enhanced growth of fungal mycelium in submerged fermentation. However media supplemented with $(\text{NH}_4)_2\text{SO}_4$ showed maximum production of biomass and amylase activity i.e. 326 U/L with 6 days of fermentation (Oshoma et al., 2019).

In many countries bread waste is easily available and disposal of these wastes is a problematic affair. To solve these issues researchers are trying to use such waste for production of enzymes i.e. amylase and protease. *Rhizopus oryzae* was used to degrade humified bread waste by SSF with minimal salt solution for production of amylase and protease. Highest amylase activity was observed 100U/g and for protease 2400U/g (Benabda et al., 2019). Since fungi have very efficient enzymatic system therefore fungi having ability to degrade lignocellulosic waste resulting in the formation of many valuable compounds that may be useful for production vast variety of useful products. Two types of extracellular enzymatic system are recognizable in fungi i.e. hydrolytic and ligninolytic system. Lignocellulosic residues from agricultural and municipal solid wastes are particularly abundant in nature and have a potential for bioconversion (Abdel-Azeem et al., 2020).

Brazil is the major producer of citrus low cost source of hesperidin a polyphenolic compound having potential to restrict anti-glycation effects (AGEs) which constitute a vast variety of compounds synthesized due to interaction between amino acids of proteins and reducing sugars which induces chronic diseases through pathogenesis. This research work concludes that inhibition of AGEs results in the reduction of amylase activity 50% proving that there is strong correlation between anti-glycation with polyphenolic content and antioxidant capacity (Fernandes et al., 2020).

CONCLUSION

Agro-industrial waste are rich in various nutrient that contain bioactive compounds makes them favorable for the growth of filamentous fungi in solid state fermentation. Composition of these products ranges from sugars minerals proteins and amino acids etc. therefore these agro-industrial residues are considered as raw material in bio-conversion processes for the production of high value industrial products. The microorganisms having unique properties to use these

solid substrate as raw materials for their growth and nutrition during fermentation processes and results in the formation a variety of biochemical of industrial application. SSF is relatively simpler but cost effective process for the industrial scale optimization and bio-processing. Alternatively, use of agro-industrial wastes as raw materials can help to reduce the production cost as well as recycling of waste make the environment more eco-friendly.

Amylase is the most widely used enzymes among different enzymes and being constantly used in different industrial and medicinal purposes. With the development of new and novel techniques in bioprocess engineering such as Response Surface Methodology (RSM), Artificial Neural Network) ANN based optimization and Bioreactor designing for SSF leads the production of amylase at large scale for reducing the cost and efficient utilization of agro-waste. This review will facilitate for establishing the large scale fermentation process with the use of appropriate fungal strain.

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