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Alternatives for Cellulase Production in Submerged Fermentation with Agroindustrial Wastes

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ABSTRACT: *This article presents a review of the alternatives for cellulase production in submerged fermentation using agroindustrial residues as carbon sources. Among the wastes that are cited, the residue of grapes shows promise for producing these enzymes. The advantages associated with this process refer to the removal of industrial waste from the environment that is associated with the same added value through the production of enzymes.*

KEYWORDS: *Aspergillus niger. Cellulase. Grape marc. Submerged fermentation*

I. INTRODUCTION

The southern region of Brazil stands out in the wine industry by volume and quality of wines. As a consequence of this economic activity, grape residues are produced in large quantity per year. These residues are rich in cellulose and can be reused as a carbon source for several processes, including the production of enzymes.

Cellulases are enzymes that form a complex capable of acting on the cellulose and promote its hydrolysis. These enzymes are commonly used in various areas of industry, including food, beer and wine, agriculture, paper, textiles, detergent and animal feed, is also an alternative for generating energy. This paper presents a review of alternatives for production of cellulases in submerged fermentation using agroindustrial residues as carbon source, emphasizing the grape waste.

1.1. Lignocellulosic Materials, Agroindustrial Wastes: Lignocellulosic materials are the most abundant organic compounds in the biosphere, representing 50% of terrestrial biomass [1], which corresponds mainly by agribusiness materials, the urban waste, and the wood of angiosperms and gymnosperms [2].

According to Castro and Pereira Jr. (2010) [2], the lignocellulosic biomass is composed of three main polymer fractions: lignin, hemicellulose, and cellulose, which are joined to each other by covalent bonds, forming a complex network resistant to microbial attacks.

The cellulose from natural materials is the world's most abundant biopolymer that is formed by residues of β-D-glucose bound together by β-1,4, bonds, and it maintains a linear and flat structure; cellobiose (Fig. 1, adapted of BON, et al., 2008), the disaccharide 4-O-β-D-glucopyranosyl-D-glucopyranose, is the repeating unit of the polymer [3] that can be hydrolyzed to glucose with the help of acids. The microbial degradation of cellulose is total and specific and has encouraged the use of cellulolytic fermentation processes by man. In nature, these processes represent the largest source of carbon to the soil [4].

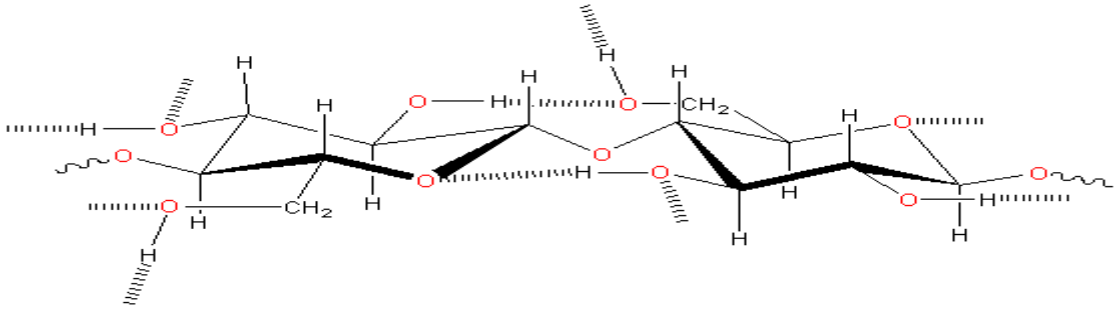

Figure1. Chemical structure of cellulose.

1.1.1. ***Agroindustrial wastes:*** Agroindustrial waste materials are rich in lignocellulosic materials that are inevitably produced by agricultural and industrial activities. The following can be cited as being among the lignocellulosic residues produced in bulk by the Brazilian agroindustrial activity: sugarcane bark, bagasse and straw, rice straw and rice bran, corn cobs and straw, chaff and bran from wheat, banana straw, cotton waste, wood scraps, and waste based on paper. Most of these materials are either partly or entirely not taken, being transformed into pollutants from the environment. Agricultural wastes contain 20–60% cellulose, 20–30% hemicellulose, and 15–30% lignin. The available quantity of these materials in the world is very large [5, 6].

Considering renewable resources, residues has a low cost as raw material for other processes and they can be purchased in regions that are located close to the local processing of the material. Due to the difficulty experienced by cellulose degradation in environmental conditions, cellulosic wastes accumulate, which makes them a nuisance to the environment. The use of lignocellulosic biomass derived from agriculture waste, forestry waste, and sewage can bring savings to the production of fuels and other products as well as reduce waste [2, 7].

1.1.1.1. Wastes from Winery: *According to Mendes and Araújo (2006) [8], more than 20% of the fermentation process of grapes for wine production involves waste that can generate environmental problems; therefore, it is necessary to search for an appropriate destination to them. Among the various solutions to this problem are composting, anaerobic digestion, animal feed processing, the use of seeds to produce oil as biofuel, soil deposition for fertilization, and pyrolysis.*

The same authors claim that in the process of making wine, every 1000 grams of grapes harvested, after crushing, generates about 350 grams of waste (bark, stems, and seeds), which are usually utilized in the soil of vineyards of the wine industry, serving as organic material for fertilization.

According to Mello (2003) [9], the Brazilian wine industry has advanced a great extent in manufactured products such as wines and juices as in the production of grapes for fresh consumption.

In 2009, grape production in Rio Grande do Sul (viniferous and regular) was 534,123,176 pounds. Being the main state producer of grapes and wines in the country, Rio Grande do Sul continues with being the largest area cultivated in Brazil, with 50 400 ha. [10, 11].

Another factor to be considered is the pruning of vines, which releases lignocellulosic material, and burning in the same field is responsible for the formation of toxic compounds emanating from the burning of lignin [6]. In this way, the use of grape marc for the production of enzymes appears as an alternative of biomass emanating from the pruning of the vines.

The grape marc waste differs from the others due to its composition, the presence of protein, starch, and lipids; while the rice husk, sugarcane bagasse, and bark of black acacia have the vast majority of lignocellulosic material composition [12].

In Table 1, we can observe some work that uses agroindustrial residues as a carbon source in the production of enzymes and other products of microbial origin.

Table 1. Agroindustrial wastes as a carbon source in the production of enzymes and other products of microbial origin.

Agroindustrial residue	**Microrganisms used**	**Authors**
Coconut shell	*Aspergillus niger*	COELHO *et al.*, (2001)
grape marc	*Aspergillus phoenicis*	SILVA, (2008)
grape marc	*Aspergillus awamori*	BOTELLA *et al.*, (2005)
grape marc	*Monascus purpureus*	DAROIT *et al.*, (2007)
grape marc	*Monascus purpureus*	SILVEIRA *et al.*, (2008)
grape marc and Orange peel	*Aspergillus awamori*	DÍAZ *et al.*, (2012)
Bagasse from sugarcane	*Aspergillus niger*	AGUIAR *et al.*, (2008)
Sawdust of *Pinus sp* and grape marc	*Pleurotus sajorcaju* PS-2001	DEON *et al.*, (2009)
Coconut shell	*Aspergillus phoenicis*	OLIVEIRA *et al.*, (2009)
Passion fruit peel	*Aspergillus niger*	SOUZA *et al.*, (2010)

As shown in Table 1, the use of waste grape has been described as an alternative substrate for the production of other products of microbial origin, which can be highlighted in the production of β-glucosidases, and pigments by the fungus Monascus purpureus and the production of hydrolytic enzymes such as exo-polygalacturonases, CMC-ase, and xylanase [12-16].

Based on the studies just cited, we can observe the high potential of grape waste as a substrate for cellulase production, with current efforts focusing on the use of agroindustrial by-products as a substrate and looking for products with high commercial value and low cost production from naturally abundant substrates in each region.

1.1.2. Enzymes and Cellulases: Enzymes: *Enzymes are proteins that exhibit catalytic activity. The enzyme complex molecular structure consists of one part of protein, but it can be connected to other molecules such as carbohydrates and lipids [17]. Enzymes are present in all living cells, which exercise the function of catalysts of the reactions that compose the anabolic and catabolic pathways of cellular metabolism [6].*

As Filho (2006) [18] explains, the great interest in the use of enzymes can be explained by several factors, including the large variety of reagents in which the same act, the complex reactions which the enzymes are capable of catalyzing on routes where the generation of waste and by-products is reduced, and that they have the capacity to operate as catalysts at high speeds in conditions of reduced energy needs (mild conditions of pressure and temperature).

The catalytic action of enzymes involves the delivery of a specific environment of the enzyme where the enzyme reaction is energetically more favorable, and this region where the reaction is called active site. The molecule that binds to the active site and acts on the enzyme is called the substrate. In general, the substrate binding site is a slot or groove on the surface of an enzyme, complementary to the shape of the substrates (geometric complementarily). In addition, the amino-acid residues that form the binding site are arranged to form specific interactions of attraction with the substrate (electronic complementarily). The reaction becomes more favorable, because the interaction between the amino-acid residues and the reactant molecules decreases the activation energy that is required for the rearrangement of covalent bonds and the performance of non-covalent interactions between the enzyme and the substrate [18, 19].

1.1.3. Cellulases: *To meet the challenge associated with degrading cellulose, cellulolytic microorganisms produce a complex mixture of enzymes called cellulases. These enzymes, which collectively have links to specific β-1, 4 cellulose, are necessary for the complete solubilization of cellulose, existing synergism between that [6].*

The complex cellulolytic enzymes are hydrolases that cleave O-glycosidic bonds and are classified by the Enzyme Commission (EC) with the coding 3.2.1.x, where the value of x varies with the cellulase evaluated. The classification of cellulases, according to their site of action in the cellulosic substrate, allows them to be categorized into three groups: endoglucanases (EnG), which cleave internal cellulosic fiber bonds; exoglucanases (ExG) or celobiohidrolases (BNG), which work in the external region of the cellulose; and β-glucosidases (BG), which hydrolyze soluble glucose oligosaccharides [2, 6].

Figure 2, adapted of LYND et al., (2002), illustrates the synergistic action between exoglucanases, endoglucanases, and β-glucosidases in the hydrolysis of cellulose fiber.

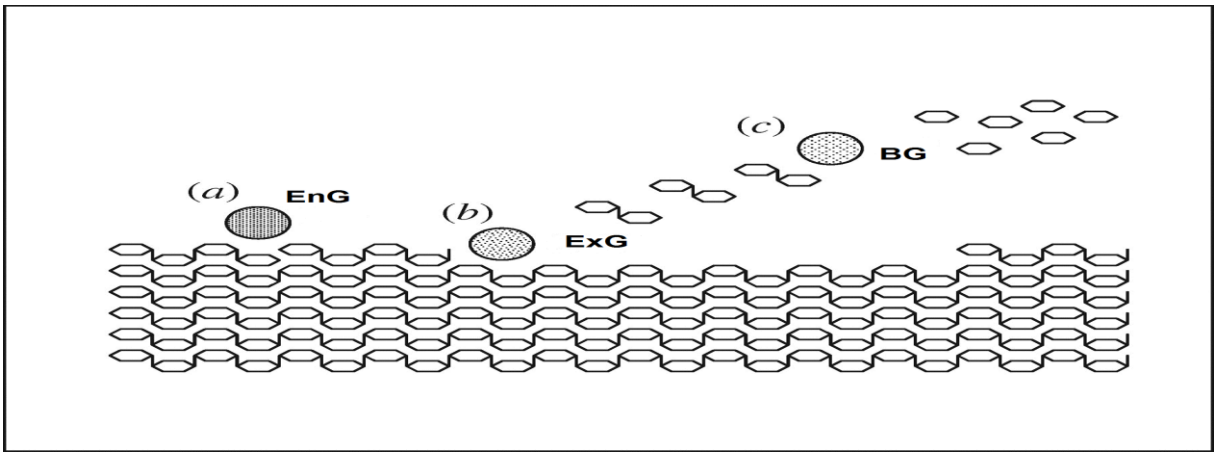

Figure 2. Mode of action of enzymes of the cellulolytic complex

1.1.3.1. Endoglucanases: *Endoglucanases are also known as endo-β-1, 4 glucanase and carboxymethyl cellulase. They catalyze the hydrolysis of internal bonds β-1, 4-D-glucosides of cellulose, randomly generating oligosaccharides of various sizes and, consequently, new chain terminals. Cellulose and xiloglicana serve as their natural substrate. They act only in the amorphous portion of cellulose, and their activity decreases along with shortening the length of the cellulose chain [20].*

1.1.3.2. Exoglucanases: *They act in a progressive way in reducers and nonreducer portions of the cellulose chains, liberating either glucose (glucanohidrolases) or cellobiose (celobiohidrolases) as the main products. They act on microcrystalline cellulose, thereby shortening the polysaccharide chains and have a limited effect on substrates such as carboxymethylcellulose (CMC) and hydroxyethylcellulose (HEC) [21].*

1.1.3.3. β-glucosidase: *This is necessary to hydrolyze short-chain oligosaccharides and soluble cellobiose into glucose, and can also be called β-D-glucoside glucohidrolase (EC 3.2.1.21). It loses activity with increasing the length of the cellulose chain and also performs the hydrolysis of terminal β-D-glucose oligosaccharides [20, 21, 22].*

When working together, the complex cellulolytic enzymes have a better yield than the sum of the individual income, that is, when acting in isolation from each other [2].

1.2. Characterization and properties of cellulases: *With the aim of using cellulases in industrial processes under the conditions of their best performance, it is essential that their properties are determined, especially with regard to kinetic factors and physical chemists [2].*

Another property of cellulolytic enzymes commonly reported in the literature is their ability to be influenced by other molecules, especially metals, and this characteristic is suffering from inhibitory or inductive effects at the moment. Among the ions studied, the ones that more often inhibit cellulases are those of mercury, copper, silver, and zinc (Hg^{+2}, Cu^{+2}, Ag+, and Zn2+), which even lead to the total loss of catalytic activity, and are present at concentrations as low as 2.0 mM [2]. For the characterization of crude cellulase preparations with regard to their activity, different substrates of endoglucanases are used, stressing, however, that the synergy between the two types of enzyme prevents a precise quantification [6]. A substituted cellulose derivative such as carboxymethyl cellulose (CMC), which is soluble, is used as a substrate for endoglucanase activity. The enzyme attacks the polymer in a random mode, producing a rapid change in the degree of polymerization. After the enzymatic reaction, the formation of reducer sugars is determined, which is known as CMCase activity. The microcrystalline cellulose is one of the substrates used in the tests for measuring the activity of exoglucanases. The measurement of enzyme activity is commonly used as a reference for determining the activity in both cellulosic global academic work and for commercial enzyme preparations [6,23].

1.3. ***Applications of cellulases:*** Cellulases have been investigated mainly with regard to their potential as an additive in the detergent industry, textile industry, and also in the bioconversion of agricultural biomass into products with commercial value [6]

In the food industry, cellulases are used in maceration processes, usually along with hemicellulases and pectinases, such as the extraction of fruit juice and oil seeds. They also have an important role in the filtration and clarification of fruit juices, which increases the effectiveness of the extraction of color and juices in the liquefaction of plant tissue, thereby allowing for a better extraction of pigments from fruits [22, 24-27].

Cellulases have great potential use in the production of glucose syrups from cellulosic materials that compete with starch and sucrose in the production of alternative sweeteners which are used in food and beverage industries. Hydrolyzed cellulose can also be used as a nutrient in fermentation for the production of various chemicals, including enzymes for food processing and food ingredients such as citric acid and acetic acid, and amino acids [6].

The animal feed industry moves a market of more than 50 billion dollars worldwide. In ruminants, the use of cellulases, along with pectinases and hemicellulases, increases the digestion of forage plants, the basis of animal nutrition, and, thus, increases the quality and digestibility of feed [22, 24, 26].

In the textile industry, cellulases are used for the removal of excess dyes in denim fabrics, a process called Bio-Stonewashing [27]. Other benefits associated with the addition of these cellulases are a reduction in the time of use of the machines involved in the process, increased productivity, improved security conditions in the work environment, and conditions of process automation [22, 24, 28, 29]. Cellulases are also present in the Biopolis tissue process, that is, the removal of surface fibrils caused by pilosity, which increases with use and washing processes. These enzymes then promote the reduction of the tendency of pellets, which is actually the formation of small balls in the tissue [6]. A major challenge associated with the application of cellulases in the textile and laundry industries is the correct use of formulations for the use of enzymes [24].

In the manufacturing of pulp, woody materials that harm the quality of pulp are removed. The use of Trichoderma cellulases in the process allows an energy savings of 20% but can reach up to 40%, depending on the time and type of the enzyme applied. The use of cellulases is present in the modification of the properties of the fibers, increasing the speed of manufacture of paper [24].

There is a worldwide trend of enzymatic hydrolysis of lignocellulosic materials, seeking to ferment sugars for the production of bioethanol on a large scale [30]. An obstacle to the use of cellulases is the cost of production, which can be overcome using genetically modified organisms (bacteria, yeasts, and plants) for the production of enzymes, and the need to produce more efficient enzymes [31]. Another option is the use of agroindustrial residues that serve as a carbon source, promoting the reduction of production costs of cellulases through the economy in the purchase of raw materials, and also collaborating with the environmental problem of waste generation from these industries.

1.4. Enzyme Production by Microorganisms: *Microorganisms are the main sources of industrial enzymes that are unique because of their wide variety of catalytic activities, the possibility of the production of enzymes by fermentation processes, and the large-scale regularity and simplicity of the nutritional requirements [6].*

1.4.1. Characteristics and nutrition: *According to Bon et al. (2008) [6], the first step in the microbial production of an enzyme of interest is the identification and acquisition of the producer organism, which can either be a wild strain or one that is modified by molecular biology techniques. The lineage of interest can be either acquired from culture collections or scientific service, or selected from samples of soil, water, air, and plant tissues such as stems or decaying fruit and other sources. Additionally, according to the same authors, ideally, the microorganism that is used in the case just mentioned should have the following characteristics: It should be safe from the biological point of view; have a high capacity for the synthesis and excretion of the enzyme; withstand environmental conditions related to osmotic pressure, temperature, and ionic strength; and be tolerant to the presence of toxic substances that can be generated during the treatment process of raw materials or by cellular metabolism.*

The maintenance and preservation of organisms are extremely important steps that ensure their viability and prevent genetic changes which lead to a reduction or loss of phenotypic properties [6].

1.4.1.1. Culture media in the production of enzymes: *The physicochemical characteristics of the culture media are of fundamental importance, not only for cell growth but also for the yield of a product. This is because the cells are able to respond to physical and chemical stimuli from the external environment through biochemical mechanisms that regulate gene expression and physiology of the organism and, by extension, its performance in the formation of the desired product [6].*

The physical and chemical constituents of the medium, such as nutrient composition, pH, temperature, dissolved oxygen, and mechanical forces, exert changes in the formation of mycelium [32]. The study of variables such as the formation of pellets is essential; it is a positive factor for citric acid production by A. niger; whereas it is harmful for the production of amylase [33].

The use of different nitrogen sources allows greater productivity in the secretion of cellulase enzymes, as seen in the work of Hanif et al., (2004) [34]. During hydrolysis, some factors can interfere by way of access to the surface area due to the porosity of the material, the presence of crystalline cellulose fibers, and the presence of lignin and hemicellulose, which hinder access to the cellulose enzyme, thus resulting in a reduced-efficiency hydrolysis process [31].

Different proportions of grape residue and culture media were analyzed by Silva (2008) [12], with the objective of determining the best ratio between the substrate and culture medium for cellulase production by Aspergillus phoenicis. For

total cellulase activity, higher activity was observed after treatment with 15 g/L of grape residue and 7.5 g/L of peptone (0.094 FPU). However, when the concentration of peptone decreased to 1.5 g/L or increased to 8.5 g/L, a low enzyme activity of 0.029 FPU occurred. The change in the concentration of grape residue of 3 g to 17 g caused a reduction in enzyme activity, averaging 0.034 FPU, which is significant for a confidence level of 95%.

In this sense, it is important to consider to what extent the increase in substrate concentration will increase the production of cellulase, without any form of inhibition, which would cause waste of raw material and growing medium, affecting the yield of the process. This is a definite factor, as the culture medium contains all the nutrients necessary for the organism to develop and generate product interest.

1.4.2. Fungal decomposers of lignocelluloses: *Fungi are one of the most important groups of microorganisms in the activity of decomposition of organic matter due to their expertise in degradation. This activity occurs mainly through their mycelium or vegetative stage. During vegetative and reproductive phases, the formation of biomass depends on the production of extracellular enzymes, which are fundamental components of the degradation of substrates, mainly lignocellulose [35].*

Cellulases as well as other extracellular enzymes that are required for hydrolysis are induced and secreted by microorganisms to grow on cellulose. Among them are cellulases produced by fungi of the genus Trichoderma, Penicillium, and Aspergillus [36].

The most common genus of filamentous fungi is Aspergillus, and it is also one of the most well studied. The species composing this genus are widely distributed worldwide and are present in the earth's surface, air, and water, in both the bodies of plants and animals, besides being associated with the deterioration of plant materials and food, especially in tropical and sub-tropical regions. Many Aspergillus species are used to obtain enzymes in the chemical biosynthesis and processing of compounds [37].

Despite the existence of pathogenic species, the genus is present in the food and industrial microbiology like positive, so that micro-organisms of the species Aspergillus oryzae and Aspergillus niger are considered safe, according to the Food and Drug Administration (FDA), an agency responsible for the control of food and medicine in the United States of America, being generally named and recognized as Safe (GRAS). Industrially, the genus is present in the production of enzymes, drugs, and antibiotics that are used in food fermentation [32, 33, 38-40]. The genus Aspergillus is widely reported in the literature as being a producer of cellulases [13, 20, 22, 41-44].

The fermentation kinetics of Aspergillus niger was studied by Aguiar et al. (2008) [45], using sugar cane bagasse as a substrate. The fungus Aspergillus niger used proved to be a producer of cellulases under the conditions studied, with activity equal to 0.408 UI. There was also a decrease in pH, due to the production of organic acids by the fungus (for example, citric acid, gluconic, oxalic, etc.).

In the study of Coelho et al. (2001) [7], Aspergillus niger was used for the production of enzymes (cellulases, xylanases, and pectinases) with green coconut shell as a substrate, because this material contains large amounts of compounds such as cellulose, hemicellulose, pectin, and others, with no need for large nutrient additions that would enhance appropriate microbial development.

However, Silva (2008) [12] observed differences in the results of enzymatic activities among isolates of the same genus, which shows that not all members of the same genus have the same potential for the production of enzymes.

As observed in the work just mentioned, Aspergillus niger is a great producer of enzymes such as cellulases, presenting a great potential for the production thereof with various cellulosic substrates, and is also very promising; therefore, the use of grape waste for the production of enzymes, given the need for regional use of this residue.

1.5. Bioprocesses for Enzymes Production: *The industrial enzymes are usually produced by microorganisms, although some of them are extracted from animal and plant tissues [6]. According to Lima et al. (2001) [46], the production of enzymes on an industrial scale is done mostly by submerged fermentation; whereas in eastern countries, there is an established tradition of using solid-state fermentation.*

In any bioprocess, biomass is a crucial parameter for the characterization of cell growth [6]. The bioprocess can be divided into two kinds: The first kind indicates the production of biomass, whereas the second kind involves the production of metabolites (primary or secondary). In the first case, the measure of biomass is very important, because it is the main objective of the process of submerged or solid-state fermentation. The production of these metabolites can be proportional to the amount of biomass. Thus, in order to improve the production of metabolites, especially a secondary metabolite, it is necessary to improve cell growth [6, 17, 42].

Environmental factors such as temperature, pH, water activity, oxygen levels, and concentrations of nutrients and products significantly affect microbial growth and product formation. In submerged cultures, environmental control is relatively simple due to the homogeneity of the suspension of microbial cells, the nutrient solution, and the products in the liquid phase [17].

1.5.1. Solid-state Fermentation: *The term solid-state fermentation (SSF) is described as the fermentation in which the growth of microorganisms in solid substrates occurs in the absence of liquid in the free form. The free water is essential to the growth of microorganisms and is either adsorbed on a solid support or complexed within a solid matrix. The SSF is considered more natural than other types of fermentation, because their processes are similar to the conditions under which most microorganisms grow in nature [17].*

1.5.2. Submerged Fermentation: *The submerged fermentation is also known as submerged culture, and its main characteristic is the use of a liquid fermentation media with soluble nutrients. This fermentation process can be performed in shaken flasks (Erlenmeyer flasks, for example), on a fermenter's bench, or on an industrial-scale fermenter [47]. In this type of fermentation, the substrate is dissolved or suspended in a water source where water is not a limiting factor [1], and it can generate different metabolites [32, 33]. Among them, it is worth mentioning enzymes, antibiotics, organic acids [32], carotenoids, biotin [33] recombinant proteins [33, 35, 48], and amino acids [49].*

Most commercial enzymes are obtained by submerged fermentation, as modern methods of control are more easily adapted to fermentation, the yields are higher, and the costs and risks of contamination are lower [12].

For the successful cultivation of filamentous fungi under water, it is necessary to study several variables. Operating parameters such as pH, temperature, oxygen consumption, and carbon dioxide formation are measured and tightly controlled [6, 50]

According to Volpato (2009) [51], submerged cultures may occur in two ways: in a rotating incubator, where the control of pH and oxygen transfer is more difficult, or in bioreactors, which enable the control of various parameters such as temperature, pH, agitation speed, and pressure, among others.

With regard to the semi-solid culture, submerged cultivation has the advantage of being able to have better rationalization and standardization of the process, which is crucial for the industry and allows a homogeneous culture system. Thus, according to Silva (2008) [12], the main advantages of the submerged processes are ease in controlling the physicochemical process, greater efficiency of nutrient absorption, and excretion of metabolites through the cells, leading to lower process times and, consequently, productivity gains.

Among the disadvantages, the following can be cited: the high cost of aeration and agitation, especially when used a reaction media with high viscosity and complex rheology; Foaming.

The submerged fermentation is used to produce several enzymes of industrial interest, as described in several studies. These can be cited in the submerged fermentation in bottle production by recombinant yeast endoxilanases P. pastoris using culture media containing "Yeast Nitrogen Base" (YNB), peptone, and yeast extract as sources of nitrogen and methanol as carbon source [52]. The lipase production in submerged cultivation allows the obtention of 3.15 U / mL in 96 hours of fermentation in synthetic medium and of 2.22 U / mL at 72 hours of fermentation in the industrial environment, with the fungus Penicilllium verrucosum [47] showing that the crop is promising for various purposes.

Among the challenges associated with submerged growth are the mechanisms of growth of the fungus, the dynamic aggregation of the mycelium, fragmentation, quantification of the morphology, and mathematical models for the growth and formation of products of cultivation [32]. A better system of homogenization with submerged growth is achieved by agitation, so that in this way, the pellets are not formed, the whole system hifal may be exposed to the environment. Therefore, the limitations imposed by lack of oxygen and reducing the rate of diffusion of nutrients can be overcome [53]; however, one should consider that the processes carried out in shaken flasks have difficulty in controlling certain parameters, such as, for example, aeration [47].

Among the studies using submerged cultivation, we can highlight Sridevi (2009) [54], who evaluated the capacity of cellulase production by Aspergillus niger in pretreated lignocellulosic residues such as sawdust, wheat straw, bagasse, and rice bran in submerged fermentation, obtaining satisfactory results, as seen in Alberton (2004) [17], who studied the use of different natural substrates in the production of xylanase, and Aguiar and Menezes (2000) [55], who studied the production of cellulase by Aspergillus niger IZ-9, grown on sugarcane bagasse chemically treated, as a substrate.

Many research groups distributed around the world have been studying strains of microorganisms with high cellulolytic activities, different lignocellulosic materials as a carbon source, and the stabilization and immobilization of these enzymes with a view to their application in large-scale processes. In this aspect, the use of grape waste as a carbon source for cellulase production proves to be very promising, possibly because of it being a residue that can be used on an industrial scale in the future. It is important to stress the need to use controls, both the ability of cellulase production by the strain chosen as the capacity of the grape marc serve as a carbon source, using data obtained from literature, to give credibility to the results obtained.

1.6. Market and Perspectives for Use of Enzymes in Brazil: *According to data from the Ministry of Development and Foreign Trade of the federal government on the enzymes market in Brazil, in 2005, the total Brazilian imports of enzymes were $ 95.7 million, while the exports were $ 5.4 million, showing that the Brazilian market is essentially an importer and also indicating strategic and technological disadvantages in terms of production and the use of enzymes in the country. Thus, the use of bagasse is an alternative to production the oxidative and hydrolytic enzymes of biotech industries to obtain a cheaper cost compared with enzymes that are available in the market [6].*

The use of enzyme catalysts in many different industries has been a growing trend worldwide, and the total market value is estimated at 2.3 billion dollars annually [27]. The market is divided into three segments: the enzyme techniques (formed by detergent, starch, textile, ethanol, pulp and paper pulp, and leather industries), enzymes in the food industry, and animal feed enzymes [27, 28].

Advances in enzyme technology in Brazil are favored by the enormous quantity and variety of renewable raw materials that can be enzymatically transformed into useful products for the strategic sectors of the economy. There are also the country's knowledge of the technologies used for the production of enzymes on a large scale, and extractive fermentation processes, as well as the greatest biodiversity as a source of biocatalysts [6].

II. CONCLUSIONS

It is possible to produce cellulases from agroindustrial residues as the residue of grapes, but larger studies are needed in order to quantify these enzymes, their separation, and the optimization of the production process, so that they could be later used in a pilot-scale production of the same or at even an industrial scale.

With regard to the environmental question, one should always stress the importance of removing the residual of the environment and adding value to it, so that industries in various sectors such as food, textiles, and beverages could later take advantage of this proposal through the use of cellulases that are obtained from grape residue. For this to happen, further studies are needed for the purification of enzymes and their marketing, so that regional demands are met by these enzymes.

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