Vol. 20(2), pp.100-107, February, 2021
DOI: 10.5897/AJB2020.17265
Article Number: 5C9A0D866176
ISSN: 1684-5315

http://www.academicjournals.org/AJB

ACADEMIC JOURNALS
expand your knowledge

African Journal of Biotechnology

Review

Aspects of mushroom cultivation to obtain polysaccharides in submerged cultivation

L. H. Campestrini* and Salles-Campos C.

Laboratorio de cultivo de fungos comestíveis, Instituto Nacional de Pesquisas da Amazônia, 13418-900, Manaus, AM, Brazil.

Received 22 October, 2020, Accepted 27 November, 2020

Medicinal and edible mushrooms have biological properties that are important in promoting health. In the last decades, an increasing number of studies have explored various aspects of the cultivation and metabolism of various species. Many isolated substances have potential for use in medicine and among them are polysaccharides, which have been shown to have physiological properties such as anti-tumor, immunomodulatory, anti-hypercholesterolemia, antiviral and antiinflammatory. The most common way of obtaining products from mushrooms is through cultivation in a solid medium. However, in this type of cultivation there is a difficulty in controlling the physical and chemical parameters and, consequently, the quality of the product. In this context, the submerged fermentation of the mycelial form of the mushroom has received attention as an alternative for the efficient production of biomass and, therefore, of polysaccharides. Technical problems and forms of cultivation are studied so that the standardization of cultivation is more effective. This review describes some aspects about the submerged fermentation technique in bottles and bioreactors, exploring the two main ones, which are the stirred tank fermentator and the air lift reactors and the species that are in these cultivated systems.

Key words: Mushrooms, polysaccharides, submersed fermentation, biorreactor.

INTRODUCTION

In recent decades, a large number of publications have presented the possibility of using mushrooms in the production of several metabolites of interest of low molar mass (Shu and Lung, 2004; Zou, 2006; Tang et al., 2007) and high molar mass, such as polysaccharides, which are shown as potent pharmacological agents with diverse activities. In addition, mushrooms have been widely consumed for centuries in the East and Europe, given their high nutritional value, with around 2000 edible species recognized and approximately 25 grown on a commercial scale (Jong and Donovick, 1989; Furnali and Godoy, 2007). In its composition, there are essential and non-essential amino acids, carbohydrates, organic acids, minerals, vitamins low lipids and high protein and fiber contents (Ghorai et al., 2009; Kawagoe et al., 2004; Sales-Campos et al., 2011a, 2013).

Special attention is given to fungal polysaccharides because they have biological activity as antioxidant, antitumor, hypoglycemic, aintinflammatory and immunomodulatory (Bai et al., 2020; Fan et al., 2007;

*Corresponding author. E-mail: lcampestrini@gmail.com.

Zhang et al., 2011; Silveira et al., 2015; Wang et al., 2019). Polysaccharides are metabolites of great interest in the cultivation of basidiomycetes. The molecular arrangement of these polysaccharides is well distributed, but most belong to the groups of β-glucans, whose main chain connections are of the type β-(13) and varying number of β-(16) branches (Seo et al., 2019). However, at least one example of a β-(13) glucan, containing β-(12) and β-(16) branches has already been described (Ruiz-Herrera and Ortiz-Castellanos, 2019).

Traditionally, mushrooms are grown on a solid substrate under controlled temperature and humidity conditions. However, this method requires a long cultivation time and low yield, in addition to the possibility that the culture is contaminated by other microorganisms (El-Enshasy et al., 2010).

In addition to solid-state crops, fungi are grown in liquid media in bottles or bioreactors. This type of cultivation presents a fast, efficient and less expensive alternative form (Wu et al., 2003, 2004; Tang and Zhong, 2002), in which organisms can be obtained in limited physical spaces under conditions controlled and optimized for the production of biomass, exopolysaccharides (EPS) and endopolysaccharides (IPS), simultaneously (Cui et al., 2006; Confortin et al., 2008; Liu and Zhang, 2019; Guillén-Navarro et al., 1998), also showing the ease of separating the mycelium from the culture medium, obtaining products of uniform quality, in addition to the possibility of being cultivated all year round (Leonowicz et al., 1991; Rosado et al., 2002).

Many species are grown in flasks of liquid medium or even in bioreactors, including *Lentinus edodes, Ganoderma, lucidum, Schizophyllum commune, Trametes versicolor, Inonotus obliquus* and *Flammulina velutipes*. For the production of polysaccharides, an important step in submerged culture is the type of bioreactor used, since it directly influences the development of the mycelium. When it comes to flasks or stirred tank reactors, even optimized conditions can behave differently (Burns et al., 1994; Maziero et al., 1999; Rosado et al., 2003; Zhang et al., 2003).

Many studies are carried out with the aim of optimizing culture media for the production of fungal metabolites using statistical techniques (Fan et al., 2007; Joo et al., 2004; Assis et al., 2013). Studies, such as the one carried out using the fungus *Grifola frondosa*, were conducted in order to optimize the production of biomass and exopolysaccharide by response surface techniques (RSM) (Cui et al., 2006).

The bioprocesses used in the cultivation of fungi are carried out under optimal conditions such as temperature, pH, aeration, types of nutrients, pressure, type of reactor used, shear stress, the use of surfactants or defoamers and produced biomass. These processes involve complex and multiple biochemical reactions as well as a particular kinetics for each type of organism grown (Garcia-Ochoa and Gomez, 2009).

Thus, the objective of this work is to review some fungi of interest grown in a submerged manner for the production of polysaccharides, in flasks and bioreactors in agitated and pneumatic tanks.

CULTIVATION IN SUBMERGED FERMENTATION: A NEW PERSPECTIVE OF CULTIVATION

The submerged fermentation process is the process of cultivating microorganisms in liquid culture medium. This type of cultivation has several advantages over solid state fermentation. One of the main advantages is the possibility and ease of homogenization during the process. Homogenization is possible, as the medium is easily mixed, eliminating the differences that are generated as the microorganism grows. The liquid medium allows important factors, such as temperature and pH, to be easily measured and controlled. In addition, the consumption of nutrients is more complete, allowing maximum use of nutrients and maximum growth. These variables can be measured and controlled due to the greater precision of the samples taken during cultivation (Rossi et al., 2002; Elisashvili, 2012). Another advantage associated with submerged fermentation is the speed with which crops are grown, reducing production time and, consequently, the chances of contamination occurring. This is due to the ease with which nutrients are dispersed in the environment, maximizing the contact area between microorganisms and nutrients (Wu et al., 2003).

Cultivation can be carried out in static and agitated flasks, with forced aeration and also in bioreactors. The studies in small flasks aim to evaluate the best cultivation conditions so that the process can be scaled, reducing the risk of failure in the process at the industrial level. The cultivation of filamentous fungi by submerged fermentation is already a widespread practice, with a primary focus on obtaining products from primary or secondary metabolism secreted in the medium such as proteins, enzymes, acids, antibiotics, exopolysaccharides (EPS) intracellular polysaccharides (IPS) and other bioactive, medicinal and/or industrial compounds and also biomass for use as a food supplement (Saeki et al., 2011; Elisashvili, 2012; Vamanu, 2012; Singh et al., 2013).

Cultivation in flasks

The medium used for the cultivation of microorganisms must contain in a balanced way all the elements and nutrients for the synthesis of cellular substances and for the production of metabolites. Chemically defined culture media are used in laboratory research, but on an industrial scale, for economic reasons, complex substrates of variable composition are used, by-products of other industries. These substrates must be adapted for

cultivation by means of extraction, supplementation and balancing the composition of the medium in order to increase the production yield of the selected microorganism, using screening experiments prior to cultivation in bioreactors or even aiming at the production of polysaccharides in the plants themselves (Crueger and Crueger, 1990).

Submerged fermentation techniques are widely developed to be applied to most medicinal mushrooms for the purpose of mycelium propagation (Smith et al., 2002). Factors such as nutrients (macronutrients and micronutrients), temperature, pH and agitation are factors that can influence the growth rate of macromycetes, the biomass yield and the production of metabolites (Sales-Campos et al., 2011b). Thus, it is essential to optimize the parameters in advance and to maintain them during the cultivation period (Singh et al., 2013).

Fungi of the genus *Pleurotus*, are widely studied and reports for cultivation methodologies are described for several strains (Table 1).

Pleurotus sajor-caju, grown in flasks, were studied for the optimization of the medium, with the different variables being a source of nitrogen $(NH_4)_2SO_4$, yeast extract and soy peptone. For this fungus, two concentrations of $(NH_4)_2SO_4$ and yeast extract were tested in addition to the high concentration of soy peptone, which caused the EPS concentration to reach 0.60 g/L (Assis et al., 2013). The cultivation of this species served as a model for an optimization process based on the response surface methodology, taking into account three parameters, with a number of combinations of 2^3. In 500 mL flasks, Erlenmeyer flasks containing 100 mL of medium were established that the highest concentrations of soy protein and yeast extract, combined with the lowest concentration of $(NH_4)_2SO_4$, were more efficient in biomass production (Confortin et al., 2008).

Also for *Pleurotus djamour*, whose optimization of the carbon source (glucose) and pH were tested as a function of biomass and EPS production, showed that the initial combination of 40 g/L of glucose and pH 3.0 decreased the consumption time of carbohydrate when compared with the other conditions (Borges et al., 2013).

Maftoun et al. (2013) cultivated *Pleurotus ostreatus* of 250 and 50 mL of medium. 7 different means were tested in order to select the most suitable one. For the growth of biomass and generation of EPS, the medium containing glucose and corn steep liquor as a source of carbon and peptone and yeast extract as a source of nitrogen, favored the production of EPS over the other media. In this study, the kinetics of obtaining EPS showed a dependence on the carbon source, especially glucose. Another similar experiment conducted by Gern et al. (2008) studied the effect of different culture media on the production of biomass and polysaccharides in the same species. The media placed in 500 mL Erlenmeyer flasks containing 100 mL of the media showed different growth rates, affected by the interaction of the factors. Differently from what was previously reported, the growth rate is affected by the interaction between the factors, with organic nitrogen increasing the studied variables and the inorganic nitrogen having a negative effect, except in the global polysaccharide productivity.

In two other strains, *P. ostreatus* “Florida” and *Pleurotus ostreatoroseus*, Rosado et al. (2003) used a medium containing a high concentration of glucose as a carbon and peptone source, yeast extract, K_2HPO_4, $MgSO_4 \cdot 7H_2O$, $(NH_4)_2SO_4$ in pH 6.0, in 500 mL flasks with 100 mL of medium. The results showed that for this medium, low concentrations of $(NH_4)_2SO_4$ and high concentration of glucose favored both fungi with higher EPS production, 22.8 g/L for *P. ostreatus* “Florida” and 16.8 g/L for *P. ostreatoroseus*.

The cultivation of *Agaricus brasiliensis* was performed, aiming at the production of EPS, in which sucrose, yeast extract, pH at 6.1 and temperature of 30°C were the most effective in increasing EPS production (Fan et al., 2007). This type of behavior shows that different species have different cultivation behavior and, therefore, there is a need to adapt the culture medium.

Ganoderma lucidum, widely cultivated for its nutritional characteristics, is also among the most cultivated by the submerged environment. In flask cultivation, to develop effective and economical solid seeds suitable for use in submerged culture for the production of EPS, four supplements were examined as sources of nitrogen for the formulation of substrate with sawdust as the basal ingredient. The higher biomass and EPS yield obtained for the four solid test seeds on the liquid seed were attributed to the formation of a large number of growth points of hyphae in cultures, resulting from the dissociation of inoculated solid seeds into numerous tiny particles (Liu and Zhang, 2019).

Cultivation in biorreactors

Larger-scale crops use bioreactors, which are closed growing equipment, in which fermentation processes take place. The size of the equipment varies according to its use and the object of use, since they are adapted to the research and industrial scale. The term fermenters is used because primarily, bacteria and yeasts were cultivated and as a rule, almost all are anaerobic (Oosterhuis et al., 2013, Camelini et al., 2014).

In the same way that flask culture media are optimized for the production of the metabolites of interest and for switching to production in a bioreactor, in the latter, there will also be a need for scaling in relation to thermodynamic aspects, fluid dynamics, transfers of gases and mass, need for aeration and agitation must be carried out (Schmidell, 2001).

Within the universe of cultivation in bioreactors, there is a series of equipment that can be used for the cultivation of fungi. The materials from which they are manufactured

Table 1. Current state of cultivation of mushrooms and polysaccharides production in submerged culture.

Mushroom	Cultive type	Result	Reference
Agaricus brasiliensis	Flasks 250 - 50 mL medium	382 mg/L	Fan et al. ,(2007)
Agaricus brasiliensis	Stirred tank bioreactor - 5 L working volume	1.67 g/L	Zou (2006)
Agaricus brasiliensis	Stirred tank bioreactor (Inceltech) - 1 L	321.2 mg/L	Fan et al. ,(2007)
Agaricus blaezei	Flasks 500 - 100 mL medium	0.84 and 1.269 g/L	Hamedi et al., (2007)
Agrocybe cylindraceaa	Stirred tank reactor (KoBiotech - South Korea) - 5 L	3.0 g/L	Kim et al. ,(2005a)
Antrodia camphorata	Stirred tank reactor (Biostat B, B. Braum, Germany) - 5 L	5.05 mg/g	Shu and Lung (2004)
Auricularia polytricha	Stirred tank reactor (KoBiotech - South Korea) - 5 L	3.1 g/L	Xu and Yun (2003)
Auricularia polytricha	Flasks 250 - 50 mL médium	2.12 g/L	Xu and Yun (2003)
Ganoderma lucidum	Flasks 250 - 50 mL medium	1.33 g/L	Liu and Zhang (2019)
Ganoderma lucidum	Biorreactor in lab scale	5.23 mg/mL	Petre et al., (2010)
Ganoderma lucidum	Flasks 250 - 40 mL medium	IPS: 0.130 g/L; EPS:1.20 g/L	Fang and Zhong (2002)
Grifola frondosa	Stirred tank reactor (KoBiotech - South Korea) - 5 L	EPS: 7.2 g/L	Kim et al., (2007)
Grifola frondosa	Reactor with three six blade discs (Biostat C10-3, Germany) - 15 L	EPS: 1.326 g/L	Cui et al., (2006)
Inonutus obliquus	Stirred tank reactor (KoBiotech - South Korea) - 5 L	0.495 g/L	Kim et al., (2005b)
Lentinula edodes	Stirred tank reactor (Inaltec) - 1 L	1.44 g/L	Garcia-Cruz et al., (2020)
Lentinula edodes	Biorreactor in lab scale	4.75 mg/mL	Petre et al., (2010)
Lentinus edodes	Biorreactor – 10L	Not informed	Wang et al., 2019
Phellinus gilvus	Stirred tank reactor (KoBiotech – South Korea) - 5 L	5.3 g/L	Hwang et al. , (2004)
Phellinus baumii	Stirred tank reactor (KoBiotech – South Korea) - 5 L	3.59 g/L	Hwang et al., (2004)
Phellinus linteus	Stirred tank reactor (KoBiotech – South Korea) - 5 L	2.43 g/L	Hwang et al., (2004)
Pleurotus Sajor-caju	Flasks 2 L	3.84 g/L per h	Assis et al., (2013)
Pleurotus Sajor-caju	Stirred tank reactor (Biostat B, B. Braum, Germany) - 5 L	0.94 g/L	Silveira et al., (2015)
Pleutorus djamour	Stirred tank reactor (Biostat B, B. Braum, Germany) - 5 L	32.2 mg/L h	Borges et al.,(2013)
Pleurotus ostreatus	BioFlo 310 biorreactor (New Brunswick) - 5L	IPS: 1.15 g/L; EPS: 2.0 g/L	Vamanu (2012)
Pleurotus ostreatus	Biorreactor in lab scale	5.10 mg/mL	Petre et al., (2010)
Pleurotus ostreatus	Flasks 250 - 50 mL medium	1.48 a 1.52 g/L	Maftoun et al., (2013)
Pleurotus ostreatus	Stirred tank reactor 16L (Bioengeneering, Switzerland) - 8 L	EPS: 0.55g g/g	Maftoun et al., (2013)
Pleurotus ostreatus	Flasks 500 - 100 mL medium	20.05 mg/L	Gern et al., (2008)
Pleurotus ostreatus	Stirred tank reactor (Biostat C15 L, Germany) - 15 L	0.69 g/L	El-Enshasy et al.,(2010)
Pleurotus ostreatus	Flasks 250 - 50 mL medium	2.1 g/L	El-Enshasy et al., (2010)
Sarcodon aspratus	Stirred tank reactor (KoBiotech - South Korea) - 5 L	2.68 g/L	Joo et al., (2004)
Tuber melanosporum	Flasks 250 - 180 mL medium	EPS: 7.09 g/L; IPS: 4.43 g/L	Liu et al., (2009)
Tuber sinense	Flasks 250 - 180 mL medium	EPS: 5.45 g/L; IPS: 2.40 g/L	Tang et al., (2008)

also vary from stainless steel to glass, which can withstand variations in pressure and temperature, especially at the time of sterilization. Another advantage of bioreactors over flask culture lies in the control factor. The bioreactors are equipped with temperature, pH and oxygen availability control systems by coupling sensors, which are also sterilizable. Unlike flask cultivation, bioreactors allow sampling and thus the process

can be followed over the culture period (Oosterhuis et al., 2013; Xu et al., 2013).

As the culture is established, the rheological parameters in the medium are modified by the increase in mycelial mass and by the production of metabolites secreted into the medium. This modification also alters the transference and in the case of filamentous fungi, this viscosity determines its growth mode in the free form or forming pellets. The form of pellets is widely observed in the cultivation of fungi and this morphology helps to avoid variations in the density of the medium. At the same time, this formation interferes with the rheological properties that can cause difficulty in transferring oxygen to the interior of the pellets, generating anaerobic conditions (Prosser and Tough, 1991; Rossi et al., 2002).

In the industry, two types of bioreactors are widely used, according to the characteristics of the cultivation and the microorganism used: those with agitated tanks and tires (Chisti, 1989; Kavanagh 2005). Currently, what limits the full use of the potential of bioreactors and the scheduling of processes for some types of microorganisms is the limited knowledge of bioprocess engineering (Elisashvili, 2012).

The cultivation of fungi in bioreactors

The most mentioned type of bioreactor used in fungus cultivation is the stirred tank reactor (STR), which is equipped with a system of blades or turbines to agitate and aerate the culture medium (Table 1). This model has a sophisticated construction that includes axles, supports and blades, which influences the cost. The aseptic mode of this model also makes it a choice, since the set of the vessel, sensors and culture medium can be sterilized in a single step and remains aseptic for a long time. A problem in relation to sterilization lies in the many moving parts that increase the points of contamination and the complexity of the mechanical seals of the equipment (Nienow, 2014).

Even though they are the most common, agitated tank bioreactors are not always the most suitable. The agitation generated by the equipment's blades necessary for aeration and mass transfer can cause damage to cells due to high shear (Duobin et al., 2013).

Mushrooms like *P. sajor-caju* showed good growth in an optimized medium with glucose, soy protein, yeast extract and ammonium sulfate, producing a good amount of biomass quickly even without pH control in agitated tank bioreactors (Confortin et al., 2008). In other strains of fungi of the genus *Pleurotus* such as *P. ostreatus* grown in an 18 L volume bioreactor with an 8 L working volume, two experiments with pH 5.5 and uncontrolled were studied in terms of biomass production and EPS.

This study showed the importance of pH control in cultures, where at 5.5 there was an increase in EPS in the order of 0.445 g/g (Maftoun et al., 2013), as well as influencing glucose consumption rates (Kurosumi et al., 2006). Changes in the pH of the culture are usually caused by the presence of organic acids and other metabolites excreted in the medium, produced by the fungi themselves (Xiao et al., 2006; Fang et al., 2002; Kim et al., 2005a).

Other variables are also important in cultivation in bioreactors. This is demonstrated for *P. ostreatus* grown in a stirring bioreactor, where the cultivation conditions varied in terms of agitation (120-180 rev min^{-1}); the initial pH (pH, 4.5-5.5) and keeping the temperature fixed at 25°C. This type of test, allowed the variation of the constitution of the medium, when it is desired to study the optimization, separating them from the physical factors (Petre et al., 2010).

Bioreactors from agitated tanks of different volumes are reported in experiments in the cultivation of fungi of the genus *Agaricus* in which the optimization of exopolysaccharide production conditions was determined by response surface, in which starch was established as the best source of carbon and yeast extract as a source of nitrogen (Hamedi et al., 2007). Likewise, *Agaricus brasiliensis* showed a similar behavior when grown in the presence of glucose (Fan et al., 2007).

For other species of fungi, such as the *Ganoderma* genus, the optimization processes established in STR also show the importance of controlling the inoculum density in the growth process, biomass production and intra and extra-cellular polysaccharides. In the study conducted by Fan and Zhong (2002), the higher inoculum density promoted a stimulus in production. This species when cultivated at an initial pH 6.5 showed lower biomass and intra and extra-cellular polysaccharides compared to pH 3.5 (Fang and Zhong, 2002).

A less used type is represented by pneumatic bioreactors, which do not have mechanical agitation, as is the case of bubble column and airlifts bioreactors (AR). Pneumatic bioreactors are bioreactors with high height, usually above 10 times the diameter, in contrast to the STR that have a height of two to three times their diameter. This is necessary because the bubbles need time to contact the liquid to transfer oxygen and absorb carbon dioxide. In STR, forced agitation keeps the bubbles in contact longer with the liquid, in addition to being continuously broken, increasing the transfer area (Merchuk, 2003).

The first is made in a single part, with an internal column that receives the injection of air, whereas the airlift bioreactor is composed of a column, which is divided into the region that contains the upward flow of liquid gas (the riser) and the region containing thedownward flow of liquid gas (the downcomer) and in this model, the difference in liquid density between these regions causes the fluid to circulate in the bioreactor by an air-lift action (Znad et al., 2004; Merchuk and Yunger, 1990; Chisti, 1989). The circulation of liquid within the bioreactor is essential for its proper performance, as it is

related to the most important variables of the process, such as the turbulence that affects the size of the bubbles and the mixing time.

The cultivation of fungi in this type of bioreactor has an advantage when studying the shear force imposed on the pellets or in the cell. These biological processes, another advantage of airlift over the bubble column and agitated tank reactors is related to the shear force imposed by the turbulent field in cells or “pellets”. Numerous studies have been conducted investigating the effects of shearing on microorganisms and cells in an effort to quantify the level of shear variation that microorganisms can tolerate (Merchuk and Yunger, 1990). Thus, in biological processes the shear force generated by the turbulence in the pellets generated during the growth of the fungi is less than in the agitation tanks, causing less damage.

Many fungi of medicinal and food interest were grown in bioreactors (Table 1). According to work by Xu et al., (2013), the cultivation of fungi of the genus *Trametes* demonstrated a similar behavior, regardless of the design of the bioreactor or the agitation rate, since the yield of products was similar. Higher EPS yield was achieved in STR than AR, and higher EPS production was related to advantageous morphological characteristics of the fungus defined by lower shear forces in AR. Recently, the cultivation of *T. ochracea* was optimized according to the different carbon sources, in a bioreactor of the stirred tank type, where sucrose proved to be the best sugar for the development of the fungus and the production of IPS and EPS (Bai et al., 2020). Other species, such as *Phellinus vaninii* grown in a stirred tank bioreactor, using lactose as a carbon source, proved to be efficient, producing EPS at a rate of 3.75 g/L (Li et al., 2019).

Pneumatic systems based on airfilt-type bioreactors showed important effects on *Agaricus subrufascens* crops when compared with agitated tank bioreactors, even when pH parameters were not controlled (Camelini et al., 2013). Likewise, different formulations of culture media for *Pleurotus flabellatus*, grown in an airlift bioreactor, showed the highest biomass productivity (0.180 g/L/day), with potato extract and a β-glucan yield of 7.70±1.11 g/100 g (Mohamad et al., 2015).

In some cases, as in *Trametes trogii* cultivation, the pneumatic airlift system did not promote biomass and EPS production in 6 days of cultivation, when compared with STR cultivated for 5 days. In this case, agitation can be beneficial to the growth of fungi and performance, improving the characteristics of mass transfer in relation to substrates, products by-products and oxygen (Xu et al., 2013).

One application of pneumatic bioreactors lies in the production of mycelium for use as an inoculant for growing mushrooms. Another great advantage is that due to the simplicity of these bioreactors, they can be manufactured at a lower cost and applying only basic techniques of turning and welding in stainless steel. The reduced diameters of these equipment also imply the need for less thick walls than the STR with consequent material savings. For these simple reasons, you can easily build a cultivation system that can be used by mushroom producers or companies that work with this type of inoculant.

CONCLUSION AND PERSPECTIVES

The experimental results described for submerged fermentation methods are important to direct and plan equipment configurations, improve the culture media according to the species studied or that have not yet been cultivated using this methodology. When aiming at greater productivity in terms of biomass and polysaccharide production, the definition of the type and quantity of the culture media and the inoculum are particularly important. The submerged culture systems described in this review include fungi that have a medicinal or edible use and are cultivated in that system. In the works described, the system proved to be quite efficient when compared with what is described in solid state fermentation, regarding the control of parameters. Certainly, the results described demonstrate that much can still be improved even if other elements are inserted in the system, such as extracts, and broths.

The biotechnological processes that involve the cultivation of fungi have a potential yet to be explored. Tropical forests are a repository of fungi that can be explored for the metabolites of interest, especially polysaccharides. For these species, the described methods can be a guide to direct the cultivation of these species in order to take advantage of their maximum potential. The polysaccharides obtained from these species represent a promising and attractive field and can be used in future research projects, where they will be used against a wide range of diseases.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

This research work was supported by CAPES and Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM) [grant number 062.00143/2020] from Brazil.

REFERENCES

Assis IS, Chaves MB, Silveira MLL, Gern RMM, Wisbeck E, Furigo Junior A, Furlan SA (2013). Production of bioactive compounds with antitumor activity against Sarcoma 180 by *Pleurotus sajor-caju*. Journal of Medicinal Food 16(11):1004-1012.

Bai J, Jia X, Chen Y, Ning Z, Liu, S, Xu C (2020).. Effect of carbon source on properties and bioactivities of exopolysaccharide produced by *Trametes ochracea* (Agaricomycetes). International Journal of

Mushrooms 23(3):289-297.
Borges GM, de Barba FFM, Schiebelbein AP, Pereira BP, Chaves MB, Silveira MLL, Pinho, MLS, Furlan SA, Wisbeck E (2013). Extracellular polysaccharide production by a strain of *Pleurotus djamor* isolated in the south of Brazil and antitumor activity on sarcoma 180. Brazilian Journal of Microbiology 44(4):1059-1065.
Burns PJ, Yeo P, Keshavarz T, Roller S, Evans CS (1994). Physiological studies of exopolysaccharide production from basidiomycetes *Pleurotus* sp. Florida. Enzyme Microbiology Technology 16(7):566-572.
Camelini CM, Rezzadori K, Benedetti S, Proner MC, Fogaça L, Azambuja AA, Giachini AJ, Rossi MJ, Petrus JCC (2013). Nanofiltration of polysaccharides from *Agaricus subrufescens*. Applied Microbiology and Biotechnology 97:9993-10002.
Camelini CM, Rossi MJ. Cardozo, FTGS, Gomes A, Sales-Campos C, Giachini AJ (2014). Fungal cultivation and production of polysaccharides. In: Ramawat K, Mérillon JM (eds) Polysaccharides pp. 1-34.
Chisti MY (1989). Airlift Bioreactors. London: Elsevier Applied Sciences 345 p.
Confortin FG, Marchetto R, Bettin F, Camassola M, Salvador M, Dillon, AJP (2008). Production of *Pleurotus sajor-caju* strain PS-2001 biomass in submerged culture. Journal of Industrial Microbiology and Biotechnology 35(10):1149-1155.
Crueger W, Crueger A (1990). Substrates for industrial fermentation. In: Biotechnology: A textbook of industrial microbiology. Wisconsin: Science Tech Publishers 59-63.
Cui, FJ, Xu ZH, Xu HY, Sun K, Tao WY (2006). Optimization of the medium composition for production of mycelial biomass and exo-polymer by *Grifola frondosa* GF9801 using response surface methodology. Bioresource Technology 97(10):1209-1216.
Duobin M, Yupping MA, Lujing G, Aijing Z, Jianqiang Z, Chunping X (2013). Fermentation characteristics in stirred-tank reactor of exopolysaccharides with hypolipidemic activity produced by *Pleurotus geesteranus* 5#. Anais da Academia Brasileira de Ciências 85(4):1473-1481.
El-Enshasy H, Daba A, El Demellawy M, Ibrahim A, El Sayed S, Elabary I (2010). Bioprocess development for large scale production of anticancer exopolysaccharide by *Pleurotus ostreatus* in submerged culture. Journal of Applied Sciences 10(21):2523-2529.
Elisashvili V (2012). Submerged cultivation of medicinal mushrooms: bioprocesses and products. International Journal of Medicinal Mushrooms 14(3):211-239.
Fan L, Soccol AT, Pandey A, Soccol CR (2007). Effect of nutritional and environmental conditions on the production of exo-polysaccharide of *Agaricus brasiliensis* by submerged fermentation and its antitumor activity. LWT 40(1):30-35.
Fang, QH, Zhong JJ (2002). Effect of initial pH on production of ganoderic acid and polysaccharide by submerged fermentation of *Ganoderma lucidum.* Process Biochemistry 37(7):769-774.
Furnali, RPZ, Godoy HT (2007). Valor nutricional de cogumelos comestíveis. Ciencia e Tecnologia de Alimentos 27(1):154-157.
García-Cruz F, Durán-Páramo E, Garín-Aguilar MA, del Toro GV, Chairez I (2020). Parametric characterization of the initial pH effect on the polysaccharides production by Lentinula edodes in submerged culture. Food and Bioproducts Processing 119:170-178.
Garcia-Ochoa F, Gomez E (2009). Bioreactor scale-up and oxygen transfer rate in microbial processes: an overview. Biotechnology Advance 27(2):153-176.
Gern RMM, Wisbeck E, Rampinelli J, Ninow JL, Furlan SA (2008). Alternative medium for production of *Pleurotus ostreatus* biomass and potential antitumor polysaccharides. Bioresource Technology 99(1):76-82.
Ghorai S, Banik SP, Verma D, Chowdhury S, Mukherjee S, Khowala S (2009). Fungal biotechnology in food and feed processing. Food Research International 42(5-6):577-587.
Guillén-Navarro GK, Márquez-Rocha FJ, Sanchez-Vázquez JE (1998). Producción de biomasa y enzimas ligninolíticas por *Pleurotus ostreatus* en cultivo sumergido. Revista Iberoamericana de Mycologia 15:302-306.
Hamedi A, Vahid H, Ghanati F (2007) Optimization of the medium composition for production of mycelia biomass and exo-polysaccharide by *Agaricus blazei* Murill DPPh 131 using response surface methodology. Biotechnology 6(4):456-464.
Hwang HJ, Kim SW, XU CP, Choi JW, Yum JW (2004). Morphological and rheological properties of the three different species of basidiomycetes *Phellinus* in submerged cultures. Journal of Applied Microbiology 96(6):1296-1305.
Jong SC, Donovick R (1989). Antitumor and antiviral substances from fungi. Advances in Applied Microbiology 34:183-261.
Joo JH, Lim JM, Kim HO, Kim SW, Hwang HJ, Choi, JW, Yun JW (2004). Optimization of submerged culture conditions for exopolysaccharide production in *Sarcodon aspratus* (Berk) S. Ito TG-3. World Journal of Microbiology and Biotechnology 20:767-773.
Kavanagh K (2005). Fungal fermentation systems and products. In: Kavanagh K. Fungi: Biology and Applications. West Sussex: Wiley pp. 89-111.
Kawagoe M, Nagaoka Y, Araki M, Yamagaki K, Naoe K, Noda H (2004) Submerged culture of Agaricus blazei mycelium in a bubble column fermentor. Journal of Chemical Engineering of Japan 37(8):1056-1061.
Kim HO, Lim JM, Joo JH, Kim SW, Hwang HJ, Choi JW, Youn JW (2005a). Optimization of submerged culture condition for the production of mycelial biomass and exopolysaccharides by *Agrocybe cylindracea*. Bioresource Technology 96:1175-1182.
Kim YO, Han SB, Lee HW, Ahn HJ, Yoon YD, Jung JK, Kim HM, Shin CS (2005b). Immuno-stimulating effect of the endo-polysaccharide produced by submerged culture of *Inonotus obliquus*. Life Science 77(19):2438-2456.
Kim SW, Hwang HJ, Lee BC, Yun JW (2007). Grifola frondosa polysaccharides as cosmeceuticals, Food Technology and Biotechnology 45(3):295-305.
Kurosumi A, Kobayasi F, Mtui G, Nakamura Y (2006). Development of optimal culture method of *Sparassis crispa* mycelia and a new extraction method of antineoplastic constituent. Biochemical. Engineering Journal 30(1):109-113.
Leonowicz A, Wojias-Wasilewska M, Rogalski J, Luterek J (1991). Higher fungi as a potential feed and food source from lignocellulosic wastes. Studies in Environmetal Science 42:229-255.
Li J, Jia Z, Yao Y, Bai Y (2019). Characterization of Exopolysaccharide Produced by *Phellinus vaninii* (Agaricomycetes) and Antioxidant Potential for Meat Batter. International Journal of Mushrooms 21(5):459-467.
Liu QN, Liu RS, Wang YH, Mi ZY, Li DS, Zhong JJ (2009). Fed-batch fermentation of Tuber melanosporum for the hyperproduction of mycelia and bioactive *Tuber* polysaccharides. Bioresource Technology 100:3644-3649.
Liu SR, Zhang WR (2019). Optimization of submerged culture conditions involving a developed fine powder solid seed for exopolysaccharide production by the medicinal mushroom *Ganoderma lucidum*. Food Science and Biotechnology 28:1135-1145.
Maftoun P, Malek R, Mahmoud AS, Abdel-Sadek M, Aziz R, El Enshasy H (2013). Bioprocess for semi-industrial production of immunomodulator polysaccharide Pleuran by *Pleurotus ostreatus* in submerged culture. Journal of Scientific and Industrial Research 72(11):655-662.
Maziero R, Cavazzoni V, Bononi VLR (1999). Screening of basidiomycetes for the production of exopolysaccharide and biomass in submerged culture. Revista de Microbiologia 30:77-84.
Merchuk JC, Yunger R (1990). The role of the gas-liquid separator of airlift reactors in the mixing process. Chemical Engineering Science 45(9):2973-2976.
Merchuk JC (2003). Airlift Bioreactors: Review of Recent Advances. The Canadian Journal of Chemical Engineering 81(3):324-337.
Mohamad SA, Awang MR, Ibrahim R, Keong CY, Hamzah MY, Rashid A, Hussein S, Rahim KA, Daud F, Hamid AB, Yusoff MW (2015). Production of endopolysaccharides from Malaysia's local mushrooms in air-lift bioreactor. Advances in Bioscience and Biotechnology 6:456-462.
Nienow AW (2014). Stirring and Stirred-Tank Reactors. Chemie Ingenieur Technik 86(12):1-13.
Oosterhuis NMG, Neubauer P, Junne S (2013). Single-use bioreactors for microbial cultivation. Pharmeutical Bioprocess 1(2):167-177.
Petre M, Teodorescu A, Tuluca E, Bejan C, Andronescu A (2010).

Biotechnology of mushroom pellets producing by controlled submerged fermentation. Romanian Biotechnological Letters 15(2):50-55.
Prosser JI, Tough (1991). Growth mechanisms and growth kinetics of filamentous microorganisms. Critical Reviews in Biotechnology 10(4):253-274.
Rosado FR, Carbonero ER, Kemmelmeier C, Tischer CA, Gorin PAJ, Iacomini M (2002). A partially 3-O-methylated (14)-linked-D-galactan and alpha-D-mannan from *Pleurotus ostreatoroseus* Sing. FEMS Microbiology Letters 212:261-265.
Rosado FR, Germano S, Carbonero, ER, Da Costa SM, Iacomini M, Kemmelmeier C (2003). Biomass and exopysaccharide production in submerged cultures of *Pleurotus ostreatoroseus* Sing. and *Pleurotus ostreatus* florida (Jack.:Fr.) Kummer. Journal of Basic Microbiology 43(3):230-237.
Rossi MJ, Souza JAR, Oliveira VL (2002). Inoculum production of the ectomycorrhizal fungus *Pisolithus microcarpus* in an airlift bioreactor. Applied Microbiology and Biotechnology 59:175-181.
Ruiz-Herrera J, Ortiz-Castellanos L (2019). Cell wall glucans of fungi. A review. The Cell Surface 5:100022.
Saeki, N, Takeda, H, Tanesaka E, Yoshida M, Takeda H (2011). Induction of manganese peroxidase and laccase by *Lentinula edodes* under liquid culture conditions and their isozyme detection by enzymatic staining on native-PAGE. Mycoscience 52(2):132-136.
Sales-Campos C, Vianez BF, de Abreu RLS (2011a). Productivity and nutritional composition of *Lentinus strigosus* (Svhwinitz) fries mushroom from the Amazon region cultivated in sawdust supplemented with soy bran. Recent Trends for Enhancing the Diversity and Quality of Soybean Products pp. 227-250.
Sales-Campos C, Araújo LM, Minhoni MTDA, Andrade MCND (2011b). Physiochemical analysis and centesimal composition of *Pleurotus ostreatus* mushroom grown in residues from the Amazon. Food Science and Technology 31(2):456-461.
Sales-Campos C, Araujo LM, Minhoni MTA, Andrade MCN (2013). Centesimal composition and physical-chemistry analysis of the edible mushroom *Lentinus strigosus* occurring in the Brazilian Amaz. Anais da Academia Brasileira de Ciências 85(4):1537-1544.
Schmidell W (2001). Agitação e aeração em biorreatores. In: Schmidell W. Biotecnologia Industrial Vol II: Engenharia Bioquímica. São Paulo: Edgard Blücher Ltda. pp. 277-331.
Seo YR, Patel DK, Shin WC, Sim WS, Lee OH, Lim KT (2019). Structural Elucidation and Immune-Enhancing Effects of Novel Polysaccharide from *Grifola frondosa*. BioMed Research International 2019:1-7.
Shu CH, Lung MY (2004). Effect of pH on the production and molecular weight distribution of exopolysaccharide by *Antrodia camphorata* in batch cultures. Process Biochemistry 39(8):931-937.
Silveira MLL, Smiderle FR, Agostini F, Pereira EM, Bonatti-Chavesa M, Wisbeck E, Ruthes AC, Sassaki GL, Cipriani TR, Furlan, AS, Iacomini M (2015). Exopolysaccharide produced by *Pleurotus sajor-caju*: Its chemical structure and anti-inflammatory activity. International Journal of Biological Macromolecules 75:90-96.
Singh MP, Vishwakarma SK, Srivastava (2013). Bioremediation of direct blue 14 and extracellular ligninolytic enzyme production by white rot fungi: *Pleurotus* spp. BioMed Research International 2013:1-4.
Smith JE, Rowan NJ, Sullivan R (2002). Medicinal mushrooms: a rapidly developing area of biotechnology for cancer therapy and other bioactivities. Biotechnology Letters 24:1839-1845.
Tang YJ, Zhong JJ (2002). Fed-batch fermentation of *Ganoderma lucidum* for hyperproduction of polysaccharides and ganoderic acid. Enzyme and Microbial Technology 31(1-2):20-28.
Tang YJ, Zhu LW, Li HM, Li DS (2007). Submerged culture of mushrooms in bioreactors – Challenges, current state-of-the-art, and future prospects. Food Technology and Biotechnology 45(3):221-229.
Tang YJ, Zhu LL, Liu RS, Li HM, Li DS, Mi ZY (2008). Quantitative response of cell growth and Tuber polysaccharides biosynthesis by medicinal mushroom chinese truffle *Tuber sinense* to metal ion in culture medium Bioresource Technology 99(16):7606-7615.
Vamanu E (2012). Biological activities of the polysaccharides produced in submerged culture of two edible *Pleurotus ostreatus* mushrooms. Journal of Biomedicine and Biotechnology 2012:56597.
Wang T, He H, Liu X, Liu C, Liag Y, Mei Y (2019). Mycelial polysaccharides of *Lentinus edodes* (shiitake mushroom) in submerged culture exert immunoenhancing effect on macrophage cells via MAPK pathway. International Journal of Biological Macromolecules 130:745-754.
Wu JZ, Cheung PCK, Wong K-H, Huang N-L (2003) Studies on submerged fermentation of *Pleurotus tuber-regium* (Fr.) Singer. Part 1: physical and chemical factors affecting the rate of mycelial growth and bioconversion efficiency. Food Chemistry 81(3):389-393.
Wu JZ, Cheung PCK, Wong KH, Huang N-L (2004) Studies on submerged fermentation of *Pleurotus tuber-regium* (Fr.) Singer. Part 2: effect of carbon-to-nitrogen ratio of the culture medium on the content and composition of the mycelial dietary fibre. Food Chemistry 85(1):101-105.
Xiao JH, Chen DX, Wan WH, Hu XJ, Qi Y, Liang ZQ (2006). Enhanced simultaneous production of mycelia and intracellular polysaccharide in submerged cultivation of *Cordyceps jiangxiensis* using desirability functions. Process Biochemistry 41(8):1887-1893.
Xu CP, Yun JW (2003). Optimization of submerged-culture conditions for mycelial growth and exo-biopolymer production by *Auricularia polytricha* (wood ears fungus) using the methods of uniform design and regression analysis. Biotechnology and Applied Biochemistry. 38(2):193-199.
Xu C, Geng L, Zhang W (2013). Production of Extracellular Polysaccharides by the Medicinal Mushroom *Trametes trogii* (Higher Basidiomycetes) in Stirred-Tank and Airlift Reactors. International Journal of Medicinal Mushrooms 15(2):183-189.
Znad HV, Báleš VJ, Markoš J, Kawaseb Y (2004). Modeling and simulation of airlift bioreactors. Biochemical Engineering Journal 21(1):73-81.
Zhang M, Zhang L, Cheung PC (2003). Molecular mass and chain conformation of carboxymethylate derivatives of β-glucan from sclerotia of *Pleurotus tuber-regium.* Biopolymers 68(2):150-159.
Zhang Z, Guoying LV, Pan H, dos Santos LF, Fan M, Soccol CR, Soccol, VT, Lacerda LG, Carvalho Filho MAS, Fan L (2011). Antidiabetic activities of ethanol extract of dry matters of culture broth of *Coriolus versiolor* in submerged culture. Brazilian Archives of Biology and Technology 54(4):701-708.
Zou X (2006). Fed-batch fermentation for hyperproduction of polysaccharide and ergosterol by medicinal mushroom *Agaricus brasiliensis*, Process Biochemistry 41(4):970-974.