

# Solid-state cultivation of *Aspergillus niger*– *Trichoderma reesei* from sugarcane bagasse with vinasse in bench packed-bed column bioreactor

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## Research Article

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# Abstract

Solid-state cultivation (SSC) is microbial growth on solid supports under limited water conditions. Citric acid, one of the products obtained by SSC, is a microbial aerobic metabolic product with various industrial applications. Several wastes from agro-industries are used in SSC, such as sugarcane bagasse and vinasse. As xylanolytic enzymes of inoculum breakdown the lignocellulosic material (bagasse), mixed fungal cultures or co-cultures are used in these SSC. Thus, this study aims to evaluate the effect of inoculum (*Aspergillus niger* and *Trichoderma reesei* consortium) in the production of citric acid from sugarcane bagasse impregnated with vinasse using bench packed bed reactors (PBR). The results show the importance of *T. reesei* in inoculum with *A. niger* at a ratio of 50:50 and 25:75, suggesting the use of solid support due to the complementation of the hydrolytic enzymes. The highest concentration of approximately 1000 mg L<sup>-1</sup> of citric acid yield for 100 mm of bed height in 48 and 72 h, with the maximum yield from glucose to citric acid (2.2 mg citric acid mg glucose<sup>-1</sup>).  $k_L a$  indicates that maintaining solid moisture and liquid film thickness is important to keep the oxygen transfer in SSC.

## 1 Introduction

Solid-state cultivation (SSC) is a process in which microbial growth occurs in solid support in the presence of limited water condition (Pandey, 2003). This low-cost technique is useful in producing new biocatalysts and bioproducts using wastes from agro-industries (Ávila et al., 2019; Perez et al., 2019). In general, agro-industrial byproducts acts as physical support, which are usually impregnated with a nutrient solution and supply capable of sustaining microbial growth (Thomas et al., 2013).

To improve the use of lignocellulosic residues by complementation in hydrolytic enzymes, fungal mixed cultures have been used in SSC (Yang et al., 2004). Fungi produce cellulolytic enzymes such as endoglucanase, exoglucanase, and β-glucosidase, besides hemicellulolytic enzymes, namely, endo-xylanase, β-xylosidase α-glucuronidase, α-arabinofuranosidase, and acetyl xylan esterase (Lopez-Ramirez et al., 2018). The synergistic cooperation between cellulolytic and hemicellulolytic enzymes helps in the saccharification of lignocellulosic materials (Brijwani et al., 2010). Moreover, strains of *Trichoderma* and *Aspergillus* produce commercial xylanolytic enzymes.

Brazil, being the largest producer of sugarcane in the world, produces 25% of the global production. Sugarcane bagasse, a byproduct of sugar processing, contains cellulose, hemicellulose, lignin, simple sugars, and ashes in lower concentrations. Due to these characteristics, sugarcane bagasse is considered important solid support to produce several bioproducts by SSC (Kumar et al., 2003; Khosravi-darani and Zoghi, 2008; Oliveira et al., 2012; Bastos et al., 2015; Bastos et al., 2017; Campanhol et al., 2019; Jugwanth, et al. 2020). Similarly, vinasse is the main wastewater in the sugarcane processing, and it is generated during the ethanol distillation-fermentation in high volumes and is rich in organic matter and potassium. The major components of vinasse are organic matter, including non-fermentable substances, fermented products (glycerol and organic acids) and the yeast residues that cannot be separated by distillation (Parsaee et al., 2019). A wide range of organic compounds found in vinasse includes alcohols,

aldehydes, ketones, esters, acids, and sugars. Therefore, using vinasse to impregnate bagasse as a nutrient solution for SSC could minimize the production cost by adding two byproducts generated in the sugarcane processing.

Citric acid is an important organic acid and has many applications in the food, beverage, chemical, and metallurgical industries (Zhang et al., 2017). The demand for citric acid increases by 4% each year; therefore, it is important to look for alternative ways of its production. A new approach in stimulating the microbial production of citric acid is by using lower alcohols (ethanol or methanol). The reason behind this approach is increasing the cell permeability or altering the activity of the enzymes citrate synthase and aconitase present in the Krebs Cycle. Also, *Aspergillus niger* converts the available ethanol into acetyl-CoA, a key precursor molecule in this cycle (Barrington and Kim, 2008). SSC technique uses lower alcohol to moisten the solid particles (Dhillon et al., 2011). Thus, the use of vinasse in impregnating bagasse is an interesting approach.

Several types of bioreactors used for SSC are Petri dishes, jars, wide-mouth Erlenmeyer flasks, Roux, and roller bottles (Durand, 2003), and other types of stirred bench bioreactors (Lopez-Ramirez et al. 2018). An ORSTOM team developed lab-scale equipment composed of small columns filled with a previously inoculated medium placed in a thermoregulated water-bath, while water-saturated air passes through each column (Raimbault and Germon, 1976). These bioreactors known as "Raimbault Columns" are small packed bed reactors (PBR) and are used by many researchers because of the following reasons: (i) proper aeration of the culture, (ii) convenient for screening studies, (iii) optimization of the medium composition, and (iv) measurement of oxygen and carbon dioxide. The main feature of a PBR is forced aeration through a static bed, which helps in the replenishment of O<sub>2</sub> and moisture, and mitigates the accumulation of heat and CO<sub>2</sub> (Arora et al., 2018). Moreover, in fixed-bed bioreactors, the injection of humidified air through the low-cost solid substrates controls temperature, oxygen, and moisture (Ávila et al., 2019). These are important aspects for scale-up study of the SSC and the construction that consists of a cylindrical glass, which houses the solid medium in a thermal jacket.

In this context, this research aimed to evaluate the synergistic effect of fungus (*Aspergillus niger* and *Trichoderma reesei*) in the production of citric acid from sugarcane bagasse impregnated with vinasse in bench PBR.

## 2 Materials And Methods

### 2.1 Inoculum

The strains of *Aspergillus niger* CCT4355 and *Trichoderma reesei* CMAA1168 were maintained in medium containing 20% sucrose, 0.25% ammonium nitrate, 1% potassium phosphate (KH<sub>2</sub>PO<sub>4</sub>), 0.025% magnesium sulfate, and 0.004% copper sulfate (Kumar et al., 2003). Both strains were sub cultured at intervals of seven days in Erlenmeyer flasks containing liquid medium.

### 2.2 Sugarcane bagasse and vinasse

Sugarcane bagasse and vinasse were collected from a sugarcane processing unit (Araras, São Paulo, Brazil). A set of Tyler Mesh with 14 and 28 sieves was used to separate the sugarcane bagasse particles of an average diameter between 0.59 to 1.17 mm, being selected for the experiments. These separated particles were sterilized in polypropylene bags in an autoclave.

Raw vinasse obtained from ethanol distillation was sterilized at 121°C for 20 min in an autoclave, and pH was maintained by potentiometry, glucose content was estimated by the enzymatic method (kit LABORLAB®), and carbon and total nitrogen were analyzed using SHIMADZU® TOC-LCPN analyzer.

### **2.3 Screening of inoculum condition**

A small packed-bed column bioreactor of 30 mm in diameter and 60 mm of bed height, known as "Rimbault Columns" (Rimbault and Germon, 1976) was used for screening fungal inoculum. Continuous water-saturated air-flow at 30°C was provided. The sterilized sugarcane bagasse (solid support) was impregnated using vinasse and inoculum suspension until the initial moisture reached 80%.

To prepare the inoculum, the SSC was set up with a spore suspension of *Aspergillus niger*, *Trichoderma reesei*, and a consortium of these at different volume proportions (75% *A. niger* and 25% *T. reesei*; 25% *A. niger* and 75% *T. reesei*; and 50% of each in the inoculum suspension). Saline solution was inoculated in the impregnated bagasse to maintain the moisture level. These samples were incubated for four days, as per the previous study done by Campanhol et al. (2019).

Gravimetric analysis was done to analyze the moisture of the solid support. Crude fungal extracts were obtained by the addition of 1:15 deionized water (solid-solvent) for 45 min at 100 rpm/28°C, with the subsequent addition of acetone following the same conditions as proposed by Khosravi-Darani and Zoghi (2008) and adapted by Bastos et al. (2015). Glucose content was determined by the enzymatic glucose oxidase-peroxidase method (LABORLAB kit). Citric acid was estimated colorimetrically with a pyridine-acetic anhydride method using a commercial kit from IN VITRO® and HPLC Ultimate 3000 Dionex®, according to the method proposed by Pereira et al. (2010).

Maximum productivity of citric acid and the specific production rates were calculated considering the elemental formula  $\text{CH}_{1.72}\text{O}_{0.55}\text{N}_{0.17}$  of the fungi biomass as reported by Nielsen et al. (2003), using biomass nitrogen determined by SHIMADZU® TOC-LCPN. Moreover, citric acid yield obtained in the SSC cultures ( $Y_{P/S}$ ) is estimated to glucose consumption and compared with the stoichiometric maximum, that is, 0.8 mol of produced carbon (citric acid) per mol of carbon of the consumed substrate (Papagianni, 2007).

### **2.4 SSC in bench packed-bed column bioreactor**

SSC is a bench bioreactor (Fig. 1), in which the inoculum condition was selected previously (item 2.3). Bench packed-bed column bioreactor (50 mm diameter and 400 mm in bed height) was filled with the sterilized sugarcane bagasse (solid support) impregnated with vinasse and inoculum suspension (50% *A.*

*niger* 50% *T. reesei* and 75% *A. niger* 25% *T. reesei*), with initial solid moisture of 80%, and continuous water-saturated air and 30°C temperature (thermal jacket).

Figure 1 Schematic of bench lab-scale column bioreactor used in SSC of fungal consortium from sugarcane bagasse (Adapted from Durand, 2003)

Oxygen demand and the overall gas-liquid oxygen transfer coefficient ( $k_L a$ ) was evaluated by the mass balance in the columns, and the radial dissolved oxygen profile was estimated, respectively (Gowthaman et al., 1995). The calculation of oxygen profile concentration in the liquid film formed on the surface of the solid supports was done according to Thibault et al. (2000).

### 3 Results And Discussion

Vinasse is used in the experiments for moistening nutrient solution of sugarcane bagasse particles. The C/N ratio of vinasse is around 20, with the following average composition: total organic carbon (10,360 mg L<sup>-1</sup>), total nitrogen (502.7 mg L<sup>-1</sup>), zinc (0.69 mg L<sup>-1</sup>), copper (0.035 mg L<sup>-1</sup>), iron (14.5 mg L<sup>-1</sup>), and manganese (3.11 mg L<sup>-1</sup>). In addition, the bagasse had a total organic carbon content of 27.6 g 100 g<sup>-1</sup>.

Table 1 shows the production of citric acid in four days from bagasse impregnated with vinasse with different proportions between the fungi *Aspergillus niger* and *Trichoderma reesei* in “Raimbault Columns” bioreactors (PBR with 200 mm height and 30 mm diameter). Inoculum of *A. niger* only produced the highest yield, followed by a fungal consortium with 50% of each fungus. In fact, *A. niger* is used for the industrial production of citric acid (Kumar et al., 2003; Papagianni, 2007). However, no significant difference between these two conditions and the condition of 75% *A. niger* and 25% *T. reesei* was observed. The obtained yield shows that 50:50 fungal consortium yield a high concentration of citric acid by glucose, suggesting better use of the substrate due to the complementation of the hydrolytic enzymes of the fungi.

Table 1

Screening of inoculum condition: fungal consortium condition for citric acid production at 4-days by SSC from sugarcane bagasse and vinasse in "Raimbault Columns"

Fungal consortium	Citric acid (mg L <sup>-1</sup> )	$Y_{P/S \max}$ (mg citric acid mg glucose <sup>-1</sup> )
75% <i>A. niger</i> 25% <i>T. reesei</i>	194.14 ± 2.31	0.25
25% <i>A. niger</i> 75% <i>T. reesei</i>	171.17 ± 3.27	0.25
50% <i>A. niger</i> 50% <i>T. reesei</i>	198.76 ± 18.84	0.67
<i>A. niger</i>	212.63 ± 6.53	0.20
<i>T. reesei</i>	113.25 ± 3.24	0.08

Since some part of the microbial citric acid produced is intracellular (mostly released out of the cells as citrate), the inoculum can cause high concentrations of the product. Thus, we obtained concentrations of  $141.10 \pm 56.101$  mg L<sup>-1</sup> on using inoculum suspension containing *T. reesei*;  $1202.59 \pm 54.86$  mg L<sup>-1</sup> on using *A. niger* inoculum; and  $568.97 \pm 36.58$  mg L<sup>-1</sup> on using inoculum suspensions containing 50:50 consortium, respectively. Inoculum suspension of *A. niger* greatly affects the production of citric acid. The initial amount of citric acid precipitated from the inoculum is negligible. Thus, in such conditions, inoculum suspensions containing 50:50 consortium yielded the highest concentration of citric acid by SSC.

The maximum yield from glucose to citric acid ( $2.2$  mg citric acid mg glucose<sup>-1</sup>) was obtained at 100 mm of bed height in 48 and 72 h, with  $k_L$  indicates that maintaining solid moisture and, consequently, liquid film thickness, is important to maintain the efficiency of oxygen transfer in SSC.

The highest concentrations of approximately 1000 mg L<sup>-1</sup> of citric acid yield is obtained using 100 mm of bed height in 48 and 72 h (Fig. 2). The use of column bioreactor helps in controlling temperature and forced aeration better and maintaining the optimal relationship between air-flow and particle bed volume, which will help in the adequate development of fungi (Arora et al., 2018). Thus, increased production of citric acid was observed using Raimbault Columns, which indicates the usage of this process in bench-scale (Oliveira et al., 2012; Bastos et al., 2015; Campanhol et al., 2019). However, one limitation of this study is the bed height. We noticed that citric acid production is dependent on the bed height, especially in 48 h, suggesting a limitation in the oxygen transfer from heights greater than 100 mm. Therefore, bed height governs the phenomena of mass transfer and microbial growth in SSC in column bioreactors.

According to the axial glucose profiles (Fig. 3), reduction in levels over time and at different bed heights is observed. However, at a bed height of 100 mm in 48 and 72 h, a high amount of citric acid is produced.

A considerable degree of microbial activity is observed in the first 24 h due to the consumption of the structural polysaccharides of the bagasse and the release of hexoses. In this case, a high amount of glucose is produced, which is used in other processes. Therefore, Raimbault Columns is proposed to scale up to bench PBR to accommodate both objectives in a single bioreaction system, and also economic feasibility should be considered. Previous studies prove that the production of citric acid by *A. niger* is dependent on the availability of oxygen (Papagianni, 2007). Also, forced aeration is the main highlight in the packed-bed reactor; therefore, it is a suitable system for large-scale production of citric acid (Arora et al., 2018).

The yield obtained using different inoculum conditions have been compared and presented in Table 2. The presence of *T. reesei* in the inoculum (50–50%) is responsible for the higher production of citric acid. Although *A. niger* generates a higher amount of citric acid, the release of glucose for this conversion seems to be more linked to the growth of *T. reesei*. Moreover, the yield on using this inoculum condition was higher than that reported by Campanhol et al. (2019) and shown in Table 1. The PBR bench is the most feasible process as it supports aerobic microbial production.

Table 2

Maximum yields glucose to citric acid ( $Y_{P/S}$ ), maximum productivities ( $P_{rod\ max}$ ) and specific production rates ( $\mu_P$ ) for SSC in bench column bioreactor at different inoculum conditions

Inoculum	$Y_{P/S\ max}$ (mg citric acid mg glucose <sup>-1</sup> )	$P_{rod\ max}$ (mg citric acid L <sup>-1</sup> h <sup>-1</sup> )	$\mu_P$ (mg citric acid g biomass <sup>-1</sup> h <sup>-1</sup> )
50% <i>A. niger</i> 50% <i>T. reesei</i>	2.2	38.70	82.34
75% <i>A. niger</i> 25% <i>T. reesei</i>	0.24	5.86	12.46

Oxygen transfer in SSC depends on moisture and thickness of the liquid film. Solid moisture is maintained over time in these conditions. As reported by Bastos et al. (2015), bed height and air-flow help in maintaining moisture in SSC. The moisture content can alter the liquid film thickness formed on the surface of the solid particles, directly affecting the global gas-liquid oxygen transfer coefficient ( $K_L a$ ). Thus, optimum and constant moisture helps in reaching the highest values of  $K_L a$ , and therefore, it is important to maintain water content in solids, regardless of microbial activity. According to Arora et al., 2018, only lower reactor volume allows the absence of free water in SSC. However, the absence of free-water in the solid medium is observed in bench PBR (Pandey, 2003; Thibault et al., 2000).

The scaling-up of batch bioreactors inevitably involves swelling. In these cases, the availability of oxygen at different bed heights is monitored in SSC (Bastos et al., 2015). These studies also show a relationship between the volume of the bioreactor and the air-flow. Shorter bed heights have shorter residence time,

which may limit the availability of oxygen. Also, the highest oxygen consumption (the largest difference between the values of the column inlet and outlet) can be observed at bed heights of 100 and 200 mm, especially in the period between 48 and 72 h, agreeing with the greater production of citric acid, as shown in Fig. 4.

The industrial production of citric acid is extremely dependent on aeration, and even short intervals of reduction in dissolved oxygen can cause irreversible losses. *Aspergillus niger* presents two respiratory models during the accumulation of citric acid (Papagianni, 2007). Thus, the limitations of oxygen transfer in SSC can completely affect the production of microbial metabolites. However, it is difficult to understand the interaction between fungal growth and physical mass transfer phenomena. The nature of the solid support used, its porosity and moisture content, and oxygen, moisture and temperature gradients in column bioreactors are important for oxygen transfer (Oostra et al., 2001; Rodríguez-Fernández et al., 2012). In addition, due to the production of metabolic water the initial value of moisture content in SSC rises in most cases, which changes the properties of solid support. However, moisture in the bagasse bed remains constant, indicating that the variations in oxygen demand (Fig. 4) are associated with the metabolic activity of the fungi.

Figure 5 shows the characteristic profile of  $K_L a$  as a function of the thickness of the liquid film present on the surface of the bagasse (Gowthaman et al., 1995; Thibault et al., 2000). Similar conditions are observed for all bed heights, being calculated from experimental values (oxygen demand, particle bed volume, volumetric air-flow) and assumed values (thickness variation, oxygen diffusivity in the liquid film).

According to the estimate of the proposed by As per the study by Doran (2012), critical  $K_L a$  is estimated in which the minimum value to leading non-limiting oxygen conditions is around  $0.75 \text{ s}^{-1}$ , leading to an unfavorable situation starting at  $150 \mu\text{m}$  of liquid film thickness. However, if the moisture content is maintained, variation in liquid film thickness can be neglected, keeping the  $K_L a$  in the appropriate range.

## 4 Conclusions

This study concludes that the production of citric acid using bagasse with vinasse in bench packed-bed bioreactors can be improved by the optimization of the inoculum condition of fungal consortium.

## Declarations

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### Conflicts of interest

The authors have no financial conflicts of interest to declare.

### **Availability of data and material (data transparency)**

All data were generated from experimental tests in the laboratory coordinated by the advisor.

### **Code availability**

Not applicable

### **Authors' contributions**

Laura Macedo Rocha: responsible for the analysis and monitoring of experiments.

Beatriz Silva Campanhol: responsible for set up the tests and discussing part of the results.

Reinaldo Gaspar Bastos: general research orientation and final analysis of results.

### **Additional declarations for articles in life science journals that report the results of studies involving humans and/or animals**

Not applicable.

### **Ethics approval**

All authors agree with ethical responsibilities

### **Consent to participate**

All authors agreed to participate in the submission of this manuscript.

### **Consent for publication (include appropriate statements)**

All authors agreed with the possible publication of the research in this journal.

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## **References**

- [1] Pandey A. Solid-state fermentation. Biochemical Engineering Journal, 13. 81–84, 2003. doi: 10.1016/S1369-703X(02)00121-3.

- [2] Ávila SNS, Gutarra MLE, Fernandez-Lafuente R, Cavalcanti EDC, Freire DMG. Multipurpose fixed-bed bioreactor to simplify lipase production by solid state fermentation and application in biocatalysis. *Biochemical Engineering Journal* 144, 1–7, 2019. doi: [10.1016/j.bej.2018.12.024](https://doi.org/10.1016/j.bej.2018.12.024).
- [3] Perez CL, Perpétua Casciatori F, Thoméo JC. Strategies for scaling-up packed-bed bioreactors for solid-state fermentation: The case of cellulolytic enzymes production by a thermophilic fungus. *Chemical Engineering Journal* 361, 1142–1151, 2019. doi: [10.1016/j.cej.2018.12.169](https://doi.org/10.1016/j.cej.2018.12.169).
- [4] Thomas L, Larroche C, Pandey A. Current developments in solid-state fermentation. *Biochemical Engineering Journal* 81, 146–161, 2013. doi: [10.1016/j.bej.2013.10.013](https://doi.org/10.1016/j.bej.2013.10.013).
- [5] Yang YH, Wang BC, Wang QH, Xiang LJ, Duan CR. Research on solid-state fermentation on rice chaff with a microbial consortium. *Colloids and Surfaces B: Biointerfaces*, v. 34, n. 1, p. 1-6, 2004. doi: [10.1016/j.colsurfb.2003.10.009](https://doi.org/10.1016/j.colsurfb.2003.10.009)
- [6] Lopez-Ramirez N, Volke-Sepulveda T, Gaime-Perraud I, Saucedo-Castañeda G, Favela-Torres E. Effect of stirring on growth and cellulolytic enzymes production by *Trichoderma harzianum* in a novel bench-scale solid-state fermentation bioreactor. *Bioresource Technology* 265, 291–298, 2018. doi: [10.1016/j.biortech.2018.06.015](https://doi.org/10.1016/j.biortech.2018.06.015).
- [7] Brijwani K, Oberoi HS, Vadlani PV. Production of a cellulolytic enzyme system in mixed-culture solid-state fermentation of soybean hulls supplemented with wheat bran. *Process Biochemistry* 45, 120–128, 2010. doi: [10.1016/j.procbio.2009.08.015](https://doi.org/10.1016/j.procbio.2009.08.015)
- [8] Kumar D, Jain VK, Shanker G, Srivastava A. Citric acid production by solid state fermentation using sugarcane bagasse. *Process Biochemistry*, v. 38, n. 12, p. 1731-1738, 2003. doi: [10.1016/S0032-9592\(02\)00252-2](https://doi.org/10.1016/S0032-9592(02)00252-2).
- [9] Khosravi-Darani K, Zoghi A. Comparison of pretreatment strategies of sugarcane bagasse: Experimental design for citric acid production. *Bioresource Technology*, v. 99, n. 15, p. 6986-6993, 2008. doi: [10.1016/j.biortech.2008.01.024](https://doi.org/10.1016/j.biortech.2008.01.024).
- [10] Oliveira AF, De Carvalho VM, Bastos RG. Cultivation of *Aspergillus niger* on sugarcane bagasse with vinasse. *Bioscience Journal*, v. 28, n. 6, 2012.
- [11] Bastos RG, Morais DV, Volpi MPC. Influence of solid moisture and bed height on cultivation of *Aspergillus niger* from sugarcane bagasse with vinasse. *Brazilian Journal of Chemical Engineering*, v. 32, n. 2, p. 377-384, 2015. doi: [10.1590/0104-6632.20150322s00003423](https://doi.org/10.1590/0104-6632.20150322s00003423).
- [12] **Bastos RG**, França HCR, Campanhol BS, Castro MC, Silveira GC. Sequential process of citric acid production in sugarcane bagasse by microbial consortium and ethanol fermentation from fungal extract. In: 2017 ASABE Annual International Meeting, Spokane, WA, USA. Proceedings ASABE, 2017. doi: [10.13031/aim.201700161](https://doi.org/10.13031/aim.201700161).

- [13] Campanhol BS, Silveira GC, Castro MC, Ceccato-Antonini SR, Bastos RG. Effect of the nutrient solution in the microbial production of citric acid from sugarcane bagasse and vinasse. Biocatalysis and Agricultural Biotechnology 19 – 101147, 2019. doi: [10.1016/j.bcab.2019.101147](https://doi.org/10.1016/j.bcab.2019.101147).
- [14] Jugwanth Y, Sewsynker-Sukai EB, Gueguim K. Valorization of sugarcane bagasse for bioethanol production through simultaneous saccharification and fermentation: Optimization and kinetic studies. Fuel 262, 116552, 2020. doi: [10.1016/j.fuel.2019.116552](https://doi.org/10.1016/j.fuel.2019.116552)
- [15] Parsaee M, Kiani MKD, Karimi K. A review of biogas production from sugarcane vinasse. Biomass and Bioenergy 122, 117–125, 2019. doi: [10.1016/j.biombioe.2019.01.034](https://doi.org/10.1016/j.biombioe.2019.01.034).
- [16] Zhang H, Xu J, Su X, Bao J, Wang K, Mao Z. Citric acid production by recycling its wastewater treated with anaerobic digestion and nanofiltration. Process Biochemistry, 58, 245-251, 2017. doi: [10.1016/j.procbio.2017.04.022](https://doi.org/10.1016/j.procbio.2017.04.022).
- [17] Barrington S, Kim JW. Response surface optimization of medium components for citric acid production by *Aspergillus niger* NRRL 567 grown in peat moss. Bioresource technology, v. 99, n. 2, p. 368-377, 2008. doi: [10.1016/j.biortech.2006.12.007](https://doi.org/10.1016/j.biortech.2006.12.007).
- [18] Dhillon GS, Brar SK, Kaur S, Verma M. Bioproduction and extraction optimization of citric acid from *Aspergillus niger* by rotating drum type solid-state bioreactor. Industrial Crops and Products, 41, 78–84, 2013. doi: [10.1016/j.indcrop.2012.04.001](https://doi.org/10.1016/j.indcrop.2012.04.001).
- [19] Durand A. Bioreactor designs for solid-state fermentation. Biochemical Engineering Journal, v. 13, n. 2, p. 113-125, 2003. doi: [10.1016/S1369-703X\(02\)00124-9](https://doi.org/10.1016/S1369-703X(02)00124-9).
- [20] Raimbault M, Germon JC. Procédé d'enrichissement en protéines de produits comestibles solides, French Patent no. 76-06-677, 1976.
- [21] Arora S, Rani R, Ghosh S. Bioreactors in solid-state fermentation technology: Design, applications and engineering aspects. Journal of Biotechnology 269, 16–34. 2018. doi: [10.1016/j.jbiotec.2018.01.010](https://doi.org/10.1016/j.jbiotec.2018.01.010).
- [22] Pereira GE, De Andrade Lima TL, Rocha H. Otimização e validação de método para determinação de ácidos orgânicos em vinhos por cromatografia líquida de alta eficiência. Quim. Nova, v. 33, n. 5, p. 1186-1189, 2010.
- [23] Nielsen J, Villadsen J, Liden G. Bioreaction engineering principles, 2nd ed. Kluwer Academic/Plenum, New York. 2003.
- [24] Gowthaman MK, Raghava Rao KSMS, Ghidyal NP, Karanth NG. Estimation of  $K_L a$  in solid-state fermentation using a packed-bed bioreactor. Process Biochemistry. V.30, p.9-15. 1995. doi: [10.1016/0032-9592\(95\)87002-4](https://doi.org/10.1016/0032-9592(95)87002-4).

[25] Thibault J, Pouliot K, Agosin E, Pérez-Correa R. Reassessment of the estimation of dissolved oxygen concentration profile and  $K_L a$  in solid-state fermentation. *Process Biochemistry*. V. 36, 9-18, 2000. doi: [10.1016/S0032-9592\(00\)00156-4](https://doi.org/10.1016/S0032-9592(00)00156-4).

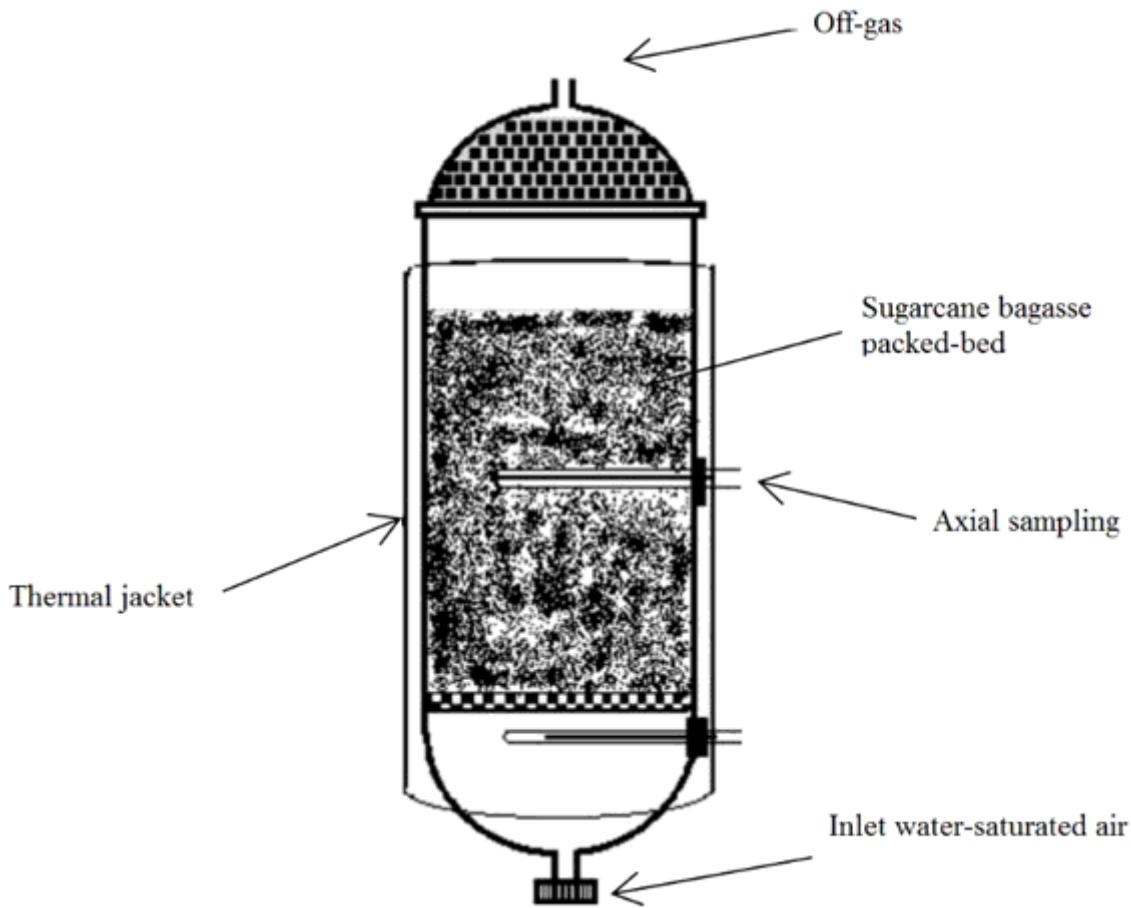
[26] Papagianni M, Advances in citric acid fermentation by *Aspergillus niger*: biochemical aspects, membrane transport and modeling. *Biotechnology advances*, v. 25, n. 3, p. 244-263, 2007. doi: [10.1016/j.biotechadv.2007.01.002](https://doi.org/10.1016/j.biotechadv.2007.01.002).

[27] Oostra J, Le Comte EP, Van Den Heuvel JC, Tramper J, Rinzema A. Intra-particle oxygen diffusion limitation in solid-state fermentation. *Biotechnology & Bioengineering*, 75, 13-24, 2001. doi: [10.1002/bit.1159](https://doi.org/10.1002/bit.1159).

[28] Rodríguez-Fernández DE, Rodríguez-León JA, De Carvalho JC, Karp SG, Sturm, W, Parada JL, Soccol CR. Influence of airflow intensity on phytase production by solid-state fermentation. *Bioresource Technology*, 118, 603–606, 2012. doi: [10.1016/j.biortech.2012.05.032](https://doi.org/10.1016/j.biortech.2012.05.032).

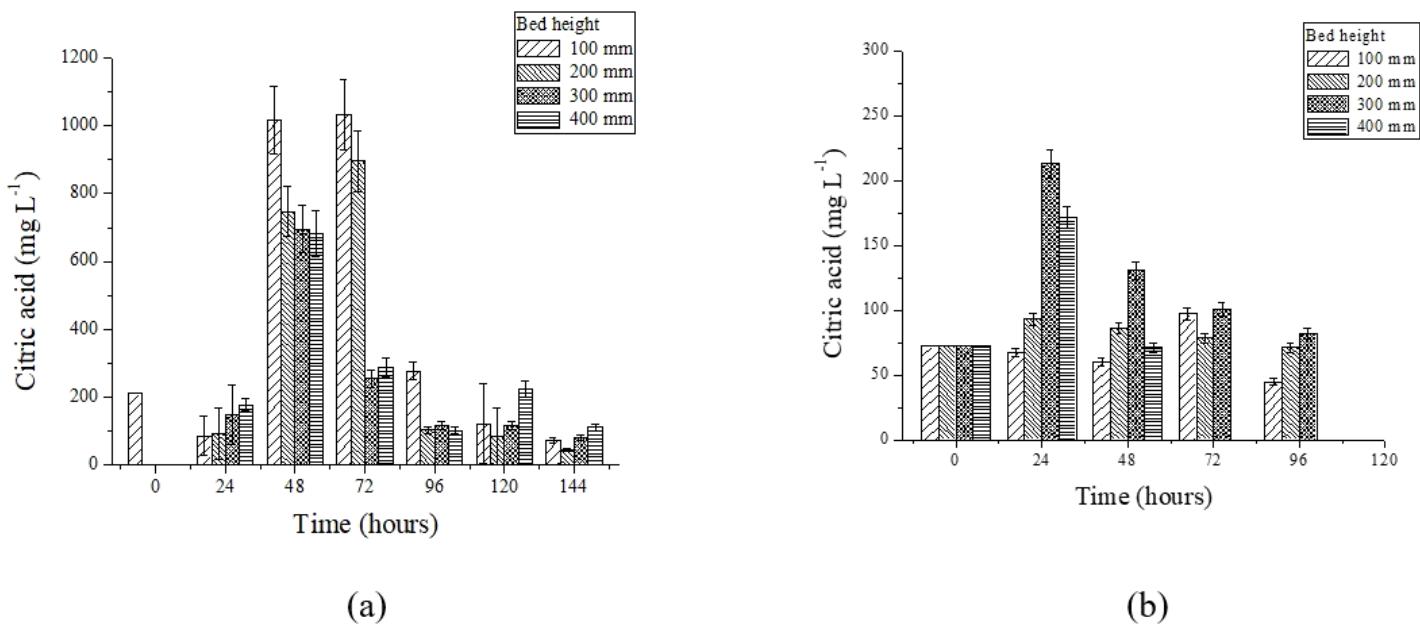
[29] Doran P. Bioprocess Engineering Principles. Second Edition. Academic Press. 926 p. 2012.

## Figures

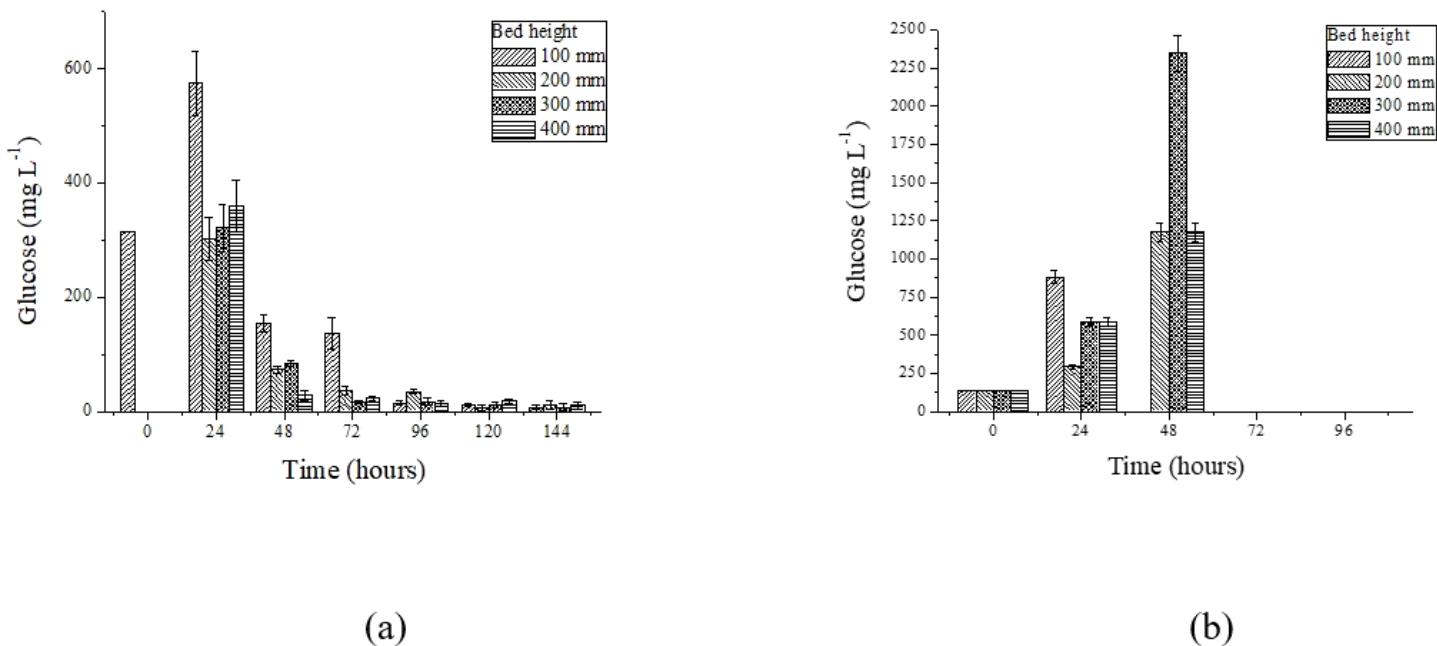


**Figure 1**

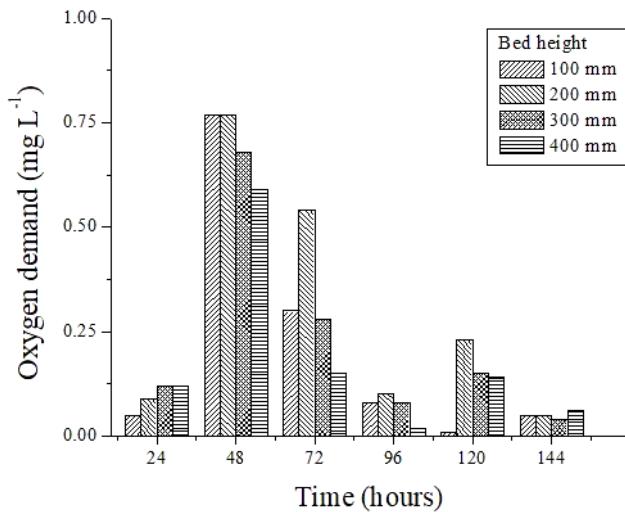
Schematic of bench lab-scale column bioreactor used in SSC of fungal consortium from sugarcane bagasse (Adapted from Durand, 2003)

**Figure 2**

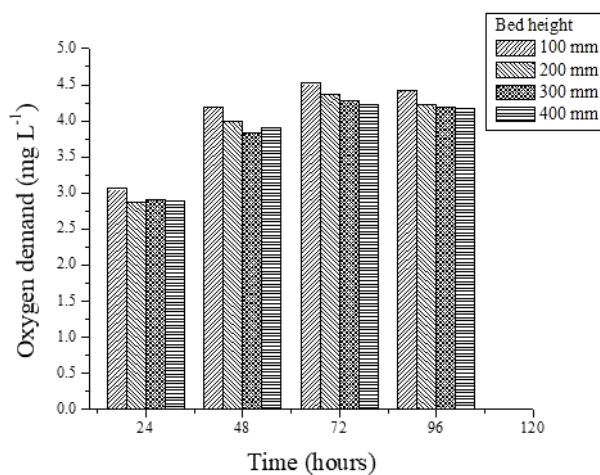
Axial profiles of citric acid for SSC in bench packed-bed bioreactor. Inoculum 50% A. niger 50% T. reesei (a) and 75% A. niger 25% T. reesei (b)

**Figure 3**

Axial profiles of glucose for SSC in bench packed-bed bioreactor. Inoculum 50% A. niger 50% T. reesei (a) and 75% A. niger 25% T. reesei (b)



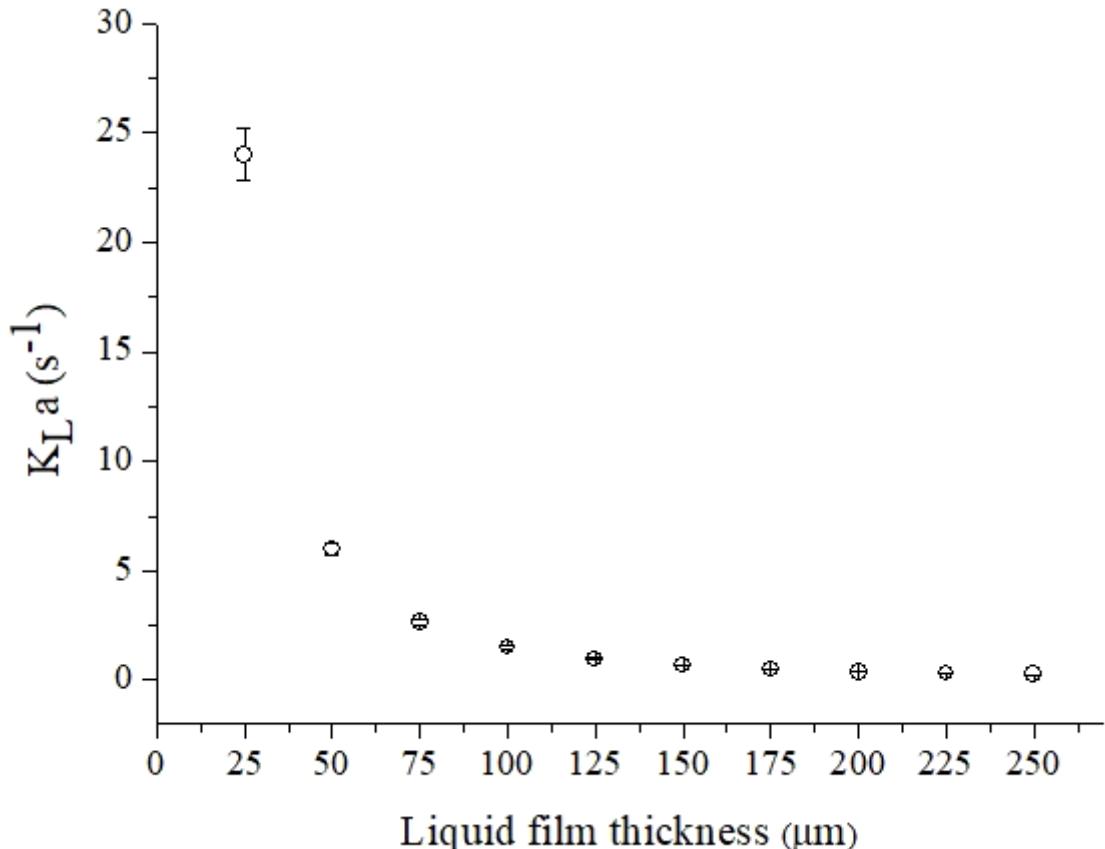
(a)



(b)

**Figure 4**

Axial profiles of oxygen demand for SSC in bench packed-bed bioreactor. Inoculum 50% A. niger 50% T. reesei (a) and 75% A. niger 25% T. reesei (b)



## **Figure 5**

Overall oxygen transfer coefficient for SSC in bench packed-bed column bioreactor with inoculum 75% *A. niger* 25% *T. reesei* (assumed thickness between 25 and 250 µm)