



## EVALUATION OF DIFFERENT PRE-SETTING CONDITIONS IN AIRLIFT BIOREACTOR TO DETERMINE THE RESPIRATORY KINETICS OF FUNGI

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### ABSTRACT

Successful forestry programs are prompted by the effects of ectomycorrhizal fungi. Ectomycorrhizal fungi can be cultivated in culture media and, therefore, used to obtain large volumes of fungal tissue to be used as inoculum. The implementation of controlled mycorrhization programs is dependent upon the production of commercially large volumes of inoculum. As of now, there is no such place around the globe where we can find people or companies that are able to achieve such goal. Information on the fungal growing kinetics is scant, and there are no studies that deal with the topic of oxygen transfer for the cultivation of these fungi (major impediment), making it hard to produce inoculum in large scales. Therefore, the current study used an airlift bioreactor to provide information on aspects, such as time of mixture, the effect of depressurization on the oxygen concentration readings and the delay of probe response, among others, that are fundamental for the commercial production of these fungal inocula. The study showed that the results obtained from the dynamic assay need adjustments prior to analytical interpretation. The data was obtained with operating specific air flow rates of 0.2, 0.36 and 0.52 vvm. In conclusion, the study provided essential information that can be used by others to continue the studies on the dynamic aspects of an airlift bioreactor operation intended for fungal biomass production.

### Indexing terms/Keywords

*Rhizopogon nigrescens*, airlift bioreactor, dynamic method

### Academic Discipline and Sub-Disciplines

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### SUBJECT CLASSIFICATION

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## INTRODUCTION

The success in development of high quality, introduced forests, lays, in part, in the fineness of the seedlings. These seedlings have not only to resist the hostile conditions found in the field, but also survive and yield large volumes of economically sustainable wood [1]. Therefore, technologies that can improve the production of such seedlings are of great interest. One such technology employs the inoculation of the seedlings with selected, more efficient ectomycorrhizal fungi [2]. These fungi can improve plant development, even in soils with low nutrient levels [3], due to their capacity to explore the neighboring soil in a mutualistic partnership with the roots of plants.

This technology initiates with the selection of more efficient strains of fungi, and evolves into a delicate process called the inoculum production [2,4-6]. With this in mind, one of the main challenges is the production of large, standardized volumes of inoculum to attend the ever-growing needs of the hundreds of nurseries spread around the world. Therefore, the development of such technology is still in its initial phases. The process is arduous and it presents a practical bottleneck, which is the intrinsically slow growing feature of ectomycorrhizal fungi. Furthermore, it also presents a series of complicated steps related to the quality and definition of the right parameters the inoculum production must have. As a result, there are virtually no studies discussing the details regarding the kinetics of the process involving these fungi, and even less discussing the specifics about mass transfers in such controlled cultivations [5,7].

During aerobic cultivations, oxygen is generally the limiting factor since it is the least soluble of all available nutrients. If the bioreactor cannot supply the demands of an actively growing microbial population, critical levels of oxygen concentration can be reached in only a few seconds during a particular cultivation [8]. The rate in which oxygen is consumed ( $Q_{O_2 \cdot X}$ ) (nomenclature for symbols are listed in Table 1) must be equal to the rate of supply [ $k_{L,a} \cdot (C_s - C)$ , ex. for stirred tank reactors] in order to keep a constant production. To determine this kinetic parameter, the transient method, which employs only one probe to measure the levels of dissolved oxygen in the medium, named dynamic assay, is normally chosen [9]. The method relies in interrupting aeration for a few seconds, and registering the fluctuations in the levels of dissolved oxygen in the medium. The experimental data adjusted to oxygen mass balance allow for the determination of the desired information.

## MATERIAL AND METHODS

### Ectomycorrhizal fungal isolate

This study employed the ectomycorrhizal fungal isolate UFSC-Rh90, *Rhizopogon nigrescens* Coker & Couch, isolated in 1993 from a managed forest of *Pinus taeda* located in the state of Santa Catarina, Southern Brazil. The isolate is available from a publicly accessible culture collection (CBMAI 1472). During the study the isolate was kept at  $25 \pm 1$  °C in MMN solid medium [10] with glucose as the sole source of carbon.

### Culture medium and inoculum production

Mycelial pellets from a previous bioreactor cultivation preserved under refrigeration ( $8 \pm 2$  °C) in a saline solution (0.85% NaCl) for three days (Figure 1), were utilized to prepare the inoculum. Inoculum viability was confirmed by the placement of ca. 40 pellets in Petri dishes containing MMN solid medium and incubated at  $25 \pm 1$  °C for 48 h. In order to promote a suitable homogeneous mycelial suspension for the bioreactor start up, 50 biomass pellets (ca. 1.5 g of dry weight) were fragmented in a blender (LAR-15 Metvisa) for 20 s at 3600 rpm in a volume of 300 mL of MMN liquid medium.



**Fig 1: Biomass pellets of the ectomycorrhizal fungus *Rhizopogon nigrescens*, isolate UFSC-Rh90, obtained in an airlift bioreactor**

For the bioreactor cultivation we used a variation of the PGK medium [11], containing (g L<sup>-1</sup>): glucose 14.0; soy peptone 3.0; malt extract 1.5; NH<sub>4</sub>NO<sub>3</sub> 1.0; KH<sub>2</sub>PO<sub>4</sub> 0.264; K<sub>2</sub>HPO<sub>4</sub> 0.628; MgSO<sub>4</sub>·7H<sub>2</sub>O 0.33; CuSO<sub>4</sub>·5H<sub>2</sub>O 0.0021; MnCl<sub>2</sub>·4H<sub>2</sub>O 0.0006; ZnSO<sub>4</sub>·7H<sub>2</sub>O 0.0005; FeSO<sub>4</sub>·7H<sub>2</sub>O 0.0004, with a C/N relation of approximately 16. The initial pH was adjusted to 5.8 with an equimolar solution of citric acid and 0.15 M sodium citrate prior to sterilization. Additionally, we added 0.25 mL L<sup>-1</sup> of polypropylene glycol to reduce foaming.



## Bioreactor operating specifications

A 5 L stainless steel airlift bioreactor with external circulation ( $A_d/A_r = 0.11$  e  $H/D=12.5$ ) [12], fitted with a InPro6000 polarographic probe (98% of the response in 45 to 90 s) and a 4100e transmitter for the dissolved oxygen (DO) readings (Mettler-Toledo International Inc.), was used. The bioreactor was sterilized (121 °C) using direct vapor created by a 25 L autoclave, connected to the bioreactor through a derivation of the air outlet. During sterilization, places like feeding, sampling, collecting, and air entrance, plus the gas exit filters, manometer and DO probe were kept at 110 °C by the depressurization created with the vapor exit. After this 30-minute phase, the bioreactor was pressurized and kept at 121 °C for an extra 30 minutes.

Under a laminar flow, 350 mL of mycelial suspension extracted from the previously produced biomass were inoculated into 4.7 L of sterile culture medium (121 °C for 30 min) using a Mariotte flask. Afterwards, 5 L of the inoculated medium were aseptically transferred to the bioreactor utilizing a sterilized connection. The bioreactor was then set for operation at  $25 \pm 1$  °C with a specific air flow rate of 0.2 vvm, determined with a gas bubble meter tube. The injected air was purified by a 0.2  $\mu\text{m}$  filtering hydrophobic membrane (Millipore Corporation, Billerica, MA, USA).

Additional determinations were done using a fungus free solution under the flow rates of 0.36 and 0.52 vvm. When these assays were performed under non-aseptic conditions, a 0.15 M NaCl solution was used to reduce the potential effects that could be caused by bacterial contamination of the medium.

## Determination of the cultivation medium oxygen solubility

Oxygen solubility in the cultivation medium, essential for the precise determination of the fungal respiration rates, was determined by the calibration of the probe while releasing a defined amount of oxygen into the medium [13]. Prior to this calibration, the medium was brought to an oxygen free state by the addition of  $\text{N}_2$ . For this, a 500 mL round bottom, 3-neck glass flask was used. The oxygen probe (InPro 6000; Mettler-Toledo International Inc.), later used in the bioreactor, was mounted in one of the necks. A second neck was used to inject the  $\text{N}_2$ , and the third to add the necessary reagents for the reactions to take place. The unit was then placed in a 25 °C water bath heater and maintained under magnetic stirring during the time the assay was conducted. The probe was previously polarized during 6 hours and calibrated in the saturated liquid reaction. The determination of oxygen solubility was performed adding 360 mL of PGK medium into the round bottom glass flask. A 140  $\text{mL min}^{-1}$  flow rate of nitrogen was then applied over the medium to avoid the transferring of oxygen via the surface. Oxygen was initially removed by bubbling  $\text{N}_2$  into the medium. Once the probe reached the zero current mark (ca. 1% of the external air, according to the manufacturer), the injection of  $\text{N}_2$  was interrupted and 100  $\mu\text{L}$  of 1.024 M  $\text{H}_2\text{O}_2$  (exact determination by titration with  $\text{KMnO}_4$ , standardized with sodium oxalate) added. The reaction of  $\text{H}_2\text{O}_2$  and added catalase enzyme (Terminox Ultra 50L; Novozymes, NC, USA) causes oxygen to be released into the medium, which was then detected by the probe. This procedure was repeated for 20, 50, 75, 100 and 150  $\mu\text{L}$  of  $\text{H}_2\text{O}_2$ , resulting in different probe responses. The precise volumes of  $\text{H}_2\text{O}_2$  necessary to saturate the medium were determined by the adjustment of a linear equation, and by the stoichiometry shown in (1). With the volume of the reaction it was possible to determine the oxygen concentration, expressing its solubility in  $\text{mgO}_2 \text{L}^{-1}$ .



## Considerations for the application of the dynamic method

The following parameters were determined when applying the dynamic method as a function of biomass sedimentation during interruption in aeration:

- Biomass speed of sedimentation:** the biomass utilized for the sedimentation assay was produced entirely in the bioreactor. 40 mL samples were placed in a 50 mL glass cylinder to determine the biomass speed of sedimentation. For that, pellets were maintained in suspension by the introduction of air at the bottom of the cylinder for a few seconds to create a homogeneous solution. Afterwards, aeration was interrupted and as soon as the air bubbles were released, the time necessary for the sedimentation of the larger pellets, from the top of the solution, to a marked line at the base of the cylinder, was recorded. The time utilized to determine the speed of sedimentation was then the mean of 10 repetitions.
- Time of mixture:** a solution of 0.15 M NaCl was used to practically fill the bioreactor. In addition, 750 mL of hot water were added to the top of the bioreactor. Following, temperatures at the top and at the bottom of the bioreactor were simultaneously monitored until stabilization was reached. Two Incoterm<sup>®</sup>, thermocouples probe digital identical thermometers (Incoterm, Porto Alegre, RS, Brazil), with a resolution of  $\pm 0.1$  °C and a reading range from -10 to 70 °C, were used to monitor temperature. In order to guarantee that the thermal inertia did not influence the readings, the thermometers time constant was measured in ca. 800 ms prior to use. This procedure was performed in each of the three air flow rates previously established (0.2, 0.36 and 0.52 vvm).
- Depressurization effect:** with the interruption in aeration, a depressurization of the bioreactor takes place, and even in the absence of biomass, it is possible to notice reductions in the readings of dissolved oxygen, as if oxygen consumption may have happened. To determine this specious consumption, a dynamic assay [14], using PGK medium, was performed in the absence of the fungus isolate in each of the air flow rates previously established. Values obtained, recorded in units equivalent to the respiration rate ( $\text{mgO}_2 \text{L}^{-1} \text{h}^{-1}$ ), were utilized to adjust the oxygen consumption levels determined when the fungal isolate was present in the medium. These values were obtained by maintaining the aeration in the bioreactor until a complete oxygen saturation was detected, followed by an interruption in aeration and further readings of the oxygen concentration by the DO probe.



d) *Probe response delay*: considering that the probe signal ( $C_p$ ) varies with time proportionally to the difference between the real dissolved oxygen concentration ( $C$ ) and the signal [15], the following equation is obtained:

$$\frac{dC_p}{dt} = k_p(C - C_p) \tag{2}$$

where  $C_p$ = probe oxygen signal readings ( $\text{mgO}_2 \text{ L}^{-1}$ ) and  $k_p$ = constant delay of probe response ( $\text{h}^{-1}$ ). The  $k_p$  was obtained through a stepwise assay as described by Schmidell [14]. Initially, the readings of the probe immersed in water were zeroed with the injection of nitrogen. The probe was then immediately immersed in culture media saturated with  $\text{O}_2$  and the readings recorded. Three such readings were performed in static culture media and three in media under agitation as a result of the media aeration. The latter reduces the effects promoted by a stagnated film that can potentially be formed in the probe's membrane under a static condition. The stepwise assay generates, from Eq. 2 with an instant  $t = 0$  and  $C = C_s$ , the following equation:

$$\frac{dC_p}{dt} = k_p(C_s - C_p) \tag{3}$$

or:

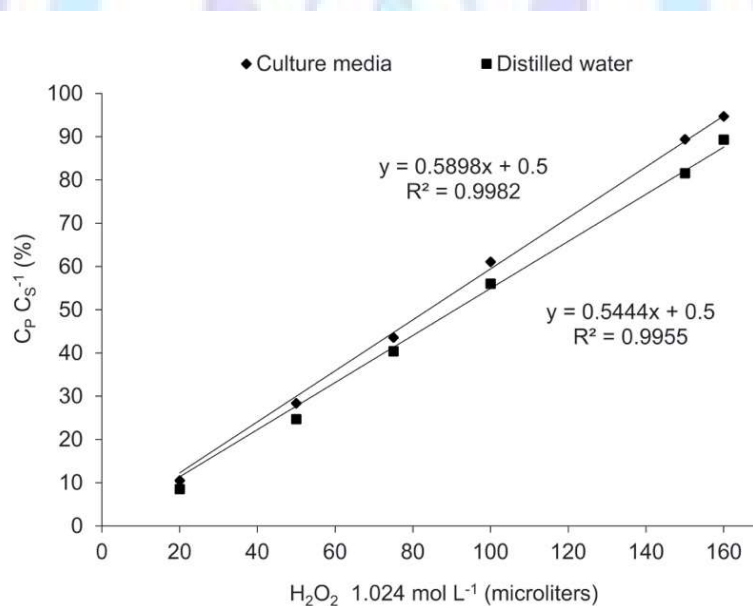
$$\ln\left(1 - \frac{C_p}{C_s}\right) = -k_p \cdot t \tag{4}$$

A slight adjustment generated the values of  $k_p$  from the values of  $\ln(1-C_p/C_s)$  as a function of the time obtained from the stepwise assay for both situations.  $C_p C_s^{-1}$  served as the probe signal itself.

## RESULTS

### Oxygen solubility in the culture media

The oxygen solubility in the culture media, determined by the correlation illustrated in Figure 2 and the stoichiometry from the  $\text{H}_2\text{O}_2$  hydrolysis (Eq. 1), was  $\approx 7.5 \text{ mg L}^{-1}$  ( $0.2348 \text{ mmol}$ ) at  $25 \text{ }^\circ\text{C}$  and at sea level atmospheric pressure. This value is approximately 8% lower than the solubility value for pure water under the same conditions. The presence of glucose and minerals reduce the liquid oxygen solubility, as already observed and reported by others [13,14,16,17].



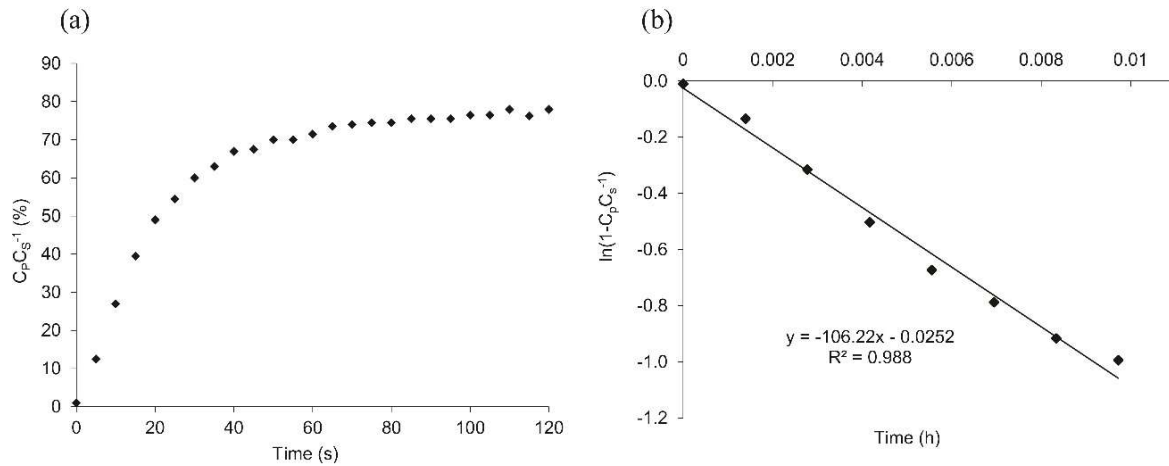
**Fig 2: Response of the oxygen probe to the addition of increasing amounts of  $\text{H}_2\text{O}_2$ , in the presence of catalase.  $C_p C_s^{-1}$  is the probe signal, calibrated under oxygen saturation by a 30 min injection of air**

### Biomass sedimentation

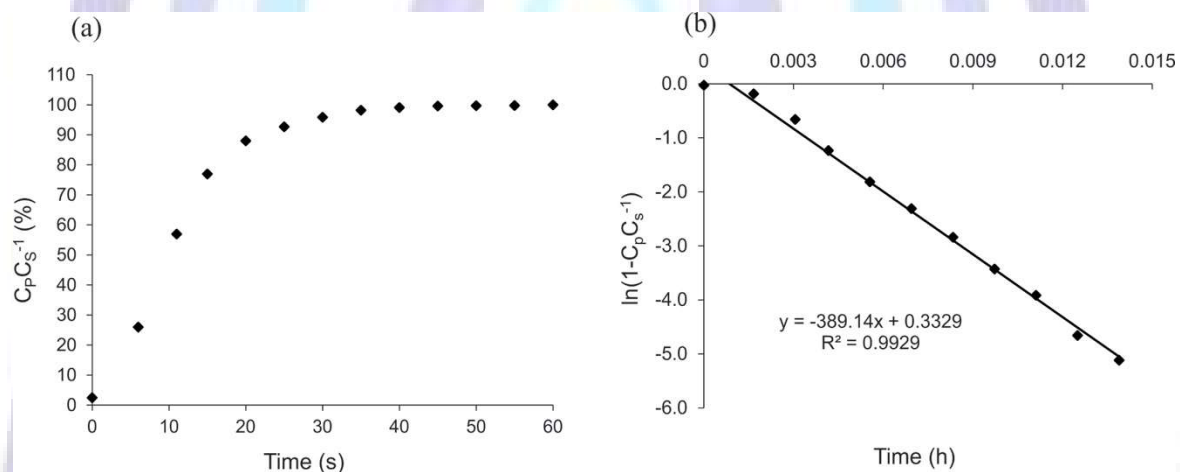
Values for pellets speed of sedimentation ranged from  $0.2$  to  $0.3 \text{ cm s}^{-1}$ . These records were obtained for large pellets with diameters of  $0.5$  to  $3 \text{ mm}$ . Considering the  $45 \text{ cm}$  distance from the surface of the liquid in the gas separator all the way to the probe, a  $150 \text{ s}$  biomass reading interval was observed. Thus, considering a safe boundary, there are ca. of  $2 \text{ min}$  for the readings to be done. If we define intervals of  $10 \text{ s}$  between each reading, we can have  $12$  experimental points to determine the specific oxygen uptake rate ( $Q_{\text{O}_2}$ ). During the dynamic assay, in the phase where the respiration rate is determined, the biomass descends while the culture medium remains practically still. Therefore, it is fundamental to evaluate the probe response time for this period.

### Oxygen probe response delay

The constant of delay for the response of the probe, obtained from the assay done under static conditions of the medium was  $106 \text{ h}^{-1}$  (Figure 3). Due to the aeration effects, this value was significantly higher ( $389 \text{ h}^{-1}$ ) for the culture medium under stirring (Figure 4). The probe manufacturer presents a time that varies from 45 to 90 s for 98% of the response, values that are definitely determined under agitation. As observed for the present study, the results obtained under stirring are in agreement with the manufacturer's specifications.



**Fig 3: Stepwise test performed with the probe initially zeroed with  $\text{N}_2$  and placed into an  $\text{O}_2$  saturated, static culture medium at  $25 \text{ }^\circ\text{C}$ . (a) probe response as a function of time and (b) probe response time determination**

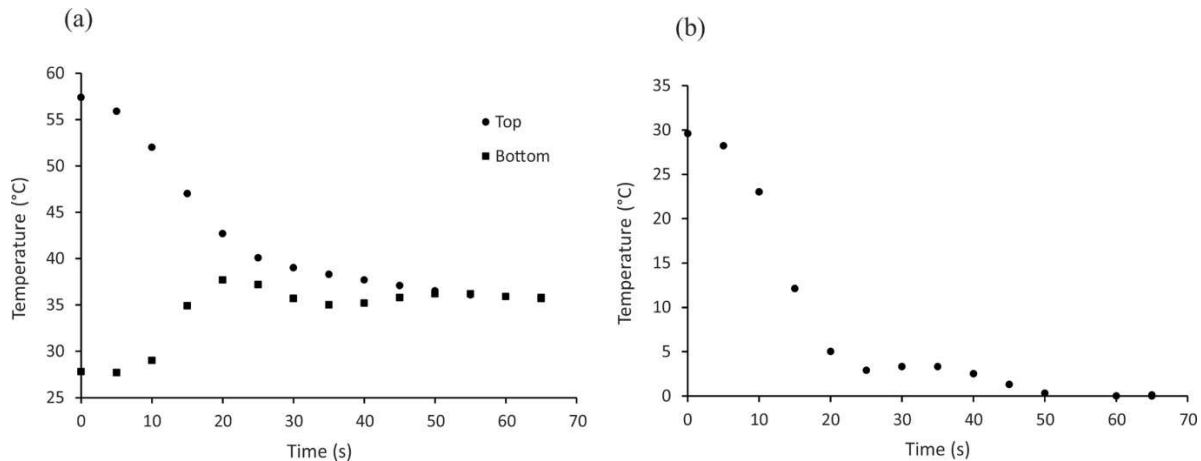


**Fig 4: Stepwise test performed with the probe initially zeroed with  $\text{N}_2$  and placed into an  $\text{O}_2$  saturated, stirred culture medium at  $25 \text{ }^\circ\text{C}$ . (a) probe response as a function of time and (b) probe response time determination**

According to Schmidell [14], fast responding probes provide 90% of the response within 20 s of reading. However, in an airlift bioreactor this time is approximately 4 times higher when dealing with readings under static conditions. This is seen for the initial phase of the dynamic assay while determining the specific oxygen uptake rate. The probe employed is the same. However, what changes is the  $\text{O}_2$  transfer resistance in the stagnated film formed between the culture medium and the probe's membrane, bringing about such variance.

### Time of mixture

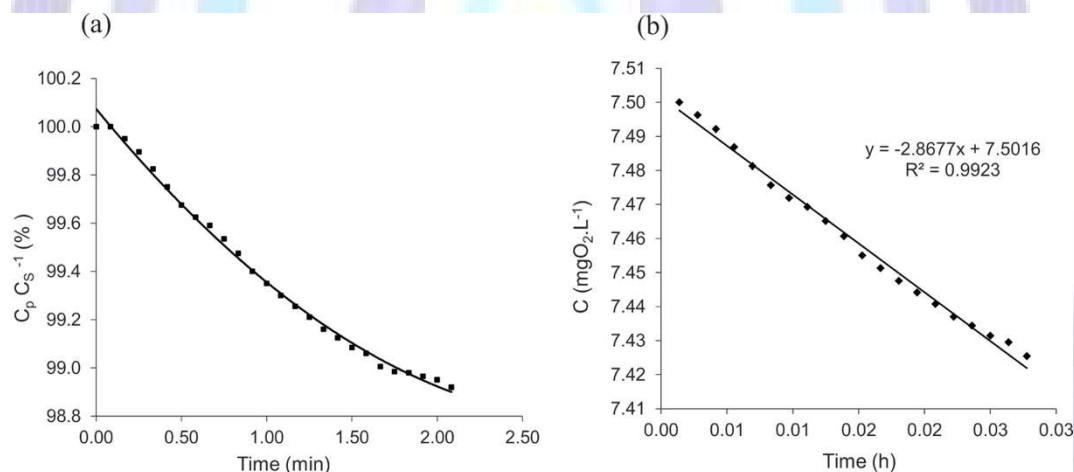
Figure 5 shows the results of an assay to determine the time of mixture. As can be seen, it takes about 50 s of mixing for a full establishment of the hydrodynamics in a flow rate of 0.36 vvm. This time is increased to 60 s and reduced to 45 s for flow rates of 0.20 and 0.52 vvm, respectively. Due to these results, in the second phase of the dynamic assay to determine the volumetric coefficient for oxygen transfer ( $k_{\text{LA}}$ ), when the flow rate was 0.2 vvm, the first 6 experimental points registered after the return of aeration (to data recorded every 10 s) were excluded from the analyses. This time was also important to reestablish the pressure in the bioreactor, and to stabilize the probe DO values, which could eliminate the needs for correction caused by the response delay in this phase.



**Fig 5: Determination of the time of mixture for an airlift bioreactor operated at a flow rate of 0.36 vvm. (a) temperature profiles for the top and the bottom and (b) temperature difference between the top and the bottom of the bioreactor**

### Oxygen specious consumption: an effect of bioreactor depressurization

Figure 6a shows the effects of depressurization in the dissolved oxygen readings, caused by the interruption in aeration while operating the bioreactor in an air flow rate of 0.2 vvm. Relative percent saturation values were transformed in concentration values utilizing an oxygen solubility value of  $7.5 \text{ mg L}^{-1}$  (Figure 6b). The angular coefficient for the data linear adjustment predicted a value equivalent to the oxygen uptake rate (specious value for product  $Q_{O_2 \cdot X}$ ) of  $2.868 \text{ mgO}_2 \text{ L}^{-1} \text{ h}^{-1}$ . This happens since the equation that foresees a linear variation for the consumption of oxygen in unlimited conditions is produced by  $C=C_0-Q_{O_2}X(t-t_0)$  [14].



**Fig 6: (a) Variation in the concentration of dissolved oxygen as a function of time, due to the interruption in aeration (0.2 vvm) and the resulting depressurization of the bioreactor. (b) determination of the effects equivalent to the oxygen uptake rate**

High flow rates create higher pressurization values, which, in turn, have a higher effect in the oxygen specious consumption. For the flow rates assessed in this study (0.36 and 0.52 vvm) we obtained oxygen specious consumption of  $3.32 \text{ mgO}_2 \text{ L}^{-1} \text{ h}^{-1}$  and  $3.62 \text{ mgO}_2 \text{ L}^{-1} \text{ h}^{-1}$ , respectively. The maximum oxygen solubility obtained in this study was  $7.5 \text{ mgO}_2 \text{ L}^{-1}$ , determined at  $25^\circ \text{C}$ . For the application in the determination of the ectomycorrhizal fungi specific oxygen uptake rate, the effects of pressure variation in the readings of  $C_p$  are considerable. It is so that we can foresee this effect also in the second phase of the dynamic assay determination, where a gradual pressurization and the reestablishment of aeration can impact the  $k_{LA}$  readings. Therefore, upon each substitution of the filtering unit in the bioreactor gas exit filtering system, there is the need to weight the material so there are no changes and, consequently, charge losses.

## DISCUSSION

For a better understanding of all processes that are related to the cultivation of ectomycorrhizal fungi intended for the production of commercial inoculum, mass transfer studies, especially for  $O_2$ , considered to be one of the most limiting factors in aerobic cultivation of microorganisms, are important. To the best of our knowledge, however, no study dealing with this phenomenon have been reported in the literature. The very few studies using airlift bioreactors [5,6] have



demonstrated the potential of this technology for the cultivation of such fungi, culminating with the mass transfer and oxygen consumption trials presented here. The dynamic methodology has proven to be adequate for this purpose, since it employs a single probe to monitor the dissolved oxygen amounts, eliminating the problems behind the addition of compounds like sulfites, which alters the physical conditions of the medium and over estimates the calculated values.

Transient conditions are commonly detected during batch cultivations. These conditions may alter the size and density of pellets and the concentration of biomass, nutrients and metabolites, among others, which can alter the growth of fungi. Additionally, the solubility of oxygen in the culture medium varies according to the nutrient consumption by the microorganism [17]. Therefore, in order to fully understand the process, it is necessary that a series of dynamic assays in different cultivation scenarios are carried out prior to any commercial application of this technology.

During the first execution phase of the dynamic assay, it is necessary to interrupt aeration. Differently from bioreactors with mechanical stirring, in airlift bioreactors this procedure causes a gravitational sedimentation of the biomass. This phenomenon creates oxygen consumption differences between the top and the bottom of the bioreactor. At the bottom of the bioreactor, there will be higher oxygen consumption caused by cell build up brought about by biomass sedimentation. An opposite effect is seen for the top of the bioreactor, which gets more cell rarefied and, therefore, lower oxygen consumption. Other outcomes are the increase in the probe response time and the hydrodynamic effects related to the DO concentration and homogenization gradients in the reestablishment of aeration.

The variation in the speed of sedimentation observed in some of the cultivations is caused mainly by microbial mycelial growth and, therefore, pellets mass increase. The different mass concentration zones that are formed as a function of the different particle sizes, typical in sedimentation assays, were not detected in the short time spans used for this assay. The time elapsed for the sedimentation of the zone presenting uniformly shaped pellets was used to determine the speeds of sedimentation. A constant cell concentration was assumed for the region where the probe was mounted, since cells leaving that particular physical placement are considered to be immediately replaced by others coming from above.

Probe response delay happens because of the time necessary for the dissolved oxygen from the liquid to be diffused through the probe membrane, which separates the liquid from the cathode surface (where oxygen is reduced generating an electron flux). Furthermore, that is the region where a stagnated film is generally formed. The film's width is dependent on the speed that the liquid flows through the surface of the probe [14]. During the first phase of the dynamic assay in an airlift bioreactor, the liquid remains practically still, with just a few subtle whirling pulses due to biomass sedimentation. On the other hand, the introduction of aeration in the second phase mixes and turns the medium. Therefore, distinct probe response delay values are seen for each phase of the dynamic assay. This implies that the response time in the interval that permits the determination of  $Q_{O_2}$  must be higher than that used to determine the  $k_{L,a}$ .

The interruption of aeration creates a depressurization in the bioreactor and a subsequent variation in the dissolved oxygen concentration. This happens both due to the oxygen partial pressure variation and the pressure effect over the probe's membrane. It works like if there was an extra consumption of oxygen. With the reestablishment of aeration, there is an initial instability in the readings due to the mixing of the liquid with different concentrations of dissolved oxygen and also due to the bioreactor's hydrodynamic (behavior of the mixture). These values can have substantial impacts for the cultivation of fungi with low oxygen rate consumption, and therefore need to be adjusted.

The specious consumption of oxygen, due to depressurization and reestablishment of the air-liquid equilibrium, must be taken into account during the dynamic assays to determine  $Q_{O_2}$ . These conditions are important since the ectomycorrhizal fungi have a low specific growth rate ( $\mu_x$ ) and, consequently, low specific oxygen uptake rate ( $Q_{O_2}$ ). The potential effect over the  $k_{L,a}$  readings during pressurization will be, in part, mitigated during the time of mixture when partial pressure stabilization is noticed. Despite this fact, during this interval the data is not considered for the analysis. A possible solution to this problem would be the building of a device that would block the exit of the gases simultaneously with the interruption in aeration. In this way the bioreactor would be kept pressurized, solving, consequently, the reading interferences caused by depressurization and pressurization.

The adaptation of the dynamic method, employing the parameters discussed earlier, was the best solution found for the determination of the specific oxygen uptake rate and the  $k_{L,a}$  during cultivation. Hybrid airlift bioreactors, like the one tested by Chisti and Jaureghi-Haza [18], did not present all these technical hitches since the biomass was maintained in suspension by stirring. Another way of keeping biomass in suspension during the period when aeration is interrupted is the utilization of an external pumping system to promote the stirring of the liquid. However, this concept is hard to idealize under aseptic conditions.

Unconventional bioreactors employed for the cultivation of slow growing microorganisms create difficulties in the determination of information about the kinetics, important for the establishing and development of the process. The establishment of the most suitable operating standards is the main objective of studies like the one presented here. These studies seek conditions that can, for example, avoid situations of critical oxygen concentration, wastes of energy, stresses caused by shearing, and oxidative environments.

The parameters evaluated in this study for the application of the dynamic method were fundamental for the determination of the coefficient of oxygen transfer, and the specific oxygen uptake rate throughout the entire cultivation cycle. Nevertheless, there are many aspects of the application of the dynamic method for airlift bioreactors that still need further investigation. Therefore, the aim of the current study is to provide insights that will stimulate additional investigations in topics such as mass transfer, to improve the comprehension about the difficulties encountered when trying to produce large amounts of ectomycorrhizal fungal biomass.



Table 1 List of symbols

Symbol	Description	Unit
$A_d$	cross-sectional area of the downcomer	$\text{cm}^2$
$A_r$	cross-sectional area of the riser	$\text{cm}^2$
$C$	oxygen concentration in the liquid phase	$\text{mgO}_2 \text{ L}^{-1}$
$C_s$	oxygen concentration in equilibrium with gas phase	$\text{mgO}_2 \text{ L}^{-1}$
$C_p$	probe oxygen signal readings	$\text{mgO}_2 \text{ L}^{-1}$
$D$	internal diameter of the riser	m
$H$	height of the riser	m
$k_{L,a}$	volumetric coefficient for oxygen transfer	$\text{s}^{-1}; \text{d}^{-1}$
$k_p$	probe response delay constant	$\text{h}^{-1}$
$Q_{O_2}$	specific oxygen uptake rate	$\text{mgO}_2 \text{ g}_{\text{cell}}^{-1} \text{ h}^{-1}$
$Q_{O_2,X}$	oxygen uptake rate by the cell	$\text{mgO}_2 \text{ L}^{-1} \text{ h}^{-1}$
$t$	Time	s, min, h
$v_{vm}$	specific air flow rate (air volume per liquid reactor volume per minute)	$\text{min}^{-1}$
$X$	biomass concentration	$\text{g L}^{-1}$
$\mu_x$	cell growth specific velocity	$\text{day}^{-1}$

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## Author' biography



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