



Unveiling thermotolerant and entomopathogenic fungi potential for solid-state fermentation: Multiscale evaluation in packed-bed bioreactor

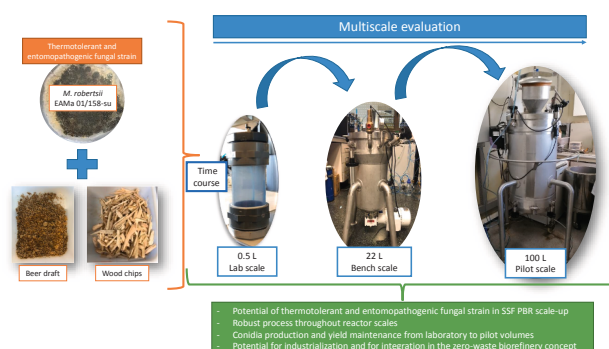
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HIGHLIGHTS

- Multiscale evaluation for conidia production by SSF in PBR up to 100 L is achieved.
- Thermotolerant and entomopathogenic *M. robertsii* strain scale-up potential is demonstrated.
- Conidia production (conidia g⁻¹BSG) was maintained from laboratory to pilot scale.
- Process robustness was achieved by maintaining product quality at all volumes.
- Results advance towards using integrated solutions to solve the SSF scale-up problem.

GRAPHICAL ABSTRACT



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ABSTRACT

This work presents the production of fungal conidia from the thermotolerant and entomopathogenic fungi *Metarhizium robertsii*, by means of solid-state fermentation (SSF) in packed-bed bioreactors (PBR) up to a pilot scale of 100 L. The *M. robertsii* strain used (EAMa 01/158-su) presents both thermotolerant and entomopathogenic properties. Its thermotolerance is an unusual trait as entomopathogenic strain, and can help to surpass heat accumulation, the main limitation when increasing scale in SSF. Using a mixture of beer spent grains (BSG) and wood chips as substrates, work is performed at 3 different scales: laboratory (0.5 L), bench (22 L) and pilot (100 L). Air-filled porosity (AFP), specific airflow (sAF) and superficial velocity (V_z) were chosen as parameters to compare across scales. At 0.5 L, production time was optimised at 5 days. At 22 L, successful batch operation yielded homogeneous production throughout the packed bed. At pilot scale of 100 L, conidia production (conidia g⁻¹BSG) and yield achieved were not significantly different from values achieved at 0.5 L, confirming the robustness of the process across reactor volumes. The results highlight the relevance of AFP, sAF and V_z as critical parameters for SSF reactor design and process evaluation and provide a basis for future industrial implementation of fungal SSF in PBR. This work validates thermotolerant entomopathogenic fungi as a viable strategy for industrial-scale SSF in packed-bed bioreactors, demonstrating the potential of thermotolerant entomopathogenic strains in fungal PBR conidia production by reaching pilot scale of 100 L.

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1. Introduction

In recent years, circular bioeconomy has gained attention as an emergent strategy to mitigate current global environmental challenges (Muscat et al., 2021). The use of organic waste as raw material for bioproduction contributes to changing the paradigm “from waste to sources” by closing the organic loop (Sánchez, 2024), representing an effective approach to the global concern of reducing waste generation (Lu et al., 2024). Apart from traditional bioprocesses that have been scaled up to industrial volumes, such as composting and anaerobic digestion, solid-state fermentation (SSF) arises as a promising complementary technology for organic waste valorisation and the development of circular bioeconomy.

SSF is a biological process that occurs in near absence of free water, in which organic waste acts as substrate to produce a specific bioproduct with the inoculation of a specific strain (bacteria, yeast or fungi) or using the autochthonous microbial population (Oiza, 2025). SSF has been explored to produce a wide range of bioproducts, including bio-surfactants (Bueno-Mancebo, 2025), enzymes, (Oliveira et al., 2020), biopesticides (Sala et al., 2021a) and biostimulants (Ghoreishi et al., 2023), emerging as a suitable and environmentally friendly technology to treat organic waste while generating marketable bioproducts (De la Cruz Quiroz et al., 2015). SSF offers interesting advantages. For example, the waste usually requires little pre-treatment before use (involving cost reduction when compared with the use of complex media) and the process needs less water, especially when compared with traditional submerged fermentation. Other advantages of SSF include cost reduction due to the use of wastes as substrates and, often, simplified downstream processing when the fermented solids containing the bioproduct can be directly used without further separation. However, SSF has challenges to overcome for its inclusion in the biorefinery scheme, particularly related to scale-up (Sánchez, 2024). The inherent complexity of SSF becomes more pronounced during scale-up, where engineering limitations (oxygen and heat transfer) are amplified (Mitchell and Ruiz, 2023). These constraints lead to irreproducibility and difficulties in obtaining a homogeneous product.

Packed bed bioreactor (PBR) is one of the most used designs when dealing with solid matrices. In PBR, microorganisms are cultivated in a static solid bed with a continuous gaseous phase flowing through the solid particles, from the bottom to the top of the reactor (Mitchell and Krieger, 2019). PBRs have great potential for converting lignocellulosic biomass and for filamentous fungi cultivation. In addition, PBR application has the potential to solve the problem of spatial use, which is not optimized in traditional SSF reactor configurations like bags or trays (Mitchell and Ruiz, 2023). However, PBRs are particularly affected from SSF scale-up constraints, hampering its use at volumes beyond bench scale. Several studies have demonstrated the formation of oxygen and heat gradients throughout the packed bed. So far, all the approaches to overcome these hindrances have been focused on the application of engineering solutions, testing different scale-up criteria (nominal superficial velocity [V_z], specific airflow [sAF], air-filled porosity [AFP], etc) or using variations of the traditional PBR designs (Sala et al., 2021a), (Mitchell and Ruiz, 2023), (Rodrigues et al., 2022), (Méndez-González et al., 2024). Although some have been relatively successful at maximizing heat removal, none has tested the use of fungi as inoculum at pilot-scale volumes (at least, 100 L), where greater temperature increments are expected. However, remarkable results at volumes up to 290 L have been achieved when using *B. thuringiensis* as inoculum (Mejías, 2020), demonstrating the potential of the PBR configuration.

Although not a scale-up strategy per se, the use of thermotolerant strains can be a complementary microbiological solution to help cope with heat accumulation during SSF scale-up. Thermotolerant organisms can survive above 40°C (Mattoon et al., 2021) and different genera have been cultivated and studied for enzymatic production (Perez et al., 2019) and their use in lignocellulosic biorefineries (Arora et al., 2015). However, thermotolerant fungi have not been used for conidia

production, even at laboratory scale. For future microbial biopesticide products, conidia of entomopathogenic fungi represent promise, however, the use of thermotolerant biopesticide strains for fungal SSF production is still unveiled. Promising thermotolerant and entomopathogenic fungal strains have been discovered and reported, however, their production strategies have not yet been studied (Yousef-Yousef et al., 2022).

This work presents a novel approach to assess heat generation and transfer in the SSF PBR volume change. A thermotolerant strain of the genus *Metarhizium* was used to mitigate the effect of heat generation in a multiscale evaluation experiment, alongside the discussion of key PBR scale-up parameters such as AFP, V_z or sAF. Successful conidia production on a conventional PBR design from laboratory scale of 0.5 L to pilot scale of 100 L was achieved.

To the best of the authors' knowledge, this is the first work to test the performance of a thermotolerant entomopathogenic fungal strain for conidia production under at different process volumes in a packed-bed SSF system up to pilot-scale conditions (100 L). The work addresses a knowledge gap on the use of thermotolerant and entomopathogenic fungal strains as strategy of heat stress mitigation in fungal SSF PBR scale-up, which is key for its implementation at industrial scale and for its integration in the scheme of waste industrial biorefineries.

2. Materials and methods

2.1. Fungal strain

A *Metarhizium robertsii* strain (EAMa 01/158-Su) was kindly provided by the Agricultural Entomology Research Group AGR 163, from the Department of Agronomy of the University of Córdoba. EAMa 01/158-Su is a thermotolerant strain and has been previously described as pathogenic against several insects (Garrido-Jurado et al., 2020; Resquín-Romero et al., 2016; Yousef-Yousef et al., 2022). The strain was cultured in potato dextrose agar (PDA) at 30 °C for 2 weeks and kept frozen in 10 % glycerol until use.

2.2. Raw materials

Brewer's spent grain (BSG, sourced from Cervesa del Montseny S.L., Sant Miquel de Balenyà, Barcelona) was used as a main substrate for fungal conidia production. The material was stored frozen until the start of the fermentations to prevent biodegradation. Wood chips (WC, Acalora, Ivars d'Urgell) were used to adjust AFP to different values depending on the proportion used, ensuring proper air distribution throughout the packed bed. Initial moisture was adjusted in the range of 50–75 %, stated as optimal for fungal fermentation by several authors (Pham et al., 2010; Sala et al., 2020; Xie et al., 2016). AFP was adjusted to values around 75 %, previously described as optimal for fungal fermentation using different fungal strains when working with a 22 L PBR (Sala et al., 2023) (Sala et al., 2021a). Inoculum volume was adjusted to 10 % of the total volume and inoculum conidia concentration was adjusted to values close to 6.5×10^6 conidia $g^{-1}dm$, optimal values found in previous works of the same authors (Sala et al., 2020). To prevent contamination, all substrates (with independence on the volume of the reactor) were autoclaved twice, and inoculation was always performed inside laminar flow chamber. Raw substrates and mixtures characterization is shown in Table 1.

2.3. Solid-state fermentation

Different SSF tests were performed using packed-bed reactors of varying volumes, as part of a multiscale evaluation of the process. Laboratory scale reactors (0.5 L) were used to determine the optimal fermentation time to maximize *M. robertsii* conidia concentration. A bench scale reactor (22 L) was then used to assess process behaviour at an intermediate volume. Finally, a 100 L reactor was employed to

Table 1

Raw substrates and mixtures characterization.

Substrate/ parameter	Beer spent grains ¹	Wood chips ¹	80/20 w/w (beer spent grains/wood chips) ²	40/60 w/w (beer spent grains/wood chips) ²
MC (%)	74.6 ± 0.7	9.3 ± 0.3	73.8 ± 1.3	53.5 ± 4.4
WHC (%)	47.5 ± 0.3	91.1 ± 1.1	45.6 ± 0.8	67.5 ± 1.9
OM (%)	94.3 ± 0.8	98.6 ± 0.7	93.9 ± 0.7	96.9 ± 0.8
pH	6.4 ± 0.3	5.3 ± 0.2	5.5 ± 0.4	5.2 ± 0.9
Carbon (%)	45.9 ± 0.5	47.4 ± 0.7	46.2 ± 0.5	46.8 ± 0.6
Hydrogen (%)	6.8 ± 0.3	5.9 ± 0.4	6.6 ± 0.5	6.3 ± 0.4
Nitrogen (%)	4.2 ± 1.4	0.3 ± 0.2	3.4 ± 0.4	1.9 ± 0.3
Sulphur (%)	0.1 ± 0.01	<0.1	<0.1	<0.1
C/N ratio	10.9 ± 3.2	158 ± 4.7	40.3 ± 2.7	99.2 ± 8.1
AFP (%)	62.4 ± 0.5	95.8 ± 0.7	75.8 ± 1.1	76.4 ± 0.7
Max sOUR (g O ₂ kg ⁻¹ dm ⁻¹)	3.5 ± 0.6	0.4 ± 0.1	3.0 ± 0.6	1.6 ± 0.3
COC ^{6D} (g O ₂ kg ⁻¹ dm ⁻¹)	17.7 ± 1.9	1.0 ± 0.2	14.1 ± 0.8	7.1 ± 0.6

MC: moisture content; WHC: water holding capacity; OM: organic matter; AFP: air-filled porosity; max: maximum; sOUR: specific oxygen uptake rate; COC: cumulative oxygen consumption; 6D: six days; dm: dry matter; w/w: weight/weight; ¹: raw material; ²: mixture.

evaluate the feasibility of the process under pilot-scale conditions. Parameters analysed across scales included AFP, sAF and V_z.

2.3.1. Experimental set-up for laboratory scale: 0.5 L reactors

Experimental set-up of this work was performed as in previous works of the same research group (Ghoreishi et al., 2023; Sala et al., 2020). Briefly, at laboratory scale, the 0.5 L reactors were made of polyvinyl chloride, cylindrical shaped of 13 cm height and 7 cm diameter. Reactors were placed in a water bath to ensure constant temperature of 30 °C. A total of 100 g of inoculated mixture (BSG + wood chips) were loaded in each reactor. Constant aeration (20 mL min⁻¹, corresponding to sAF of 0.67 – 0.74 mL min⁻¹ g⁻¹dm) was provided by means of a mass flowmeter (Mass-Stream D-6311, Bronkhorst, Netherlands). To provide humidity control over the solid matrix, the air was humidified before entering the reactors from the bottom, forcing it to flow through the mixture across all the reactor's height until reaching the top of the reactor. The air flows through a perforated grid, evenly distributed across the solid matrix at the bottom of the reactor. Exhaust gases flow through a vapour trap to prevent excess water from reaching the O₂ sensor. The oxygen percentage in the output gases was measured by an electrochemical O₂-A2 oxygen sensor (Alphasense, UK, precision of ± 0.1 % O₂ and measuring once each 0.0167 h). Data analysis was performed by a non-commercial tailor-made software Arduino® based which calculates the respiration rates (see section 2.3.7).

Humidity control and air uniformity systems were scaled and replicated both at bench and pilot scales.

2.3.2. Experimental set-up for bench scale: 22 L reactor

At bench scale, the reactor consisted of a cylindrical stainless-steel vessel with a removable basket of 48 cm height × 24.5 cm diameter, presenting a total volume of 22 L. A total of 6 kg of inoculated mixture were loaded into the basket. Air supply and data acquisition system were the same as for 0.5 L reactors (section 2.3.1). Constant aeration (2000

mL min⁻¹, corresponding to sAF of 0.67 – 0.81 mL min⁻¹ g⁻¹dm) was provided. Temperature of the solid media was monitored on-line in the lower half part of the bed by means of a temperature probe (Pt-100 sensors, Sensotrans), while also obtaining accurate temperature profiles using temperature sensors (standard Thermochron iButton device, Maxim Integrated, U.S., precision of ± 1°C and measuring once each 20 min) at different heights of the bed (0, 12, 24 and 36 cm) both at the centre of the packed bed and at the basket wall. External wall and room temperature were also monitored.

For final analysis at the end of the experiment, the fermented solid matrix was divided into three sections (superior, central or lower) based on their axial position in the reactor's bed (28–40, 12–28 and 0–12 cm height, respectively). Data reported herein for the final sample of the 22 L reactor fermentation corresponds to the average value of nine samples. Conidia concentration, moisture, pH and conidia germination were analysed at different reactor heights. In addition, the reactor was sampled twice (at days 4 and 6). The 4-days sample was obtained exclusively from the top of the 28–40 cm section, while the sample from day 6 was a mixture from the entire reactor volume.

2.3.3. Experimental set-up for pilot scale: 100 L reactor

Pilot scale reactor consisted in a cylindrical stainless-steel vessel of 80 cm height x 45 cm diameter, presenting a total volume of approximately 115 L. Size proportions were maintained with 22 L reactor. A total of 30 kg of inoculated material was loaded in the reactor, reaching a maximum height which roughly surpassed 60 cm. The reactor did not have a removable basket. Air supply was the same as in previous experimental set-ups, with constant airflow of 10 L min⁻¹, corresponding to a sAF of 0.73 – 0.83 mL min⁻¹ g⁻¹dm. A Clip Sens Series O₂ sensor (Sensotran, Spain) was connected to the output gases after the vapour trap to monitor O₂ concentration, allowing periodic O₂ % measurements at a lower frequency than in the other reactors.

Accurate temperature profiles were obtained using the same sensors as in 22 L set-up. Sensors were distributed at different heights (0, 20, 40 and 60 cm) and radial position (centre of reactor 0 cm, r/2 11.25 cm and radius at wall 22.5 cm), making a total of 16 sensors inside the reactor's packed bed. Room temperature was also monitored.

The reactor was sampled twice (at day 4 and at day 8), with sample of day 4 being obtained from the top of the 40–60 cm section. Similar to 22 L sampling, final samples of 100 L reactor were divided in three areas (superior, central or lower) depending on their axial position in the reactor's bed (40–60, 20–40 and 0–20 cm height). Of each area, three samples were analysed, corresponding to centre of the packed-bed, close to the reactor's wall and a mix. With this method, data correspondent to last sample of each reactor corresponds to the media value of nine samples. Same parameters as in 22 L scale were analysed at different reactor heights and radius, providing statistical difference between reactor areas.

2.3.4. Sterile conditions maintenance

Procedures to ensure working in conditions as sterile as possible in the bench (22 L) and pilot (100 L) reactors were as follows. Reactors were cleaned with water, bleach and ethanol before the fermentation. Inoculation was performed inside laminar flow chamber in previously cleaned tray vessels, in a maximum of 500 g of non-inoculated material for the 22 L reactor and of 2 kg for the 100 L reactor. Previous assays on moisture and AFP were performed to ensure its maintenance after reactor loading. This procedure ensured maximum homogeneity of the inoculum throughout the packed bed. For the 22 L reactor, the material was loaded into the previously autoclaved removable basket inside the laminar flow chamber, then transported to the vessel covered in autoclaved aluminium foil. For the 100 L reactor, material was directly transported to the vessel using trays covered in autoclaved aluminium foil, introduced into the vessel through the top cover and mixed again using an autoclaved ladle.

2.3.5. Time course test at 0.5 L

A time course test was performed to establish the optimal conidia concentration time in terms of productivity (conidia $\text{g}^{-1}\text{dm}^{-1}$). It consisted in 6 reactors of 0.5 L with the same initial conditions, harvested at days 2, 3, 5, 7, 9 and 12. Conidia concentration and conidia germination were evaluated as response parameters, while moisture and pH were also monitored.

2.3.6. Oxygen uptake rate

Specific oxygen uptake rate (sOUR) was calculated according to previous works of the research group (Sala et al., 2020) (Ghoreishi et al., 2023), expressed as 1 h average value (sOUR) (Equation 1).

$$\text{sOUR} = F \cdot (0.209 - y_{\text{O}_2}) \cdot \frac{P \cdot 32 \cdot 60 \cdot 10^3}{R \cdot T \cdot \text{DM}} \quad (1)$$

where: sOUR is the specific Oxygen Uptake Rate ($\text{g O}_2 \text{ kg}^{-1} \text{ dm h}^{-1}$); F, airflow (mL min^{-1}); y_{O_2} , is the oxygen molar fraction in the exhaust gases ($\text{mol O}_2 \text{ mol}^{-1}$); P, pressure of the system assumed constant at 101325 Pa; 32, oxygen molecular weight ($\text{g O}_2 \text{ mol}^{-1} \text{ O}_2$); 60, conversion factor from minute to hour; 10^3 , conversion factor mL to L; R, ideal gas constant ($8310 \text{ Pa L K}^{-1} \text{ mol}^{-1}$); T, temperature at which F is measured (K); DW, initial dry matter of solids in the reactor (g); 10^3 , conversion factor g to mg.

To provide information of the biological activity and as a direct measure of the degraded carbon, the cumulative oxygen consumption (COC) was calculated as the area below the O_2 consumption curve (Sala et al., 2021a).

When working with the 100 L set-up, sOUR was expressed as average value of 3 punctual measurements each 5 min, as it was not possible to collect enough data to obtain the same frequency achieved for the rest of fermentation set-ups.

2.4. Conidia counting and germination

Conidia production was analysed by means of conidia concentration, conidia productivity and conidia germination.

Fungal conidia concentration was determined using the method described in previous works of the authors (Sala et al., 2020). Briefly, a Neubauer chamber (BrandTM 717805) was used. 10 g of sample (conidiated mixture) were mixed with 50 mL of Tween 80 0.1 %, shaken for 20 min at 150 rpm and appropriately diluted before counting. All cell counts were performed per triplicate and related to the dry matter present in the reactor at the counting time, following Equation 2:

$$\text{Concentration} = \frac{n^\circ \text{ of conidia}}{\text{CV} \cdot \text{DF}} \cdot \frac{\text{EV}}{\text{DM}} \quad (2)$$

Where: Concentration is the conidia concentration in the initial tube (conidia g^{-1}dm); n° of conidia, the counted conidia in the Neubauer chamber at a known dilution; CV, Neubauer chamber counting volume (mL); DF, dilution factor of the counting tube; EV, extraction volume (mL); DM, dry matter in the sample (g dm).

Conidia productivity (conidia $\text{g}^{-1}\text{dm}^{-1}$) was calculated by dividing conidia concentration (conidia g^{-1}dm) by fermentation time (in days) at the moment of the counting.

Volumetric conidia concentration (conidia L^{-1}) was calculated by multiplying conidia concentration (conidia g^{-1}dm) by the grams of dry matter present in the reactor and diving by the total volume of each reactor.

Conidia production (conidia g^{-1}BSG) was calculated by subtracting the initial conidia present in the inoculum, multiplying conidia concentration (conidia g^{-1}dm) by the grams of dry matter present in the reactor and dividing by the grams of BSG present in the reactor. Conidia production values were used to obtain the product/inoculum yield, expressed as a fraction of the conidia present in the initial mixture. For these calculations, BSG was considered as substrate, and wood chips were considered as support material.

Conidial viability was expressed as the percentage of germinated conidia. Conidia germination tests were performed prior to the use of all suspensions (inoculums) and for all samples obtained during fermenta-

tion, as described in previous works of the same authors (Sala et al., 2023). Germination serves as a quantitative indicator of the quality of the conidia. Briefly, after conidia counting, 100 μL of 10^6 diluted conidia suspension were transferred to a PDA plate and incubated at 30°C for 48 h. Following, three glass cover slips were placed on the agar surface of each plate, and 100 conidia were counted under each cover slip, distinguishing between germinated (Gc) and non-germinated (NGc) conidia by identification of presence of germ tube. Conidia germination (%) under each cover slip was calculated following Equation 3.

$$\text{CG}(\%) = 100 \cdot \frac{G_c}{(G_c + \text{NGC})} \quad (3)$$

Where: CG: conidia germination (%); GC: germinated conidia; NGC: non-germinated conidia.

To ensure uniform quality, all inoculum used in this work presented conidia germination percentages higher than 95 %

2.5. Analytical methods

Standardized methods have been used to determine moisture (%), dry matter (%), organic matter (%) and pH (Leege, 1998). Chemical elemental analysis was used to perform C/N analyses to all initial samples. Results are shown in Table 1. Elemental analysis includes carbon, nitrogen, hydrogen and sulphur analyses for raw substrates and mixtures used in the presented fermentations.

AFP, V_z , and sAF were chosen as parameters to compare in the change of volume, due to their relevance in SSF scale-up process, especially when working with the packed-bed configuration (Mitchell and Ruiz, 2023).

AFP was calculated according to Equation 4 as presented by Richard et al. (Richard et al., 2004):

$$\text{AFP} = 1 - \text{BD}_t \left(\left(\frac{1 - \text{DM}}{\text{D}_w} \right) + \frac{\text{DM} \cdot \text{OM}}{\text{PD}_{\text{OM}}} + \left(\frac{\text{DM}(1 - \text{OM})}{\text{PD}_{\text{ash}}} \right) \right) \quad (4)$$

Where: AFP, air-filled porosity (%); BD_t , total bulk density on a wet basis in the correspondent reactor configuration (kg m^{-3}); dm, dry matter on a wet basis (%); OM, organic matter on a dry basis (%); D_w , water density (1000 kg m^{-3}); PD_{OM} , organic fraction particle density (1600 kg m^{-3}) and PD_{ash} , ash particle density (2500 kg m^{-3}).

V_z (cm min^{-1}) was calculated by dividing the airflow rate ($\text{cm}^3 \text{ min}^{-1}$) by the cross-sectional area of the empty bioreactor (cm^2) (Mitchell and Ruiz, 2023).

sAF ($\text{mL min}^{-1} \text{ g}^{-1}\text{dm}$) was calculated by dividing the airflow rate (mL min^{-1}) by the grams of dry matter (gdm) present in the reactor at the time of calculation.

Water holding capacity of the substrates and mixtures used was calculated following Equation 5:

$$\text{WHC}(\%) = 100 \cdot \frac{(W_s - W_0)}{W_0} \quad (5)$$

Where: WHC: water holding capacity (%); W_s : water saturated weight of the solid sample after 2 h soaked on water (g); W_0 : dry weight of the solid sample (g).

2.6. Statistical analyses

Statistical difference between samples was analysed by means of a one-way ANOVA ($p < 0.05$ confidence) with the Tukey test. Results were classified in letter groups. Those with different letter groups were significantly different.

All statistical analyses were done using Minitab 17 (Minitab Ltd) software.

3. Results and discussion

3.1. Phase 1: Determination of the optimal fermentation time for *Metarhizium robertsii* EAMa 01/158-Su SSF in 0.5 L PBR

A time course experiment of the fungal SSF was conducted at a constant temperature of 30°C to monitor the evolution of the main

parameters during the fermentation process. Maximum productivity was observed on day 3 (Fig. 1). However, there were no significant differences (p-value 0.163) between productivity values on days 3 and 5, which were $4.5 \times 10^9 \pm 0.4 \times 10^9$ and $4.0 \times 10^9 \pm 0.6 \times 10^9$ conidia $\text{g}^{-1} \text{dm} \text{d}^{-1}$, respectively. Conidia concentration showed no significant differences between day 5 and 7 ($2.0 \times 10^{10} \pm 0.3 \times 10^{10}$ vs $2.1 \times 10^{10} \pm 0.2 \times 10^{10}$ conidia $\text{g}^{-1} \text{dm}$). The product/inoculum yield obtained herein was 2922 ± 154 .

Most fungal SSF works at laboratory scale using a similar reactor configuration reached values lower than 1×10^{10} conidia $\text{g}^{-1} \text{dm}$, with higher values achieved only when using industrial configurations like polypropylene bags or trays (Muñiz-Paredes et al., 2017). Prior to this work, *M. robertsii* strain EAMa 01/158-Su was grown by submerged fermentation (SmF) in flasks using a defined medium (Yousef-Yousef et al., 2022). In that study, the fermentation process required 8 days, compared to the 5–7 days observed here to reach maximum conidia concentration (Fig. 1), highlighting the potential of SSF for efficient fungal propagule production. Other *M. robertsii* strains fermented in packed-bed reactors show conidia concentration around 3×10^9 conidia $\text{g}^{-1} \text{dm}$ within 8–10 d fermentation (Méndez-González et al., 2020). SmF in flasks has been broadly used to obtain propagules of several *Metarhizium* strains (García Riaño et al., 2024; de Lira et al., 2020).

Conidia germination remained stable around 95 % across all samples, ensuring sample quality throughout the whole fermentation period.

Maximum sOUR of $4.9 \text{ g O}_2 \text{ kg}^{-1} \text{dm h}^{-1}$ was reached 1.75 d after the start of the fermentation, obtaining higher values than those previously described with a fungal monoculture (Méndez-González et al., 2020), (Sala et al., 2021b). Although sOUR values heavily depend on the biodegradability of the used substrate, maximum values obtained using similar substrates were typically on the range of $2\text{--}4 \text{ g O}_2 \text{ kg}^{-1} \text{dm h}^{-1}$ (Sala et al., 2021b). Temperature difference might also have affected the overall performance, although difference between this work and previous works was of only 5°C (25 vs 30°C), which should imply little effect due to differences in thermotolerance between used strains.

3.2. Phase 2: Conidia production of *Metarhizium robertsii* EAMa 01/158-Su at bench scale of 22 L PBR

To maintain an appropriate AFP value, due to the relevance of this parameter when scaling fungal SSF, BSG/WC ratio was changed to 40/60 w/w instead of 80/20 used at 0.5 L, resulting in AFP between 75 and 80 % (see Table 1). The proposed value was reported as high enough to ensure proper air distribution throughout a 22 L packed bed in our previous works using different fungal strains and substrates (Sala et al., 2021a),(Sala et al., 2023).

Fig. 2a shows the evolution of process parameters. Maximum concentration ($8.0 \times 10^9 \pm 1.0 \times 10^9$ conidia g^{-1}) and productivity (1.33×10^9

$\pm 0.58 \times 10^9$ conidia $\text{g}^{-1} \text{dm d}^{-1}$) were achieved at day 6, being significantly different from day 4 ($5.7 \times 10^8 \pm 0.6 \times 10^8$ conidia $\text{g}^{-1} \text{dm d}^{-1}$ p-value 0.032). Lag phase of roughly 1.5 d, being longer than at 0.5 L, caused maximum productivity to occur later at 22 L. Obtained values were close to half of the ones yielded when working at 0.5 L volume. These results were expected due to the use of a different BSG/WC proportion. The change from 80/20 to 40/60 (w/w) implies a diminishment in the total amount of easily available carbon and nitrogen for fungal growth and sporulation. Substrate mixture change is also exemplified by COC^{6D} , presenting higher values when working at 0.5 L in comparison to 22 L (14.0 vs $5.4 \text{ g O}_2 \text{ kg}^{-1} \text{dm h}^{-1}$). Lower carbon availability, coupled with higher temperatures achieved in the reactor, results in hyphal growth reduction and, ultimately, less sporulation. As previously stated, AFP adjustment is mandatory to ensure proper air distribution through the packed bed, which would not be possible at 22 L if working with 80/20 w/w proportion (Sala et al., 2021a). Hence, for a proper evaluation of the results between different reactor volumes, conidia production must be corrected by the quantity of BSG used (conidia $\text{g}^{-1} \text{BSG}$) in each fermentation. This discussion will be presented in section 3.4.

The product/inoculum yield obtained in the 22 L fermentation was 4819.5 ± 410.5 . These values are almost double the yield values obtained when working at 0.5 L volume.

Conidia germination presented a stable value around 95 % from day 4 until the end of the experiment, maintaining the quality observed at laboratory scale from day 4 onwards.

Maximum sOUR of $2.2 \text{ g O}_2 \text{ kg}^{-1} \text{dm h}^{-1}$ was reached 2.9 d after the start of the fermentation. In consonance with conidia concentration, sOUR was delayed (approx. 0.5 d) and reduced (from 4.9 to $2.2 \text{ g O}_2 \text{ kg}^{-1} \text{dm h}^{-1}$) when comparing to 0.5 L SSF. This behaviour has been previously reported when scaling several fungal SSF processes (Sala et al., 2023),(Méndez-González et al., 2024),(Melikoglu et al., 2015). As oxygen and carbon consumption are linked, sOUR differences seem to be caused by substrate proportions in the matrix.

Fig. 2b shows mean temperature profiles evolution at different reactor positions in the radial axis. Despite temperature differences, the thermotolerant strain *M. robertsii* EAMa 01/158-Su was capable of homogeneously grow throughout the entirety of the packed-bed. These results highlighted the strain's capability of withstanding high temperatures without decrease in conidia yield, demonstrating the robustness of the new production strategy across different scales. A detailed multiscale evaluation is provided in section 3.4, including a comparison of production performance across the various reactor sections.

3.3. Phase 3: Conidia production of *Metarhizium robertsii* EAMa 01/158-Su at pilot scale of 100 L PBR

The evaluation at pilot scale was conducted using a 100 L PBR batch bioreactor. The evolution of process parameters at this scale is shown in

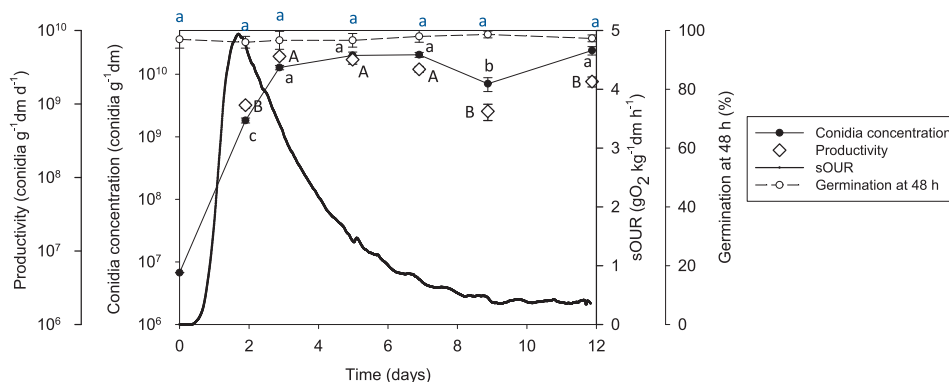


Fig. 1. Conidia concentration, productivity, specific oxygen uptake rate and % conidia germination at 48 h profiles obtained in time course test at 0.5 L scale. When shown, statistical difference is presented in lowercase (conidia production), uppercase (productivity) and lowercase in blue (germination).

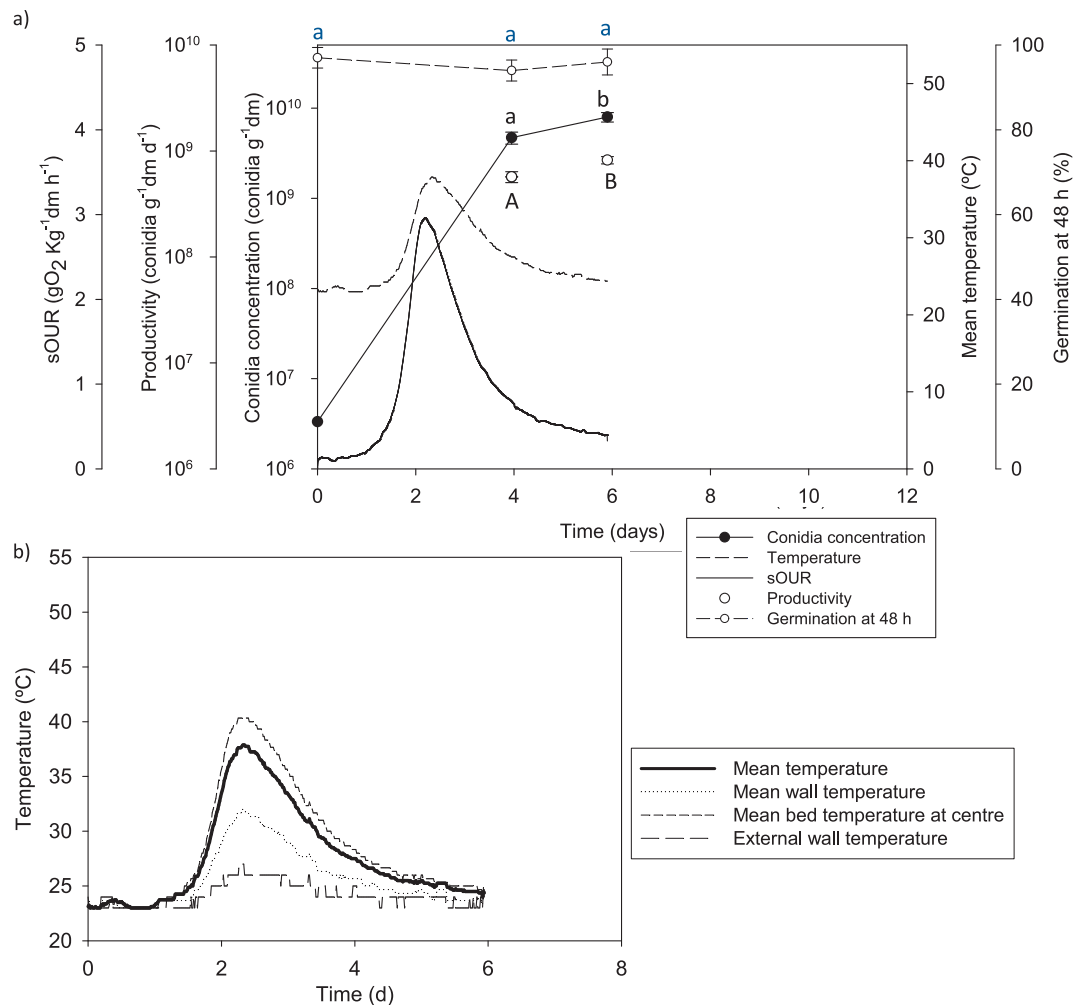


Fig. 2. Process parameters evolution at bench scale (22 L). a) Conidia concentration, productivity, specific oxygen uptake rate, mean temperature and conidia germination at 48 h, b) Radial temperature profiles. When shown, statistical difference is presented in lowercase (conidia production), uppercase (productivity) and lowercase in blue (germination).

Fig. 3a.

Maximum conidia concentration was obtained in day 8, corresponding to a concentration value of $4.6 \times 10^9 \pm 1.6 \times 10^9$ conidia g⁻¹dm and to a productivity of $5.75 \times 10^8 \pm 2.0 \times 10^8$ conidia g⁻¹dm d⁻¹. Both values were significantly different from the values obtained at day 4. These values are close to half the concentration obtained at the previous scale ($8.0 \times 10^9 \pm 1.0 \times 10^9$ vs $4.6 \times 10^9 \pm 1.6 \times 10^9$ conidia g⁻¹dm). Both sOUR and temperature peaks occur later when comparing to 22 L batch. This increase in lag phase displaces maximum productivity further in time, both at 22 and 100 L, when comparing to laboratory scale.

Maximum sOUR values were of $2.4 \text{ g O}_2 \text{ kg}^{-1} \text{dm h}^{-1}$, close to the maximum of $2.2 \text{ g O}_2 \text{ kg}^{-1} \text{dm h}^{-1}$ obtained in the 22 L batch.

As for product/inoculum yield, it amounted to 2468.5 ± 207.5 , not significantly different from values reported at 0.5 L (2922 ± 154). Herein, conidia concentration in the product was maintained at 3 orders of magnitude above conidia concentration in the inoculum in all reactor scales tested.

Fig. 3b and 3c show mean axial and radial temperatures, respectively, in the 100 L reactor. After an initial lag phase of 1.5 days, temperature rose rapidly due to fungal activity, peaking at 2.9 days, and gradually decreasing thereafter. The packed bed exhibited a vertical temperature gradient, with lower temperatures between 20–30 °C at the bottom and progressively higher temperatures with increasing height (Fig. 3b). The temperature gradient was significant in the lower half of the bed and much smaller in the upper half. This behaviour is

attributed to the cold air entering the reactor, which warms up and gets saturated as it flows through the bed, cooling and drying the bed along its path. This cooling effect is null in the upper half since air is already warm and saturated and thus no longer contributes to the dissipation of metabolic heat.

Heat dissipation along the radial axis occurred through conduction across the steel wall and convection with the surroundings. As shown in Fig. 3c, solids in contact with the reactor wall displayed temperatures close to those of the external wall due to the low thermal resistance of the steel wall. When the reactor wall was exposed to sunlight, temperature quickly increased both outside and inside the bioreactor. However, these mechanisms were insufficient to maintain low temperatures across the packed bed, as evidenced by the similarly high temperatures reached both at the centre and at $r/2$.

Maximum temperature achieved in the reactor was 55 °C. Mean temperatures higher than 40 °C were maintained for approximately 2 days, being higher than 50 °C in most of the reactor volume from 20 to 60 cm height (except walls) for more than 1 day. Despite these harsh conditions, *M. robertsii* EAMA 01/158-su strain was capable of growth and sporulation throughout the reactor, although visual differences were observed when comparing the bottom (0–20 cm) with the rest of the reactors' height (20–60 cm), with more hyphae growth and conidia dispersion observed at the bottom of the reactor's height. Visual differences were also observed between different radius, with higher growth and sporulation located closer to the wall (between $r/2$ and r).

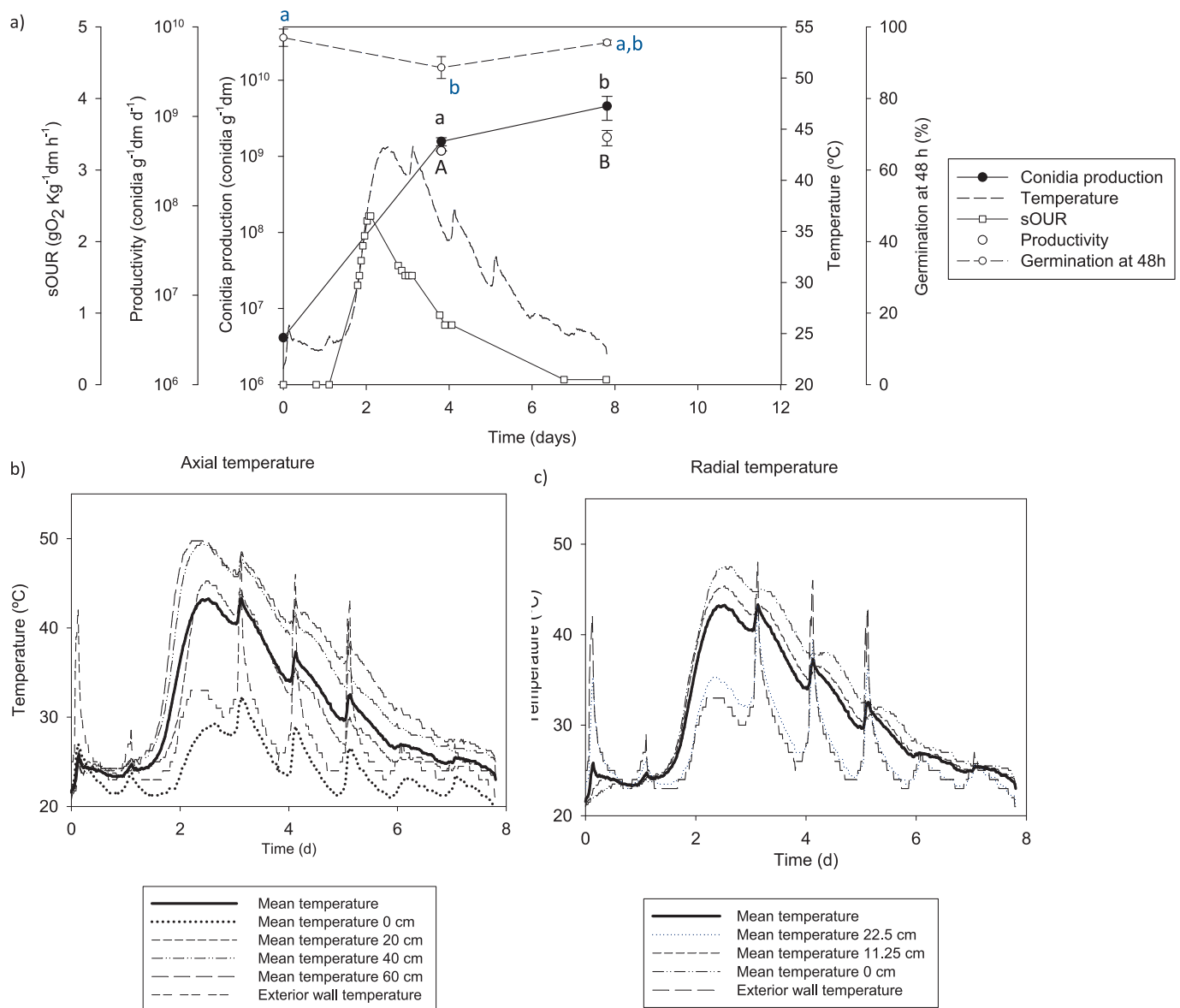


Fig. 3. Process parameters evolution at pilot scale (100 L). a) Conidia concentration, productivity, specific oxygen uptake rate, mean temperature and conidia germination at 48 h, b) Axial temperature profiles, c) Radial temperature profiles. When shown, statistical difference is presented in lowercase (conidia production), uppercase (productivity) and lowercase in blue (germination).

Despite visual differences between reactor sections, the use of the thermotolerant and entomopathogenic strain *M. robertsii* EAMa 01/158-su, capable of withstanding the achieved temperatures, was key for the success of the bioprocess when working at 100 L volume via the proposed packed-bed configuration. In depth analysis of differences between reactor sections is presented in the following global analysis section.

3.4. Multiscale comparative analysis: Packed-bed performance from 0.5 to 100 L

Comparative results across all tested reactor volumes are presented in Table 2. Process parameters of relevance for multiscale evaluation in SSF packed-bed reactors, namely superficial airflow (sAF), air-filled porosity (AFP), and superficial air velocity (V_z), are highlighted in bold.

Fig. 4 shows mean values and standard deviations of process parameters analysed at different bed heights for both 22 and 100 L reactors, including results from the 0.5 L scale as a reference. Significant differences compare values obtained both at bench at pilot scales.

Conidia concentration (Fig. 4a), was homogeneous along the bed in the 22 L reactor, showing no dependence on bed height. Similar values were obtained in the lower section of the 100 L packed bed; however, production was lower in the upper half due to higher temperatures (50–55 $^{\circ}\text{C}$) held for longer times. Interestingly, production in the upper half of the pilot reactor was not significantly different from production obtained in all bench reactor sections. Same behaviour (albeit with lower temperatures) has been previously observed in the 22 L reactor under similar conditions but using non-thermotolerant fungi (Sala et al., 2021a),(Sala et al., 2023). These results identify heat dissipation as a key parameter for future optimization at the pilot scale, particularly to improve conidia yield in the upper bed layers (Pitol et al., 2017).

Despite the presence of a temperature gradient, moisture (Fig. 4b) and pH remained homogeneous across all bed heights and reactor volumes (pH range 7.0–7.2), indicating process robustness with respect to these parameters. Particularly, moisture was not significantly different between 22 and 100 L volumes, however, it was different from 0.5 L for the substrate mixture used varied (from 80/20 0.5 L to 40/60 BSG/WC w/w at both 22 and 100 L). Despite moisture differences, the fungal

Table 2
Multiscale evaluation comparative results.

Parameter /Reactor	0.5 L	22 L	100 L
Airflow (mL min ⁻¹)	20	2000	10,000
sAF (mL min ⁻¹ g ⁻¹ dm)	0.705 ± 0.035	0.74 ± 0.07	0.785 ± 0.045
Mixture (BSG/WC) (w/w)	80/20	40/60	40/60
AFP (%)	75	76	72
V _z (cm min ⁻¹)	0.54	4.2	6.6
Conidia max. conc. (conidia g ⁻¹ dm)*	2.0x10 ¹⁰ ± 0.3x10 ¹⁰ a	8.0x10 ⁹ ± 1.0 x10 ⁹ b	4.6x10 ⁹ ± 1.6x10 ⁹ c
Conidia max. conc. time (d)	5	6	8
Productivity (conidia g ⁻¹ dm d ⁻¹)	4.0x10 ⁹ ± 0.6x10 ⁹ a	1.33x10 ⁹ ± 0.58x10 ⁹ b	5.75x10 ⁸ ± 0.2x10 ⁸ c
Conidia production (conidia g ⁻¹ BSG)	7.15x10 ⁹ ± 0.35 x10 ⁹ a	1.2x10 ¹⁰ ± 0.1 x10 ¹⁰ b	6.0x10 ⁹ ± 0.5 x10 ⁹ a
Total conidia produced L ⁻¹	1.15 x10 ¹² ± 0.05 x10 ¹² a	8.55 x10 ¹¹ ± 0.75 x10 ¹¹ b	7.0 x10 ¹¹ ± 0.6 x10 ¹¹ b
Product/inoculum yield	2922 ± 154 a	4819.5 ± 410.5 ^b	2468.5 ± 207.5 ^a
Conidia germ. at 48 h (%)	>95	>95	>95
Max media temp (°C)	28 (fixed)	38	44
Max temp (°C)	28 (fixed)	43	55
Max sOUR (g O ₂ kg ⁻¹ dm h ⁻¹)	4.9	2.9	2.4**
Max sOUR time (d)	2.0	2.1	2.1**
COC ^{6D} (g O ₂ kg ⁻¹ dm h ⁻¹)	14.0	5.6	***
pH range	5.5 – 6.8	5.6 – 7.1	4.5 – 7.2
Moisture range (%)	70—73	52—59	55—60
WHC (%)	45–47	64–68	61–65

sAF: specific airflow; BSG: beer draf; WC: wood chips; w/w: weight/weight; AFP: air filled porosity; conc: concentration; dm: dry matter; temp: temperature; max: maximum; sOUR: specific oxygen uptake rate; germ: germination; V_z: superficial velocity; cons: constant; 6D: six days; WHC: water holding capacity; *: corresponds to media value from 9 samples in 22 and 100 L reactors; **: different O₂ sensor; ***: calculation not possible. In bold: parameters to compare in the volume change (Marked in the Parameter column). When applicable, significant differences are indicated with lowercase letters a, b or c.

strain could grow at all volumes, highlighting its robustness.

The thermotolerant and entomopathogenic strain *M. robertsii* EAMA 01/158-Su demonstrated homogeneous growth and sporulation at temperatures up to 43 °C in the 22 L reactor, without requiring active metabolic heat removal thanks to the biological heat tolerance exhibited by the fungal strain. Although no active cooling was required to ensure process viability at 100 L, implementing improved heat management strategies could further enhance conidia productivity and process robustness (Mattoon et al., 2021).

Temperature profiles have been reported in literature similar to the ones described herein. Rodrigues et al. (2022) reached a maximum of 47 °C at all analysed heights of their reactor, a multilayer packed bed of 15 cm diameter and 90 cm height, working with the thermophilic fungus *M. thermophila*. Similarly, Méndez-González et al. (2022) also reported stable temperatures from 10 cm height, although maximum height of their column was only of 30 cm. Zanelato et al. (2012) achieved temperatures around 50 °C, also working with *M. thermophila* in a packed bed of 7.6 cm diameter and 10 cm length (although in this work, high temperatures were necessary to ensure optimal enzyme production conditions). In contrast, attempts to use water jackets for thermal control proved inefficient (Rodrigues et al., 2022), (Oliveira et al., 2020). As in this work, agitation was not used by any of these authors, as it hampers filamentous fungi growth and lengthens the fermentation, although it has been reported as a heat dissipation strategy when working with other microorganisms in SSF (Mejías, 2020).

Different strategies have been suggested to improve heat dissipation in PBR such as improving air distribution by means of perforated pipes

inside the reactor (Mitchell and Ruiz, 2023); reducing inlet air temperature (Pitol et al., 2016) or modifying wall surface in contact with the fungi to rise conidia concentration, but in general these attempts have shown limited efficiency (Méndez-González et al., 2024) and none of these solutions have been performed at a pilot scale. Besides, it is noteworthy to mention that all these works were focused on the production of enzymes, which is the main application of PBR when working with thermophilic strains. No other work has reported the production of fungal conidia by SSF using a thermotolerant and entomopathogenic strain.

A clear pattern emerged when comparing conidia production across scales. Specific conidia concentration (conidia g⁻¹dm) decreased approximately by half with each volume increase (0.5 to 22 L and 22 to 100 L). However, the conidia concentrations achieved still exceeded values reported by other authors working with similar bench-scale volumes (da Cunha et al., 2020), (Méndez-González et al., 2022). Results differ when analysing volumetric conidia concentration (conidia L⁻¹): highest values are obtained at 0.5 L (1.15x10¹² ± 0.05x10¹²), while 22 and 100 L were not significantly different (8.55x10¹¹ ± 0.75x10¹¹ for 22 L vs 7.0x10¹¹ ± 0.6x10¹¹ for 100 L). An interesting pattern is observed when comparing production results (conidia g⁻¹BSG): maximum values are obtained at 22 L (1.2x10¹⁰ ± 0.1x10¹⁰), whereas values both at 0.5 L (7.15x10⁹ ± 0.35x10⁹) and 100 L (6.0x10⁹ ± 0.5x10⁹) were not significantly different. This pattern is also observed in product/inoculum yield, with highest values for 22 L (4819.5 ± 410.5) and lower but not significantly different values for 0.5 (2922 ± 154) and 100 L (2468.5 ± 207.5). Both conidia production and yield values indicate the performance of an optimized 22 L batch process, obtaining the highest values among all reactor volumes tested. However, values obtained for both responses at 100 L were not significantly different from values obtained at 0.5 L, validating the robustness of the process at larger volumes despite reduction when compared with 22 L results.

In terms of product quality, conidia germination in samples corresponding to maximum conidia concentration time was maintained at values > 95 % in all volumes and in all reactor volumes and sections (0.5, 22 and 100 L). This value serves as an indicator for product quality and consistency between reactor scales. While germination is widely used as a primary quality parameter (Solano Porras, 2026), additional studies on virulence, infectivity and storage stability would further characterize conidial quality after scale-up, particularly for the product obtained at pilot scale.

As of sOUR comparison, temperature maximum time was delayed with the increase in reactor volume, however, sOUR maximum times were almost similar at both 0.5 and 22 L (2.1 vs 2.0, respectively). sOUR respiration profiles up to bench scale confirm that respiration was linked with conidia production and productivity, being consistent with previous results by several authors working with other fungal strains (Sala et al., 2020), (da Cunha et al., 2020). Despite reduced measurements, profile obtained at 100 L followed a similar pattern, with sOUR peaking at proximal times as in lower volumes (Table 2). Moreover, conidia production raised to its peak after O₂ started to decrease, behaviour shared at all reactor volumes at similar times.

Cleaning and sterility risks must be taken into consideration when changing scale. As stated in section 2.3.4, when possible, procedures for cleaning and sterility were maintained between volumes. Despite hot-spot formation (as seen in Fig. 3b and 3c), contamination was not observed at pilot scale, neither by visual reactor examination nor at Neubauer chamber. These observations enhance the potential of our proposal at industrial levels, where strict cleaning and sterility protocols should be applied.

Due to previous experience of the research group revealing AFP and sAF relevance when working at bench volumes (22 L) (Sala et al., 2021a), (Sala et al., 2023), and to the impossibility of maintaining sAF and V_z constant simultaneously, AFP, sAF and V_z were chosen as parameters to be compared across scales. AFP and sAF values were chosen

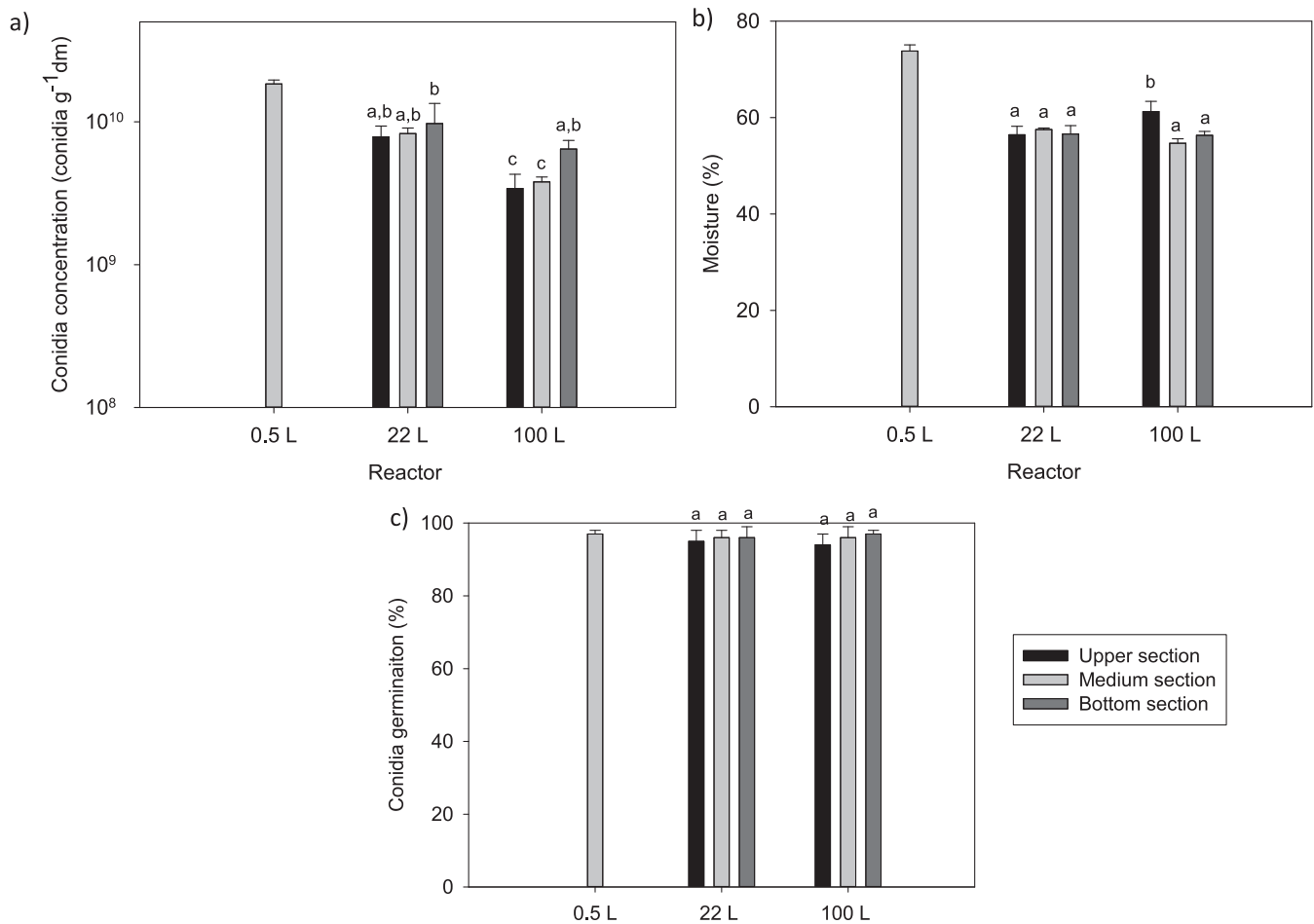


Fig. 4. Mean values (all reactor volumes) and standard deviations for all samples collected at the end of all 22 and 100 L batches. a) Conidia concentration, b) Moisture, c) Conidia germination. Significant differences correspond to values obtained from both reactors, comparing the performance of the 2 reactor volumes.

according to the results obtained in previous works working with other fungal strains in a 22 L packed bed. V_z was analysed due to its demonstrated relevance in heat transfer and dispersion (Perez et al., 2019). Besides, all three parameters have demonstrated relevance in fungal SSF scale-up by several authors, using different combinations of substrate and fungal strains (Méndez-González et al., 2024), (Barrera et al., 2019), (Biz et al., 2016), (Karimi et al., 2014) and at least by working volumes similar to the ones used at bench scale in this work (Rodrigues et al., 2022), (Shahryari et al., 2020), (Pitol et al., 2017).

sAF was maintained in the range of 0.67 – 0.83 mL min⁻¹ g⁻¹dm, while AFP ranged between 73 and 76 %. This implied the variation of V_z since the PBR geometry does not allow for both sAF and V_z to be simultaneously maintained, as both are proportional to airflow, but V_z is also inversely proportional to the cross-sectional area of the PBR. Higher AFP and sAF could contribute at increasing heat removal when working at pilot scale, and a compromise is needed to keep volumetric productivity and energy costs at reasonable levels.

When working at large cross sections, it is advisable to perform analyses on spore detachment efficiency, fungal morphology changes and on conidia attrition caused by high gas velocities, as they can influence the quantity and quality of the final product (Han et al., 2026). They were not performed in this work as they heavily depend on the reactor airflow. Even at pilot scale of 100 L, superficial air velocity reached 6.6 cm min⁻¹, which is still low in comparison to other works at volumes similar to our bench scale reactor (Pitol et al., 2016; Barrera et al., 2019; Rodrigues et al., 2022). Besides, according to Han et al. (2026) spore detachment efficiency critical values are located around 1.25 m s⁻¹, a much higher value than the superficial velocity used in our pilot reactor

(0.0011 m s⁻¹). This approach fits with our vision for product application, which would be to apply the obtained product directly to soil, reducing costs related to downstream processing.

Nevertheless, conidia production was successful at all scales while working in a relatively low V_z value. Hence, heat removal, while advisable, was not mandatory to ensure a successful SSF process when working with a thermotolerant and entomopathogenic fungal strain. Even when comparing to pilot scale, were V_z reached 6.6 cm min⁻¹, while typical V_z values range from 5 to close to 100 times higher (Mitchell and Ruiz, 2023). High V_z values have been used to improve heat dissipation by several authors (Pitol et al., 2017; Rodrigues et al., 2022; Shahryari et al., 2020), sometimes in conjunction with other parameters such as AFP (Biz et al., 2016). V_z values presented by all these authors correspond to much higher values than the ones reported in this work. It is noteworthy to mention that higher airflow values imply higher production costs, which highlights our results' value as they were obtained with a considerably low airflow, highlighting that acceptable yields can be achieved even under low airflow conditions, while still leaving room for improvement through targeted thermal control strategies. Further work shall address the effect of V_z on overall thermal dynamics and, thus, in process yields at pilot scale.

4. Conclusions

A multiscale evaluation of solid-state fermentation conidia production using the thermotolerant and entomopathogenic *Metarhizium robertsii* strain EAMa 01/158-su in packed-bed reactors has been successfully conducted from laboratory (0.5 L) to pilot scale (100 L).

Process robustness across volumes was enabled by the thermotolerant and entomopathogenic *M. robertsii* strain used, which can overcome the effects of heat generation inside the reactor despite operating without temperature control due to its innate biological tolerance. *M. robertsii* strain EAMa 01/158-su shows potential as fungal conidia producer, being capable of withstanding temperatures up to 55 °C for 2 days. Values obtained for conidia production (conidia g⁻¹BSG) and yields in the 100 L reactor were not significantly different from those obtained under controlled laboratory conditions, confirming the feasibility of applying the microbiological strategy at pilot scale. Conidia quality measured as conidia germination has been consistently above 95 % at all reactor volumes, demonstrating the robustness of the process. More work should be conducted at pilot scale to assess the effect of classical scale-up parameters such as superficial velocity and targeted thermal control strategies on conidia production.

Overall, results serve as a first report of pilot solid-state fermentation in a packed bed reactor using a thermotolerant and entomopathogenic fungal strain. They pave the way for the application of integrated solutions for the solid-state fermentation scale-up problem, which should be addressed via combined use of thermotolerant strains and engineering approaches. Finally, they serve as foundation to present thermotolerant and entomopathogenic strains potential in fungal solid-state fermentation industrialization.

CRediT authorship contribution statement

Arnau Sala: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Conceptualization. **Adriana Artola:** Writing – review & editing, Supervision, Conceptualization. **Teresa Gea:** Writing – review & editing, Funding acquisition. **Raquel Barrena:** Writing – review & editing, Supervision, Conceptualization.

Data availability statement

Dataset is available at: <https://doi.org/10.34810/DATA3482>.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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