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Production of dried *Beauveria bassiana* conidia in packed-column bioreactor using agro-industrial palm oil residues

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ABSTRACT

Agro-industrial waste byproducts are commonly inexpensive organic nutritional sources for production of filamentous fungi, and selection of an optimal bioreactor is a key factor for attaining satisfactory biomass yield and quality. The insect-pathogenic fungus *Beauveria bassiana* (Hypocreales: Cordycipitaceae) has been extensively mass produced by solid-state fermentation using precooked cereal grains. Here, we propose low-cost palm oil residues as the main substrate using a cylindrical packed-column aerobic bioreactor prototype designed for conidial production of *B. bassiana*. Fermentation treatments using *B. bassiana* strain CG1229 assessed the impact of temperature, air flow, substrate moisture content, and ratio between palm fiber (PF) and palm kernel cake (PKC) on conidial yield and desiccation tolerance. Results showed significant enhanced productivity (2×10^{10} conidia g^{-1} dry matter) and reduced fermentation time (from 168 h to 120 h) by this method compared with the conventional tray bioreactor. Optimal substrate conditions were 60% initial moisture and 30% PF + 70% PKC content, fermentation at 26 °C with aeration rate $\geq$ 0.2 L min^{-1}. Air drying conidia inside the column yielded > 95% germination. Maximum spore production was achieved in 120 h. Our findings demonstrate the feasibility of using a packed-column bioreactor for mass production of *B. bassiana* conidia using low-cost agricultural residues, which contributes to a sustainable production method of this biopesticide in Brazil.

1. Introduction

There is growing interest to use microorganisms in agricultural systems to promote plant protection and health while reducing chemical inputs. Among these examples, the use of insect pathogens for the biological control of arthropod pests in integrated pest management programs is well documented [1]. Fungal biopesticides for phytophagous insects and mites are mainly represented by entomopathogenic fungi belonging to Hypocreales in Ascomycota phylum. Most of these fungi produce environmentally resistant asexual spores (aerial conidia) for dispersion in the environment and subsequent infection in arthropod populations [2]. An important step in developing commercial fungal bioinsecticides concerns the large-scale economic production of high quality conidia using low-cost solid substrates. Fungal growth by solid-state fermentation (SSF) is frequently employed to produce aerial conidia, which are the infective stage of many entomopathogenic fungi used in biopesticides. In general, conidia produced by SSF are considered more robust and storage stable compared to other propagules produced by submerged liquid fermentation [3].

Historically, rice is the main raw material employed for SSF of entomopathogenic fungi in many developing countries [4]. However, the use of rice in SSF (a staple food of many populations) enhances opportunity costs relative to food security. As an alternative, using agro-industrial residues in SSF is economically advantageous and also promotes valorization of these materials within the context of a circular economy [5,6]. Agro-industrial residues or by-products are reported as promising multi-purpose "green" low-cost materials that can serve as a nutrient source for fungal growth [7]. For instance, various organic

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agricultural by-products, including whey from cheese and casein production [8] or plant and animal manures [9–11] can increase spore yields of entomopathogenic fungi in relation to cereal grains. Additionally, various growth matrices can be added to the nutritional substrate in SSF to increase porosity and gaseous exchange [12–14]. Despite the growing availability of residues from palm oil industry in Brazil, few studies have evaluated palm kernel cake (PKC) and palm fiber (PF) as nutritional substrates or support growth matrices in the production system of fungal biopesticides [15]. The palm oil market is expected to grow at an annual rate of 6% from 2023 to 2028. According to the Brazilian Association of Palm Oil Producers (Abrapalma), Brazil had around 240 thousand hectares of palm plantations in 2018 [16], and current annual production of palm kernel oil is 66 kton, generating around 2 kton of PKC per year [17].

Bioreactor design during SSF can directly affect the growth and conidial production and quality. In tray-type bioreactors, conidial production is facilitated through high exposure of the mycelial mat to oxygen and the great number of conidiogenous cells formed on a wider substrate surface [18]. However, considering a static fermentation system with reduced air circulation, heat may accumulate during the vegetative stage, causing humidity loss and thermal stress along the fermentative bed. Reduced air exchange also occurs when fermentation is carried out in polypropylene (plastic) bag bioreactor, widely employed in SSF for industrial conidia production of fungal biopesticides [19].

Given this context, we evaluated a prototype based on an aerated packed-column bioreactor for SSF of *B. bassiana* using residues derived from the palm processing industry in Brazil. This bioreactor allows two-in-one step process that subsequently includes drying the spores by purging dry air flow through the fungus-colonized substrate for further formulation or direct application. We assessed several production parameters (i.e., substrate moisture content, aeration rate and substrate composition) on growth kinetic, yield and viability of conidia produced by *B. bassiana*. This research is important towards the replacement of rice as the main substrate in SSF of entomopathogenic fungi and to optimize yield and efficiency for conidia production using inexpensive agro-industrial residues that are widely available in Brazil.

2. Materials and methods

2.1. Microorganism

The entomopathogenic fungus *B. bassiana* (strain CG1229) was isolated from *Rupela albinella* Stoll (Lepidoptera: Crambidae) larvae collected from rice fields in Arari, MA, Brazil, in 2011 [20]. Our previous studies have shown the insecticidal effectiveness of this fungal strain against economically important agricultural pests, such as whiteflies [15,20,21], which make this fungus a promising candidate as a bioinsecticide. This fungal strain has been registered under the Brazilian Genetic Heritage – Sisgen – protocol ABC8EC6 and preserved in liquid nitrogen in the Invertebrate-Associated Fungal Collection (CFI) at EMBRAPA Genetic Resources and Biotechnology (Brasilia, DF, Brazil).

2.2. Liquid inoculum

Conidia from a 10-day old fungal culture grown on potato dextrose agar (PDA, Sigma®, St. Louis, MO, USA) were collected by surface scraping and cells were added to 10 mL of a sterile aqueous solution with surfactant (0.01% Tween® 80, Merck, Darmstadt, Germany). Erlenmeyer flasks containing 100 mL of liquid medium (36.0 g dextrose, 3.6 g yeast extract, 4.0 g KH_2PO4, 0.8 g $CaCl_2.2H_2O$, 0.6 g $MgSO_4.7H_2O$, 0.1 g $FeSO_4.7H_2O$, 16 mg $MnSO_4{\cdot}H_2O$, and 14 mg $ZnSO_4.7H_2O$ per liter of distilled water) were inoculated with the conidial suspension (10% v/v), delivering a final concentration of 10^6 conidia mL^{-1}. Following a diphasic (two-stage) fermentation method, the liquid culture was maintained for 3 days in an orbital shaker (28 ± 0.5 °C) and used to inoculate the standard substrate (10^7 blastospores g^{-1} of substrate) [22].

2.3. SSF using fixed-bed bioreactor

A cylindrical packed-column aerobic bioreactor prototype was previously designed to promote a high conidial production and biomass drying in a single process [23]. Packed-bed bioreactor consists in a cylindrical glass column with 20 cm length and 4 cm of diameter, providing 200 cm^3 of internal volume and water flow was set to countercurrent in the jacket (Fig. 1). The system was assembled with up-flow aeration controlled by a rotameter and with a cooling glass jacket, allowing the gaseous exchange and the temperature to be controlled during the fermentation and drying. Inlet air humidity near to saturation was obtained by passing the air flow through a column of water.

Solid residues derived from palm oil extraction consisted of palm kernel cake (PKC – particles with diameter < 1.18 mm) and palm fiber (PF - length < 2.8 mm) (Agropalma S.A, Belém, PA, Brazil) and were further used as nutrient source and support matrix for fungal growth. In the first experiment, the standard substrate (100% PKC) without any nutritional supplementation and a mixture of PKC and PF (70% + 30%, respectively) were tested. Palm fiber was added to optimize aeration and avoid heat accumulation during fermentation. Both substrates were prepared with two different proportions of water (60% and 65% of dry substrate w/w). The soaked substrates were autoclaved at 120 °C for 15 min and inoculated, following a biphasic fermentation process, with a liquid culture of *B. bassiana* inside a microbiological laminar flow cabinet after cooling the substrate.

The autoclaved bioreactor columns with approximately 200 cm^3 were loaded with 30 g of the inoculated substrates without compression. Column-type bioreactor conditions were incubated at 32 °C with an aeration rate at 0.5 L min^{-1}. Three independent replicates (runs) were performed per substrate type.

2.4. Conidia analysis

Aerial conidia were enumerated from aliquots of 1 g fungus-colonized substrates collected after 144 h of fermentation Conidia yield was evaluated by washing the spores using sterile solution of Tween 80® at 0.1% (v/v). The fungus-water mixture (sample of fungus-

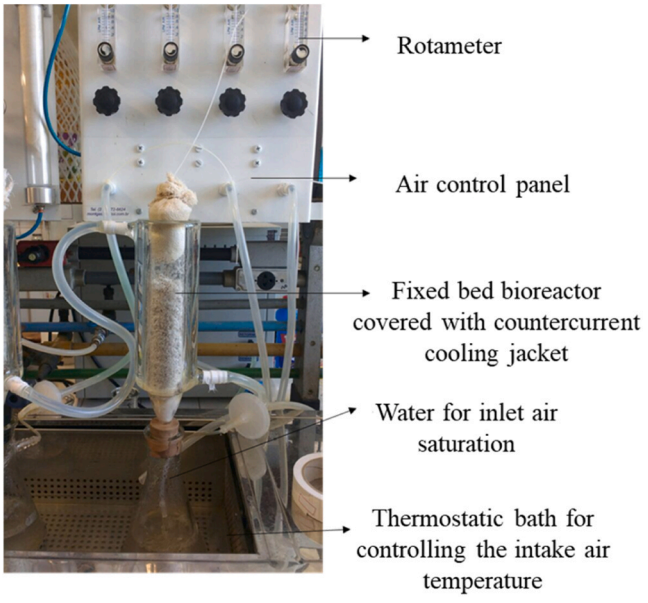

Fig. 1. Prototype of a cylindrical packed-column aerobic bioreactor (height = 20 cm, diameter = 4 cm) with glass jackets for temperature control and forced bottom-up aeration used in our studies of solid-state fermentation.

colonized substrate in 10 mL of surfactant solution) was placed into Erlenmeyer flasks and agitated for 30 min in a rotary incubator shaker (G24, New Brunswick Scientific, New Jersey, NY, USA) at 30 ± 0.5 °C and 200 rpm. The number of conidia per volume of substrate was determined by counting the cells with a Neubauer chamber at 400X phase contrast magnification. Water activity (aw) and final moisture content of the fungus-colonized substrates grown in the bioreactor were determined using an electronic hygrometer (AquaLab, Series 3TE, Decagon Devices Inc., USA) and by the dry weight method, respectively [24].

The proteolytic activity expressed by this fungus was determined measuring the absorbance of enzymatic extract obtained from the colonized substrate. For this, 20 g of the substrate were mixed with 100 mL of phosphate buffer solution (0.1 M PBS and pH 7.0) in Erlenmeyer flasks. Flasks were placed in an orbital shaker (200 rpm) for 20 min at 35 °C and the solids removed by filtration and centrifugation [25]. The crude enzymatic extract (50 mL) was then mixed with azocasein solution for 5 min at 40 °C and, after this period, the chemical reaction was interrupted by adding hydrochloric acid (1.0 M). Extract samples were centrifuged (2 min at 11,000 g) and absorbance (U g^{-1}) determined at 345 nm [26]. Samples were measured in triplicate and sterile water was used as a negative control.

2.5. Assessment of fermentation parameters for maximum conidia production

Conidial yield over time (fungal growth kinetic test) was performed to determine the optimal time for sample collections. Runs were carried out at 32 °C and aeration rate at 0.5 L min^{-1}, using PKC (70%) + PF (30%) with 65% of initial moisture content. Samples were collected daily between 96 h and 168 h (24 h intervals) and conidial yield evaluated as described above. Conidial viability was determined on PDA amended with 10 μL L^{-1} of the chemical fungicide carbendazim (Sigma®) using plastic Petri plates (6 × 1.5 cm). Conidia suspension (100 μL) containing approximately 10^5 conidia mL^{-1} was inoculated on medium surface and air dried for 15 min. Conidia were deemed germinated (i.e., viable) if they had germ tube longer than their diameter after 16 h incubation at 26 ± 0.5 °C with 12 h photoperiod [27].

A full factorial experimental design was then established for the assessment of different set parameters in the column-type bioreactor on the conidia production by SSF using PKC + PF substrate. The variables temperature, aeration rate, initial moisture content and palm fiber ratio were evaluated. Bioreactor conditions were set in two levels (−1 and 1) and the central point (zero) for all four variables (Table 1), totaling 20 runs in a multiple factors study. Inoculum preparation, substrate inoculation and bioreactor load were performed as already described. The fermentation factors investigated during SSF in packed column bioreactor were chosen according to our previous work using the tray method for production of this fungus [15], with the only exception that now aeration rate was included as a new variable in the production system. Conidia yield was evaluated by harvesting spores from fungus-colonized substrate on different time intervals of the fermentation course, as previously described. In this case, samples from the fungus-colonized substrates were collected after 120 h to measure the growth kinetics based on conidial concentration. Conidial viability was also determined as previously described. Results for production yields by SSF were expressed as viable conidia per gram of dry matter.

Table 1
Levels of variables used in a full factorial experimental design (2^4) to study *Beauveria bassiana* conidia production and viability by solid state fermentation conducted in a packed-column aerobic bioreactor prototype.

Variables	Levels		
	-1	0	1
Aeration rate (L min^{-1})	0.2	0.5	0.8
Inlet temperature (°C)	26	29	32
Moisture content in the substrate (%)	60	65	70
Fiber concentration in PKC (%)	10	20	30

An additional experiment on fungal growth kinetic in the fixed-bed bioreactor was carried out in order to validate the best conditions determined in the study with different abiotic variables. In this case, bioreactor was set to run up to 192 h at 26 °C, aeration rate at 0.2 L min^{-1}, 60% of substrate moisture and 10% of PF in PKC mixture, and the same parameters (conidia production and viability) were evaluated. Samples from the fungus-colonized substrates were collected daily between 120 h and 192 h (24 h interval).

2.6. Data analysis

Conidia production by *B. bassiana* on PKC with two different proportions of water and mixed or not with PF in the fixed-bed bioreactor and the proteolytic activity of the fungus were analyzed by a Design of Experiments (DoE) platform. In order to determine the most significant parameters in the process, a two-level full factorial 2^4 design was performed. In this type of analysis, it is implied sixteen runs without counting replications or center point runs (Table 1). Statistica® 7.0 (Stat Soft. Inc., Tulsa, OK, USA) software estimated independent factors and interactions. The standardized effects of variables (*t*-values) and the test of significance (*P* value at 10% significance level) were used to evaluate the effects of temperature, initial moisture, and PKC on conidia production [28].

The multivariate equation that describes the response model is given by:

$$Y = a_0 + \sum_{i=0}^{n} a_i * x_i + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} a_{ij} * x_i * x_j$$

where Y is the response variable, a_0 is the term correspondent to the mean, a_i is the linear effect coefficient, a_{ij} is the interaction effect coefficient, and x_i and x_j are independent factors.

Conidial viability data were fitted to a generalized linear model with binomial distribution and logistic link function with fermentation time as the only effect in the predictor.

3. Results

3.1. Effect of substrate and initial moisture content on bioreactor yield

Conidia yield, proteolytic activity and the final moisture content of the strain CG1229 of *B. bassiana* varied based on the substrate and initial moisture content (Table 2). A significant increase on the number of conidia was detected when PF was added to the standard substrate PKC for both moisture content ($F_{1,4} = 19.76$, $p = 0.0113$ and $F_{1,4} = 25.65$, $p = 0.0071$), but an interaction between substrate and moisture content was not detected ($F_{1,4} = 0.83$, $p = 0.4136$). Conidia yield was 3.2 and 2.1 times higher when the fungus was cultivated with PKC + PF than with 100% PKC and with 60% and 65% of initial moisture, respectively. Inversely, proteolytic activity was greater on 100% PKC than the substrate mixed with the palm fiber, reaching ca. 76.3 U g^{-1} for 65% of moisture and 88.7 U g^{-1} for 60% of moisture ($F_{1,4} = 11.35$, $p = 0.0281$). The interaction between substrate and moisture content was not detected ($F_{1,4} = 0.11$, $p = 0.7593$), and moisture had no influence on the proteolytic activity during fermentation ($F_{1,4} = 1.11$, $p = 0.3503$). Differences in the final moisture between the two substrates and the two moisture contents were not observed ($p > 0.05$).

3.2. Assessment of abiotic variables for maximum conidia production in a column-type bioreactor

The fungal growth kinetic in the preliminary test indicated the time between 120 and 168 h as the best interval for sample collections and for the assessment of different parameters tested in the packed-column

Table 2
Beauveria basssiana conidia yield and protease activity on palm kernel cake (PKC) and palm fiber (PF) substrates after 144 h using a column fixed-bed bioreactor at 32 °C, 60% or 65% initial moisture content and air flow of 0.5 L min^{-1}.

	Initial moisture content (%)					
	60	65	60	65	60	65
Raw material (substrate)	Yield (conidia g^{-1} of dry substrate)		Proteolytic activity (U g^{-1})		Final moisture (%)	
100% PKC	$1.47 \pm 0.01 \times 10^9$ aA	$5.45 \pm 0.65 \times 10^9$ bB	88.66 ± 6.96 aA	76.33 ± 13.26 aA	38.38 ± 3.03 aA	55.36 ± 2.08 aA
70% PKC + 30% PF	$4.65 \pm 1.45 \times 10^9$ bA	$1.17 \pm 0.34 \times 10^{10}$ aB	56.55 ± 8.81 bA	50.22 ± 0.38 bA	51.42 ± 8.40 aA	59.65 ± 0.75 aA

Values (± standard errors) followed by the same letter between columns (upper case) or rows (lower case) in each parameter are not different (n = 2 independent assays). Contrast between means at $p<0.05$ was performed with Tukey HSD test.

bioreactor. Conidiation was still very low at 96 h of incubation and increased exponentially between 120 and 168 h. After 168 h of incubation, conidia yield reached an average of 1.2×10^{10} conidia g^{-1} dry matter, suggesting that the fungal fermentation reached its production peak. The period of 120 h was then chosen to assess the influence of the different growth parameters on conidia production with the aim to optimize spore production and fermentation time (Fig. 2).

Table 3 describes conidia yield and viability (germination) after 120 h of growth in the packed-column bioreactor, under different fermentation conditions. The fitted model describing conidia production by moisture content and palm fiber concentration in the substrate is significant ($F_{3,\ 15} = 26.01$, $p < 0.001$, $R^2 = 0.76$), indicating that better yields can be achieved with minor fiber concentrations. Similarly to the analysis performed on conidia production, conidial viability was modeled using a factorial analysis enabling meaningful predictors and their interactions to be detected ($F_{4,\ 14} = 7.72$, $p = 0.0008$, $R^2 = 0.69$) (Fig. 3). At a significance level of 10% ($p < 0.1$), only the moisture content of the substrate and PF concentration added to the substrate had a statistically significant effect on the *B. bassiana* production in this type of bioreactor (Supplementary Table S1). This analysis clearly indicates that, when those variables go from its higher level (70% and 10%, respectively) to the lower level (60% and 10%, respectively), promotes higher yields on conidia production (Supplementary Fig. S1a). Initial moisture content of the substrate amended with PF at lower concentration (10% w/w), temperature and aeration rate exhibited significant effects on conidial viability of *B. bassiana* (Supplementary Fig. S1b). Contour plot generated by the model describes conidia production and viability in terms of the abiotic variables tested (Fig. 4).

The kinetic assay performed after factorial study using the most promising conditions for SSF in the bioreactor validates the predicted results based on the experimental design and enabled the optimization of the conidia yield within 120 h of fermentation (approximately 2×10^{10} conidia g^{-1} dry matter). Optimal conditions settled in the bioreactor induced higher conidia yields (1.35×10^8 viable conidia g^{-1} dry matter h^{-1}) within the first 120 h than those found after 168 h (6.96×10^7 viable conidia g^{-1} dry substrate h^{-1}) in the preliminary kinetics performed before the factorial design study (Fig. 5).

4. Discussion

This study demonstrates the technical feasibility in producing aerial conidia of *B. bassiana* strain CG1229 using palm oil residues with a packed-column bioreactor. Our results indicate greater conidia yield per gram of raw material (1.2×10^{10} conidia g^{-1} dry matter) obtained with the column-type bioreactor than the tray-type bioreactor reported previously by Silva et al. (2018) using the same substrate [15]. According to these authors, the stationary fermentation on trays enabled yields up to 7.65×10^9 conidia g^{-1} dry matter of PKC after 144 h of incubation, which is almost 2-fold lower than that found in the present study under similar conditions (32 °C, 65% of initial moisture content) and when palm fiber was added. In addition, adjustments in initial moisture content of the substrate, aeration rate, inlet temperature and PF concentration further increased conidia yield and reduced fermentation time (2×10^{10} conidia g^{-1} dry matter after 120 h) in the column-type bioreactor, indicating that it might have superior performance over the conventional stationary tray bioreactor. As a limitation of the present study, the lab-scale packed-reactor requires the inoculum to be prepared for each batch, which could pose a difficult challenge for industrial production using this method. Further research should direct efforts in analyzing the scalability and cost-benefits of employing sequential batch or fed-batch reactors for SSF of *B. bassiana* and probably other related fungal biocontrol agents.

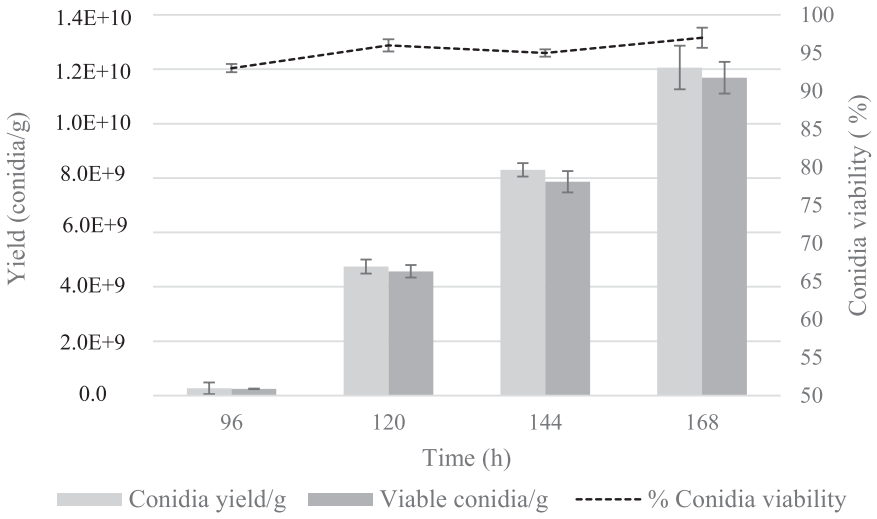

Fig. 2. Conidia production kinetics and spore viability of *Beauveria bassiana* in a fixed bed bioreactor under growth conditions set to 32 °C, aeration rate at 0.5 L min^{-1}, 65% of substrate moisture and 30% of palm fiber (PF) in palm kernel cake (PKC) mixture, before multi factorial experiment.

Table 3
Conidial production by *Beauveria bassiana* achieved at 120 h of fermentation for different experimental conditions carried out according to a full factorial experimental design.

Runs	Aeration rate (L/min) (X_1)	Temperature (°C) (X_2)	Moisture content (%) (X_3)	Fiber concentration (%) (X_4)	Spore yield ($\times 10^9$con g^{-1}) (Y_1)	Spore viability (%) (Y_2)
1	0.2	26	60	10	20.5	90.44
2	0.8	26	60	10	8.38	88.64
3	0.2	32	60	10	13.5	89.00
4	0.8	32	60	10	12.3	88.11
5	0.2	26	70	10	3.62	78.85
6	0.8	26	70	10	2.04	74.59
7	0.2	32	70	10	1.11	85.00
8	0.8	32	70	10	3.41	87.31
9	0.2	26	60	30	2.93	88.08
10	0.8	26	60	30	2.64	86.30
11	0.2	32	60	30	4.96	90.44
12	0.8	32	60	30	1.00	91.59
13	0.2	26	70	30	2.15	88.33
14	0.8	26	70	30	1.94	71.11
15	0.2	32	70	30	0.73	84.74
16	0.8	32	70	30	2.72	82.16
17	0.5	29	65	20	8.63	86.18
18	0.5	29	65	20	9.05	84.8
19	0.5	29	65	20	9.40	90.30
20	0.5	29	65	20	11.5	91.30

The specific type of agricultural residue may influence the conidial virulence factors and enzymatic potential produced in a given bioreactor. In our study, proteolytic activity expressed by *B. bassiana* revealed that 100% PKC induced higher production of proteases per dry matter of substrate than when this fungus was grown with PKC and PF mixture, as the fiber from the latter cannot be metabolized by the fungus. The production of protease occurred during the course of fungal growth and it was strongly stimulated by the presence of substrates rich in protein, such as the palm kernel (PKC). Despite the absence of virulence bioassays that could reveal the insecticidal potential of *B. bassiana* proteases, it is reasonable to state that this higher activity of proteases found in *B. bassiana* grown only with PKC may be desirable in terms of virulence. Previous studies substantiate the importance of proteolytic enzymes associated with the fungus virulence against insects [29–32].

Moisture and water activity are among the main variables to be controlled for optimization of entomopathogenic fungi growth [33]. It is also known that the interaction of moisture and aeration can affect sporulation more than these factors alone [34]. The excess of moisture in the substrate leads to yield reduction, once the water promotes bed compaction and occupies the air circulation space, which otherwise should be free for better heat removal and aeration during fungal growth [35], and thus allowing the process less prone to bacterial contamination. Moreover, substrate particles can clump together when water is added to reduce the surface area to volume ratio, thus limiting the space where sporulation can occur [36]. The use of inert materials to ensure a high porosity is very well described in SSF of filamentous fungi [37–39]. The higher the bed porosity, the better the oxygenation and water evaporation, which also leads to a greater thermal exchange and oxygen diffusion throughout the bed mass. Such condition is critical to promote balanced and optimal growth in aerobic SSF by filamentous fungi, especially when working with packed-column bioreactors where there is a gradient of moisture and aeration along the column. Plant fibers used as a filler may help to increase space between particles and thus enhancing gas exchange through the biomass + substrate matrix. Palm fiber (PF) promoted higher bed porosity, facilitating air circulation and heat removal, moisture maintenance, and thus providing better conditions to the fermentation process, as confirmed by the higher conidia yield observed in our packed-column bioreactor. On other hand, fiber may cause water loss, thus reducing water activity along the fermentative bed [40]. Interestingly, our data showed no significant difference regarding the final moisture content of substrates containing either 100% PKC or a mixture between 70% PKC and 30% PF in the fermentative bed when the initial moisture content was $> 60\%$. Although the reduced space between particles in the fermentative bed may hamper the heat exchange and then increase the temperature and the water evaporation from the substrate [38,41], the water activity in the fermentative bed was kept favorable for fungal development with or without the use of PF. The size of the particles is also important in providing high superficial area, hence facilitating nutrient access by mycelium during fungal growth [35]. Our results suggest that the optimum substrate (PKC)-inert (PF) ratio can be achieved with low inert concentration, reflecting in greater spore yields for this fungus.

Forced aeration in fixed-bed or packed-column bioreactors may promote better control of operating conditions, especially temperature, as it allows controlling the incoming air temperature and flow [42]. Generally, entomopathogenic fungi grow optimally within a range of 20–30 °C [43], although some *B. bassiana* strains have an optimal range of 30–35 °C, while other strains of *M. anisopliae* are able to maintain high germination rates when exposed to temperatures above 40 °C [44]. This disparity of thermo-preference in these entomopathogenic fungi is strongly explained by the wide genetic varibility among strains, whose phenotype plasticity is greatly shaped by their ecological niche and geographical origin. For the *B. bassiana* strain used in the present study, greater yield was obtained with a mild inlet temperature (26 °C) at minimum aeration rate (0.2 L min^{-1}), resulting in efficient heat removal from the fermentative bed. The reduction in air flow also enables to work with smaller humidity ranges without great water loss and irregular moisture gradients along the fermentative bed during all stage of the fermentation process, which diminished substrate compression and favored substrate porosity. However, heat exchange by forced aeration is challenging in larger packed-column aerobic bioreactors loaded with larger volumes of substrate and further studies with more controlled cooling systems are required. Another advantage of this packed-reactor for conidia production resides in its dual function of fermentation and subsequently air drying the spores with the substrate matrix. After drying, the spores can be mechanically separated from the substrate through sieving and vacuum [23,45].

5. Conclusions

Solid state fermentation process using a packed-column bioreactor is a promising strategy for the production of high quality of viable conidia. For the first time, we demonstrated the use of low-cost palm oil residues as substrate in this type of biorreactor for production of *B. bassiana*

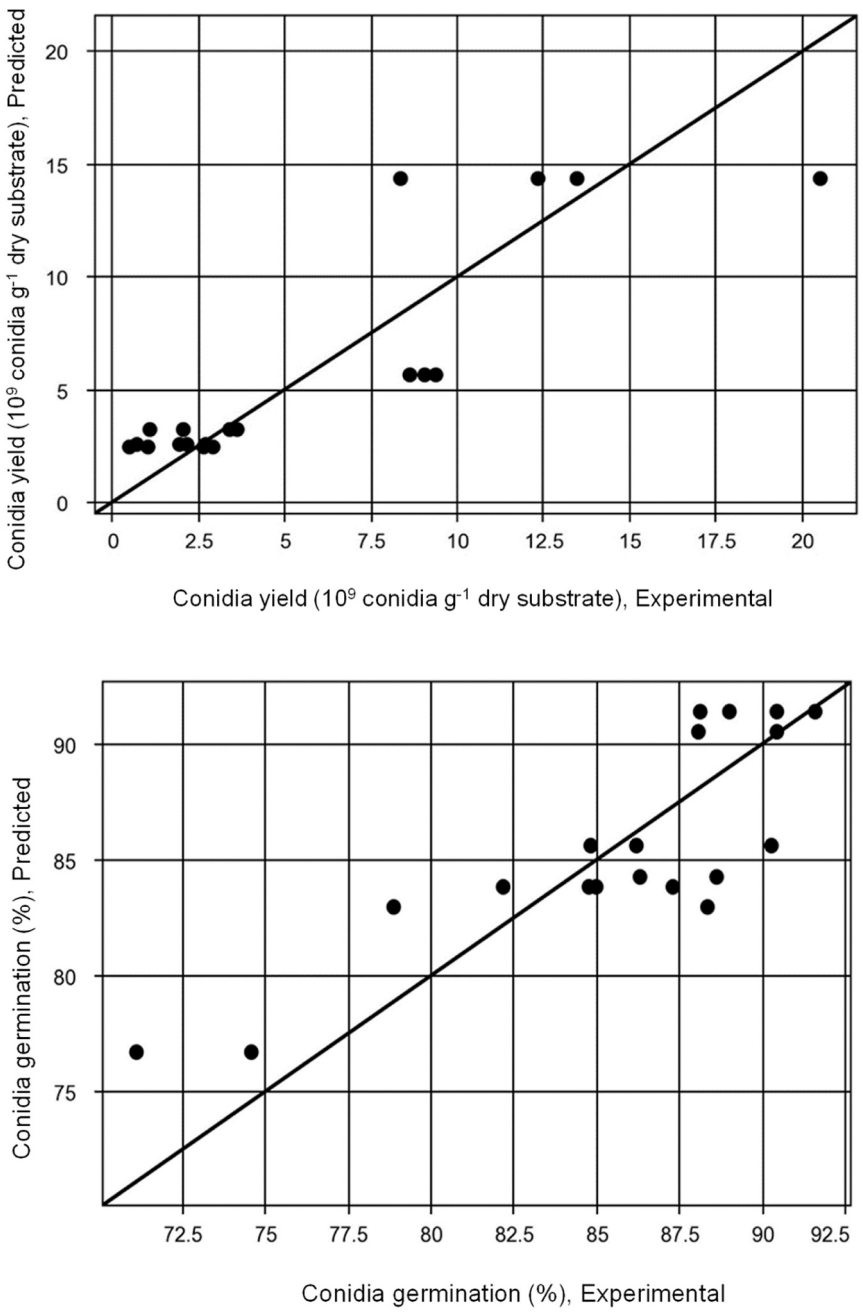

Fig. 3. Observed versus predicted values for conidia yield and conidial viability of *Beauveria bassiana* produced by a packed-column bioreactor after 120 h of growth.

conidia, showing the superior performance of the system over the conventional stationary tray bioreactor documented in our previous work [15]. A well defined operating conditions (inlet temperature and aeration rate) and physical characteristics of the organic matrix (initial moisture content and PF concentration) enabled the use of a nutrient rich agro-industrial residue (PKC). We note that this method is potentially applicable to other beneficial filamentous fungi, such as *Trichoderma*. On-farm production of these fungi in Brazil may benefit from larger bioreactors of this type. Nevertheless, further validation studies with in larger packed-column aerobic bioreactors with automated control settings during fermentation are crucially necessary to better assess the feasibility and consistency of spore production and quality by *B. bassiana* at an industrial scale, and additionally compare costs and benefits with the traditional SSF.

CRediT authorship contribution statement

JNS, GMM and DMGF conceptualized and designed the research. JNS conducted the experiments. GMM performed the statistical analyses. GMM, RBL and DMGF contributed to the writing and editing of the manuscript. All authors approved the manuscript.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Gabriel Moura Mascarin reports financial support was provided by Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro - FAPERJ.

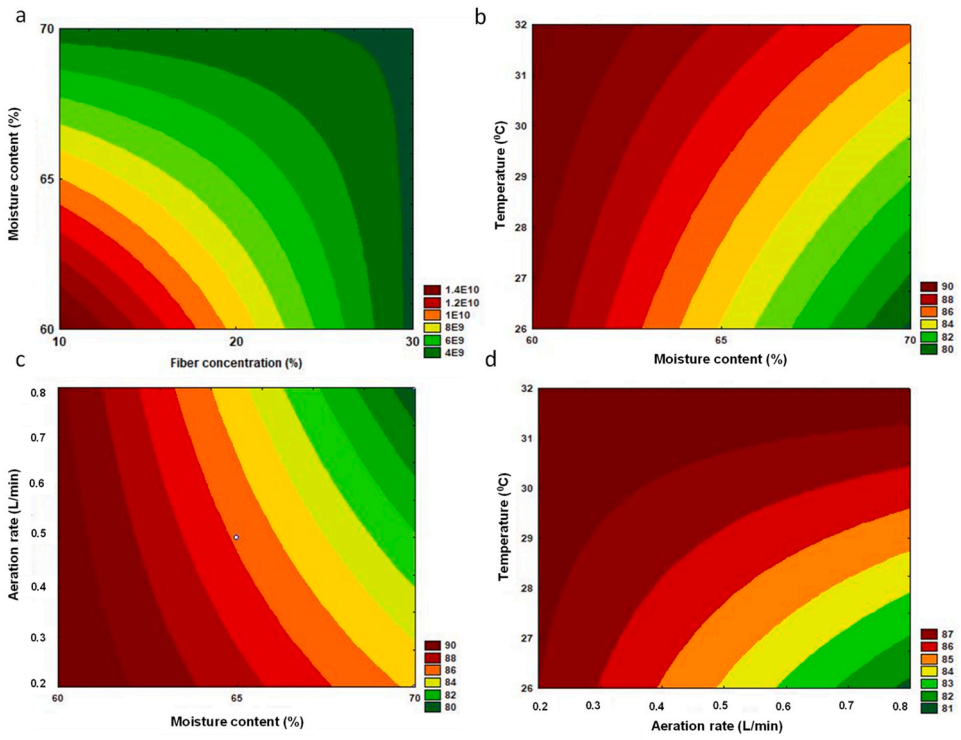

Fig. 4. Contour plot for *Beauveria bassiana* conidia production (a – correlation between moisture content and fiber concentration) and conidial viability (b – correlation between temperature and moisture content; c - correlation between aeration and moisture content; d - correlation between temperature and aeration) after 144 h in the packed-column bioreactor.

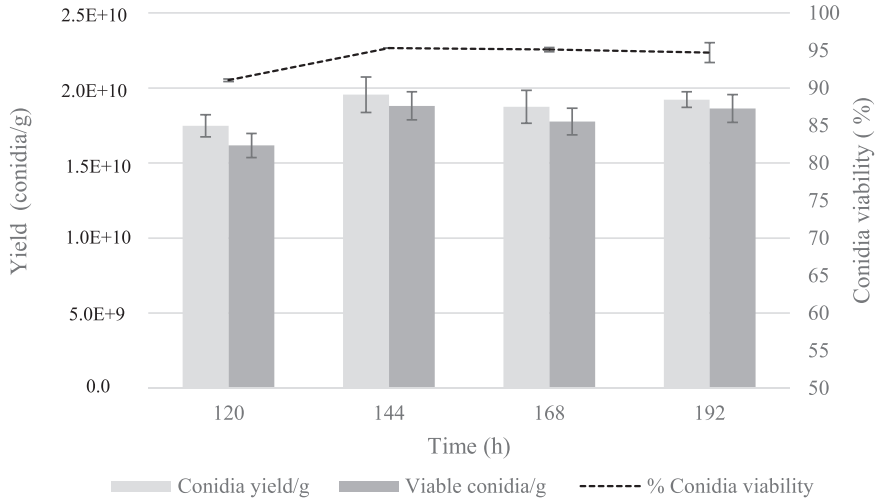

Fig. 5. Conidia production kinetics and spore viability of *Beauveria bassiana* in a packed-column bioreactor with growth conditions set to 26 °C, aeration rate at 0.2 L min^{-1}, 60% of substrate moisture and 10% of palm fiber (PF) in palm kernel cake (PKC) mixture, after multi factorial experiment (optimized conditions).

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bej.2023.109022.

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