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Diversity and enzymatic activity of the microbiota isolated from compost based on restaurant waste and yard trimmings

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Introduction: The bad management of organic waste negatively affects environmental quality and composting has been a viable recycling alternative. Microorganisms are responsible for waste degradation during the composting process and, consequently, for transforming this waste into natural fertilizer. This work aimed to analyze and identify the biodiversity of yeasts and filamentous fungi throughout a composting process based on organic residues under different treatments (commercial inoculum, non-commercial inoculum, and control treatment) and to investigate the enzymatic activity of these microorganisms.

Methods: Microorganisms were isolated and identified from samples at 0, 5, 10, 20, 40, 60, and 120 days. Filamentous fungi were identified according to their macroscopic and microscopic characteristics, and yeasts were identified by sequencing the 18S rDNA region. All identified strains were evaluated for ligninolytic, cellulolytic, hemicellulolytic, amylolytic, pectinolytic, proteolytic, lipolytic, and ammonification. During the composting phases, the filamentous fungi were higher than the yeast population.

Results and discussion: At the beginning of the process, a higher species diversity was observed, and the population of yeasts and filamentous fungi was, on average, 6.50 log CFU g^{-1}. The microbial communities were similar throughout the process in the two inoculated treatments, which showed more significant microbial activity, diversity, and efficiency in the transformation of organic matter, and consequently, advantages in terms of the final product quality compared to the control treatment. The yeasts *Pichia kudriavzevii, Pichia farinosa, Issatchenkia orientalis,* and the filamentous fungi of the genus Aspergillus spp. proved to have high biotechnological value and could be used as starter cultures to accelerate the composting process.

KEYWORDS

DNA sequencing, enzymes, gardening waste, microbiota, molecular techniques, organic residues

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1. Introduction

In the last few decades, the generation of different types of waste has been growing at an accelerated and exponential rate, with an emphasis on solid organic waste, which for the most part is the result of the waste of restaurants, especially those located in large urban centers (Zhang and Sun, 2019). This disposal implies several problems both at a social level–related to public health and world hunger–and an environmental if done improperly (Huang et al., 2022).

The proper treatment and disposal of this waste are essential due to the environmental problems that it can generate (Palaniveloo et al., 2020). Numerous research and studies have been carried out worldwide, aiming to find new methods of treatment or disposal of this waste (Kim et al., 2021; Torrijos et al., 2021; Huang et al., 2022). The composting technologies have stood out as a highly viable and practical approach to treating organic waste since it is a low-cost process whose scope is environmental sustainability (Rempel et al., 2018; Huzairi et al., 2022). Composting involves the transformation of organic matter through the action of various microorganisms and results in natural fertilizers, closing a sustainable cycle (Zhang and Sun, 2019; Huang et al., 2022). Composting is a degradation process that, in addition to microbiological activity, it involves other parameters such as C:N ratio, aeration, and moisture, which determine the proper development of specific groups of microorganisms, which ensure the quality of compost (Chen et al., 2021; Wang et al., 2021). Therefore, everything must be harmonious and proportioned to produce efficient and quality fertilizer (Fan et al., 2018; Torrijos et al., 2021).

Composting occurs with natural microbiota, including bacteria, actinobacteria, yeasts, and filamentous fungi, which are usually involved in the degradation of primary components during aerobic composting (Huzairi et al., 2022). These microorganisms, including yeasts (and fungi), are beneficial in the outcome of compost (Kuznetsova et al., 2022) and knowledge of these at the species level becomes important, as many within these groups have a decisive impact on plant growth promotion and disease suppression (Corato, 2020). The diversity of the microorganisms present throughout the process interferes with compost quality (Palaniveloo et al., 2020; Kim et al., 2021). Therefore, it is essential to monitor and identify the microbial species present in the different phases related to temperature, namely: mesophilic (around 40°C), thermophilic (around 70°C), maturation (around 30°C), and cooling (>20°C) (López-González et al., 2013; Oviedo-Ocaña et al., 2019). The evolution of the microbial population is related to temperature variations and other physical-chemical and biological parameters, such as aeration, humidity, and C: N ratio (Bohacz, 2016; Puspitaloka et al., 2022). The addition of selected inoculants can contribute significantly to the improvement in the degradation of recalcitrant residues (Awasthi et al., 2020) and, consequently, reduce the final time with the acceleration of the process (Onwosi et al., 2017; Liu et al., 2018).

Microorganisms actively degrade residues (Bohacz, 2016; Zhao et al., 2022), and some genera of microorganisms are commonly found in various composting processes, such as *Candida*, *Aspergillus*, *Bacillus*, and *Fusarium* (Di Piazza et al., 2020; Du et al., 2021; Wang Y. et al., 2022). However, the microbial species changes due to several factors and depends on the material to be composted, process conditions, the use or not of starter cultures, and reactors (Niu et al., 2019). Thus, composting is considered an auspicious process at an environmental and biotechnological level since isolated and identified microorganisms can have industrial applications for use as inoculants and have valuable and interesting metabolites and enzymes (López-González et al., 2015a; Zhang and Sun, 2019).

This study aimed to analyze and identify the biodiversity of yeasts and filamentous fungi isolated during restaurant waste-based composting conducted with different treatments [commercial inoculum (CI), non-commercial inoculum (EM) and control treatment] and to investigate the enzymatic activity and biotechnological potential of these microorganisms.

TABLE 1 Initial characterization of residues.

Material	Carbon	Nitrogen	Relation C/N
Garden waste	39	0.6	65/1
Organic waste (RU)	35.16	2.93	12/1

RU, University restaurant.

TABLE 2 Samples collected during the composting process.

Sample	Sampling days	Phase	Temperature (C°)
Control	0	M	37.0
EM	0	M	37.0
CI	0	M	37.0
Control	5	THER	57.9
EM	5	THER	60.3
CI	5	THER	57.5
Control	10	THER	65.4
EM	10	THER	62.8
CI	10	THER	55.1
Control	20	THER	54.1
EM	20	THER	56.2
CI	20	MES	43.3
Control	40	MES	40.8
EM	40	MES	41.9
CI	40	MES	36.2
Control	60	COL	41.7
EM	60	COL	40.7
CI	60	COL	36.6
Control	120	FP	39.5
EM	120	FP	36.3
CI	120	FP	35.5

M, initial mix; THER, thermophilic; MES, mesophilic; COL, cooling; FP, final product.

2. Materials and methods

2.1. Composting and sampling

Composting was performed by mixing organic waste from the University Restaurant (UR) and gardening waste from the Federal University of Lavras–UFLA, Lavras, Minas Gerais, Brazil. The research was conducted from 2017 to 2018 in Lavras, Minas Gerais, Brazil.

The initial physicochemical characterization of both residues was carried out so that the initial C: N ratio was calculated (Table 1) and, consequently, the ideal proportion (30:1) of each residue to be composted to start the process in an ideal and satisfactory way

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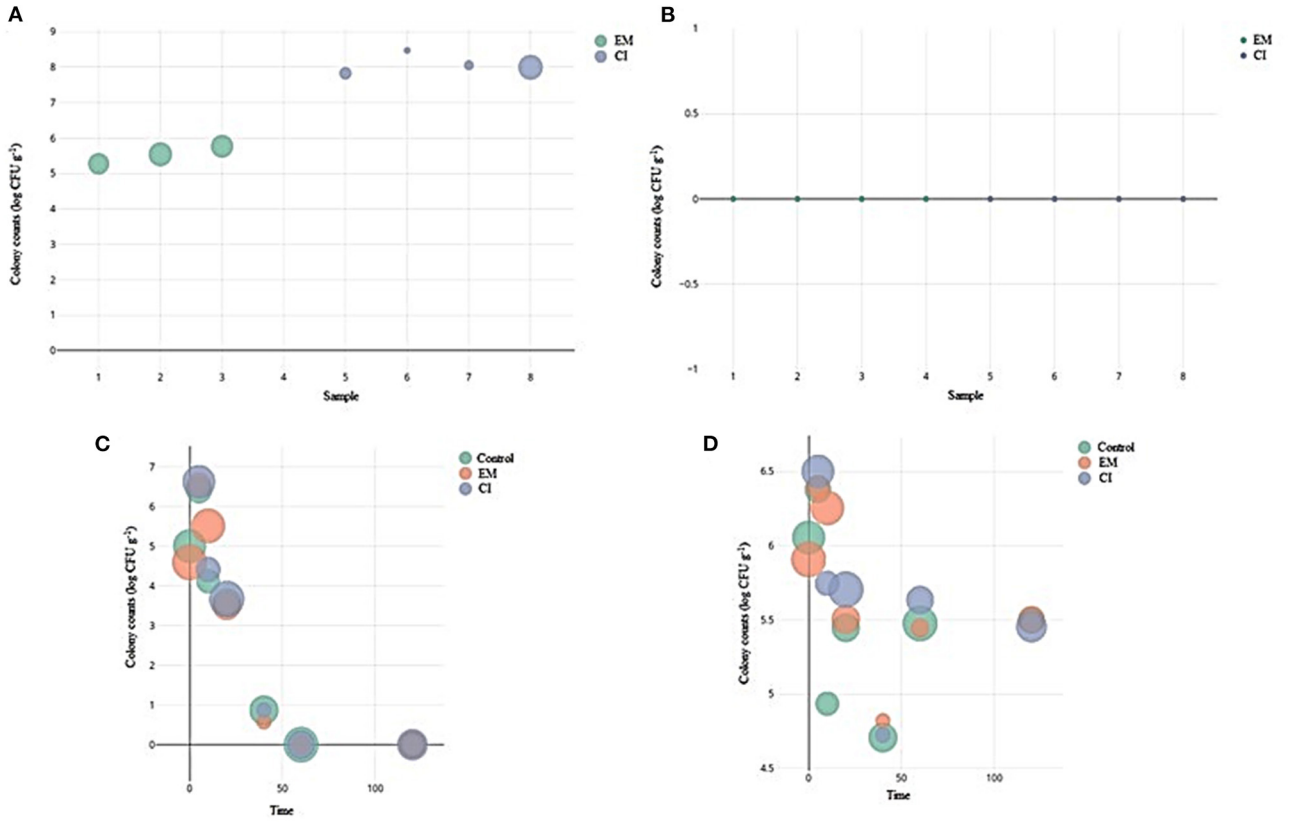

FIGURE 1
Colony counts (log CFU g^{-1}) and relative abundance [(counts of isolates/total counts of groups) × 100] of the isolates present in each treatment throughout the composting process. Bubble sizes (diameters) represent relative abundance (%); a bubble representing the maximum abundance is included as a reference. Each chart shows the isolated population from each inoculum and the groups of microorganisms analyzed in each treatment throughout the composting process. **(A)** Yeast population in the inoculum; **(B)** Filamentous fungi population in the inoculum; **(C)** Yeast for the composting process; **(D)** Filamentous fungi for the composting process.

(Pisa et al., 2020). The waste from the restaurant is composed of leftover rice, beans, vegetables, lettuce leaves, purple cabbage, carrots, and zucchini pieces, among others in smaller quantities. Residues from the landscaping management of the university campus such as leaves from the trees that are swept and residue from tree and lawn pruning were also used to balance de C: N ratio.

The experiment was carried out using two different inoculants: a non-commercial (EM) and a commercial (CI) biological product composed of *Lactobacillus plantarum* 10^4 CFU/mL, *Saccharomyces cerevisiae* 10^3 CFU/mL, sugarcane molasses and water. EM was prepared according to Sharma et al. (2017) by cooking 700 g of unsalted rice in distilled water as a non-specific substrate for capturing different microorganisms in native forests, rich in beneficial microorganisms. After cooking, the rice was distributed in small amounts in a disposable plastic container (500 g), covered with perforated plastic, and placed under the burlap in the forest. Five disposable plastic bottles were distributed in the forest in a radius of 20 meters., for the growth of natural microorganisms, for 15 days, where the temperature and humidity were on average 19°C and 80%, respectively. Then, the microorganisms were deposited in a 20-liter bottle, homogenized with 1 liter of sterile sugar cane juice as a carbon source, and later, the volume of the bottle was filled with distilled water in a ratio of 20:1. The bottle was stored in a ventilated and cool place for 20 days, a period sufficient for multiplication and fermentation of the natural microbiota (Kwon et al., 2011) to obtain the inoculum EM. We did not identify the microbiota present in the inoculum since there was no growth in the culture medium.

The commercial inoculum was prepared and activated according to the manufacturer's instructions technical sheet. The recommended amount of distilled water and commercial sugar was added to the product, and the fermentation took place for 20 days in a conditioned place at room temperature (21–26°C). The compost without the addition of inoculants was used as a control treatment.

The waste was crushed separately into small particles (1–2.5 cm) with the help of a shredder. After being crushed, the organic waste from the UR was added directly to the gardening waste in a proportion of 1:2.5, respectively. Then the wastes were mixed with the help of a tractor, resulting in a homogeneous mixture. The present work used a bioreactor (1 m^3) designed with an automatic air injection system and built with masonry blocks (Supplementary Figure 1). After homogenization, microbial inoculation was applied once, at the beginning of the experiment. In each experimental unit, 5 liters of inoculum (EM or CI) and 15 liters of water were used and properly identified. In the control treatment, only water was added. In the control treatment, only water was added.

Samples were collected based on the changes in the composting phases, which were monitored by temperature measurements at intervals of 0, 5, 10, 20, 40, 60, and 120 days, as seen in Table 2.

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TABLE 3 Yeast present in EM and CI inoculum, quantification, and Genbank access code.

Inoculum	Yeasts	Percentages (%)	Access code at GenBank
EM	*Pichia Farinosa*	44.4	KY104532.1
	Pichia kudriavzevii	22.2	KP171557.1
	Candida tropicalis	14.8	KY981526.1
	Candida inconspicua	11.1	TID31406.1
	Blastobotrys adeninivorans	7.5	KY101745.1
CI	*Pichia kudriavzevii*	40.7	KY911185.1
	Hanseniaspora opuntiae	33.3	KY497945.1
	Wickerhamomyces anomalus	26.0	JQ241273.1

EM, non-commercial inoculum; CI, commercial inoculum.

TABLE 4 Genera and species of microorganisms identified in the composting process in both treatments during the composting process.

Microorganisms	Genera and species
Yeast	*Issatchenkia, Pichia, Candida, Hanseniaspora, Wickerhamomyces, Blastobotrys,* and *Bipolaris*
Filamentous fungi	*Aspergillus niger*, *Aspergillus parasiticus*, *Aspergillus ochraceus*, *Aspergillus oryzae*, *Aspergillus caepitosus*, *Penicillium solitum*, *Rhizopus* and *Cladosporium* spp.

To ensure a representative sample, subsamples were taken from nine distinct locations of each composter, being 3 surface samples, 3 interior samples, and 3 depth samples. The subsamples were mixed in equal amounts for each treatment to obtain a final sample weight of ∼500 grams.

2.2. Isolation and identification of yeasts and filamentous fungi

Twenty-five g of each sample was added to 225 ml of sterile peptone water on an orbital shaker at 120 revolutions per minute (rpm), for 30 min, at room temperature, on average of 25°C (López-González et al., 2013). The samples were mixed in a stomacher at an average speed of the 60's, and 10-fold dilutions were prepared. The microbial population was evaluated using four different culture media. YEPG (Yeast Extract-Peptone-Dextrose) and DRBC (Dichloran Rose Bengal Chloramphenicol) media at 28°C for 48 h for isolating yeasts (Nathaniel et al., 2020) and PDA (Potato Dextrose Agar) and DG18 (Dicloran Glycerol Agar) media at 25°C for 7 days for isolating filamentous fungi (Rempel et al., 2018).

For morphological analysis and grouping of filamentous fungi, the following macroscopic characteristics were observed: (1) color of the colony; (2) mycelium color; (3) color of the back of the petri dish, and (4) absence or presence of streaks. After grouping based on macroscopic characteristics, the fungi were identified based on microscopic characteristics, such as (1) septation of hyphae; (2) shape and size of vesicles and conidia, and (3) length and texture of the stipe observed on fresh slides stained with methylene blue and observed under a microscope. Subsequently, these fungi were picked on specific media according to the genera found. The fungi identified as belonging to the genus *Cladosporium* were picked in MEA medium (in g L-1: 20 g malt extract, 1 g bacteriological peptone, 20 g glucose, 20 g agar) and incubated at 25°C for 7 days. The fungi identified as the genera *Aspergillus* and *Penicillium* were picked in CYA medium (in g L-1: 1g K_2HPO_4, 10mL, Czapek concentrate, 5 g yeast extract, 1 mL metallic solution, 20 g agar) and incubated with MEA at 25°C and 37°C for 7 days.

2.3. Phenotypic identification of filamentous fungi

The identification based on the microscopic characteristics of the filamentous fungi of the Aspergillus, Penicillium, and Cladosporium species was based on the keys proposed by Klich (2002), Bensch et al. (2012), and Visagie et al. (2014), respectively, and the analyzes were conducted in the laboratory of mycology and mycotoxins of the Department of Food Science of UFLA.

After incubation, the isolates were further classified according to their macroscopic and microscopic characteristics. For taxonomic identification at the species level, identification keys for the genera *Aspergillus* and *Penicillium* and the descriptions of the genus *Cladosporium* by Bensch et al. (2012) were used.

2.4. Identification of yeasts by sequencing of the 18S rDNA region

For the extraction of yeast DNA, colonies were suspended in 500 μL of sterile ultrapure water, and the DNA was extracted using the QIAamp DNA Micro Kit (Qiagen, Germany). For amplification, PCR was performed in a final reaction volume of 25 μL, which contained: 17.2 μL of sterile MilliQ water, 5 μL of MasterMix 5X buffer (Promega), 0.5 μL of dNTP, 0.5 μL of each primer: ITS1 (5' TCCGTAGGTGAACCTGCGG 3') and ITS4 (5' TCCTCCGCTTATTGATATGC 3'), 0.1 μL Taq DNA polymerase (Invitrogen), 0.25 μL MgCl2 and 1 μL DNA. The thermal cycler was programmed for an initial denaturation of 5 min at 95°C, followed by 35 steps of denaturation at 95°C for 1 minute, primer annealing at 55°C for 1 min, and DNA strand extension at 72°C for 1 min. The final extension took place at 72°C for 7 min and the storage temperature was 4°C.

PCR products were analyzed for purity and subjected to denaturing gradient gel electrophoresis (DGGE) using the vertical DCode system (Bio-Rad). The PCR products were applied in 10 μL aliquots, added with 10 μL of running dye (2% bromophenol blue, 2% xylene cyanol, glycerol, and MilliQ water) on a polyacrylamide gel (8% w/v) in TAE buffer (0.5X) with denaturation gradient ranging from 40 to 60% (100% corresponding to 7 M urea and 40% (v/v) formamide). Electrophoresis was carried out at a constant voltage of 150 V for 4 hours, with a constant temperature of 60°C. After electrophoresis, the gels were stained with SYBR-Green I (Molecular Probes1:10,000 v/v), and the image was captured using a transilluminator. Sequencing was performed based on the DGGE bands, where the different and constant bands were excised with

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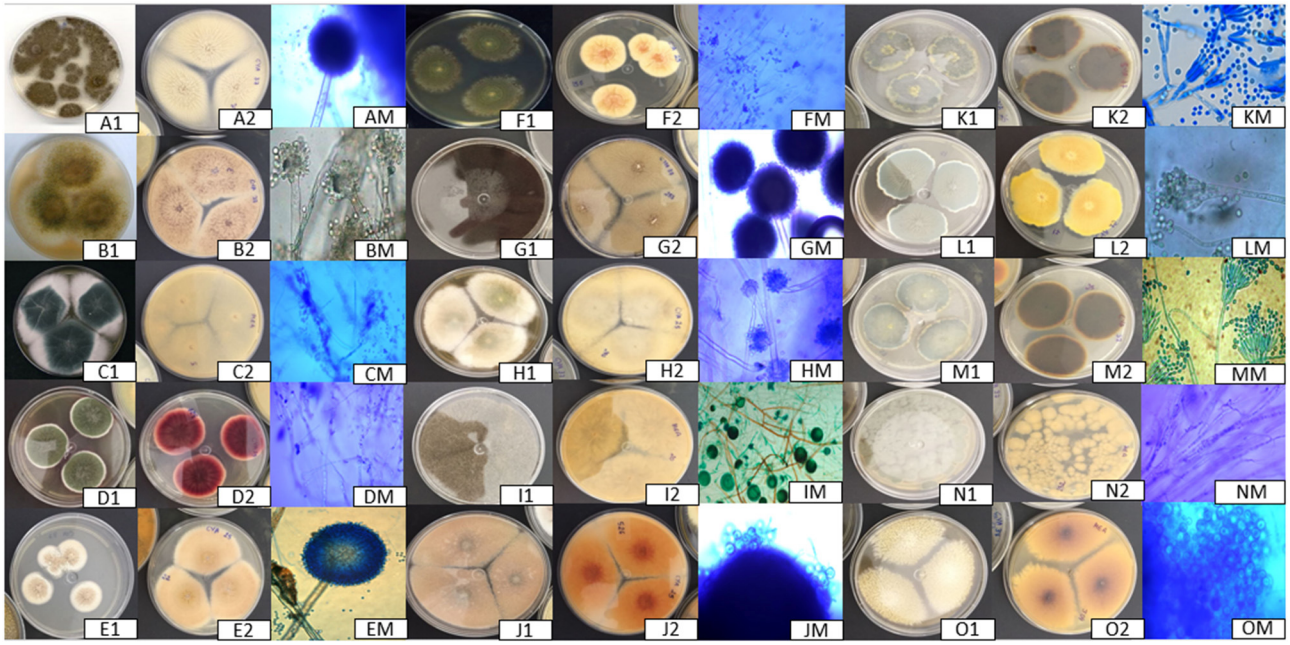

FIGURE 2
Photographs of isolated filamentous fungi. 1, Front view of the colony; 2, Back view of the colony; M, Microscopy image. A, *Aspergillus tubigiensis*; B, *Aspergillus oryzae*; C, *Aspergillus fumigatus*; D, *Aspergillus* sp.; E, *Aspergillus ochraceus*; F, *Aspergillus caepitosus*; G, *Aspergillus niger*; H, *Aspergillus parasiticus*; I, *Rhizopus*; J, *Emericella* sp.; K, *Penicillium citrinum*; L, *Penicillium solitum*; M, *Penicillium implicatum*; N, *Penicillium* sp.; O, *Cladosporium*.

the aid of a sterile blade, and the DNA was eluted in sterile ultrapure water. The DNA was reamplified in PCR under the same conditions as above. The products of the second PCR were sequenced by Macrogen and the sequences obtained were aligned with the GenBank database, with the aid of the BLAST software (National Center for Biotechnology Information, Maryland, USA). Multiple sequence alignments were performed using the MEGA-X software with the Clustal-W package. The Hasegawa-Kisinho-Yano nucleotide substitution method was used with a gamma distribution. The maximum Likelihood Tree test and the bootstrap method with 500 bootstrap replications were used for the phylogenetic analysis.

2.5. Enzymatic activity analyses

A total of 318 yeast and 41 filamentous fungus isolates were used in the assay for the activity of eight different enzymes, specifically ligninolytic (Widiastuti and Wulaningtyas, 2008), cellulolytic and hemicellulolytic (Kasana et al., 2008), amylolytic (Rempel et al., 2018), and pectinolytic (Ferreira et al., 2013), proteolytic (Scatamburlo et al., 2015), lipolytic (Fuciños et al., 2005) and ammonification (Jana and Roy, 1985) activities. The tests were performed on Petri dishes with solid media containing the appropriate substrate for each enzyme. Each yeast isolate (incubated in YEPG medium at 28°C for 48 hours) was normalized to a concentration of 10^7 cells/mL in 3 mL of sterile saline (0.9% NaCl), according to the McFarland scale. Petri dishes for each enzyme to be evaluated were inoculated with 10 μL aliquots of the yeast cells at 10^7. For the filamentous fungi, 6-mm diameter disks from a colony grown for 3 days on MA medium were placed in the center of the Petri dishes. The inoculated media were then incubated at 28 and 25°C for the yeasts and filamentous fungi.

Iodine solution was used to confirm the cellulolytic, hemicellulolytic amylolytic, and the results were classified based on the presence or absence of a clear halo around the yeast and filamentous fungi colonies. Discoloration around the colonies in the presence of Azure-B was used for assessing ligninolytic activity. A solution of 1% hexadecyltrimethylammonium bromide was used for the pectinolytic activity. The lipolytic activity was evaluated by forming a clear, crystallized halo around the colonies, and ammonification activity was assessed based on the formation of a pink halo. Extracellular protease production (Strauss et al., 2001) was determined after 5 days at 30°C in a YPD medium by the presence of a clear zone around the colony.

The enzymatic index (EI) was determined using Equation 1 (Eq 1), which is the ratio of the mean diameter of the degradation halo to that of the colony (Hankin and Anagnostakis, 1975).

$$EI = \frac{mean\ diameter\ of\ the\ degradation\ halo}{\text{mean diameter of the colony}} \quad (1)$$

The isolates exhibiting the highest EI (>2.0) had the highest extracellular enzymatic activity (Oliveira et al., 2012).

2.6. Experimental design

The experimental design was completely randomized; the entire experiment was conducted in triplicate. The efficiency of the composting process was statistically proven and conducted according to an already published article with physicochemical data, by Gaspar et al. (2022).

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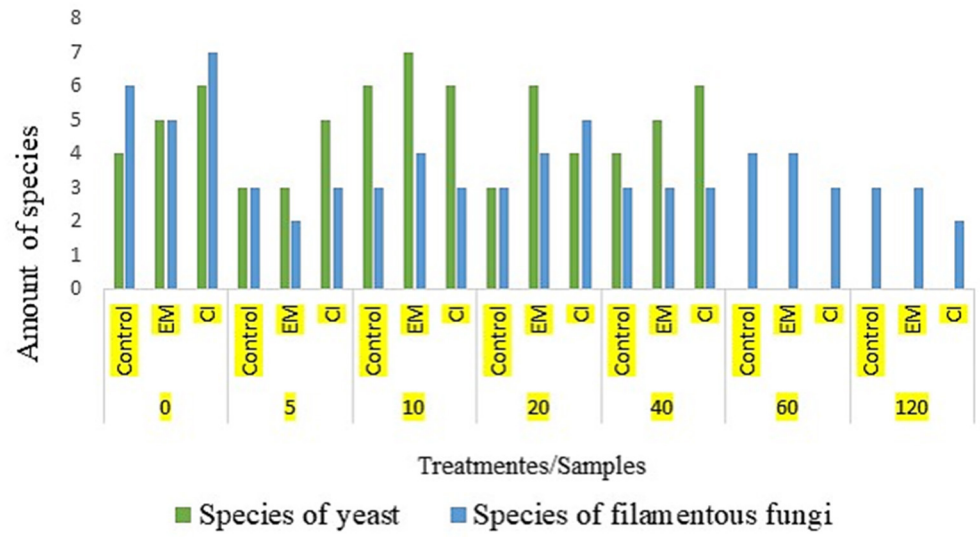

FIGURE 3
Species richness in each phase and each treatment.

3. Results and discussion

3.1. Population dynamics of yeast and filamentous fungi

The population of yeasts and filamentous fungi in the inoculum was evaluated before being added to the compost. The number of yeasts in the EM inoculum was approximately 5.53 log CFU g^{-1}, smaller than the 8.09 log CFU g^{-1} population in the CI inoculum (Figure 1A). However, filamentous fungi were not observed in any inoculum evaluated (Figure 1B). The yeasts found in each inoculum were sequenced, identified, and presented in Table 3, together with their respective quantifications in each inoculum.

Monitoring the different microbial populations present throughout the composting process is extremely important to determine the rate of particle biodegradation and the quality of the final product (Jurado M. et al., 2014; Oviedo-Ocaña et al., 2019). After the addition of inoculants in the composters and over the time of the composting process, it was observed that the total yeast population decreased during the process and became null in the cooling and maturation phases, with the highest values of diversity and sizes obtained during the thermophilic phase (Table 5). In the thermophilic phase, the yeast population reached 6.4, 6.56, and 6.63 log CFU g^{-1} for the control, EM, and CI treatments, respectively (Figure 1C). Different yeast species are typically associated with the beginning of the composting process due to the environmental factors favorable to these microorganisms, such as the high availability of O_2 and low pH (Langarica-Fuentes et al., 2014; Nakasaki and Hirai, 2017). The population of filamentous fungi was higher than the yeasts at the beginning of the process, mainly during the thermophilic phase. During this phase, a larger filamentous fungi population with 6.38, 6.39, and 6.50 log CFU g^{-1} in control, EM, and CI treatments, respectively (Figure 1D), may be present because these microorganisms produce spores, and consequently are more resistant to high temperatures (Nakasaki and Hirai, 2017; Wang Y. et al., 2022). During the cooling and maturation phases, the population of filamentous fungi increased and remained constant, which may result from favorable environmental factors and waste degradation (Meng et al., 2018; Huzairi et al., 2022).

3.2. Population dynamics of microorganisms

Several yeasts and filamentous fungi were identified throughout the composting process in all treatments and are shown in Table 4 and Figure 2.

At the beginning of the composting process, a substantial number of isolates and species diversity were observed in both yeast and filamentous fungi in the CI treatment. However, throughout the process, this number was higher and constant in the EM treatment, showing higher microbial activity and efficiency in the transformation of organic matter in the EM and IC treatments compared to the control treatment (Figure 3).

Inoculation of compost with starter cultures has several benefits related to microbial diversity, a reduction in composting time, and the highest and fastest rate of particle degradation (Manu et al., 2017; Jia et al., 2021; Huzairi et al., 2022). Yeasts perform essential roles in the composting process, *P. kudriavzevii*, for example, acts on the degradation of organic acids, which consequently causes an increase in pH and promotes bacterial growth that contributes significantly to the waste degradation (Ngoc et al., 2015). In general, filamentous fungi are considered fundamental in the degradation of recalcitrant fractions of agricultural residues to be composted (Sundberg et al., 2011; Huzairi et al., 2022).

Although some studies have shown that microbial inoculation can improve composting, mainly by reducing the processing time, increasing the enzymatic activity, and several other factors can affect the process (Gou et al., 2017; Singh et al., 2021), some microorganisms naturally present in the initial residues can

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TABLE 5 Persistent microorganisms isolated during different phases of the composting process.

Treatment	Species	Composting phases						Enzymatic activity							
		M	MES	TER	COL	MAT	FP	LIG	CEL	HEM	AMY	PRO	PEC	LIP	AMM
Yeasts															
Control	*I. orientalis*	+	+	+	-	-	-	+	-	-	+	-	-	-	-
	P. farinosa	-	-	+	-	-	-	-	-	+	-	+	-	+	+
	B. sorokiniana	-	-	+	-	-	-	-	-	-	-	-	-	-	-
	C. tropicalis	-	-	+	-	-	-	+	+	-	-	-	-	+	+
	B. raffinosifermentans	-	-	-	+	-	-	-	-	-	-	-	-	-	-
	B. adeninivorans	-	+	+	-	-	-	-	-	-	-	-	-	-	-
	P. kudriavzevii	-	+	+	+	-	-	-	-	-	-	-	-	-	-
EM	*B. raffinosifermentans*	-	-	-	+	-	-	-	-	-	-	-	-	-	-
	B. adeninivorans	-	+	+	-	-	-	-	-	-	-	-	-	-	-
	P. farinosa	+	+	+	-	-	-	-	-	+	-	+	-	+	+
	I. orientalis	+	+	+	-	-	-	+	-	-	+	-	-	-	-
	K. marxianus	-	-	+	-	-	-	-	-	-	-	-	-	-	-
	H. opuntiae	-	-	+	-	-	-	-	-	-	-	-	-	-	+
	W. anomalus	-	-	+	-	-	-	-	-	-	-	-	-	-	+
CI	*P. fermentans*	-	+	-	-	-	-	-	-	-	-	-	-	-	-
	P. kudriavzevii	-	+	+	+	-	-	-	-	-	-	-	-	-	-
	I. orientalis	+	+	+	-	-	-	+	-	-	+	-	-	-	-
	C. tropicalis	+	+	+	-	-	-	+	+	-	-	-	-	+	+
	P. farinosa	-	-	+	-	-	-	-	-	+	-	+	-	+	+
	W. anomalus	-	-	+	-	-	-	-	-	-	-	-	-	-	+
Fungi															
Control	*Aspergillus* sp.	+	+	+	+	+	+	-	+	-	-	-	+	-	+
	P. citrina	+	+	-	-	-	-	-	+	-	-	-	+	-	-
	M. sterilia	+	+	-	-	-	-	-	-	-	-	+	-	-	-

(Continued)

TABLE 5 (Continued)

Treatment	Species	Composting phases						Enzymatic activity							
		M	MES	TER	COL	MAT	FP	LIG	CEL	HEM	AMY	PRO	PEC	LIP	AMM
	P. solitum	+	+	-	-	-	-	-	+	-	-	+	+	-	+
	Rhizopus sp.	+	+	-	-	+	-	-	+	-	-	-	+	-	-
	Penicillium (sexual phase)	+	+	-	-	-	-	-	+	-	-	-	+	-	-
	A. caepitosus	-	-	+	-	-	+	-	+	-	-	-	+	-	+
	Emericella sp.	-	-	-	+	+	-	-	+		-	-	+	-	+
EM	*Aspergillus* sp.	-	-	+	+	+	-	-	+	-	-	-	+	-	+
	A. oryzae	+	+	+	+	+	+	-	+	-	-	+	+	-	+
	Aspergillus (sexual phase)	+	+	-	-	+	-	-	-	-	-	-	-	-	-
	A. fumigatus	+	+	+	+	-	+	-	+	-	-	-	-	-	+
	Rhizopus sp.	+	+	+	+	+	+	-	+	-	-	-	+	-	-
	Aspergillus sp.	-	-	+	-	-	-	-	+	-	-	-	+	-	+
	Aspergillus niger	-	-	+	+	-	+	-	+	-	-	+	+	-	+
CI	*A. ochraceus*	+	+	+	-	-	+	-	+	-	-	-	+	-	+
	P. implicatum	+	+	-	+	-	-	-	-	-	-	-	-	-	-
	Penicillium (sexual phase)	+	+	-	-	-	+	-	+	-	-	-	+	-	-
	A. fumigatus	-	-	+	-	-	-	-	+	-	-	-	-	-	+
	A. parasiticus	-	-	+	-	-	-	-	+	-	-	-	+	-	+

EM, non-commercial inoculum; CI: commercial inoculum.
Phases in which an isolate was detected; M, initial mixture; MES, mesophylic; TER, thermophilic; COL, cooling; MAT, maturation; FP, final product.
LIG, Ligninolytic; CEL, Cellulolytic; HEM, Hemicellulolytic; AMY, Amylolytic; PRO, Proteolytic; PEC, Pectinolytic; LIP, Lipolytic; AMM, ammonification.

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TABLE 6 Enzymatic activities and their respective culture media, incubation times and development mode.

Activity	Medium (g×L^{-1})	IT(days)	Revelation	Author
Lignolytic	1 g KH_2PO_4; 0.5 g $C_4H_{12}N_2O_6$; 0.5 g $MgSO_{40.7}H_2O$; 0.01$CaCl_{20.2}H_2O$; 0.01 g yeast extract; 0.001 g $CuSO_{40.5}H_2O$; 0.001 g $Fe_2(SO_4)_3$; 0.001 g $MnSO_{40.4}H_2O$; 0.1 g Azure-B; 20 g agar.	7	Discoloration around colony grown with Azure-B	(Widiastuti and Wulaningtyas, 2008)
Cellulolytic	2 g carboxymethylcellulose; 2 g $NaNO_3$; 0.5 g $MgSO_4$; 1 g K_2HPO_4; 0.5 g KCl; 0.2 g peptone; 20 g agar	7	iodine solution	(Kasana et al., 2008)
Hemicellulolytic	6.7 g YNB; 10 g xylan; 20 g agar	5	Iodine solution	(Kasana et al., 2008)
Amylolytic	10 g peptone, 10 g yeast extract, 20 g soluble acid, 20 g agar	3	Iodine solution	(Rempel et al., 2018)
Pectinolytic	5 g glucose, 5 g polygalacturonic acid, 6 g KH_2PO_4; 1 g yeast extract; 2 g $(NH_4)SO_4$; 15 g agar + 1 ml/1L ($FeSO_4$; $MgSO_4$; $CaCl_2$; H_3BO_3; $MnSO_4$; $ZnSO_{40.7}H_2O$; $CuSO_{40.5}H_2O$; MoO_3)	5	Bromide solution hexadecyltrimethylammonium 1%	(Ferreira et al., 2013)
Proteolytic	10 g yeast extract; 20 g glucose; 20 g peptone; 20 g agar; 20 g casein	3	Clear halo around the colony	(Strauss et al., 2001)
Lipolytic	10 g peptone; 5 g NaCl; 0.01 $CaCl_2$; 20 g agar	5	Clear and crystallized halo	(Fuciños et al., 2005)
Ammonification	CYA + 10 g asparagine; 0.09 g phenol red	15	rosy coloring halo	(Jana and Roy, 1985)

be competitive or resistant to different environmental changes (López-González et al., 2015b; Huzairi et al., 2022). Nakasaki and Hirai (2017) observed that, despite stimulating the naturally occurring microbiota, the use of *P. kudriavzevii* as an organic waste compost inoculant did not positively influence the quality of the final compost.

3.3. Identification of yeast and filamentous fungi species

The criteria used to select for persistent microorganisms in each treatment was the detection of the same specie at 2 or more sampling times during the composting process because it shows that he appeared more than once and in different phases. Table 5 shows the yeast and filamentous fungi species identified from each treatment.

Among the yeast species isolated from the compound inoculated with efficient microorganisms (EM) and commercial inoculum (CI), the genus *Pichia* corresponded to 66.6 and 40%, respectively. While the genera *Candida* and *Blastobotrys* were exclusive to treatments inoculated with EM, with 25.9 and 7.5% respectively. On the other hand, the genera *Hanseniaspora* and *Wickerhanomyces* corresponded to 33.3 and 26% of the compost with commercial inoculum (CI). Among the species of filamentous fungi, those of the genus *Aspergillus* were the most frequent, also occurring in the presence of the genera *Penicillium*, *Rhizopus,* and *Cladosporium*. In the control compost, the appearance of yeasts *I. orientalis* and *P. kudriavzevii* was constant between the composting phases, as well as filamentous fungi of the genus *Aspergillus* and *Penicillium*.

Each composting process has unique characteristics (Niu et al., 2019), and some yeast species, such as *Blastobotrys adeninivorans*, *Blastobotrys raffinosifermentans,* and *Bipolaris sorokiniana*, have not been previously reported in other composting processes. As an organic compost, several microorganisms, even phytopathogenic organisms, may be present in the early stages, such as *B. sorokiniana* (Song et al., 2018; Puspitaloka et al., 2022). However, this yeast was found only in the control treatment and during the thermophilic phase (Table 5), after which it was eliminated in the final composting stages.

The identified filamentous fungi mainly represented the *Aspergillus*, were present in both treatments and predominated at all sampling times (Table 5). This genus of filamentous fungi produces spores that are resistant to high temperatures (Wang S.-K. et al., 2022), and although it was widely detected throughout the composting process, it may not have participated in all stages since what could be present were the spores. In a previous study on composting organic waste, species of the genus *Aspergillus* were also detected (Zhao et al., 2017; Roca-Barcelo et al., 2020). *Aspergillus* and *Penicillium* species were detected during the composting of wheat straw for mushroom production (Zhang et al., 2014). *Penicillium* species were detected in composting from white wine distillers' grains (Yu et al., 2021). In composting organic waste, Di Piazza et al. (2020) reported having identified species of *Penicillium* spp., *Rhizopus* spp., and *Aspergillus* spp. Different fungi species have been isolated from different composting methods, including *A. fumigatus, Chaetomonium thermophilum, Humicola grisea, Humicola insolens, Mucor pusilus, Talaromyces duponti, Scytalidium thermophilium* (Yim et al., 2019). Species of the genus *Rhizopus* spp. were also reported by Huzairi et al. (2022). Additionally, filamentous fungi belonging to the genus *Aspergillus* have been detected during composting with bovine manure and construction wastes (Holman et al., 2016).

The genera *Candida* and the species *A. fumigatus* have also been detected during lignocellulosic material compost (Silva et al., 2009; Jurado M. et al., 2014). *P. farinosa* was the most representative yeast, as it was identified during the thermophilic phase of all the treatments (Table 5) and in the EM inoculum. When analyzing the lignocellulosic composting process, López-González et al. (2015a)

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TABLE 7 Enzymatic activities of yeasts and fungi.

Enzyme activity	Microorganism	EI[a]
Yeasts		
Ligninolytic	*I. orientalis*	4.2
	I. orientalis	4.0
	I. orientalis	2.0
Cellulolytic	*C. tropicalis*	4.7
	B. raffinosifermentans	2.0
Hemicellulolytic	*P. farinosa*	2.0
	B. adeninivorans	2.0
Proteolytic	*P. farinosa*	4.5
	P. kudriavzevii	4.0
	P. kudriavzevii	3.7
Lipolytic	*C. tropicalis*	2.1
	P. farinosa	3.3
	C. tropicalis	2.0
	C. tropicalis	2.1
	C. tropicalis	2.0
	C. tropicalis	2.0
Ammonification	*C. tropicalis*	2.4
	C. tropicalis	2.0
	C. tropicalis	2.8
Fungi		
Cellulolytic	*Aspergillus sp. (sexual phase)*	2.0
	Rhizopus sp.	2.7
	P. citrinum	2.0
	Rhizopus sp.	2,3
	A. ochraceus	2.2
	P. implicatum	2.2
	Aspergillus sp.	2.0
	A. caepitosus	3.0
	Aspergillus sp.	2.3
	A. parasiticus	2.3
	Aspergillus. sp.	5.0
	Aspergillus sp.	2.6
	Aspergillus sp.	3.4

[a]EI, Enzymatic index.

identified the yeast species *Candida railenenses*, *Candida sequanensis*, *Pichia fermentans* and the filamentous fungi species *A. fumigatus*, *P. solitum,* and *P. citrinum* during the early composting phases.

In metaproteomic studies with two types of raw materials prepared by mixing commercial rabbit food with cooked rice at ratios of 7:3 and 5:5, the most abundant genera were *Saccharomyces*, *Candida,* and *Schizosaccharomyces;* species of the genera *Kluyveromyces, Pichia*, and *Aspergillus* were also detected (Nakasaki and Hirai, 2017).

Alternaria, Aspergillus, Candida, Cladospodium, Fusarium, Gibellulopsis, Ochrocladosporium, Penicillium, Plectosphaerella, and *Scopulariopsis* were also isolated previously by Jurado M. et al. (2014).

3.4. Characterization of the enzymatic activity of the identified organisms

Microbial enzymes are essential in the composting process (Huzairi et al., 2022), and according to Di Piazza et al. (2020), the mesophilic phase of the composting process is characterized by the most intense metabolic activity and the highest enzyme synthesis.

All microorganisms in the study were tested for enzyme activities and those with EI >2.0 were described and correlated with the taxonomy in Table 5.

The isolated yeasts showed a higher diversity of enzymatic activity compared to filamentous fungi among the inoculated treatments during the initial 40 days of the composting process. Among the yeasts, 7.84% have the ligninolytic capacity, 5.88% cellulolytic, 11.80% hemicellulolytic, 9.80% amylolytic, 5.88% proteolytic, 35.3% lipolytic, and 23.50% ammonification capacity.

At the end of composting, filamentous fungi predominated and yeasts were found to be absent in the culture medium. None of the isolated filamentous fungi exhibited the lipolytic, hemicellulolytic, amylolytic, or lipolytic activities tested. The filamentous fungi showed 33.0% cellulolytic, 31.90% pectinolytic, 28.60% ammonification, and 6.50% proteolytic capacity.

Many species showed some enzymatic activity, but only a few were potential enzyme producers, such as yeast species of the *Pichia* and *Issatchenkia* genera, with an enzymatic index of lignolytic, cellulolytic and proteolytic activities >4.0, and filamentous fungi of the genus *Aspergillus*, with an enzymatic index for cellulolytic activity reaching 5.0. Among isolates of the same species, differences in enzyme production were found, showing different biotechnological potentials and differences in the same species. The isolates with high potential for enzyme production (i.e., EI > 2.0) are presented in Table 6. *Issatchenkia orientalis* was the yeast species with the highest enzymatic potential for ligninolytic activity (EI = 4.2). Regarding the cellulolytic and amylolytic activities, *C. tropicalis* stood out, with EIs of 4.7 and 2.8, respectively. *P. farinosa* presented EIs of 2.0, 4.5, and 3.3 for hemicellulolytic, proteolytic, and lipolytic activities, respectively. *Aspergillus* spp. presented high enzymatic potentials for cellulolytic activity with EIs = 5.0. Thus, the results obtained allow us to identify the *I. orientalis*, *C. tropicalis*, *P. farinosa,* and *Aspergillus* spp. isolates as promising enzyme producers (Table 7).

In composting, enzymes are important because the biotransformation of organic materials occurs under the action of the enzymatic activity of the microorganisms that can improve and accelerate the degradation processes, directly reflecting on the final product (Zhang and Sun, 2016; Chen et al., 2021). Applying compost with a microorganism with good enzymatic activity can help improve soil fertility (Huzairi et al., 2022). In this sense, the presence of enzyme-producing microorganisms in composting is essential for the degradation of solid waste and, subsequently, when applied in the form of compost, for maintaining soil dynamics and in cycles of nitrogen, carbon, or phosphorus (Deus et al., 2017;

Zhang et al., 2021). *I. orientalis* strains can hydrolyze phytates in the soil, which improves the absorption of phosphorus by plants and, consequently, reduces the concentration of fertilizers added to the soil (Mezeli et al., 2017). Species of the *Aspergillus* genus can degrade residual herbicides that are extremely dangerous to the environment (Sondhia et al., 2016).

Composting has stood out among the most viable alternatives for the use of waste because it is a sustainable technology that adds value to organic waste (Zhang et al., 2021). Besides, the final product allows for the recycling of organic waste—however, the type of waste in composting changes the microbiota present in each process (Bohacz, 2016; Torrijos et al., 2021; Huzairi et al., 2022).

In general, isolates that present some biotechnologically relevant enzymatic activity has a range of metabolic activities and physiological capabilities (i.e., such as the production of enzymes and improvements in the composting process, including shorter processing time) that make them potentially suitable for a wide variety of applications (Jurado M. M. et al., 2014; Sajid et al., 2022).

4. Conclusions

Several species of yeasts and filamentous fungi act in the process of organic waste degradation, and the use of inoculants during the composting process directly affects microbiological diversity. The microbial communities were similar throughout the process in the two inoculated treatments, which showed more significant microbial activity, diversity, and efficiency in the transformation of organic matter, and consequently, advantages in terms of the final product quality compared to the control treatment.

The microorganisms with the highest enzymatic indices were *Issatchenkia orientalis*, *Candida tropicalis*, *Pichia farinosa*, and *Aspergillus* spp. The yeast species *Pichia kudriavzevii*, *Pichia farinosa*, *Issatchenkia orientalis*, and the filamentous fungi of the genus *Aspergillus* spp., proved to be persistent and with enzymatic activities of high biotechnological value. Although each restaurant has varied organic residues, the good values obtained in this study show that these results can be extended to other contexts. For example, the persistent species with high enzymatic indexes identified in this study could be cultivated in the laboratory to formulate a possible cocktail to be used as an inoculant and accelerator of the composting process, with initial populations of 9.0 log UFC g-1 for process efficiency and viability.

In general, yeast and filamentous fungi isolates exhibit metabolic capabilities that can have biotechnological applications, and this study reaffirms the potential of composting as a source of biotechnologically relevant microorganisms.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

Author contributions

SS: conceptualization, data curation, investigation, methodology, project administration, validation, visualization, roles, and writing-original draft. LA: conceptualization, investigation, and methodology. MPe: formal analysis and methodology. MPr: investigation. VB and GM: data curation, software, and writing-review and editing. VB, RS, and MPa: conceptualization, formal analysis, funding acquisition, methodology, project administration, resources, supervision, validation, visualization, roles, writing-original draft, and writing-review and editing. JD, MPa, ER, and RC: conceptualization, funding acquisition, methodology, project administration, resources, and supervision. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2023.1013361/full#supplementary-material

SUPPLEMENTARY FIGURE 1
(A–C) Graphical representations of masonry composers. **(D)** Installation of the experiment. Source **(A–C)**: Passos (2014).

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